

ALMA MATER STUDIORUM
UNIVERSITA' DI BOLOGNA

SCUOLA DI SCIENZE

Corso di Laurea Magistrale in
BIOLOGIA MARINA

**A first survey on the status of the *Posidonia oceanica* meadow
in Calpe Bay (Alicante, Spain)**

Tesi di laurea in
Alterazione e conservazione degli habitat marini

Relatore:

Prof. MARCO ABBIATI

Presentata da:

MARTINA SCANU

Correlatore

Dott. JOSE RAFAEL GARCIA-MARCH

Anno Accademico 2016/2017

SUMMARY

- 1. Introduction.....3**
 - 1.1 Evolutionary history.....3
 - 1.2 Habitat distribution.....4
 - 1.3 The plant.....5
 - 1.4 Ecology.....8
 - 1.5 Structure of meadows.....9
 - 1.6 The role of *Posidonia oceanica* meadows in coastal systems.....9
 - 1.6.1 Ecological role.....9
 - 1.6.2 Physical role.....12
 - 1.6.3 Economical role.....12
 - 1.6.4 Bioindicator role.....13
 - 1.7 The causes of regression.....16
 - 1.8 Policies applying to *Posidonia oceanica* meadows.....18
- 2. Work prupose.....21**
- 3. Materials and methods.....22**
 - 3.1 Study area.....22
 - 3.1.2 Geological context.....23
 - 3.1.3 Marine context.....24
 - 3.2 Physical descriptors.....25
 - 3.2.1 Water conditions.....25
 - 3.2.2 Sediment analysis.....26
 - 3.3 Monitoring *Posidonia oceanica*.....29
 - 3.3.1 Statistical analysis.....33

4. Results	34
4.1 Physical descriptors.....	34
4.1.1 Water conditions.....	34
4.1.2 Sediment analysis.....	38
4.2 <i>P. oceanica</i> monitoring results.....	44
5. Discussion	53
6. Conclusion	60
7. References	59

1. Introduction

Posidonia oceanica (Linnaeus) Delile, (1813) is a marine plant, with roots, vascular trunks, leaves and flowers. The name “Posidonia” was put in honor of Poseidon, god of the sea, while the Latin name “oceanica” was given by Linnaeus, however is misleading since this species is not found in the Atlantic ocean (Cabioch et al., 1995).

Taxonomic classification	
Empire	Eukaryota
Kingdom	Plantae
Phylum	Tracheophyta
Subphylum	Euphyllophytina
Infraphylum	Spermatophytae
Superclass	Angiospermae
Class	Monocots
Order	Alismatales
Family	Posidoniaceae
Genus	Posidonia
Species	oceanica

Table 1.1 Taxonomic classification of *P. oceanica*

1.1 Evolutionary history

About 120-110 million years (Ma) ago, in the Cretaceous period, some continental angiosperms, the Phanerogams, returned to the marine environment. Further back in time, about 475 Ma ago, in the Ordovician (Primary era), their distant ancestors had left the marine environment to colonize the continents (Wellman et al., 2003).

By the number of their species in today’s natural environment, marine angiosperms represent rather a small group: 13 genera and 60 species (Kuo et al., 2011). For purposes of comparison, there are 234 000 angiosperms species (almost all continental) and about ~2.2 million (60.18 million SE) marine species of fauna and flora together (Fredj et al., 1992, Heip et al., 1998, Mora et al., 2011). There are three hypothesis that could explain this big difference in the marine and terrestrial number: (1) the predominance of vegetative reproduction compared to the sexual one for marine angiosperms, excludes genetic recombination and turn off the evolutionary motor (Romero 2004). (2) The absence of mutualistic symbiosis with insects for pollination reduces the speciation events (Romero 2004). (3) Lastly, the competitive advantage the marine angiosperms have over other marine primary producers is so great that competition has not driven evolution (Boudouresque 2012).

However, although the marine angiosperms are not numerous, their ecological role is considerable in coastal environments: many of them are ecosystem engineers or at least key species. The ecosystems they build up or in which they are the major actors, play an essential role in many parts of the world. This is true for the Mediterranean sea (Boudouresque 2012), where in addition to *P. oceanica* there are other species of seagrasses whose (Table 1.2).

Species	Distribution	Habitat	Maximum depth (m)	Rhizome diameter (mm)	Leaf length (cm)	Leaf width (cm)	Hypogea biomass (gdw m ⁻²)
<i>Posidonia oceanica</i>	Mediterranean Sea, stops at the border line where Mediterranean and Atlantic waters mix	Common on sandy bottoms, is often found on rock and coralline	50	10	115	1.2	6526
<i>Cymodocea nodosa</i>	Widely distributed throughout the Mediterranean, around the Canary Islands and down the North African coast	Common on sandy bottoms, it prefers calm hydrodynamic environments	40	2	40	0.36	324
<i>Zostera marina</i>	Limited and pointform; reported in Italy	Common on sandy and muddy bottoms, it suits low-salt waters, low tolerance to desiccation	7	3.5	60	0.6	Min 21 Max 161
<i>Zostera noltii</i>	Widely distributed throughout the Mediterranean	Common on sandy and muddy bottoms, it suits low-salt waters	6	1.3	40	0.10	Min 31 Max 62
<i>Halophila stipulacea</i>	Present in the eastern Mediterranean, reported in Italy	Common on sandy bottoms	30	1.3	7	0.7	-

Table 1.2 Summary of the main characteristics of the Mediterranean seagrasses (modified from Buia et al., 2003)

1.2 Habitat distribution

Currently, the genus *Posidonia* presents a bipolar distribution: of the 9 known species, *P. oceanica* is endemic to the Mediterranean Sea, while the other 8 species are widespread in the seas of Australia. *P. oceanica* is present almost throughout the Mediterranean sea. Its range of distribution finishes just before the Strait of Gibraltar on the west side (Conde Poyales 1989), and it is absent from the Egyptian coast to Lebanon (Por 1978) on the east side (Fig. 1.1).

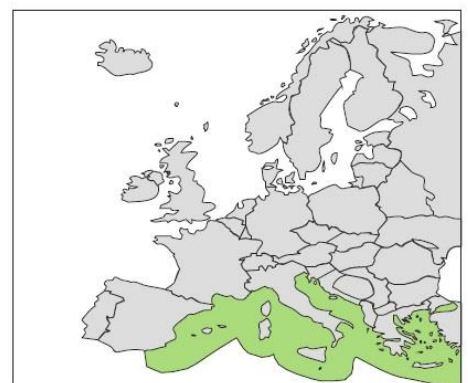


Figure 1.1. Geographical distribution of *P. oceanica* in European coastal waters. (Borum et al., 2004).

It is known that most of the Mediterranean sea dried up in the Messinian period, 5.6 to 5.3 million years ago, because of the closing of the Strait of Gibraltar, but is unknown how *P. oceanica* survived this crisis. Maybe refuge areas existed, from which it could recolonize the Mediterranean after the Gibraltar Strait reopened (Boudouresque et al., 2012).

P. oceanica is the only seagrass colonizing in a continuous way the coastal zone between the surface and the 40 m depth (Buia et al., 2003), is unique among seagrasses in that it can thrive on either rock or sand (Giovannetti et al., 2008).

1.3 The plant

P. oceanica is made of horizontal and vertical stems, more or less woody, usually buried in the sediment, called rhizomes. They can be of two types: the horizontal called plagiotropic, and the erect orthotropic (Fig. 1.2); there is not a functional differentiation between these rhizomes, and the environmental conditions can determine the growth pattern. The vertical growth is favored in areas where sediment deposition is very intense, whereas the horizontal one promote the extension of the seagrass beds and plant tends to colonize or re-colonize the substratum.

Regardless of the type, the rhizomes support shoots (Fig 1.3) made by a little group of leaves, generally 4-8. Leaves are 8-11 mm wide and may reach 150 cm in length. In the shoot, they present an alternate disposition, being the central leaves the youngest and the external the oldest. Leaf growth zone (meristem) lies at its base. Every leaf consists of a base (petiole) and of the limbo. The limbo is the visible part of the leaf, is from green color to dun, according to the leaf age, and it can be densely covered by organisms that are

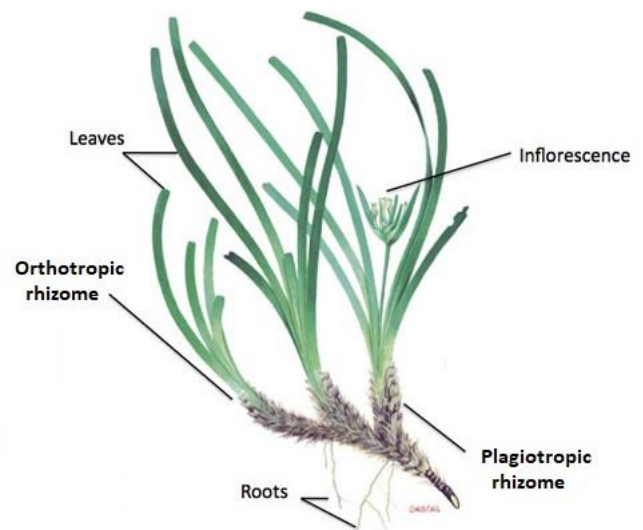


Figure 1.2. Anatomical details.

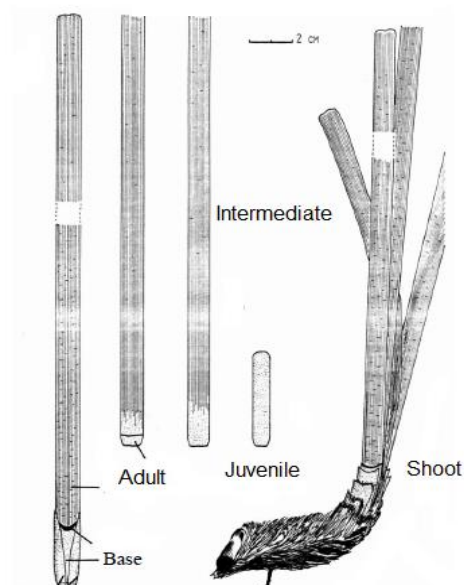


Figure 1.3. Shoots and leaves details (Buia et al. 2003).

fixed on, the epiphytes. The petiole is almost not pigmented, it is fibrous and the real leaf appears after it. It can measure up to 5 cm and is separated from the limbo by a semicircular mark (lanula), which is where the split takes place in the fall of the leaf. The morphological features allow to classify leaves in: juveniles less than 5 cm long, intermediate longer than 5 cm and adult when a petiole is formed (Giraud et al., 1979, Boudouresque et al 1983).

When they die, the leaves do not fall off at all, the basal leaf (petiole), remains attached to the rhizome (Fig. 1.4). The vegetative season is all year, leaves are formed and drop all year round (Pergent et al., 1991). Detached leaves are not very putrescible, and they can last for several centuries, creating a peculiar habitat for detritivorous invertebrates. The analysis of the age and growth of the plant based on leaf structure is known as lepidochronology (Crouzet et al., 1983). This is a powerful tool for measuring the speed of growth, past primary production, old pollutant levels, etc. (Pergent et al., 1990).



Figure 1.4. Characteristic rhizomes, consisting largely of petiole, basal part of the leaves.

Generally leaves live between 5 and 8 months and more rarely up to 13 months. This species presents a peculiar life cycle, with a few marked seasonal variations, being in the leaves better where it is estimated. It is possible to speak about several phases. The juvenile phase, or of hard latency, from October to February. During this period new, short leaves are formed with few epiphytes. The phase of maturity or activity is from March to June, and though new leaves are not generated, the existing ones present a very rapid growth. The phase of old age or of sluggish growth lasts from June to September, and during this period the growth is diminishing to the minimum; in the center of the bundle, a great number of leaves appear. At the same time, the existing leaves are aging and reach their maximum heights, and are loaded with epiphytes, reducing its photosynthetic efficiency. Though the loss of the leaves



Figure 1.5. Large amount of dead leaves dropped at the end of summer.

is continued all the year round, it is more intense from August, when it is possible to say that the fall begins (Fig. 1.5), being with the big autumns storms with the maximum loss of leaves will take place. The same storms drag up to the coast the fallen leaves, forming they typical accumulations on the beaches, called “banquettes” (Calvo 1995) (Fig. 1.6).

Figure 1.6. "Banquettes" formations along La Fossa beach, Calpe.



Being a real plant, *P. oceanica* produces flowers, but does it rarely. Usually, less than 1 flower is produced per 10 square meters per year, but flowering may be more frequent during warm years (Borum 2004). The flowers are hermaphrodites and are grouped in inflorescences that

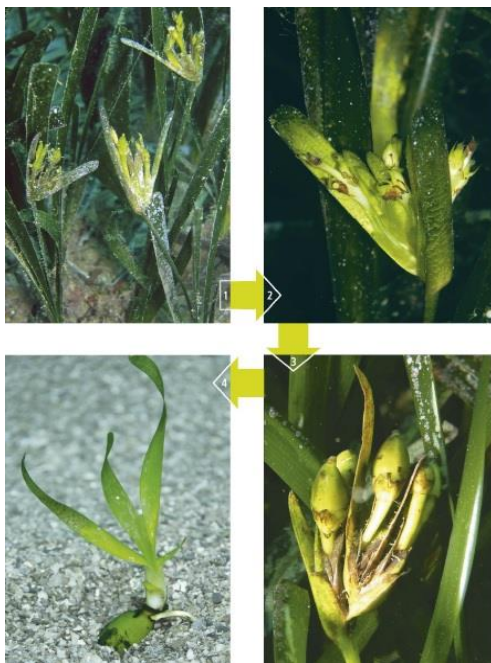


Figure 1.7. *P. oceanica* flower phases: (1) apariencia, (2) ovule formation and several dehiscent anthers, (3) fertilized ovule, (4) seed, germinating producing first leaves, small rhizome and a root to be fixed to the sediment.

arise in the center of the bundle, which impedes its observation (Fig. 1.7). The pollen forms viscous filaments that drift. The fruits of *P. oceanica* have the shape and the size of an olive; they are generally dark green and have just one seed (Hartog 1970). They require 6-9 months to ripen. Between May and July, they drop off and float for a certain time. According to the direction of the currents, they may be washed up on beaches in great quantities. However, *P. oceanica* reproduces mainly in a vegetative way; this reproduction strategy is called “stolonization”, and occurs by dispersion of rhizome fragments and its horizontally increasing by producing lateral ramifications.

P. oceanica's low genetic variability could be a weakening factor for this species (Raniello et al., 2002). The rarity of the flowering and, especially, seed production, as well as self-pollination, and inversely the frequency of vegetative reproduction could explain this feature of low variability. The little investment and low success of sexual reproduction, combined with the extremely slow clonal spread of *P. oceanica* explains the extremely slow colonization rate of this species. Numerical models simulating the occupation of space by *P. oceanica* meadow suggest that it would need 600 years to cover 66 % of the space available. Similar colonization timescales have been calculated based on patch size and patch growth rate in patchy *P. oceanica*

meadows. The very long time scales for colonization of this species indicate that recovery of disturbed *P. oceanica* meadows, where important plant losses have occurred, would involve several centuries (Borum 2004). *P. oceanica* is a long-living seagrass, with shoots able to live for at least 30 yr. *P. oceanica* recruited shoots at low rates (0.02 to 0.5 In units yr⁻¹) which did not balance the mortality rates (0.06 to 0.5 In units yr⁻¹) found in most (57 %) of the Spanish meadows. If the present disturbance and decline rates are maintained, shoot density is predicted to decline by 50% over the coming 2 to 24 yr. Because *P. oceanica* rhizomes grow very slowly (1 to 6 cm yr⁻¹), maintenance of existing meadows is essential, and our results suggest bad future prospects for *P. oceanica* in the Spanish Mediterranean Sea like most other seagrass species in the world oceans (Marbà et al., 1996).

1.4 Ecology

In calm conditions, *P. oceanica* can develop very near average sea level: its leaves then spread out on the surface, while the maximum depth depends on water transparency. In fact, light is one of the most important factors for the distribution and density of *P. oceanica* regulating the lower depth distribution (Elkalay et al., 2003).

P. oceanica dislikes low salinity. It dies off immediately below 33 ‰. The species appears to resist high salinity levels more successfully, although Ben Alaya (1972) has shown that 41 ‰ constitutes its upper tolerance limit. In fact, for example, it is present in the hypersaline lagoons of Tunisia (Bahiret el Biban, 46‰ in August) and Libya (Farwa: 39-44‰ according to the season); in these lagoons its vitality, as number of leaves produced per year, seems identical to or above what is observed out at sea (Romero et al., 1992, Pergent et al., 2000).

The extremes of temperature measured in a *P. oceanica* meadow are 9.0 and 29.2°C (Augier et al., 1980). It is, however, possible that low and high temperatures are only exceptionally borne. *P. oceanica*'s absence from the Levantine coast and its scarcity in the northern Adriatic could be due respectively to the summer and winter temperatures. Also, in deep meadow, Mayot et al., (2005) suggest that the increase in seawater temperature that is currently observed, could have a harmful effect on this seagrass. The temperature is therefore considered the overall parameter controlling the geographical distribution of the species in Europe.

Moreover, *P. oceanica* dislikes too intense hydrodinamism, and exposure is the most important factor regulating the upper depth distribution of a meadow. In general, it is estimated that

seagrasses do not exist at flow velocities above 1.5 m per second or at very exposed shores. Currents and wave action prevent seagrass growth and distribution by causing resuspension and transport of the sediment. Besides affecting the general light climate of the water column, erosion can expose roots and rhizomes causing the seagrasses to detach from the sediment. Additionally, very strong currents or wave action may tear up entire plants or prevent new shoots from being established (Borum 2004). Storms tear off shoots and can also erode the “matte”, either directly, or by leaching the sediment, which weakens the meadows. That is why in rough sea conditions the meadow grows no nearer the surface than 1 or 2 meters.

1.5 Structure of meadows

Seagrasses deeply alter the environments they colonize, giving rise to specific systems, known as the most diverse, complex and productive meadows present along the coastal zone of almost all the oceans and seas (Short and Coles 2001). The leaves and rhizomes of *P. oceanica* support many flora and fauna, some of which are calcified. When they die, their remains fall off and form an autochthonous sediment. Moreover, because of their density and distribution, the leaves of *P. oceanica* reduce the speed of the current; this reduces the kinetic energy of the sedimentary particles carried by the water, which then deposits on the seabed, constituting the allochthonous sediment (Boudouresque 2012).

P. oceanica rhizomes grow in height, even in the absence of sedimentation. To resist being buried, they are capable of speeding up their growth (Boudouresque et al., 1994).

The “matte” is the whole mass composed of rhizomes, leaves, roots and the sediment that fills the interstices. All complex is not very putrescible and is conserved for several centuries or even thousands of years (Boudouresque et al., 1983). Over the course of time, the “matte” rises to the surface, Moliner and Picard (1952) measured a seabed elevation of 1 meter per century. The “matte”’s elevation, in some conditions, can reach the surface, forming a barrier reef.

1.6 The role of *Posidonia oceanica* meadows in coastal systems

1.6.1 Ecological role

Darwin (1859) was the first to say that marine seagrasses can constitute a food base for many species of macro-herbivores.

As with forests in the terrestrial environment, *P. oceanica* meadows are the climax community and their presence attests to a relatively stable environment. In numerous Mediterranean coastal water ecosystems, *P. oceanica* plays an important role in a number of key geomorphological and ecological processes such as nutrient recycling (reducing the degree of water movement, thus improving sediment stability), provision of food for herbivorous fauna, shelter and nursery areas for many organisms (e.g. fishes, crustaceans) (Francour et al., 1999). These meadows are also a unique biodiversity hotspot (Boudouresque 2004).

Just inside the meadow it is possible to distinguish two populations: one photophilous and adapted to the expiration of the leaves on which it is installed, and another sciophilous and more



Figure 1.8. Dense epiphytic community on *P. oceanica* leaves.

stable installed in the stems and rhizomes. The leaves are occupied by small species of epiphytes (Fig. 1.8), of very short life and very fast crescent, which are adapted to the rapid and particular growth of the substrate in which they are fixed. In the rhizomes, appropriate species of the rocky bottoms

are installed. Thus, if the density of the meadow is high, the light that reaches the rhizomes is scarce and the species that appear belong to the precoraligenous community or the sciaphilic algae of calm regime, whereas if the density is low, the light that arrives at the rhizomes is enough so that the species of the infralittoral photophilic algae community can calmly settle down. In the buried part of the rhizomes, a typical fauna of soft bottoms is installed, rich in bivalves, polychaetes and small crustaceans.

A basic feature of the *P. oceanica* ecosystem is the combination of two kinds of primary production; at global level, only marine seagrasses ecosystems present this special feature (Boudouresque et al., 1996). (1) The primary production from *P. oceanica* is rich in cellulose and lignin, compounds that are not used much by herbivores, and in phenolic compounds, one of whose roles is to dissuade potential consumers. The net primary production of *P. oceanica* is on average between 420-13000 g DM/m²/year (DM = dry mass); it drops in relation to depth (Pergent et al.,

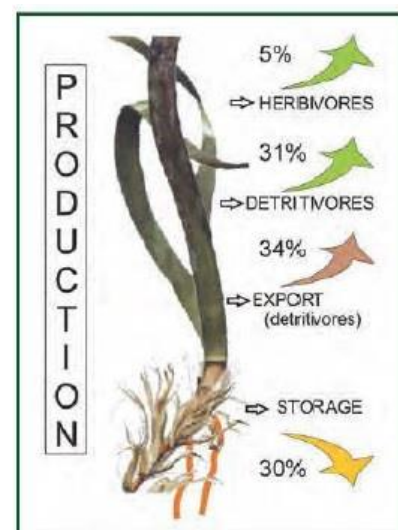


Figure 1.9. Production processes (Boudouresque et al. 2012).

1997). (2) The primary production from the Multicellular Photosynthetic Organisms (MPOs) leaf epibiota is composed of very palatable Rhodobionta and Chromobionta, thus easily usable by herbivores; it is between 100 and 500 g DM/m²/year (Giorgi and Thelin 1983). All in all, the *P. oceanica* ecosystem is one of the planet's most productive ecosystems (Boudouresque 2012). Vegetal biomass is also very high and decreases with depth (Mazzella et al., 1992). No other marine ecosystem, except mangroves, presents such high vegetal biomass. This is due to the storage of the biomass over a long period of time in the "matte". Little (less than 10%) of the primary production is used by herbivores. These are mainly the fish *Sarpa salpa* (Fig. 1.10), the sea urchin *Paracentrotus lividus*, isopod crustaceans *Idotea hectica*, spider crabs *Pisa mucosa* and *P. nodipes* (Boudouresque and Verlaque 2001). The modest grazing role of *S. salpa*



Figure 1.10. Individuals of *S. salpa* grazing on *P. oceanica*.

could constitute an artifact linked to human action; indeed, in a certain number of Marine Protected Areas (Tabarca and Medes Islands in Spain, Port-Cros in France, El Kala in Algeria) overgrazing by *S. salpa* was observed (Boudouresque et al., 2012). On the other hand, the leaf epibiota is widely used, in particular by gastropods. Much of the primary production (24 to 85 %) is exported in the form of dead leaves;

they may constitute up to 40% of the digestive contents of sea urchin *P. lividus* in a hard substratum community some hundreds of meters away from the nearest meadow (Cebrian and Duarte 2001).

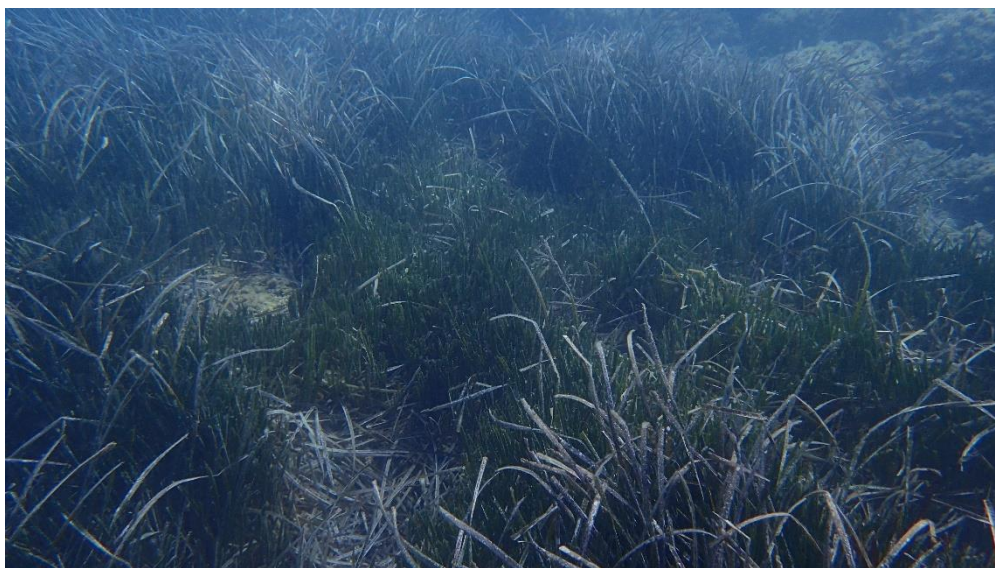


Figure 1.11. Meadow patch with clear signs of herbivorous *S. salpa*.

1.6.2 Physical role

On the coastal seabed, *P. oceanica* meadows are real plant barriers that encourage the decantation and sedimentation of particles in suspension in the water column (trapping sediment) (Fig. 1.12). The meadow plays a similar role to European beachgrass and pine trees that stabilize the coastal sand dunes. It should also be noticed that sediment settling and trapping in the “matte” increase the transparency of the coastal water (Boudouresque and Meinesz 1982). The *P. oceanica* meadow’s considerable vegetal biomass also acts as a kind of barrier which

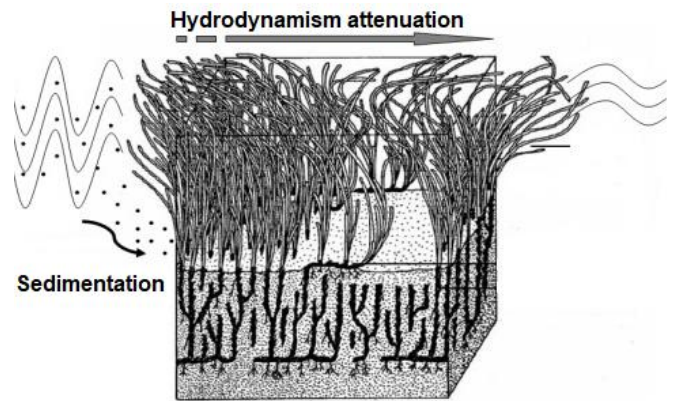


Figure 1.12. Physical role of *P. oceanica* meadow, modified from Bourdouresque and Meinesz. (1982).

slows and effectively absorbs hydrodynamism at the seabed. Some experiments in laboratory and in situ showed that a meadow can reduce hydrodynamism from 10 to 75% the under the cover of leaves and 20% above the canopy (Gacia and Duarte 2001). This hydrodynamism reduction is particularly visible behind the *P. oceanica* barrier reefs, where the presence of these plant barriers enables sheltered lagoons to be established (Boudouresque et al 1994) and more generally the reduction in waves and currents is likely to protect from coastal erosion and helps stabilize the shoreline.

1.6.3 Economical role

It is the result of the ecological and physical importance in the coastal system. It obviously affects the management of living resources via its high biological production, the protection from predators it offers to young fish and young organisms, and it also continues a much sought-after spawning ground for many species of commercial interest (Connelly et al., 2005). It also affects the development of tourism and seaside activities, by helping maintain water quality, and especially by stabilizing the shoreline that it defends by protecting it from erosion. Furthermore, even if the meadows are not always the “spots” sought by divers, they are at the origin of significant exported biological richness (in terms of species and food) to other more sought-after habitats (Boudouresque 2012). Moreover, *P. oceanica* leaves dead can be very useful. Use of the leaves inside mattresses, or as bedding for animals, went on for a long time; indeed, “vermin” never entered the mass, certainly because of the phenolic acid contained in

the leaves (Bourdouresque et al., 2012). For centuries, when there was not yet any bubble wrap or expanded polystyrene, *P. oceanica* leaves were used by the Venetians to wrap up and transport their famous, delicate glasswork, to the extent that these leaves were known as “Venetian straw”. In North Africa, in the early 20th century the coastal populations were still using dried *P. oceanica* leaves to build roofs, while in Corsica, Sicily and Greece these were used for thermic and sound insulation. Dead *P. oceanica* leaves were also used for a long time as compost by farmers on the Mediterranean coast.

1.6.4 Bioindicator role

For several years now, the use of seagrasses for environmental monitoring, to assess the evolution of impact, or more generally to manage coastal ecosystems has been envisaged. The use of these species, known as “bioindicators”, seems to be a quick and effective way of assessing the quality of the environment (Bellan 1993).

Seagrasses in general, and *P. oceanica* meadows in particular, are considered to be appropriate for biomonitoring because of their wide geographical distribution, reasonable size, sedentary habit, longevity, permanence over the seasons, easy collection and abundance and sensitivity to modifications of the littoral zone. Reasoned management, on the scale of the whole Mediterranean basin, requires standardized methods of study, to be applied by both researchers and administrators, enabling comparable results to be obtained (Pergent-Martini et al., 2005). In particular, *P. oceanica* meadow constitutes a powerful integration of overall marine water quality, it demonstrates by its presence and its vitality the quality of the water above it. Moreover, their rhizomes concentrate radioactive, synthetic chemicals and heavy metals, recording the environmental levels of such persistent contaminants.

In summary *P. oceanica* seems to be a reliable bioindicator according to: (1) their sensitivity to disturbances, as demonstrated by a number of reports of meadow regression due to various causes; (2) its wide distribution along the Mediterranean coast and (3) the good knowledge about specific response of the plant and of its associated ecosystem to specific impact (Tab. 1.3). Furthermore, as said, this species is able to inform about present and past level of trace metals in the environment (Pergent-Martini, 1998).

TYPE	VARIABLE	IMPACT OR STRESS	RESPONSE
1	Shoot density (shoot/m ²)	Reduction of light availability. Burial. Direct elimination due to trawl fishing, boat anchoring, coastal construction, etc.	Shoot density decrease (shoot mortality)
	Cover (%)	Reduction of light availability. Burial. Direct elimination due to trawl fishing, boat anchoring, coastal construction, etc.	Vegetal coverage decrease
	"Matte" structure	Disturbance on sedimentary flux. Burial. Boat anchoring, coastal construction, etc.	Fragilization of the "matte" structure
	Epiphyte biomass (mg/cm ²)	Reduction of light availability	Epiphyte biomass decrease
		Eutrophication	Epiphyte biomass increase, except if compensated by herbivores.
N content in epiphyte species associated to meadow	Eutrophication Reduction of light availability. Burial. Direct elimination due to trawl fishing, boat anchoring, coastal construction, etc.	Increase in tissues Decrease of biodiversity	
2	Foliar area (cm ² / shoot)	Reduction of light availability Eutrophication (turbidity increase due to phytoplankton development)	Leaf surface increase to optimize photosynthetic productivity
	Foliar necrosis (% leaves with necrosis /shoot)	Eutrophication	Possible consumption by herbivores due to nutrient increase
		Reduction of light availability. Prolonged anoxia (burial). Other stress factors (e.g. toxins, pathogens, hypersalinity)	Increase in necrosis in tissues
	Lepidochronology	Reduction of light availability, sediment resuspension...	Changes in foliar productivity and the growth rate of the rhizomes
Plagiotropic rhizomes (%)	Reduction of light availability, sediment resuspension...	Decrease of percentage of plagiotropic rhizomes	
3	Sucrose content	Reduction of light availability due to eutrophication, sediment resuspension...Burial	Decrease of carbon reserves in rhizomes
	Nitrogen and phosphorus content	Eutrophication	Increase of nutrient content in plant
		Reduction of light availability	Increase of nutrient content (nitrogen) in leaves due to low growth under low light conditions
		Anoxia in sediment	Decrease of nutrients accumulated in rhizomes due to negative effects on the availability and uptake of nutrients by roots
	δ ¹⁵ N and δ ³⁴ S	Eutrophication	Increase of δ ¹⁵ N
		Anoxia in sediment	Increase of δ ³⁴ S
Metal traces: Cu, Zn and Pb	Metal contamination	Increase of metals in tissues. Effects on photosynthetic activity (Cu, Pb, Zn)	

Table 1.3. List of main descriptors of *P.oceanica* used in monitoring and their responses to different impacts, 1: community descriptors, 2: individual descriptors, 3: physiological descriptors (Pergent-Martini et al., 2005; Boudouresque et al., 2006; Romero et al., 2007).

Moreover, *P. oceanica* meadows, from a political point of view, are included in a law issued at European level. In fact, the use of seagrasses as a tool for an ecological evaluation of coastal waters has recently been highlighted in European legislation by the Water Framework Directive (WFD 2000/60/EC). The Directive establishes the basis for monitoring, protection and enhancement of all aquatic ecosystems in the European Member States (MS), by setting ecological quality objectives (i.e. "good water status" for all European waters by 2015) which require to assess water quality status using a combination of ecological indicators in priority. To assess the good status of water bodies, the WFD requires that monitoring programmes are

implemented for the ensemble of aquatic environments concerned. This monitoring must be based on “biological quality elements” (BQE). For coastal waters Angiosperms, together with phytoplankton, macroalgae and benthic fauna (Devlin et al., 2007), are one of the four BQEs required for the evaluation of ecological status because of, as mentioned above, its specific responses to anthropogenic disturbance are acknowledged (Balestri et al., 2004; Ruiz and Romero, 2003; Leoni et al., 2006).

Using some descriptors, have been therefore elaborated, from the scientific community, a series of indexes (Tab. 1.4), on the *P. oceanica* metrics, so that the evaluation of the quality of the waters can be simple, replicable in space and time, and can have a reasonable cost.

<i>Index</i>	<i>Metrics</i>	<i>Methods</i>	<i>Reference</i>
Posware	-shoot density, -width of the intermediate leaf, - leaf production, -rhizome production, -rhizome elongation	combined in a datawarehouse (Autoclass C, miner 3D, and fuzzy clustering analyses)	Buia et al., 2005
POMI	-shoot density, -meadow cover, -percentage plagiotropic rhizomes, -shoot leaf surface, -percentage foliar necrosis, P, N and sucrose content in rhizomes, - $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ isotopic ratio in rhizomes, -N content in epiphytes, -Cu, Pb, and Zn content in rhizomes	combined using Principal Component Analysis (PCA)	Romero et al., 2007
Valencian CS	-shoot density, -meadow cover, -dead matte cover, -percentage of plagiotropic rhizomes, -rhizome baring/burial, -shoot leaf surface area, -percentage of foliar necrosis, -herbivore pressure, -leaf epiphyte biomass.	combined using Principal Component Analysis (PCA)	Fernandez Torquemada et al., 2006,2008

Table 1.4. Three methods based on *Posidonia oceanica* in the WFD to assess the ecological status of Mediterranean coastal waters (Gobert et al. 2009).

Recently, in addition to the plant itself, the composition and abundance of the leaves epiphytic community is beginning to be used as a BQEs. In fact, the deterioration of the water quality and the increased amount of dissolved nutrients, due to the rising human pressure on coastal waters in the last century (UNEP/GPA, 2006), have been shown to affect the growth of epiphytes on seagrass leaf blades (Duarte, 2002). Increases in the epiphyte biomass, differences in their spatial heterogeneity and shifts in species composition have been observed under human disturbance regimes. Moreover, differently from the descriptors commonly adopted for

evaluating the health status of *P. oceanica* ecosystem, the epiphyte community structure was able to detect alterations in the water quality already after 4 months (Giovannetti et al., 2010).

1.7 The causes of regression

Seagrass communities, which are widely considered as key ecosystems in coastal waters (Short et al., 1996), are now challenged with hasty environmental alterations resulting from human-induced disturbance (Boudouresque et al., 2006). Worldwide seagrass loss has been reported due to a variety of both direct and indirect human impacts (Short et al., 1996; Hemminga et al., 2000). In the Mediterranean Sea, the major sources of large-scale seagrass regression include environmental pollution, eutrophication, increased water turbidity, bioinvasion, and climate changes (Montefalcone et al., 2007; Occhipinti- Ambrogi, 2007), but there are also sources of regression of little-scale.

When water transparency decreases this has a direct effect on *P. oceanica* meadows. Compensation depth (the depth at which the losses due to respiration balance out with photosynthesis production) becomes shallower, and with it becomes shallower the limit of the meadow. Ruiz and Romero (2001) have demonstrated experimentally, placing screens above a meadow, that a reduction in light reduces both growth rate, shoot biomass and storage of starch in the rhizomes; and after one year, when normal light intensity has been reestablished there is still no sign of recuperation.

For example, a harbor construction or building coastal facilities can locally change swells and currents and alterate the normal sedimentation dynamic (erosion or accumulation). All that can, consequently, cause a clear increase in sediment organic matter and in sediment deposition rates, especially fine sand. For this reason, light availability could be reduced due to suspended sediments. Seagrass structural indicators respond unequivocally to these environmental changes, with clear reductions in shoot density (Roca et al., 2014).

Also coastal rivers can have an impact on *P. oceanica* meadows by: desalination to which the plant is very sensitive, nutrient and sediment inputs. The correction of watercourses can modify the granulometric features of the sediment carried down in favor of the finest particles, generating great turbidity in the water column which restricts the photosynthetic action of *P. oceanica*.

Also nutrient inputs resulting from fish farming activities located in the vicinity of seagrass meadows can potentially alter the structure and affect the functioning of these ecosystems. The presence of suspended matter in the water column can cause a reduction in the amount of light reaching the seagrass, while elevated concentrations of dissolved nutrients can promote a high epiphytic cover on the leaves. The overall result is a reduction in growth rate of the seagrass and gross changes in shoot morphology (Dimech et al., 2002). In a particular way, Cabaço et al., (2013) showed that biomass and density tended to increase simultaneously under high nutrient levels in short-term experimental studies, whereas they tended to decrease simultaneously in descriptive studies where the seagrass populations were exposed to the long-term effects of nutrient increase.

Anthropogenic waste, as well as greatly changing the sedimentary balance of the coastal water, also implies a wide range of contaminants that affect the vitality of the *P. oceanica* meadows. The effect of soluble substances is rapid, but insoluble substances can also have a very negative impact; being relatively stable they can accumulate to reach concentrations that are toxic to flora and fauna. The presence of a high contaminant concentration can determine the alteration of the biosynthetic pigments: along with an increasing pollution gradient, the photosynthetic pigment content in *P. oceanica* leaves decreases (Augier et al., 1979).

Moreover *P. oceanica* meadows, ranging in depth from the surface down to about 40 m, cover seabed areas that coincide with the ideal sites for pleasure-boats anchoring (Montefalcone et al., 2008). Among the various types of human activities, the mechanical damages resulting from uncontrolled pleasure boats anchoring (Fig. 1.13) in shallow coastal waters would appear to be responsible for localized regressions of *P. oceanica* meadows (Montefalcone et al., 2006). The impacts of anchoring systems on *P. oceanica* have been shown to be recorded at two different levels: the individual level (the plant), where mechanical damage is the direct cause of pulling up leaves and rhizomes (Ceccherelli et al., 2007), and the population level (the meadow), where mechanical damage reduces shoot density and cover of the meadow (Francour et al., 1999). Experimental evaluations of the impact of anchors of small vessels have shown that each anchoring can on average damage up to 6 shoots of *P. oceanica*, removing



Figure 1.13. Impact of an anchor of leisure boat on *P. oceanica* meadow.

small amount of biomass and, at the same time, interrupting continuity among shoot, and the disturbance is higher where the substratum is highly penetrable (Ceccherelli et al., 2007). The frequency of anchoring at some visited sites, in terms of number of boats and extension of the visitation period, could make this chronic disturbance very stressful to the growth of the seagrass over several years; compared with the slow clonal growth of *P. oceanica*, estimated at about 1–7 cm yr⁻¹, recovery may take centuries. Notwithstanding the protection measures undertaken by the European Community for their conservation, *P. oceanica* meadows keep on being affected by this kind of local impacts, which are hardly controlled but within marine protected areas (González- Correa et al., 2007). An alternative to direct anchoring is the deployment of anchoring chain systems to which buoys – and subsequently boats – are moored (Montefalcone et al., 2008).

Another cause of regression of the *P. oceanica* meadows is the competition with two Chlorobionta introduced species: *Caulerpa taxifolia* and *C. racemosa (cylindracea)*. *C. taxifolia* and *cylindracea* are native to Australia and were accidentally introduced into the north-western Mediterranean at the beginning of the 90's, since when its geographical expansion was relatively rapid. *C. taxifolia* is able to colonize almost all kinds of substrate, particularly the “dead matte”, so stressed and degrades meadows are an extremely favourable environment for this species (Boudouresque et al., 2012), while *C. cylindracea* colonizes bottoms bordering *P. oceanica*, and its growth in height is a function of the *P. oceanica* shoot density and the orientation of the meadow (Ceccherelli et al., 2000).

It is not always easy to distinguish between natural and human factors, or between the different kinds of human-induced factors, which almost act simultaneously and certainly have synergic effects, should not hide a robust scientific certainty: man really has been responsible for most of the regressions observed in the second half of the 20th century.

1.8 Policies applying to *Posidonia oceanica* meadows

For all the reasons mentioned above and due to the fact that *P. oceanica* meadows have great short-term recovery difficulties, this led to enhanced efforts in protecting legally the species in many Mediterranean countries (Platini 2000).

It is useful differentiate between direct legal protection measures either concerning the species *P. oceanica* or the habitats it constitutes, and regulatory measures which, without directly aiming to protect the meadows, can indirectly encourage their conservation.

In policy terms, the ecosystem approach is a relatively recent one and only international conventions signed after 1990, or those were produced before this date but were updated, possibly take *P. oceanica* meadows into account. This is the case of the Bern Convention (Convention on the conservation of European wildlife and natural habitats), signed in 1979 under the auspices of the Europea Council by several Mediterranean countries. Indeed, while it did not initially mention any marine plant species, its Annexes were modified to add three of the seagrasses species of the Mediterranean Sea (*Cymodocea nodosa*, *Posidonia oceanica* and *Zostera marina*). The same holds good for the Barcelona Convention, adopted in 1976, which was the key convention for the protection of the areas and species in the Mediterranean. A legal tool of the Mediterranean Action Plan (MAP), launched by the UNEP (United Nations Environment Programme), for the protection of regional seas, the convention initially focused on the marine pollution, but in 1982 the 20 signatory countries showed their interest in protecting marine habitats adopting the Mediterranean Specially Protected Areas Protocol. However it was only in 1999, when a new protocol was adopted, that *P. oceanica* is specifically mentioned in a list of endangered or threatened species and also the ecosystem that it built is recognized of “special interest at the scientific, aesthetic, cultural or educational levels”, and then introduced in the SPAMI (Specially Protected Areas of Mediterranean Importance) list. These provisions were enhanced when in October 1999 and Action Plan for the conservation of marine vegetation in the Mediterranean was adopted. It identifies priority actions at national and regional level, such as ensuring the conservation of species and plant formations by developing legal measures of protection, avoiding the loss and degradation of marine meadows and of other plant formations and habitats for marine species, and ensuring the conservation of formation as *P. oceanica* barrier reefs, considered natural monuments.

To these international conventions should be added the Habitats Directive of 21 May 1992 (92/43 EEC/Natural Habitats), at European level. This Directive has six Annexes, and in the first one appear the *P. oceanica* meadows which are classified as a priority habitat.

Several countries now have specific laws about meadows or envisage such procedures, if only to make the provisions mentioned in the international conventions they have signed or ratified.

For what concerns indirect ways to protect marine meadows is possible to recall all the policy measures that aim at restricting pollutant waste, ensuring the treatment of urban waste, fighting against water eutrophication, banning certain fishing techniques and fighting against the introduction of invasive species. Also setting up Marine Protected Areas can be a way of protectin *P. oceanica* meadows even if conserving spaces do not specifically refer to the meadows.

2. Work purpose

The main purpose of this work is, through a first survey, to provide baseline data for the monitoring scheme of *Posidonia oceanica* meadows at Calpe Bay. The features and status of a seagrass bed have been assessed by physical, physiographical, structural and functional descriptors.

3. Materials and methods

3.1 Study area

This study was carried out in the waters of the “Parque Natural del Peñón de Ifach”. Ifach's Crag (Penyal d'Ifac in Valencian) is located in the region of the High Sea-coast in Calpe's municipality in the province of Alicante (Spain) (Fig 3.1).

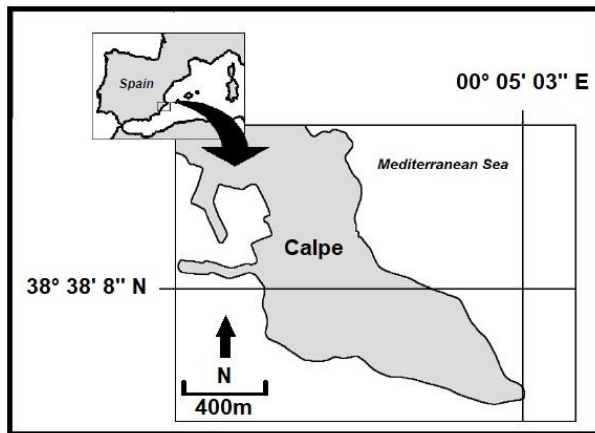


Figure 3.1. Geographic location of the study area.



Figure 3.2. View of the Peñón de Ifach from the sea.

The Crag is 332 m above the sea level and has an area of 50.000 m² (Fig. 3.2). This calcareous mountain was declared Nature reserve by the Decree 1/1987, January 19, of the Valencian Government. Only in 1993 has definitely been approved the plan of management of the park that currently extends for a total of 53,3 acre and it is entirely terrestrial. The surrounding marine area to the mountain has recently been declared special protection area (SPA) under the Birds Directive (79/409/CEE), while no protection plans have been implemented for the marine area.

On both sides of the natural reserve, there are opened beaches of thin sand: "Levante" or "La Fossa" towards North; "Cantal Roig" and "Arenal-Bol" towards South, which, together with "Morro de Toix", shape the coastline of Calpe's bay (Fig. 3.3).



Figure 3.3. Calpe bay from above.

3.1.2 Geological context

Ifach's Crag is the last spur of the Baetica mountain chain (Andalusia) and is part of the internal delimiting of the mountain chain called Prebética. The compressive efforts in the Prebetic sector formed the basin that was refilled by Miocene' marine materials among those who inserted big fallen blocks (olistoliths) proceeding from the top part of the folds of Oltà's Saw. The olistolith of Ifach's Crag (limestones eoceno-oligocenas) finished resting on the younger miocenas marls.

In the Eocene - Oligocene, approximately 65 millions of years ago, the place that today occupies the Crag, was a marine slightly deep platform in which were going settling the remains of fossils. 14 million years ago an intense orogenic phase took place forming big faults of orientation, responsible for the collapse under the sea of the sectors placed between Morro de Toix and Ifach's Crag. In addition, big blocks of land emerged to the surface (olistoliths). The most recent materials are a consequence of the coastal deposition in conditions of marine slow and continued regression, these are dune cords that do not appear on the surface. The crag is joined to the continent by a detrital isthmus, which is a sedimentary environment recent littoral. Approximately two million years ago, the sediment proceeding from the rivers and from the erosion of the cliffs formed a tongue connection for the action of the coastal dynamics (Fig. 3.4). Between the island and continent there existed a slightly deep strait that went away for sandy contributions brought by the currents parallel to the coast, giving origin to two sandy tops of the coastal arrow (now cemented sandstones). At the same time the wind action accumulated

contributing materials forming a dune cord that isolated in the interior a zone flooded with salt waters, result of the mixing of continental waters with the sea. Therefore the Salt mine is a part of a former bay closed by the formation of this transverse cord dune to the surge. Nowadays it is possible to see this barrier of fossil dunes between Arenal and Cantal Roig beach. Another part of the barrier would be in the La Fossa beach.



Figure 3.4. Formation process of the Salt mine and the isthmus that connects the Peñón de Ifach to the land (from García-March et al., 2015).

3.1.3 Marine context

The seabed adjacent to the mountain is very heterogeneous in relation to the mountain orientation (Fig. 3.5).

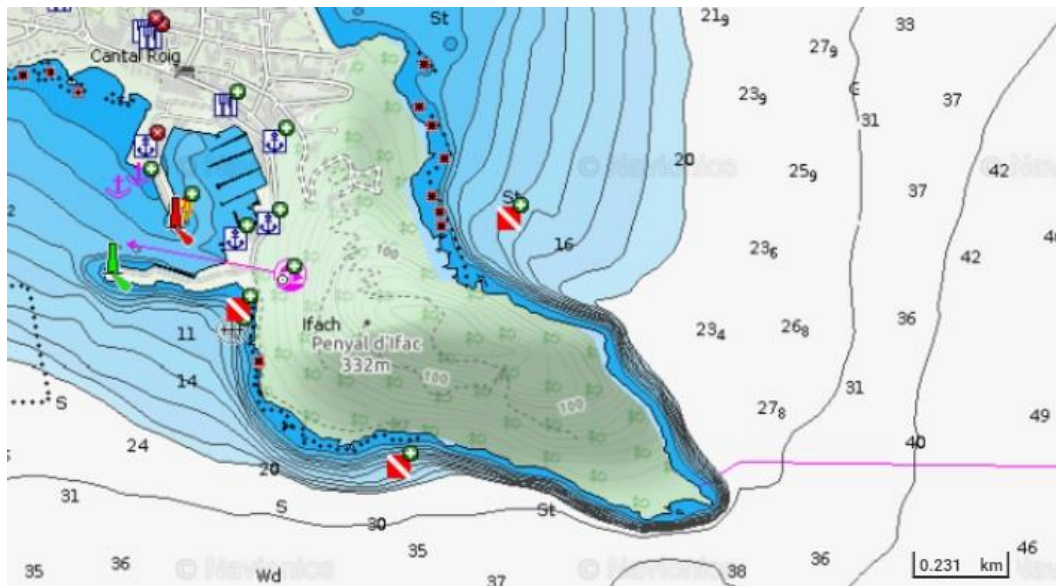


Figure 3.5. Bottom bathymetry from Marinetráfico.com.

The depth gradient is really marked in the south/southwest zone of the Peñón de Ifach, where it quickly reaches -35 m. On this side of the mountain, there is a beach of pebbles (Racò Bay) along the depth gradient gives gradually step to different biocenosis of thin sands. On this side of the cape, a great *P. oceanica* meadow is present, which extends from -6 m to -22 m depth. The sector north of the Peñón de Ifach, however, has vertical walls at the base of which great calcareous blocks are located coming from the detachment of the walls of the same mountain. On this side, there is a really big sandy beach (La fossa beach) beginning from which the sea floor goes deeper very slowly. From this side of the mountain, there are two different meadows of *P. oceanica*, one directly in front of the beach constituting a structure like a reef, while the other is situated on the north slope of the mountain.

The waters that surround the Peñón de Ifach are protected from wind and surge principally in his west and north faces due to the geographical protection that he is awarded by the saws of Oltá, Bernia and Mascarat. In turn, the sectors south and North-East are the most exposed to climatic phenomena of this type being the most frequent proceeding from this side.

The whole area is affected by a variety of anthropic pressures, most of which are related to tourism. From June, the number of tourists that reach the area and that consequently crowd the beaches, reaches exorbitant numbers. Also the number of boats that anchor in the bays increase

very much in summer, going to constitute a probable threat for the *P. oceanica* meadows, also because there are no buoys to attach to, instead of anchoring.

Behind Cala Racò bay is located a fishing port that does not directly affect the area, but has done it in the past. The fishermen comment that up to a couple of decades containers did not exist to throw the garbage in the fishing port and that the garbage of major size, gathered by the trawlers, was beginning to be thrown into the Cala Racò area, the so-called “Brut de Ifach” (García-March et al., 2015) (Fig 3.6). In this way, for many years there was spilled all kinds of residues of large size, from ropes up to girders, containers and fishing tackles. Some remains have joined the bottom and on them, numerous incrusting organisms and *P. oceanica* have grown, but the majority perceive it as a problem from the environmental and aesthetic point of view. It would suit to solve by means of a professional cleanliness of the funds, coordinated with scientists, to minimize the ecological impact of the own extraction of the garbage.



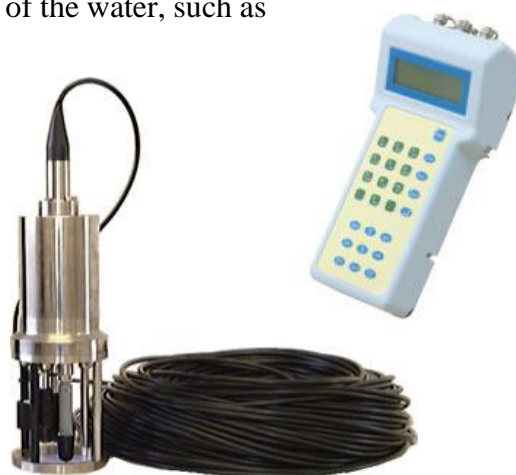
Figure 3.6. "Brut de Ifach" mapping from García-March et al. (2015).

fishing tackles. Some remains have joined the bottom and on them, numerous incrusting organisms and *P. oceanica* have grown, but the majority perceive it as a problem from the environmental and aesthetic point of view. It would suit to solve by means of a professional cleanliness of the funds, coordinated with scientists, to minimize the ecological impact of the own extraction of the garbage.

3.2 Physical descriptors

3.2.1 Water conditions

To monitoring the physical-chemical parameters of the water, such as temperature, turbidity and dissolved oxygen a multi-parameter CTD probe has been used, AAQ-RINKO (LFE Advantech Co., Ltd.) (Fig. 3.7). It is connected, through a cable, to a hand-held unit that records the measured parameters, returning the profiles along the water column.



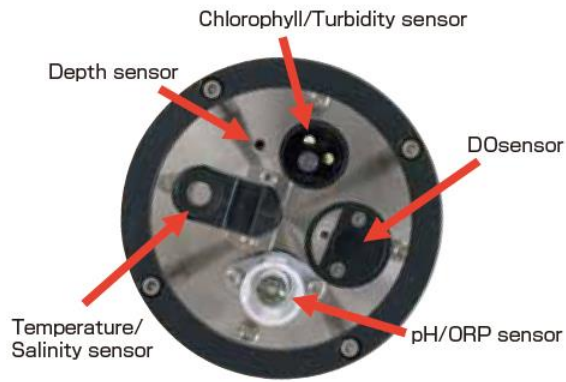


Figure 3.8. CTD probe sensors.

Conventional water quality profilers with a slow response optical sensor require holding the instrument for a certain period at the measurement depths. The one we used, with an optical fast response, makes vertical measurements possible with a profiling speed of 0.5 m/s, thereby significantly reducing the observation time.

The data were collected once a month, for one year, up to -30 m depth, to have information related to the whole range in which the meadow of *P. oceanica* is distributed.

3.2.2 Sediment analysis

From October 2016, samples of sediment of around 300g were collected monthly, in three different stations (C01 -5m, C02 -10m, C03 -15m) of the Cala Racò meadow (Fig. 3.9.), with the purpose to analyze its particles size and to quantify in its variation in relation to the seasons, related to hydrodynamic conditions.



Figure 3.9. Sediment monitoring station in Cala Racò meadow.

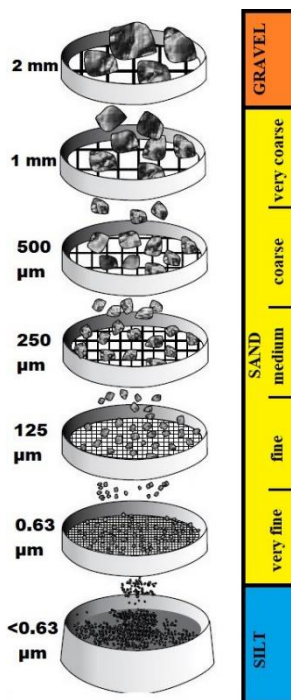


Figure 3.10. Sieves illustration and relatives and dimension of the holes.

To do so each sample has previously been prepared: rinsed with distilled water to avoid that the crystals of salt created adhesion among the grains and they brought to a distorted result. Subsequently to this step, the samples were set in heater for at least 24 hours to 70°C. A special battery of sieves (CISA) was used, in which the mesh size decreases downward progressively, reducing itself of ½ every level (Fig. 3.10). The whole battery was placed on a mechanical agitator (CISA sieve shaker Mod. RP08 (Fig. 3.11)) for a period of about 30 minutes, with the purpose to get the complete sifting of the material. Subsequently, every fraction held back inside every sieve appraising was weighted and the percentage of it in comparison to the total weight for every single granulometric class of affiliation was calculated. The values of every single weighed so gotten are

reported to the classes belonging on the Wentworth's scale. All the sediment samples were analyzed fresh, whereas it was not possible, they were frozen with the purpose to maintain their integrity.



Figure 3.11. Agitator and sieves photo.

Since granulometric distributions can be assimilated to Gaussian curves, the dimensional spectrum description follows the rules of classical statistical parameters. These "classics" can be integrated by a different index, which can be revealed graphically or by analytical methods from cumulative curves. These curves were constructed by adding each size class to the previous one, on the abscissa there were the values of the sieve diameter on a logarithmic scale (\emptyset notation = $-\log_2(\text{mm})$, Tab. 3.1).

From these curves, after calculating the values for the percentiles, it was possible to obtain the descriptive parameters (Folk and Ward 1957).

mm	\emptyset notation
2	-1
1	0
0.5	1
0.25	2
0.125	3
0.063	4
<0.063	5

Table 3.1 Conversion scale from mm to \emptyset

NAME	MEANING	FORMULA (Falk & Ward 1957)
Md_ø	Average diameter.	$\frac{\phi_{16} + \phi_{50} + \phi_{84}}{3}$
σ	Sorting or standard deviation of the sediment, represents the measure of dimensional variability within the sample. It gives information about the sediment classation.	$\sigma = \frac{\phi_{84} - \phi_{16}}{4} + \frac{\phi_{95} - \phi_5}{6.6}$
Sk	Skewness, it represents the measurement of distribution symmetry. The values can range from -1.0 to +1.0. It provides information on the energy to which the sediment is subjected, and hence to the relationships between the dimensional classes that make it up.	$Sk = \frac{\phi_{84} + \phi_{16} - 2\phi_{50}}{2(\phi_{84} - \phi_{16})} + \frac{\phi_{95} + \phi_5 - 2\phi_{50}}{2(\phi_{95} - \phi_5)}$
K	It represents the measure of the sluggish of distribution in relation to sorting. This parameter has a poor geological significance.	$K = \frac{\phi_{95} - \phi_5}{2.44(\phi_{75} - \phi_{25})}$
Fh	Hydrodynamic factor, allows estimating the mean value of the currents.	$Fh = \frac{\phi_{95} - \phi_5}{2.44(\phi_{75} - \phi_{25})} - \frac{\phi_{75} - \phi_{25}}{2}$

Table 3.2 Folk & Ward parameters and their meaning.

3.3 Monitoring *Posidonia oceanica*

Six monitoring stations were realized, four of which located in a single meadow on the South slope of the Peñón de Ifach mountain, and two situated in lists stains on the North side of the same mountain (Fig 3.12).



Figure 3.12. *P. oceanica* monitoring stations.

Every station of monitoring consisted of 6 points of sampling, in each of which a SCUBA diver team realized the corresponding measurements, along a transect 10 meters long (Fig 3.14).



Figure 3.13. Members of the SCUBA diver team.

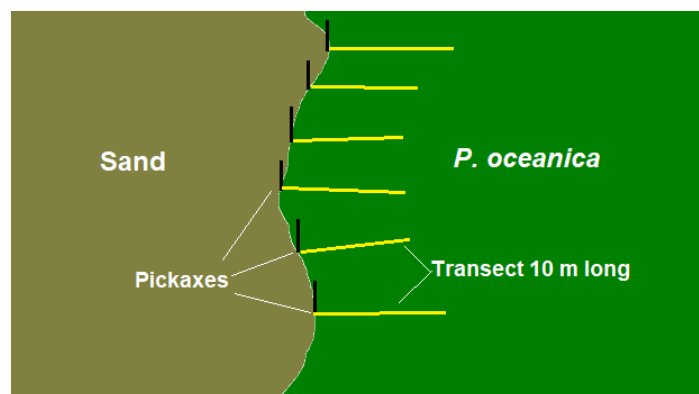


Figure 3.14. Schematization of the structure of a sampling station.

The position of every sampling point were indicated by a metallic picket of 1,5 m of total length, fixed in the sediment and provided with a small buoy that stands out 1 meter above the leaves (Fig 3.14).



Figure 3.15. Reference buoy connected to a picket.

The pickets were numbered and distance between two adjacent pickets ranged between 3 and 6 meters (Sandulli et al., 1998), following the limits of the meadow, when presents, so that they could be used as reference for the monitoring regarding the expansion or the regression of the limit of the meadow during the years. The examples brought in literature to monitoring the lower limit of *P. oceanica* meadows foresee the use of bodies of reference, e.g. blocks of cement or metallic rods, that don't have the possibility to be moved by the hydrodynamic action (Buia et al., 2003). For each transect, the direction (in °) was recorded, in this way, every measurement can be repeated in the same place, at the time. The majority of the stations were located in correspondence to the lower

limit of the meadow to show, eventually, first signs of degradation of the system. The typology of border has been defined following the classification of Meinesz and Laurent (1978) and Pergent et al., (1995).

Furthermore, for each meadow (A, B and C) it has been classified the Bed typology that together with the Limit typology constitute the physiographical descriptors of seagrasses meadows.

The ecological status of the *P. oceanica* meadows was assessed investigating some structural descriptors:

- Shoot density: is the number of seagrass shoots m^{-2} and thereby provides a measure of seagrass abundance along depth gradients. This descriptor was measured along every single transect three times, for a total of 18 measures each station, using a 25x25 cm PVC frame. These values were reconducted to a square meter. This variable represents one of the most important descriptors to appraise the status of a meadow, above all if measured on a multiannual temporal scale (Buia et al., 2003).

- Covering: is a measure of abundance, but expressed as a percentage. It consists in visual estimation of the percentage of the surface of the bottom occupied by the shoots of *P. oceanica* on a known area. Ten measures of this variable were conducted on each transect (one each meter), for a total of 60 measures in every station, using a 40x40 cm PVC frame. To facilitate the sampling, frames were subdivided into 4 subsquares of 25x25 cm. To each subsquare a value ranging from 0 % to 100 % was assigned according to the proportion of surface that was

occupied by the shoots (Fig. 3.16). All measures were done by two previously inter-calibrated operators.

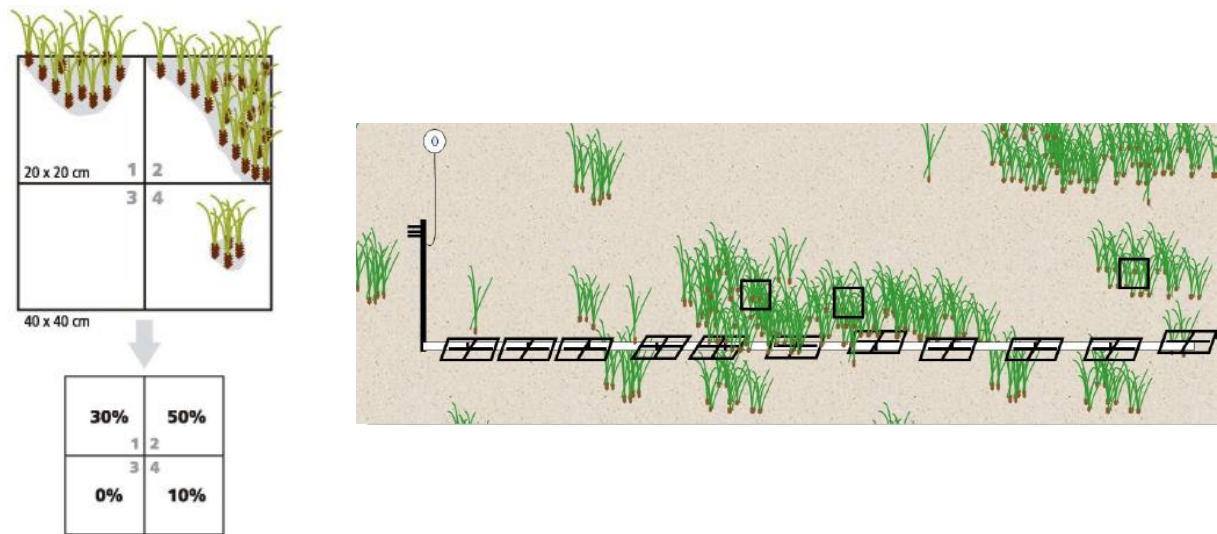


Figure 3.16. Estimate of Covering structural descriptors of the *P. oceanica* meadow, along a transect of 10 m length (Marbà et al. 2015).

From these two descriptors the Global density was estimated as the result of the product of the average density inside *P. oceanica* patches and the proportion covered by the meadow (Romero 1985).

- Burial: it is the vertical distance (in centimeters) between the level of the sediment and the ligule of the most external leaf of the shoot of *P. oceanica*. The value and sign of this distance changes depending on the balance among the vertical growth of the plant and the dynamics of the sediments in every site: it is positive (sediment level below the ligule line) if erosion processes are predominant, and negative (sediment level above the ligula) if an excessive accumulation of sediments exists on the meadow. The burial of the vertical shoots of *P. oceanica* then is a symptom of erosion of the meadow. Both the erosion and the deposition of sediment provokes an increase in the mortality of shoots of the meadow (Cabaço et al., 2008). The meadow to be healthy, the sediment must be stable or accumulate at low rates, so that the meadow could respond with an elevation rate of the substratum of few cm year^{-1} (Gacia and Duarte 2001). High rates of sediment deposition provoke the burial of the meadows. Accumulations of sediment superior to 10 cm provoke the mortality of shoots of 50 %, and when the burial of the rhizome exceeds 14-15 cm, the mortality of shoots is 100 % (Cabaço et al., 2008). To evaluate the burial of the shoots on the selected stations, the vertical distance (in centimeters) between the sediment and the lígula (meristem of growth) was measured in one of

the external leaves of the bundle of *P. oceanica*. In every station these measures were realized 12 times each transect.

Furthermore, in each station 30 shoots were collected to proceed with the analysis of the morphology of the leaves. In this case, the functional descriptors analyzed were:

-Number of leaves/shoot

-Leaves length

-Leaves width: measured around the middle of the leaf.

-Apex status: it can appear entire, broken or bitten from some herbivorous. It can be distinguished from the shape like in Figure 3.17.

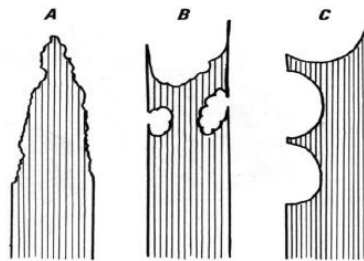


Figure 3.17. Herbivorous traces on *P. oceanica* leaves (Buia et al. 2003)

From this synthetic descriptors can calculate the derived descriptors. They are:

- Leaf area average for shoot ($\text{cm}^2 \text{shoot}^{-1}$): for every shoot, the surfaces of all the leaf (an only face is considered) edges are calculated, therefore the gotten values are added for having the total area of the shoot and they are finally mediated for the number of sampled shoots.

- Herbivorous rate: how many leaves result vices herbivorous on the total one, directly derived from the analysis of the apex status.

- Leaf Area Index ($\text{m}^2 \text{m}^{-2}$): it is calculated multiplying the surface leaf average for shoot for the density of the meadow for each square meter.

- Coefficient "A": percentage of adult and intermediary leaves that has the apexes broken; it is calculated dividing the total number of adult and intermediary leaves with broken apexes for the total number of the observed leaves. The value of the coefficient "A" it is more often the result of some factors like the hydrodynamism, the age of the leaves and the level of presence of herbivores.

3.3.1 Statistical analysis

With the aim of verifying the small-scale variability, and therefore between the transects of each station, for the structural parameters, an ANOVA two-way nested was performed following the sampling design, using R statistical software. This statistical analysis was conducted after verifying the normality of data distribution with the Shapiro-Wilk test ($p > 0.05$) and the Levene test was used to confirm homogeneity of variances ($p > 0.05$).

4. Results

4.1 Physical descriptors

4.1.1 Water conditions

The water temperatures recorded in the water column throughout the year showed the seasonal pattern typical of temperate seas, with a minimum temperature of 14°C and a maximal one of 30.9°C (Fig. 4.1).

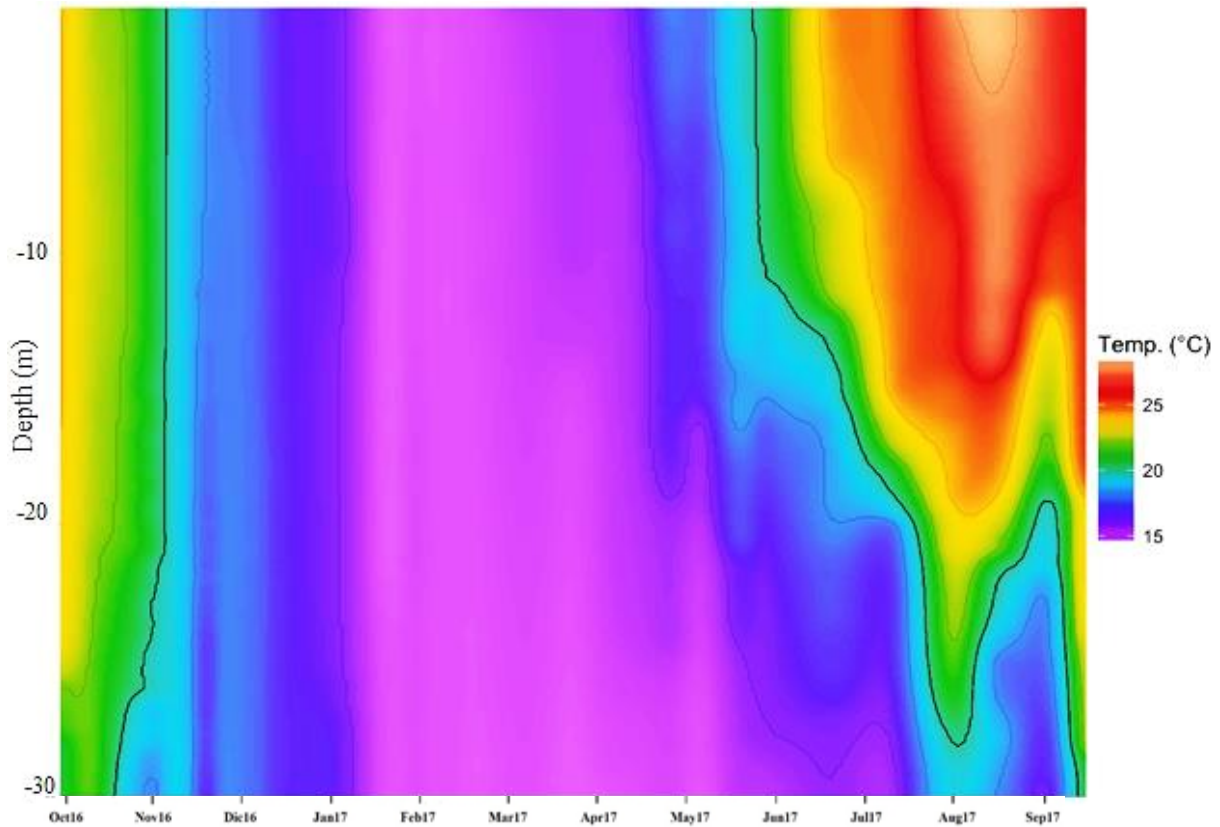


Figure 4.1. Temperature time series along bathymetric gradient.

From October to February the temperatures recorded in the water column are homogeneous. Bearing in mind that there is no fresh water supply and having verified that at different depths even salinity remains homogeneous along the water column, it could be stated that during the winter season the water masses had equivalent density. Considering the condition of high hydrodynamism characteristic of that season, it could, therefore, be assumed that there was a complete reshuffle affecting the entire water column.

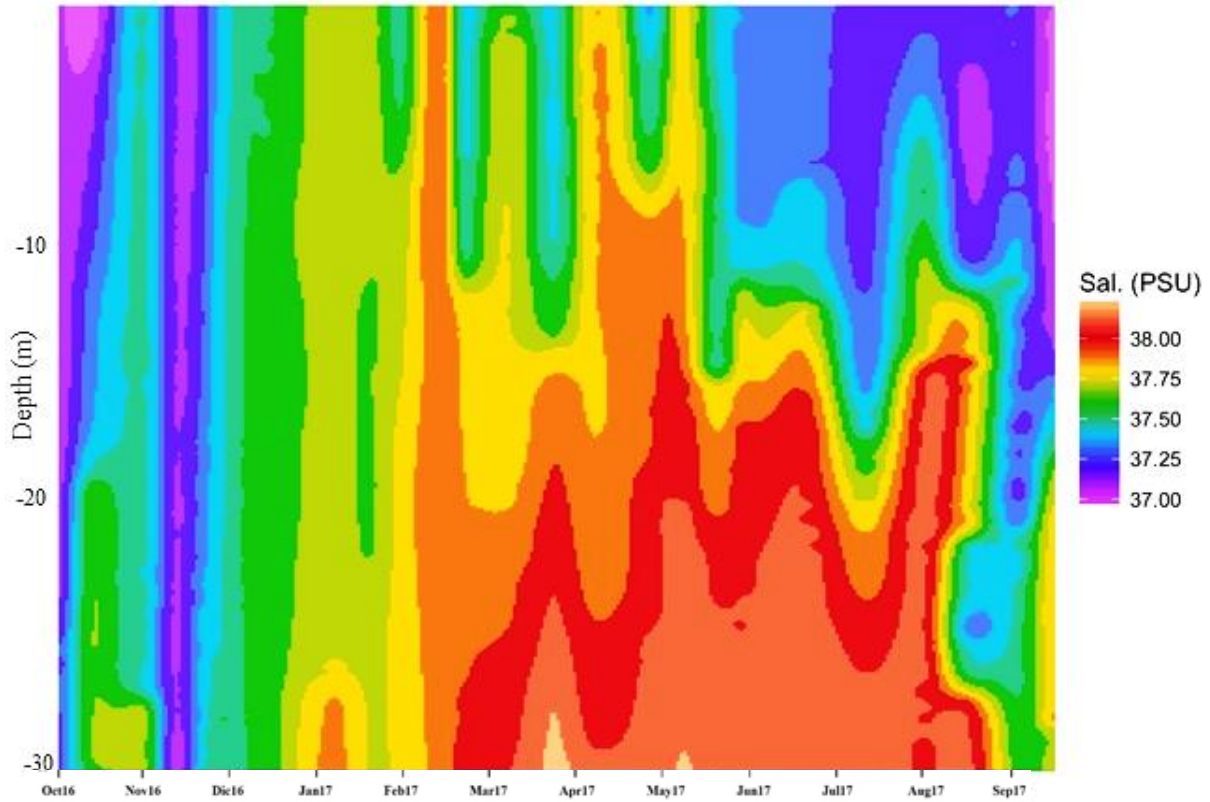


Figure 4.2. Salinity time series along bathymetric gradient.

From February, with the advent of spring and hence rising air temperature, the structure of the water masses changed. In fact, from this time of year, a stratification process of water masses occurs, due to the superficial warming, which becomes increasingly evident in the summer season, where it became clear the thermocline at a depth around -15m (Fig 4.3).

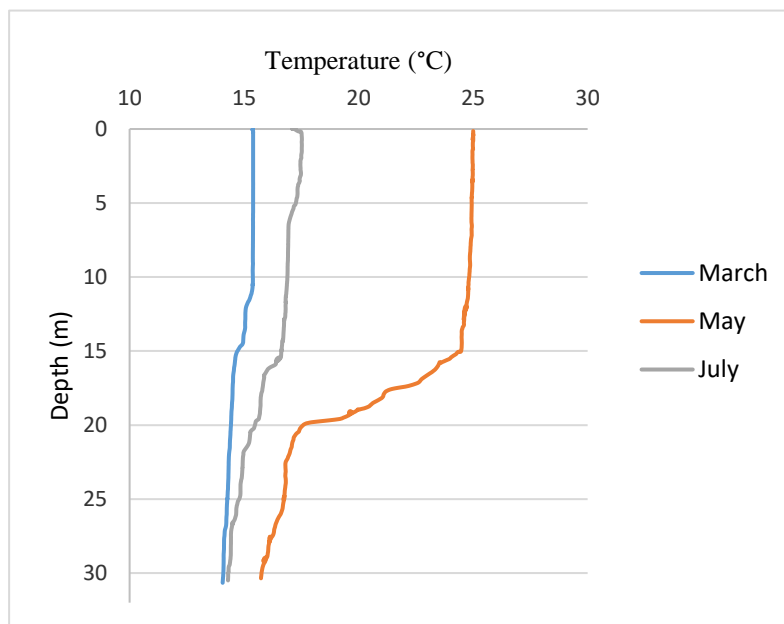


Figure 4.3. Temperature profiles, thermocline formation process.

The establishment of the thermocline, together with the abundant rains of the early months of the year, induces a change in the density of water masses preventing its mixing; this is also evident in the salinity chart (Fig. 4.2) two distinct water bodies can be identified above and below -15m. There are hot waters with less salinity, less dense in the surface layer, and more dense deeper, colder and saltier waters below. At the end of the summer season, it is perceived by the data as the situation set up during the hottest period of the year, tends to return to the winter homogeneity with the thermocline breaking due to the strong hydrodynamic regime and the overall lowering of temperatures.

Another of the physical parameters under consideration was turbidity, in fact, under high conditions, it is one of the drivers that guide the distribution and density of the *P. oceanica* meadows. Values of this variable measured throughout the year were not high and were unlikely to be able to affect the health of the plant. The only spikes that occur during the winter season were related to isolated climatic events that have caused the particulate fraction present in the sediment to be brought back into suspension (Fig. 4.4).

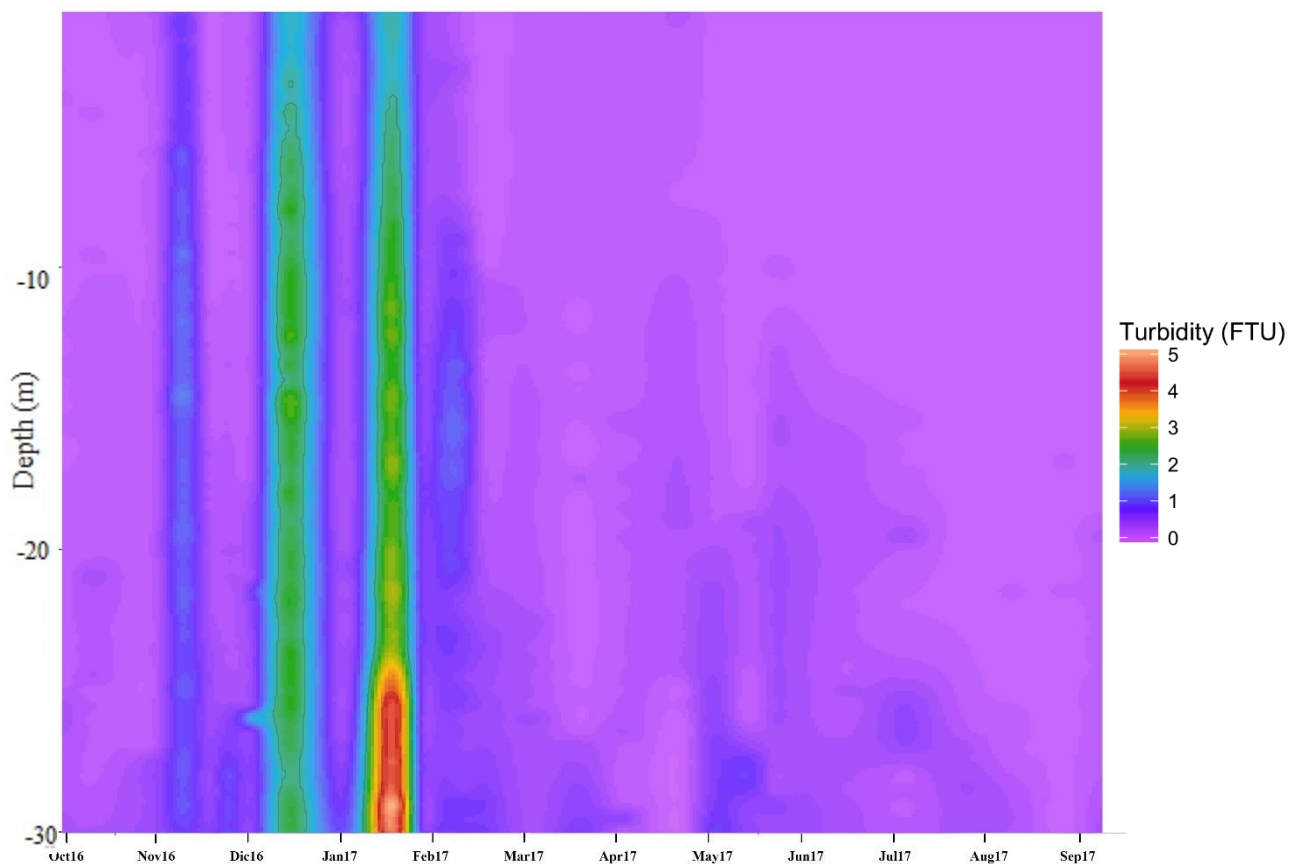


Figure 4.4. Turbidity time series along bathymetric gradient.

Concerning dissolved oxygen values, two types of patterns could be observed (Fig. 4.5). Except for November, in accordance with the above, a uniformity of values for the entire water column occurred. The situation began to diffract with the approach of spring and hence the growth of phytoplankton on the shallower layers.

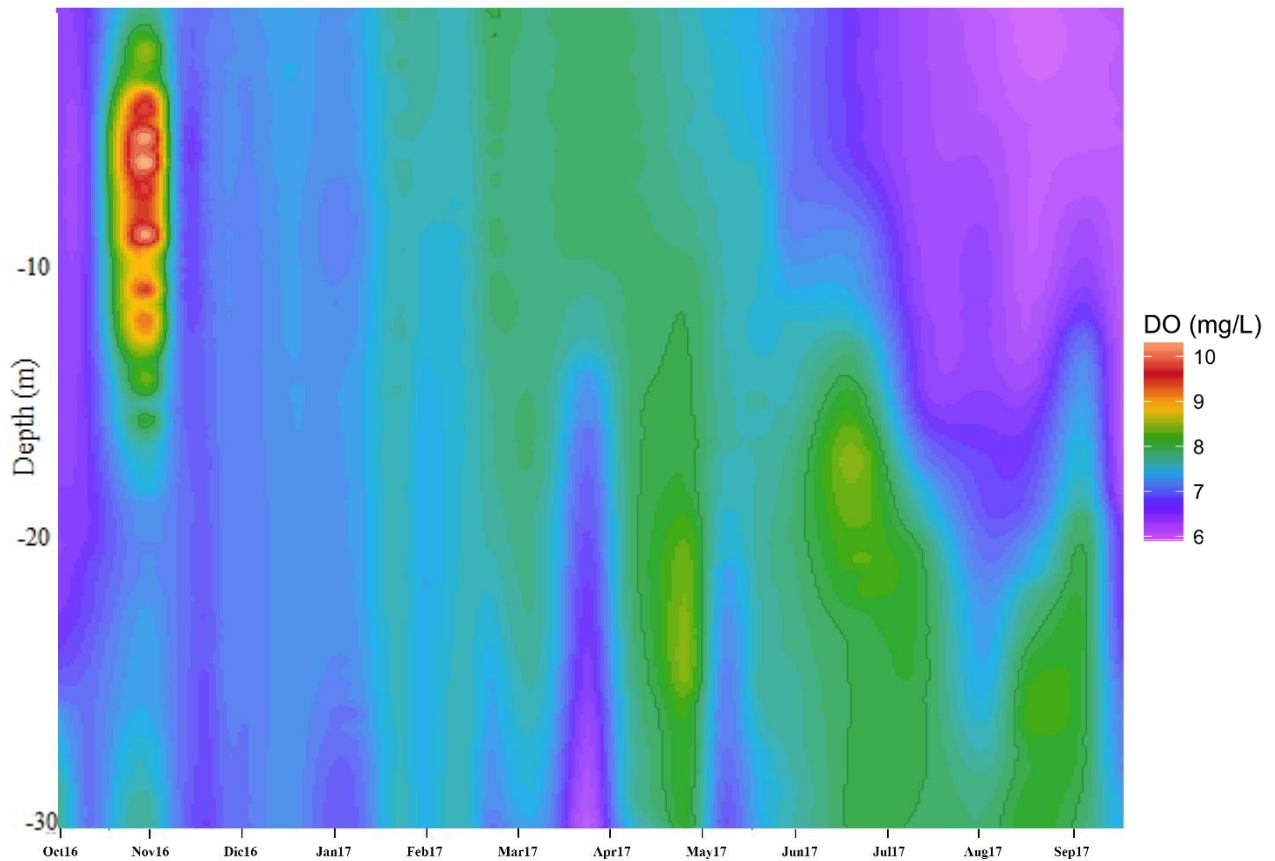


Figure 4.5 Dissolved oxygen time series.

In fact, a large number of primary producers attracts its direct predators, zooplankton. These microorganisms, located in the same bathymetric band of their prey, tend to consume large amounts of oxygen, resulting in a decrease of it in surface concentrations (Gilly et al., 2013). The great difference with the deep oxygen concentrations during the summer season was always due to the presence of the thermocline that prevented the mixing of water, but was further accentuated by *P. oceanica*'s oxygen production peaks that are highest in the summer.

4.1.2 Sediment analysis

From a preliminary visualization of the results gotten by the size particle analysis of the annual series of the samples of sediment, it emerges that in all the three stations, the dominant dimensional class is sand (Wentworth scale 2-0.063 mm).

The analysis of the annual series of sediment samples has initially been investigated through the use of the ternary diagram of Shepard (1954), graphically represented using Grapher 9 software, that brings on the sides of a triangle the data ponder them some different granulometric fractions and it has the purpose to classify the sediment in groups that have different ecological meaning in relation to where they are placed. With this typology of analysis the picked samples in the three stations lasting the whole arc of the year show to be very similar among them and to belong all to the category of sands (Fig. 4.6, 4.7, 4.8).

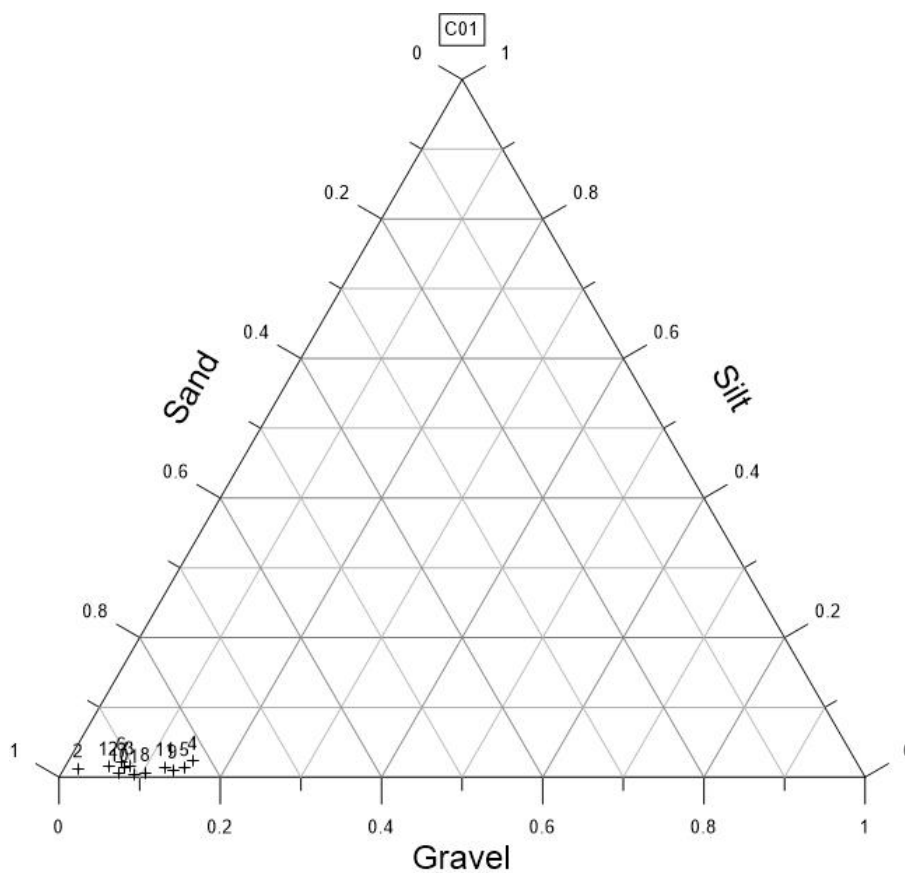


Figure 4.6. Shepard triangle for C01 sampling station.

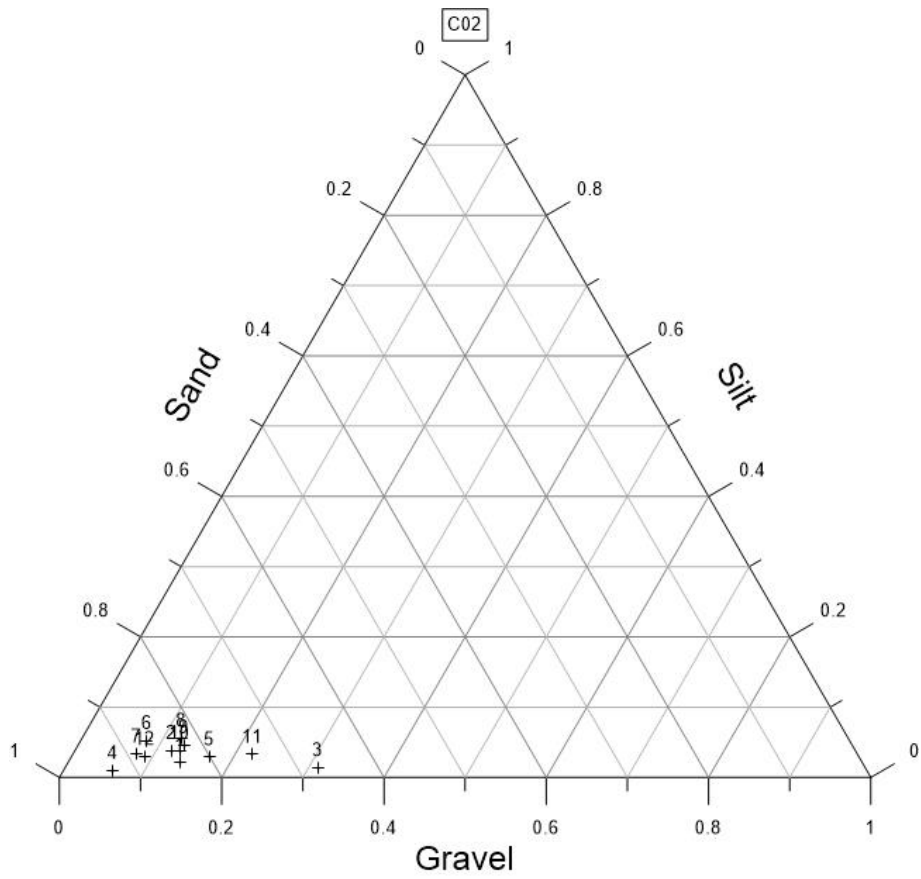


Figure 4.7. Shepard triangle for C02 sampling station.

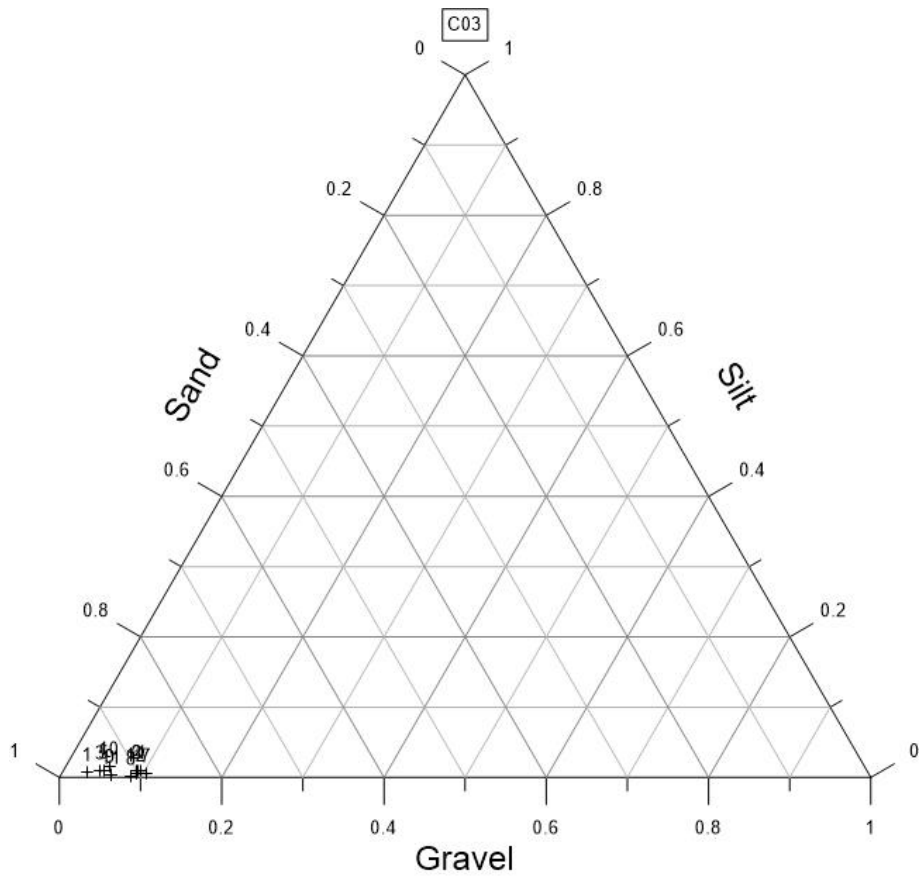


Figure 4.8. Shepard triangle for C03 sampling station.

The following ternary diagram has been made, derived by the first one, which brings a different classification, with the purpose to show the possible heterogeneities among samples, therefore having a pure descriptive value. The weights of each dimensional class separated with the sieves were declassified in different dimensional categories within the group of sands (Coarse sand >1mm, medium sand 1-0.250mm, fine sand < 0.250mm), considering that they constitute around the 90% of the total weight in all samples. Also in this case samples are results to be very homogeneous inside the same stations during the whole year (Fig. 4.9, 4.10, 4.11).

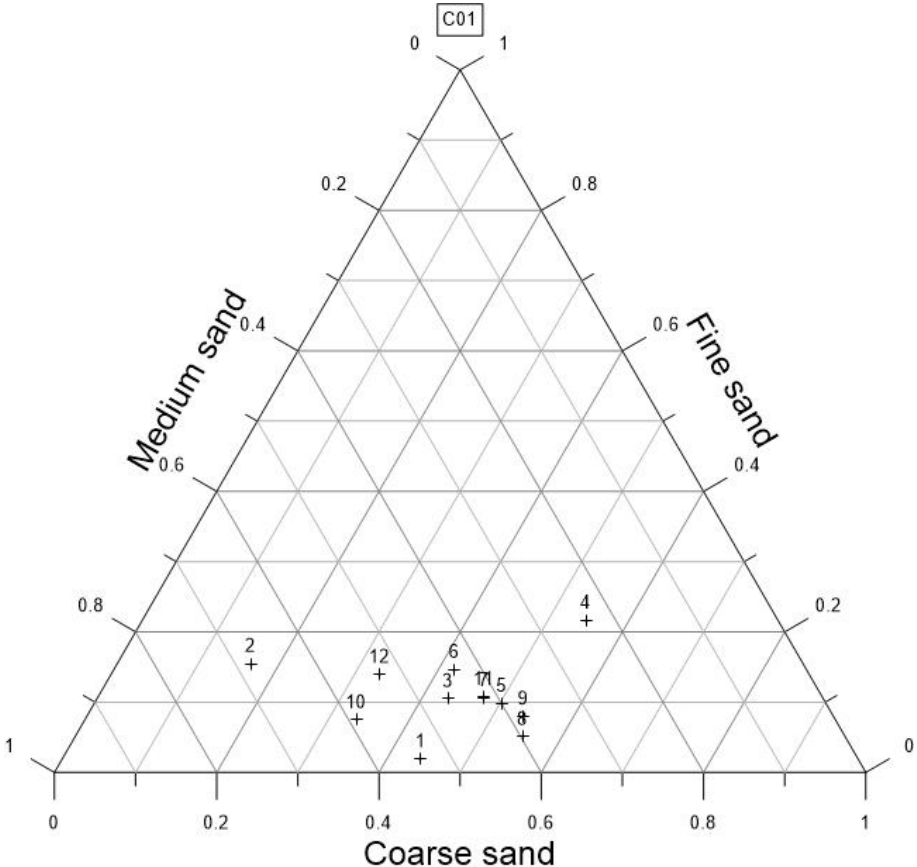


Figure 4.9. Sands classification triangle for C01 sampling station.

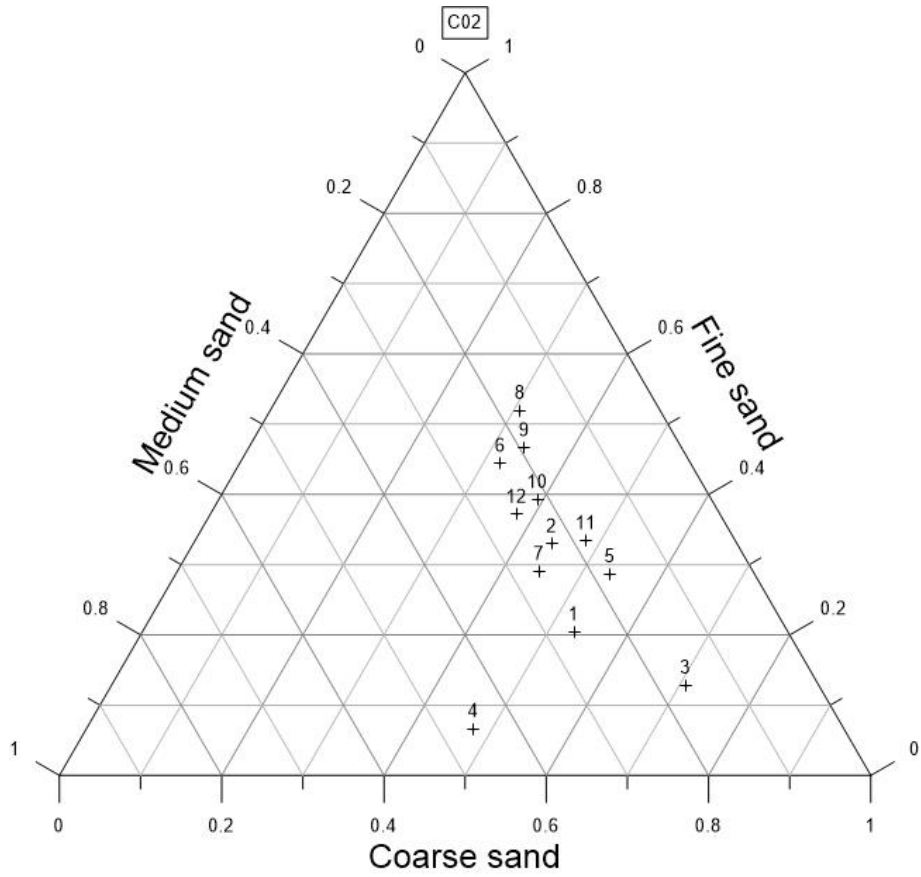


Figure 4.10. Sands classification triangle for C02 sampling station.

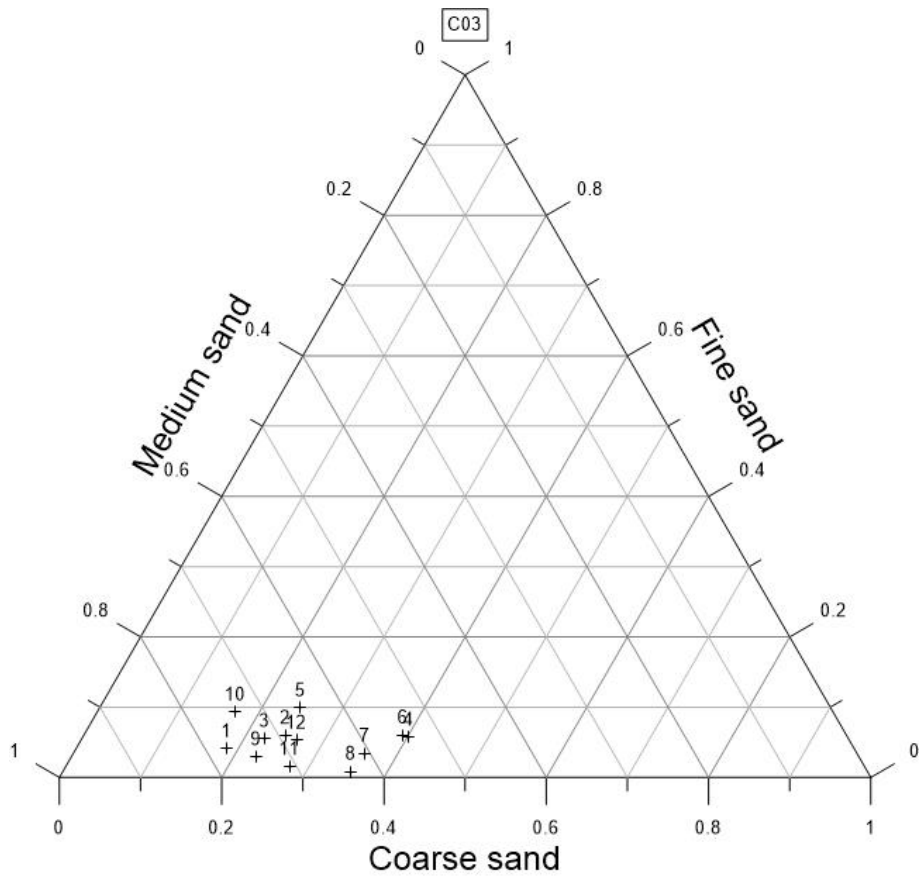


Figure 4.11. Sands classification triangle for C03 sampling station.

The variability during the year, inside the stations at different depth, has been investigated using the weights related to every level of the battery of sieves in relationship to the time. With such subdivision, following the calculation of the Pearson's correlation index, meaningful tendencies to temporal variation does not appear, for the C01 and C03 station, while instead meaningful results are gotten by the analysis of the C02 station. At C02 a positive correlation between time and sediment fraction of 0.5mm, and a negative correlation between time and sediment >0.5mm was found. During the year, from the winter toward the summer, there is, therefore, a situation of increase of thin sand and a diminution of coarse sand.

Everything seems to point out that in the C01 and C03 stations, in the whole arc of the year, there is a rather stable sediment structure suggesting that hydrodynamism is not particularly subject to seasonal variations, while the C02 station, show a correlation between time and different dimensional classes and therefore a temporal pattern.

The initial hypothesis of seasonal variation in the sediment particle size, seems denial for the superficial and deep station, but not for the intermediary one, but to affirm it with certainty further studies replicated in time are needed.

Proceeding with the results of the granulometric parameters, giving them information on the hydrodynamic conditions, they confirm a condition of elevated/medium hydrodynamism in the first and third station and a scarce/weak hydrodynamism in the intermedite one during the whole year. Besides, it appears clear that in the three stations the absence of a selective agent, contributes to a bad classation of the sediments (Tab. 4.1).

The peculiar hydrodynamic situation in the zone C02 should subsequently be investigated. It could be that *P. oceanica* meadow, reducing the energy of the waves, already less intense in the summer period, facilitates an appropriation of the thin particles in this zone.

Station	Sample	Mdφ	σ	Sk	K	Fh		
C01	1	0.11	0.76	-0.23	asymmetrical very negative	0.88 platicurtic	0.31	medium or strong hydrodynamism
C01	2	0.57	1.14	0.09	asymmetrical very positive	high energy	1.61	medium or strong hydrodynamism
C01	3	0.15	1.15	-0.09	symmetrical	low energy	0.98	medium or strong hydrodynamism
C01	4	-0.12	1.77	0.32	asymmetrical very positive	high energy	0.40	medium or strong hydrodynamism
C01	5	-0.01	1.23	-0.06	symmetrical	high energy	0.86	medium or strong hydrodynamism
C01	6	0.19	1.21	0.01	symmetrical	low energy	0.96	medium or strong hydrodynamism
C01	7	0.06	1.15	0.00	symmetrical	low energy	0.95	medium or strong hydrodynamism
C01	8	-0.12	0.95	0.14	asymmetrical positive	low energy	0.63	medium or strong hydrodynamism
C01	9	-0.10	1.16	-0.04	symmetrical	high energy	0.84	medium or strong hydrodynamism
C01	10	0.30	1.03	0.01	symmetrical	low energy	1.05	medium or strong hydrodynamism
C01	11	0.06	1.21	-0.08	symmetrical	high energy	0.87	medium or strong hydrodynamism
C01	12	0.34	1.17	-0.05	symmetrical	high energy	1.12	medium or strong hydrodynamism
C02	1	-0.08	1.71	0.47	asymmetrical very positive	low energy	0.50	medium or strong hydrodynamism
C02	2	0.31	1.90	0.38	asymmetrical very positive	low energy	-1.03	hydrodynamism almost nil
C02	3	-0.52	1.41	0.35	asymmetrical very positive	low energy	0.73	medium or strong hydrodynamism
C02	4	0.05	1.03	0.19	asymmetrical positive	low energy	0.89	medium or strong hydrodynamism
C02	5	-0.10	1.93	0.49	asymmetrical very positive	low energy	-0.92	medium or strong hydrodynamism
C02	6	0.93	1.89	0.20	asymmetrical positive	low energy	-1.16	hydrodynamism almost nil
C02	7	0.24	1.81	0.44	asymmetrical very positive	low energy	-0.77	hydrodynamism almost nil
C02	8	2.64	1.98	-0.51	asymmetrical very negative	high energy	-1.22	hydrodynamism almost nil
C02	9	1.40	1.96	-0.05	symmetrical	high energy	-1.23	hydrodynamism almost nil
C02	10	0.65	1.94	0.24	asymmetrical positive	low energy	-1.21	hydrodynamism almost nil
C02	11	0.13	2.01	0.36	asymmetrical very positive	low energy	-1.18	hydrodynamism almost nil
C02	12	0.61	1.85	0.29	asymmetrical positive	low energy	-1.07	hydrodynamism almost nil
C03	1	0.48	0.73	-0.02	symmetrical	high energy	1.10	medium or strong hydrodynamism
C03	2	0.50	1.09	-0.03	symmetrical	high energy	1.16	medium or strong hydrodynamism
C03	3	0.49	0.90	0.02	symmetrical	low energy	1.11	medium or strong hydrodynamism
C03	4	0.36	1.04	-0.10	symmetrical	high energy	0.56	medium or strong hydrodynamism
C03	5	0.61	1.13	0.08	symmetrical	low energy	1.42	medium or strong hydrodynamism
C03	6	0.37	1.03	-0.12	asymmetrical negative	high energy	0.67	medium or strong hydrodynamism
C03	7	0.52	0.95	-0.35	asymmetrical very negative	high energy	0.52	medium or strong hydrodynamism
C03	8	0.49	0.86	-0.39	asymmetrical very negative	high energy	0.53	medium or strong hydrodynamism
C03	9	0.62	0.88	-0.20	asymmetrical negative	high energy	0.98	medium or strong hydrodynamism
C03	10	0.57	1.08	0.24	asymmetrical positive	high energy	1.25	medium or strong hydrodynamism
C03	11	0.39	0.84	-0.14	asymmetrical negative	high energy	0.78	medium or strong hydrodynamism
C03	12	0.52	1.02	-0.15	asymmetrical negative	high energy	0.96	medium or strong hydrodynamism

Table 4.1. Falk&Ward parameters results.

4.3 *P. oceanica* monitoring results

The results obtained for each descriptor of the *P. oceanica* meadows are presented as explained in the section Materials and methods.

Passing through the results of the **physiographical** descriptors, from a bathymetric point of view, the meadow of Cala Racò is located within the depth range of 5-22 m, and was characterized by a predominantly sandy bottom, with large rocky blocks. In this location, the meadow showed some signs of discontinuities and clearings of various form and size, within one of which there was a wreck of a small fishing boat. Since it is characterized by the presence of a continuous and homogeneous mat, the bed can be classified as “flat and continuous” (“*herbier de plain*”) (Pergent and Pergent-Martini, 1995). The lower limit was fairly sinuous and develops mainly along the isobath of -22 meters and was classified as a “net limit” as it was characterized by a sharp meadow break, the presence of orthotropic and plagiotropic rhizomes and the lack of “matte” (Fig. 4.12).



Figure 4.12. Limit of the meadow of Cala Racò (A3 station).

Instead, with regard to the north side of the Peñón de Ifach, the patch of *P. oceanica* in which station B was placed was entirely developed at -24m, on a sandy bottom where there were huge stone blocks on which *P. oceanica* tufts were present. Here it is possible to identify an hilly bed (“*herbier de colline*”), characterized by patches (“small islands”) of mat, covered with green vegetation which rise up from the surrounding sandy areas that are devoid of vegetation (Boudouresque et al., 1985). Here the lower limit (Fig. 4.13) was classified as a “regressive limit” characterized by the presence of dead mat, on which isolated live shoots persist. A limit of this type indicates a regression of the meadow which is often related to increased turbidity.



Figure 4.13. Limit of the patch of *P. oceanica* on the north side, B station.

The reef-meadow extended along the entire coastline in the bathymetric band ranging from -5m to -9m; in this case station C was placed at the lower limit of the meadow and it was classified as "erosion limit" (Fig. 4.14) as it was characterized by the presence of a clear "matte" step (Fig 4.15), with prevalence of orthotropic rhizomes.



Figure 4.14. Limit of the reef-meadow, C station.



Figure 4.15. Detail of the step of "matte" in C station's limit.

Between the **structural** descriptors, the shoot density values of the stations were heterogeneous, they ranged from a minimum mean value of 261.33 ± 14.11 shoot m^{-2} , in the second transect of B station, and maximum mean value of 1237.33 ± 244.93 shoot m^{-2} in the fourth transect of C station, as shown in Figure 4.16.

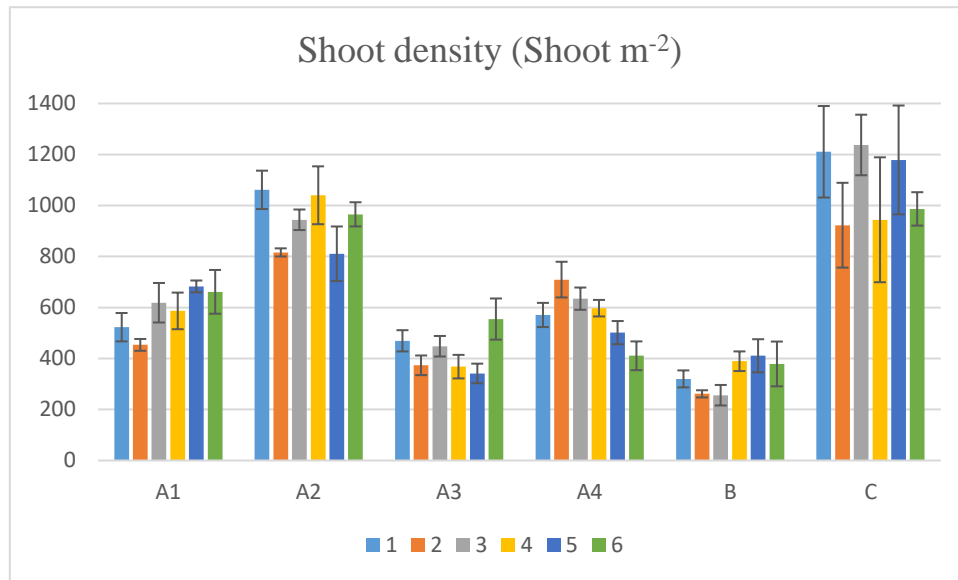


Figure 4.16. Mean Shoot density values for each transect of each station.

The average of Covering for each station during the study period is graphically represented in figure 4.17, with its relative standard error. The covering values in these stations ranged between a minimum mean value of 6.94 ± 1.93 % in the last transect of the deepest station (B) on the north side of the Peñón de Ifach and maximum mean value of 66.69 ± 4.83 % in the second transect of the shallower station in Cala Racò (A1).

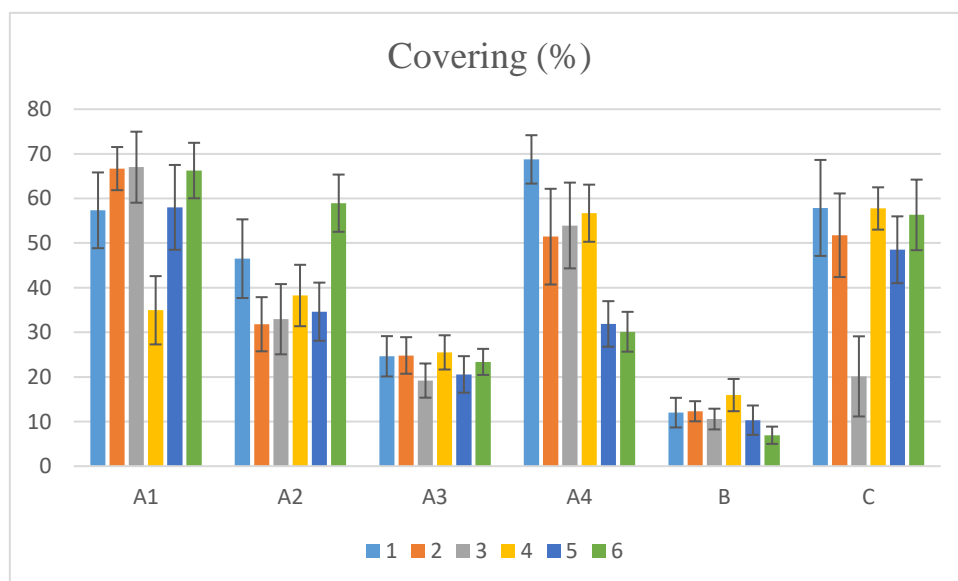


Figure 4.18. Mean Covering values for each transect of each station.

The values of Global Density, calculated from the density and covering values, as presented in the previous section 3.3, ranged between minimum mean value of 26.27 ± 6.09 shoot m^{-2} , in B station and maximum mean value of 700.67 ± 103.85 shoot m^{-2} at C station, following the pattern of the maximum density previously exposed (Fig 4.18).

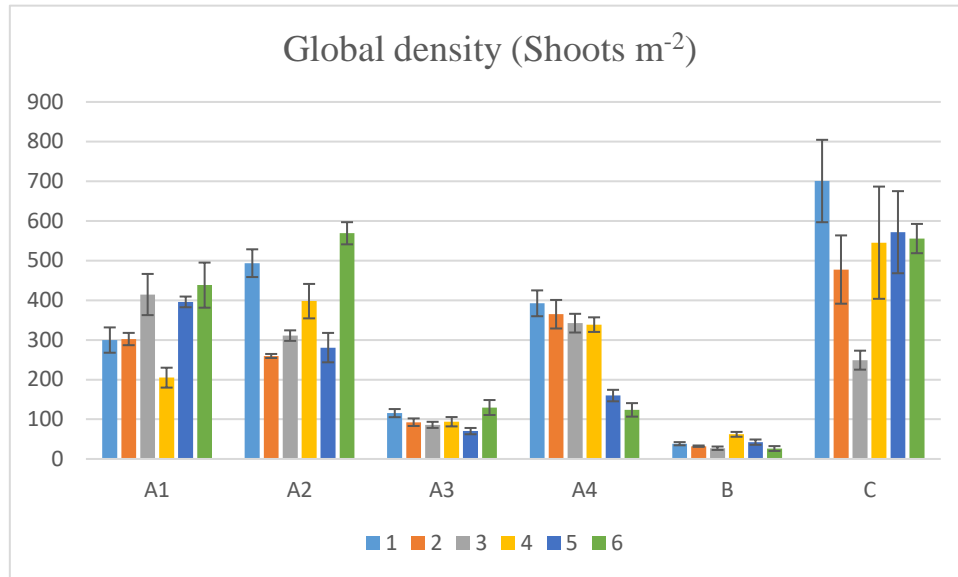


Figure 4.18. Mean Global density values for each transect of each station.

Subsequently to the calculation of the average global density for each station, it was possible to compare the data with those reported in the literature by Pergent et al., (1995). In fact, these authors formulated a broad classification of the health status of *P. oceanica* meadows. Density is related to many environmental parameters: depth, turbidity, anthropogenic disturbance, etc. In this classification "balanced meadows", where the density is standard or exceptional, are distinguished from "disturbed meadows" or "heavily disturbed meadows", where density, limited by several factors, is low (Fig 4.19).

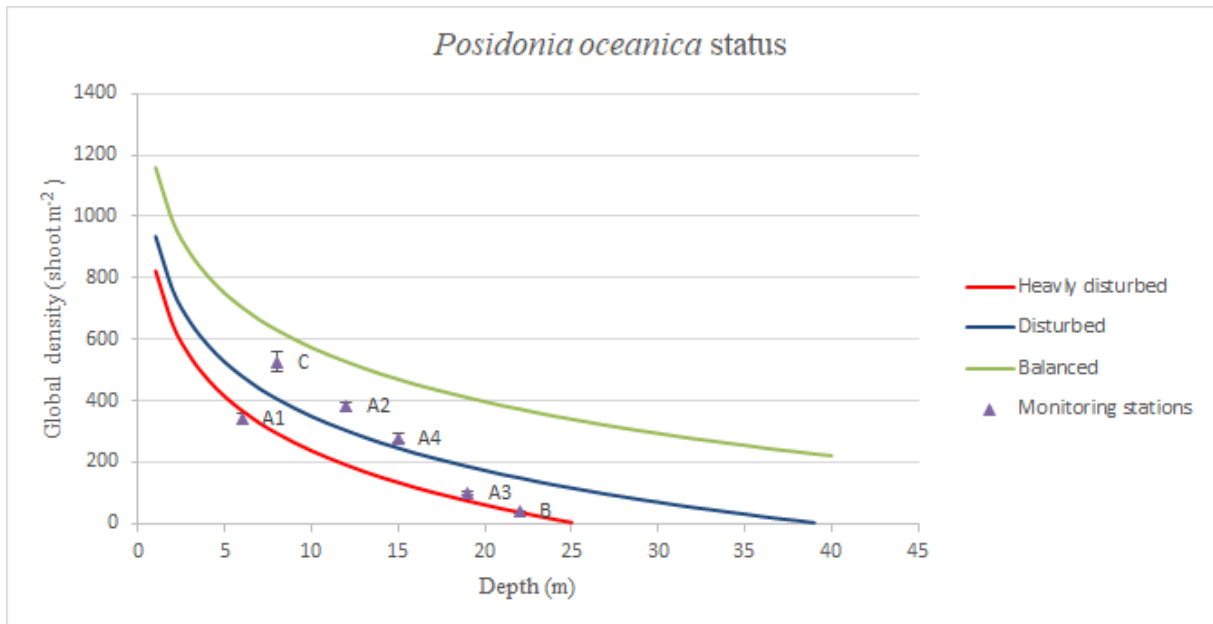


Figure 4.19. *P. oceanica* status, modified from Pergent et al. 1995.

It turns out that half of the density values measured at the stations were in the standard, while the others showed very low or unusual values. Reporting the density values thus analyzed, to the meadow to which they belong, it could be said that the Cala Racò (A1, A2, A3, A4) meadow was ranked as "balanced" at intermediate bathymetry and "disturbed" both in surface and in the lower limit; while the meadow on the north side of the Peñón de Ifach, the one in which B station was placed was at the boundary between "disturbed" and "heavily disturbed" while the one in which C station was localized was "balanced".

The last structural descriptor analyzed was the burial of the shoots. These measures, done in all the transect of each station, are shown in Figure 4.20 with its standard error. In all stations, a

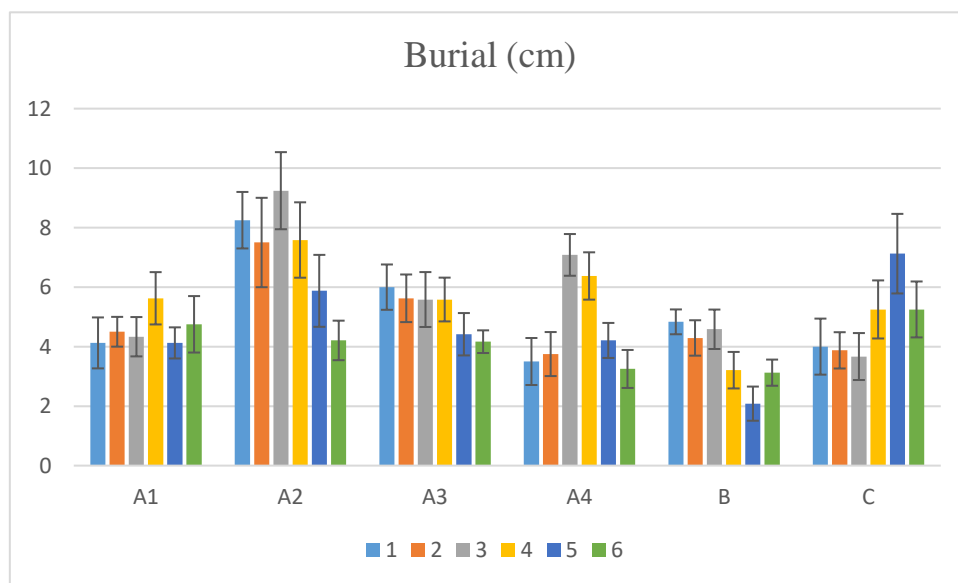


Figure 4.20. Mean Burial values for each transect of each station.

positive trend of the parameter was evident, which coincides with a slight disintegration of the shoots.

For all structural descriptors, due to the nature of the sampling design, a two-way nested ANOVA was performed, which revealed significant differences only between stations, but not among the transects conducted within them (Tab. 4.2); therefore it was possible to exclude a significant variation among transects within stations.

ANOVA Density			Alpha		0.05	
	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>p-value</i>	<i>sig</i>
Transects	133668.9	5	26733.77	0.21732	0.952354	no
Stations	3690468	30	123015.6	21.38628	3.05E-25	yes
Within	414149.8	72	5752.081			
Total	4238287	107	39610.15			

ANOVA Covering			Alpha		0.05	
	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>p-value</i>	<i>sig</i>
Transects	4941.152	5	988.2304	0.235562	0.943742	no
Stations	125856	30	4195.201	9.784884	2.55E-30	yes
Within	138912.7	324	428.743			
Total	269709.9	359	751.281			

ANOVA Burial			Alpha		0.05	
	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>p-value</i>	<i>sig</i>
Transects	132.2681	5	26.45361	0.870643	0.512325	no
Stations	911.5194	30	30.38398	3.49386	7.34E-09	yes
Within	3443.772	396	8.696393			
Total	4487.559	431	10.41197			

Table 4.2. ANOVA results for (1) Shoot density, (2) Covering and (3) Burial.

Below are the results relating to the **functional** descriptors, thus all those obtained from the phenological analysis of leaves and shoots, taken at each station.

The average number of leaves per shoot was measured at all stations and it is shown in Figure 4.21 with its relative standard error. As is possible to see, the number of leaves ranges between a minimum of 4.6 ± 0.13 recorded at station A1, to a maximum value of 6.67 ± 0.34 at B station. Data were compared to what was reported in the literature, through a t-test, but no homogeneity was found. In fact, the average number of leaves per shoot reported from Buia et al., (2003) is of 8 leaves in shallow zones (less than -15 m) and 7 leaves in deep zones (more than -15 m). Our data do not comply with the pattern described in the literature, which foresees a decrease in the number of leaves per shoot along the bathymetric gradient, but it seems to be opposed as shown in Figure 4.22.

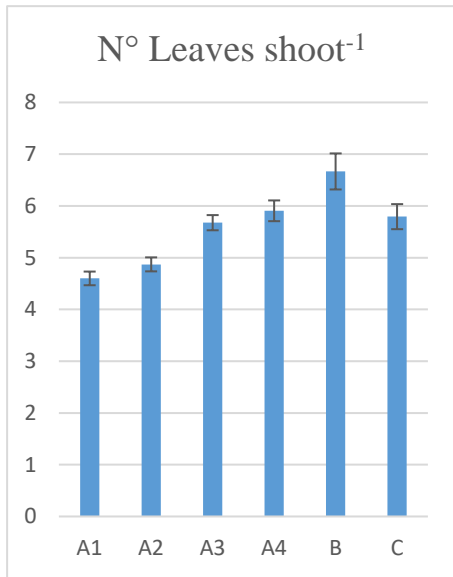


Figure 4.21. Mean values of number of leaves per shoot.

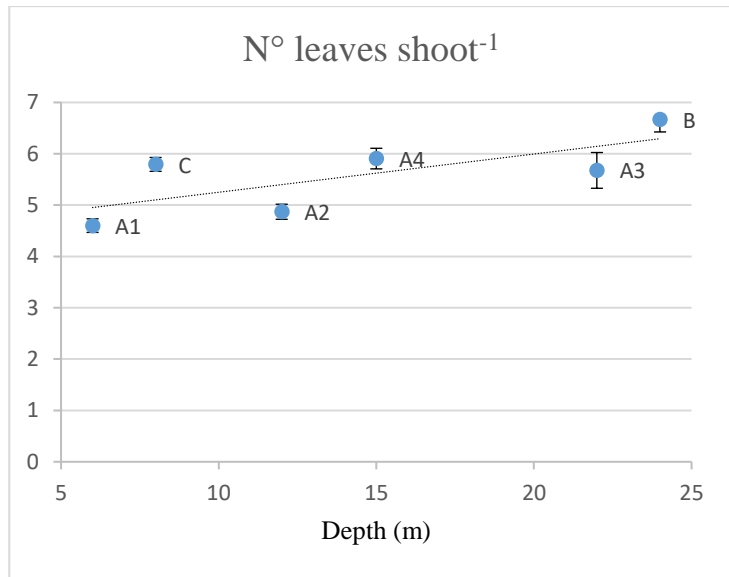


Figure 4.22. Number of leaves per shoot, along bathymetric gradient.

The average values of *P. oceanica* leaf length in the investigated meadows ranged between a minimum mean value of 34.08 ± 2.47 cm in the deepest station (B); to the maximum mean value of 68.32 ± 2.34 cm in the shallowest station (A1). All values are listed in Figure 4.23 with their standard errors. In this case, the pattern found complies with what is reported in the literature. In fact, the leaf length decreased along the bathymetric gradient as shown in Figure 4.24. Moreover, Pearson's correlation index showed a very high degree of correlation between the measured variable and the environmental variable ($r = -0.83$, $\rho < 0.05$).

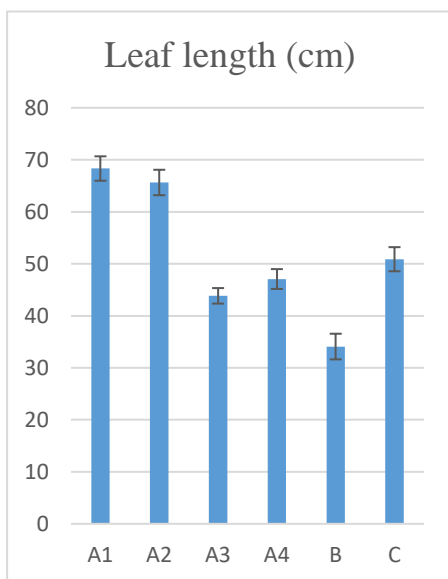


Figure 4.24. Mean Leaf length values in each station.

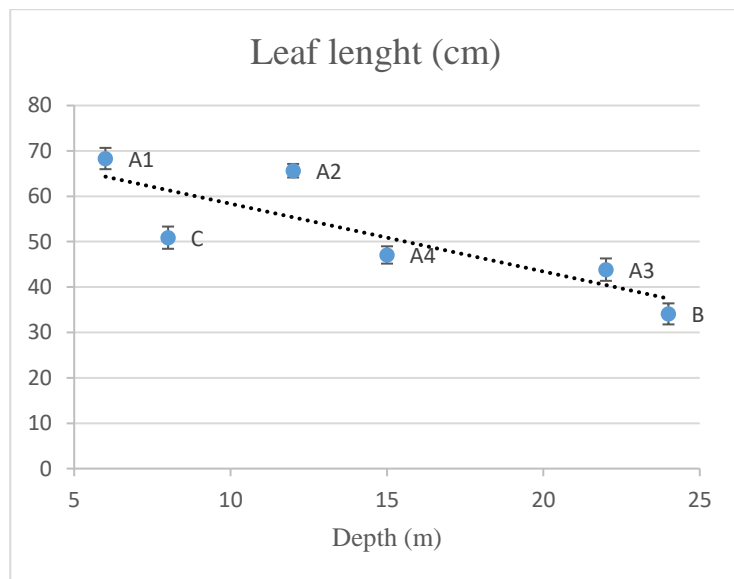


Figure 4.25. Leaf length along bathymetric gradient.

About leaves width values, they ranged between 0.8 and 1.1 cm, with an average value of 0.95 cm in all stations; a t-test showed homogeneity with what is reported in the literature.

For what concerns the derived descriptors, calculated values for the mean leaf surface parameter for shoot are shown in Figure 4.26 with the corresponding standard errors; these ranged from a minimum of $226.74 \pm 10.77 \text{ cm}^2 \text{ shoot}^{-1}$ (B) up to a maximum of $319.70 \pm 14.68 \text{ cm}^2 \text{ shoot}^{-1}$ (A2). Also in this case, the trend of the variable along the bathymetric gradient has been studied to verify consistency with the pattern described in the literature, which shows a decrease as the depth increases. This was verified, as can be seen in Figure 4.26, and subsequently the calculation of the Pearson correlation index, showed that the two variables were closely related ($r = -0.84, \rho < 0.05$).

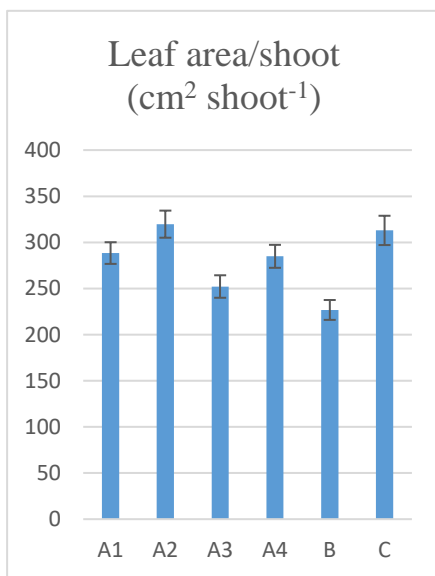


Figure 4.25. Mean Leaf area/shoot values in each station.

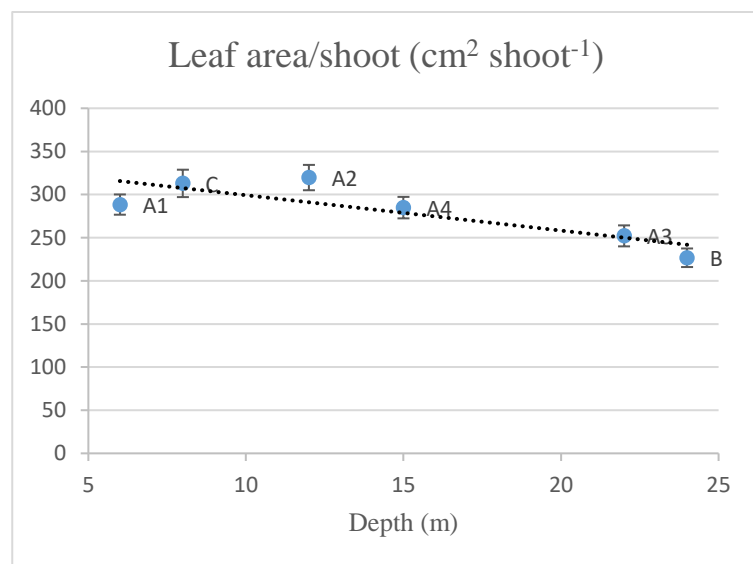


Figure 4.26. Leaf area/shoot values along bathymetric gradient

Concerning the measures regarding the status of leaf apex, it showed great heterogeneity between the stations (fig), with maximum values of leaves whose apex seemed to be broken because of the action of some herbivore reaching $70.25 \pm 4.07\%$ in the superficial station A1, as shown in Figure 4.27. Later, the values of the herbivorous rate were compared to the bathymetric gradient. Even in this case, it was possible to detect a negative relationship between the two variables; in fact, as can be seen in Figure 4.28, as the depth increased, decreased the number of leaves showing traces due to the herbivorous activity. Between the two variables, a no correlation was found, always tested by the Pearson correlation index.

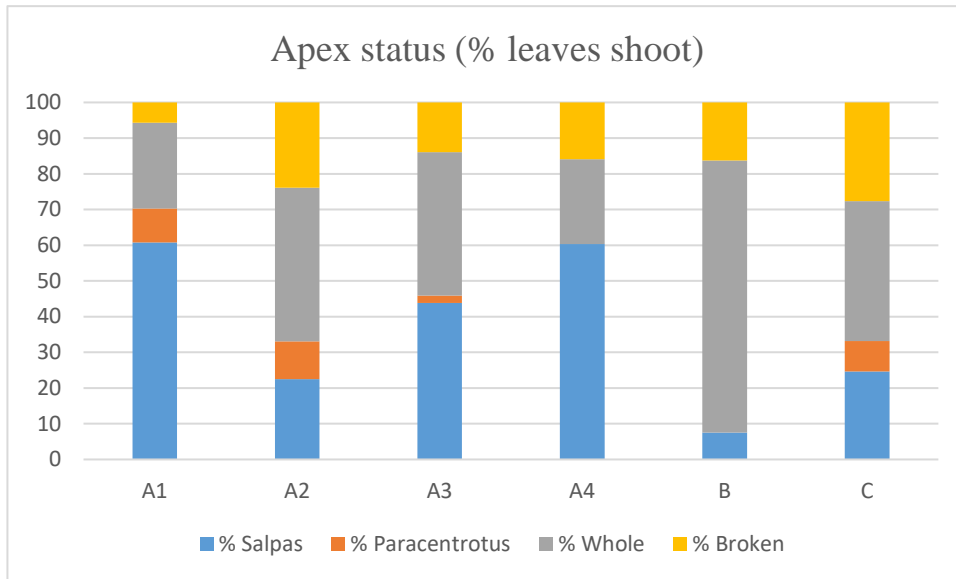


Figure 4.27. Apex status in each station.

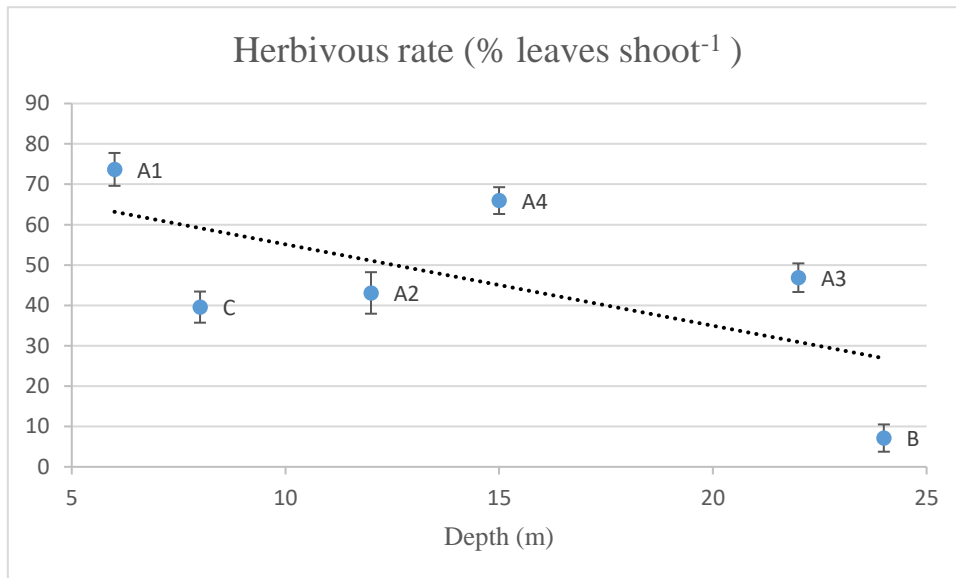


Figure 4.29. Herbivorous rate along bathymetric gradient.

Lastly, the values of the two derived descriptors, LAI and Coefficient "A", calculated in each station are given in Table 4.3. For all stations, the LAI values were consistent with those reported in the literature ($1.1 < \text{LAI} < 29$ (Buia et al., 2003)), except for station B showing a rather low value.

Stations	A1	A2	A3	A4	B	C
Leaf Area Index ($\text{m}^2 \text{m}^{-2}$)	9.890576	12.1686	2.470087	7.933146	0.864226	16.46578
Coefficient "A"	0.769231	0.574324	0.616216	0.857143	0.267606	0.695402

Table 4.3. LAI and Coefficient "A" values in each station.

5. Discussion

By analyzing the **physical descriptors**, the physiochemical parameters of the water, such as temperature, salinity and turbidity, and comparing them with what is reported in the literature, it can be stated that the waters surrounding the Peñón de Ifach Natural Reserve fit with the expectation for a non overdeveloped Mediterranean coastal area. The salinity values recorded are consistent with the optimal conditions for plant life, the latter being able to tolerate a wide variation range, provided that it is above 33 ‰. As for turbidity, which apart from individual seasonal events does not exceed 0.5 FTU, it can be concluded that its value does not deteriorate the state of the meadow. As far as temperature is concerned, variations were recorded between a minimum of 14 °C in January up to a maximum of 30.9 °C in August. These values fall within the temperature range tolerated by *P. oceanica* which, according to observations conducted by Augier et al., (1980), is able to withstand conditions between 9 °C and 29.2 °C. However, it can be noted that the maximum surface temperature recorded in the summer months is close to the maximum tolerable from the plant. Mayot et al. (2005) suggest that the increase in seawater temperature that is currently observed throughout the world (Salat et Pascual 2002) could have a harmful effect on *P. oceanica*. Temperature affects almost every aspect of seagrass metabolism, growth and reproduction, and also has important implications for geographic patterns of seagrass species abundance and distribution. The rise in temperature in the next 25 years will result in a projected increase in sea level between 10 and 15 cm, mostly because of thermal expansion of the ocean waters and, to a lesser extent, because of melting glaciers and ice sheets. The rise in sea level may have numerous implications for circulation, tidal amplitude, current and salinity regimes, coastal erosion and water turbidity, each of which could have major negative impacts on local seagrass performance (Borum 2004). Long-term forecasts regarding the change in salinity in relation to global temperature rise suggest that in the Mediterranean, where evaporation processes are predominant to precipitation, an increase of about 0.5 PSU in ten years may be expected. These predictions, unlike those for temperature, are not ecologically worrisome for *P. oceanica* meadows; in fact, it has been shown that they can survive even in hypersalinity conditions such as Tunisian lagoons where salinity is around 44 ‰ (Boudouresque et al., 2012).

About the hydrodynamic conditions, investigated by sediment sample analysis, they show no particular criticality for the survival of *P. oceanica* meadows in this area. Although a seasonal pattern in sediment dynamics is evident, neither the winter nor the summer season are characterized by a hydrodynamism that significantly impacts on the nature of the substrate and

then on the growth of the plant. As evident in the results of CO₂ monitoring station, the meadow is able to mitigate the energy of the wave motion in the summer season fulfilling its role in effectively absorb hydrodynamism. Field experiments have shown that a meadow is able to reduce by the 20 % the velocity of currents above it (Gacia and Duarte 2001).

Despite the results of the physical descriptors regarding the environmental condition highlight an acceptable situation for the growth of *P. oceanica*, on the basis of the observations made in this study on the physiographical, structural and functional descriptors of the seagrass it is noted that ecological status varies among the examined meadows.

With regard to the **physiographical descriptors**, on the south side of Peñón de Ifach, where stations A are located, the upper limit is the direct consequence of the geomorphology and the rocky nature of the seabed in its closest part to the coast. On the north side, however, where the station B is located, the presence of clearances and low densities could be related to the shape of the emerged coast of the natural park, which is characterized by vertical walls shading the backdrops, probably preventing the expansion of the meadow towards the coast. As for the reef-meadow in which station C is located, the upper limit is the consequence of the probability of the meadow being exposed to drying. The “net”, “regressive” and “erosive” lower limits for the A, B, C monitored meadows are relatively shallow, despite the remarkable transparency of the water, but no historical observations on the meadows exist and we can not made any consideration on the current dynamics of these limits.

In relation to the **structural parameters**, Global density recorded varies among stations, however, the southern and reef meadow can be classified as "dense", while the one named B as a “semi-meadow”. By comparing the densities observed at each station with the corresponding depths (Pergent et al., 1995) (Fig. 4.19), the stations C, A2 and A3 are defined as “in equilibrium” state, while A1, A3 and B are on the limits between a “disturbed” and “very disturbed” condition. Within the same meadow (Cala Racò A) different status were observed in separate patches. This highlights the possibility of disturbances acting on the meadow health at very small scale (e.g. anchoring activity), as already supposed by Guillén et al., (2013). “Disturbed” conditions of B station were observed in a shaded area where it is probably due to the low solar radiance. In relation to the values obtained regarding *P. oceanica* shoots burial in all the stations, it can be stated that, although they are always positive, they are not such to permit the predominance of erosive processes in place, but rather indicate a dynamic balance between sedimentation processes and growth of the plant.

According to Buia et al., (2003) on the values of **functional descriptors** (leaves/shoots, leaves length and leaf area/shoot) including derived descriptors(leaf surface index (LAI)), taking into account the normality ranges reported from the point of view of leaf growth, stations can be considered in optimum conditions, with the only exception of station B. This station, in fact, reports a value of LAI rather low compare to what is reported in the literature. This may still be the result of lower solar radiation in the area where the meadow is located that may affect the photosynthetic activity of the plant. The coefficient "A", which mainly expresses the combined effect of the mechanical action of hydrodynamism on leaves and grazing of herbivores, is consistent with expected values related to the depth of the stations. In fact, the percentage of broken apices or bites is higher in the more superficial stations and decreases along the bathymetric gradient. Overall the status of *P. oceanica* meadows in Calpe bay is not worrying. The results obtained and the evaluations made in this study provide a valuable baseline for future studies and comparisons, in the framework of the monitoring studies needed to keep track of the dynamics of the Calpe bay meadows. The results revealed that the ecological status of the Cala Racò meadow is not particularly good, but this is not surprising. Guillén et al., (2013) in relation to a ten-year monitoring network, reported that *P. oceanica* meadows located in Calpe are in less than optimal conditions. Despite this, there is a clear trend of an improvement, identified by these authors using the Global density and Covering parameters until 2011, although it is very slow (Fig 5.1).

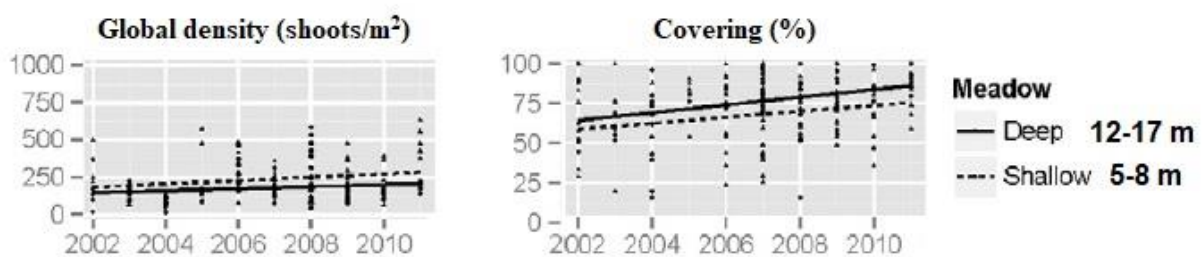


Figure 5.1. Trends of Global density and Covering in the period 2002-2011 in Cala Racò meadow. (Modified from Guillén et al. 2013)

The present study seems to confirm the positive trend highlighted by Guillén et al., (2013), showing even higher values for the two parameters considered. It can, therefore, be stated that *P. oceanica* meadow in Calpe bay, from more than 15 years, are facing a recovery process following a disturbance event in the past. These events could consist of the construction of the “El Racò” fishing port, dating back to the first half of the 20th century, or the dumping of waste in the sea-front just outside the harbor, occurring up about 20 years ago. Due to the low resilience of *P. oceanica* (Procaccini et al., 1996), the species keeps a strong and very long

biological memory of past events of regression: the recovery of a regressed *P. oceanica* meadow may take several years (Boudouresque et al., 2006), independently from the quality of the water.

Data collected in this study were compared with those collected by Guillén et al., (2013), while this is not always possible due to the heterogeneity of the methodologies often used. Since different efforts have been made to carry out monitoring studies in several countries in order to protect legally *P. oceanica*, it would be desirable to have a major homogeneity in the protocols to be used, at least at European level. Proper management of the *P. oceanica* meadows, in fact, would require standardized methodologies of study, to be applied by both researchers and administrators, enabling comparable results on the scale of the whole Mediterranean basin. Considering the enormous variety of the methodologies, Pergent-Martini et al., (2005) (Fig 5.2)

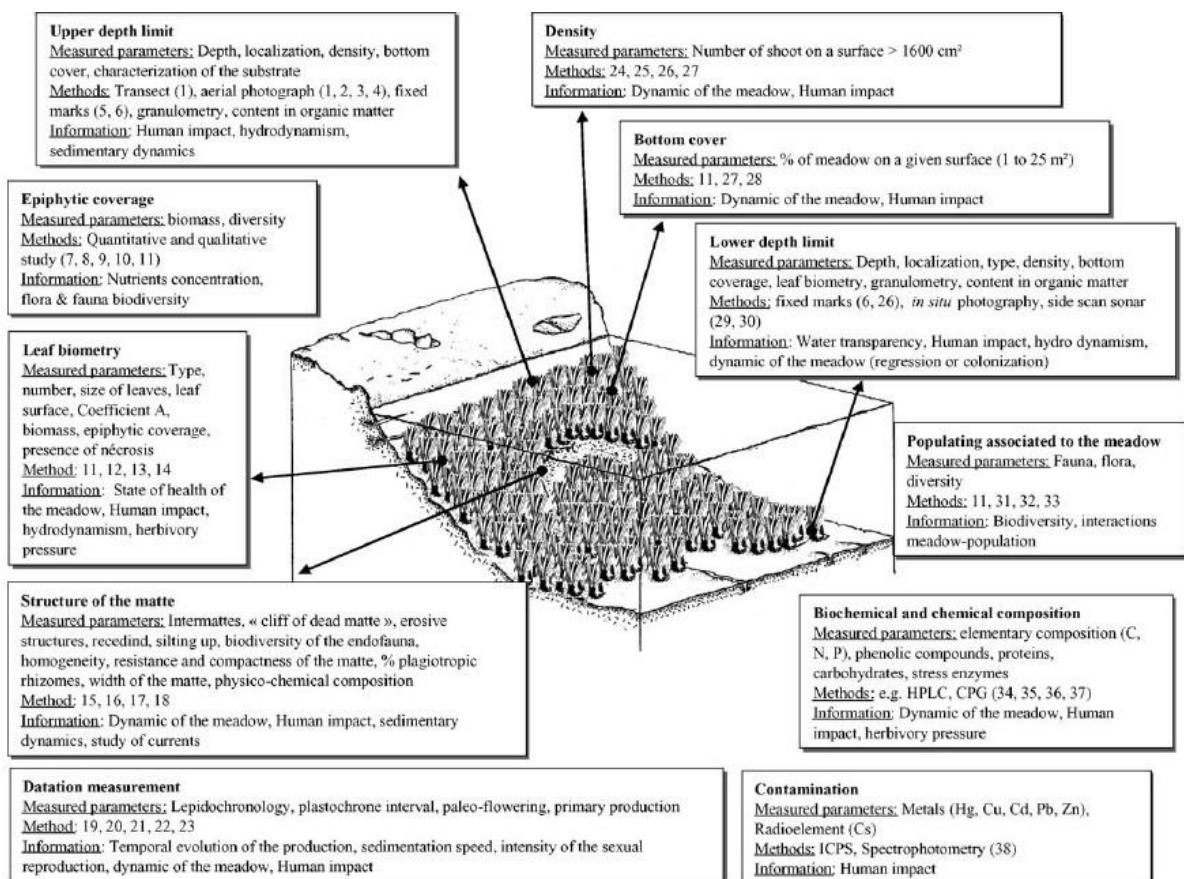


Figure 5.2. Recapitulative plan of the main descriptors of *Posidonia oceanica*, with the measured parameters the methods of investigation: (1) Meinesz et al. (1988); (2) Lefevre et al. (1984); (3) Pasqualini et al. (1997); (4) Mc Kenzie et al. (2003); (5) Augier et al. (1984); (6) Boudouresque et al. (2000); (7) Balduzzi et al. (1981); (8) Dauby and Poulicek (1995); (9) Cinelli et al. (1984); (10) Morri (1991); (11) Buia et al. (2003); (12) Giraud (1977); (13) Giraud (1979); (14) Drew and Jupp (1976); (15) Blanc (1956); (16) Clairefond and Jeudy De Grissac (1979); (17) Willsie (1987); (18) Pergent et al. (1995); (19) Pergent (1990); (20) Duarte (1991b); (21) Cebrian et al. (1994); (22) Mateo et al. (1997); (23) Pergent et al. (1989); (24) Panayotidis et al. (1981); (25) Romero (1986); (26) Meinesz and Laurent (1978); (27) Duarte and Kirkman (2003); (28) Francour et al. (1999); (29) Ramos-Martos and Ramos-Espla (1989); (30) Pasqualini et al. (2000); (31) Blanc-Vernet (1984); (32) Russo and Vinci (1991); (33) Harmelin-Vivien and Francour (1992); (34) Hamoutene et al. (1995); (35) Ferrat et al. (2002); (36) Mateo and Sabate (1993); (37) Gobert et al. (1995) and (38) Romeo et al. (1995). From Pergent-Martini et al. (2005).

made a review on the main descriptors of *P. oceanica*, with the measured parameters and the methods of investigation, used in 41 laboratories situated all over the Mediterranean coasts.

They showed that some descriptors, as density and bathymetric positions of the meadow, benefit from a protocol that is applied, quite homogeneously, by all the laboratories, but this is not the case for all descriptors. However, concerning the measurement of meadows density, the type of quadrat and/or the number of replications varied from one laboratory to another.

Anyway, the acquisition of a descriptor is only the first step in its use: an interpretation scale is required to make the descriptor effective.

With respect to density, for example, in the literature there is a large number of indicators used to classify the status of the meadows. The scale of Giraud (1977) remains in common use because it is simple to implement (six classes with precisely defined markers) (Pergent-Martini et al., 2005). Nevertheless, it should be noticed that this scale does not take into consideration the normal decrease in meadow density in function of depth or the type of substrate, which are the most important parameters driving the distribution of this plant. The attempt of classifying proposed by Romero-Martinengo (1985), which introduced the effect of depth, was difficult to apply because of the lack of some of the parameters needed to calculate it (e.g. coefficient of light attenuation). The scale proposed by Pergent et al., (1995) and used in this work, includes the depth parameter, by means of a logarithmic factor, on the basis of bibliographical data concerning stations submitted to different levels of human pressure. Its aim is both to compare stations situated at different depths (this cannot be done with the scale of Giraud, 1977) and to evaluate the “normal” level of density and the quality of the environment. However, it needs to be improved by incorporating a greater quantity of features and also by a more precise determination of the human characteristics of the site and the type of bottom (e.g. soft or hard) (Pergent-Martini et al., 2005). The sampling design may be also a critical issue for the validity of the results obtained. Because some descriptors (such as meadow cover or shoot density) may demonstrate a high variability at small or medium spatial scales (Panayotidis et al., 1981; Balestri et al., 2003), Fernández-Torquemada et al., (2008) recommend utilizing a nested sampling design with an adequate spatial replication. Furthermore, seasonal variation at the community, population, and individual plant level must be taken into account. Descriptors such as shoot foliar surface and epiphyte biomass should be sampled during a fixed period of the year to avoid any confounding effect of seasonality.

For a selection of metrics to be applied in future monitoring programmes, especially for those on large spatial scale, also cost-effectiveness is an important issue, and therefore the metrics should be easily measured and applied. Obviously, the most convenient techniques vary in relation to the research goals. When the scope is to provide detailed data on distribution and change in seagrass areas or to estimate the biomass, the best choice is high-resolution mapping. Aerial photography is the most common remote sensing method for seagrass mapping studies and for monitoring over time, while satellite data are valued for large-scale localization investigations. Airborne scanners may also provide a high accuracy and this technique is becoming gradually more competitive (Krause-Jensen et al., 2004). However, when the aims of the monitoring programme are to obtain higher resolution data or samples collection, it is necessary to have a SCUBA diving team. To lower the management costs of such a team, for example, some Spanish communities (e. g. Región de Murcia) have used volunteer groups to carry out annual monitoring of meadows. The participation of voluntary divers in biological monitoring programs must guarantee an acceptable data quality and reliability so that they can be used for monitoring purposes and in the political decision processes that affect the management of human activity on the coast. But, to achieve this, these projects must gather a series of basic conditions: (a) provide an adequate theoretical and practical training of the volunteers, (b) encourage the interest and motivation of the volunteer to be rigorous with the tasks performed, (c) use simple and robust measurements, (d) apply control measures to quantify the error of the measurements made by the voluntary, (e) be coordinated by groups or entities with recognized scientific training.

Involvement of volunteers provides a future potential for a participatory monitoring network, supporting the implementation of scientific knowledge on marine ecosystems. Moreover, it also contributes to their future conservation, since this may be achieved only with the support of the citizens and of the society. The Monitoring Networks have, therefore, the commitment to become vehicles for transmitting scientific knowledge and awareness on current problems that threaten our marine ecosystems. In addition, through their active participation, sports diver are getting involved directly in tasks of conservation of habitats and species that have aroused their admiration. Finally, in the medium and long term, the role that this type of activities can have is the diversification of underwater tourism. Recreational diving has undergone overcrowding and its concentration in marine "sanctuaries" may cause the deterioration of the natural values of the marine protected areas, as has already been demonstrated in many places (Ruiz et al.,

2012). In this sense, the education of the sports diver, in particular, and the citizen, in general, in environmental issues is a fundamental aspect.

6. Conclusion

With the aim of identifying and then sustaining the health status of the meadows of Calpe Bay, it was considered appropriate to set up a monitoring network as presented in this paper.

The underwater meadows of *Posidonia oceanica* are especially vulnerable to the impact of human activities, a property that, together with its wide geographical distribution, has made them excellent candidates as a biological indicator of the state of conservation of coastal marine ecosystems. It is also one of the most important ecological habitats in the Mediterranean and, paradoxically, of the most threatened due to the development of human activity on the coast. Consequently, they benefit from a protection status in most of the Autonomous Communities that make up the Spanish Mediterranean coast and are considered priority protection habitats in the Community Directive and in the Barcelona Convention.

For this reason, it is important to create monitoring networks that have adequate characteristics. A Monitoring Network for the *P. oceanica* meadows should consist of a series of fixed points (sampling stations), distributed along the coast, which are visited periodically to make a series of measurements in order to know the health status of the habitat or populations targeted study and its long-term evolution. For a project of these characteristics to be viable, a series of basic requirements must be met:

1. Cover broad temporary scales (minimum 10 years).
2. Contemplate a wide network of points representative of the environmental conditions in which the habitat is located and that allow us to infer changes originated by human activity from those that are caused by natural factors.
3. Use easy-to-obtain measurements with effective, robust and standardized methods that allow the comparison of results obtained by different samplers, in different geographical areas and in different years, with little error.

7. References

- Alaya, H. B. (1972). Répartition et condition d'installation de *Posidonia oceanica* Delile et *Cymodocea nodosa* Ascherson dans le golfe de Tunis. *Bull. Instit. Nation. Peche de Salammbô*, 2, 331-416.
- Augier, H., & Maudinas, B. (1979). Influence of the pollution on the photosynthetic pigments of the marine phanerogam *Posidonia oceanica* collected from different polluted areas of the Region of Marseille (Mediterranean sea, France). *Oecologia plantarum*.
- Augier, H., Robert, P., & Maffre, R. (1980). Etude du régime thermique annuel des eaux au niveau des peuplements de Phanérogames marines de la baie de Port-Cros (Iles d'Hyères, Méditerranée, France). *Trav. sci. Parc nation. Port-Cros*, 6, 69-131.
- Balestri, E., Benedetti-Cecchi, L., & Lardicci, C. (2004). Variability in patterns of growth and morphology of *Posidonia oceanica* exposed to urban and industrial wastes: contrasts with two reference locations. *Journal of Experimental Marine Biology and Ecology*, 308(1), 1-21.
- Balestri, E., Cinelli, F., & Lardicci, C. (2003). Spatial variation in *Posidonia oceanica* structural, morphological and dynamic features in a northwestern Mediterranean coastal area: a multi-scale analysis. *Marine Ecology Progress Series*, 250, 51-60.
- Borum, J., Duarte, C. M., Greve, T. M., & Krause-Jensen, D. (Eds.). (2004). *European seagrasses: an introduction to monitoring and management* (p. 2006). M&MS project.
- Boudouresque, C. F. (2004). Marine biodiversity in the Mediterranean: status of species, populations and communities. *Travaux scientifiques du Parc national de Port-Cros*, 20, 97-146.
- Boudouresque, C. F., & Meinesz, A. (1983). *Découverte de l'herbier de Posidonie*. Parc National de Port-Cros.
- Boudouresque, C. F., & Verlaque, M. (2001). Ecology of *Paracentrotus lividus*. *Developments in aquaculture and fisheries science*, 32, 177-216.
- Boudouresque, C. F., Bernard, G., Bonhomme, P., Charbonnel, E., Diviacco, G., Meinesz, A., ... & Tunesi, L. (2012). Protection and conservation of *Posidonia oceanica* meadows.
- Boudouresque, C. F., Bertrand, M. C., Bouladier, E., Foret, P., Meinesz, A., Pergent, G., & Vitiello, P. (1990). Le réseau de surveillance des herbiers de Posidonies mis en place en région Provence-Alpes-Côte d'Azur (France). *CIESM*, 32(1), 1-11.

- Boudouresque, C. F., Crouzet, A., & Pergent, G. (1983). Un nouvel outil au service de l'étude des herbiers à *Posidonia oceanica*: la lépidochronologie. *Rapp PV Réun Commiss Internatl Explor Sci Médit*, 28(3), 111-112.
- Boudouresque, C. F., Gravez, V., Meinesz, A., Molenaar, H., Pergent, G., & Vitiello, P. (1994, April). L'herbier à *Posidonia oceanica* en Méditerranée: protection légale et gestion. In *Actes du colloque scientifique OKEANOS, Maison de l'Environnement de Montpellier publ., France* (pp. 209-220).
- Boudouresque, C. F., Mayot, N., & Pergent, G. (2006). The outstanding traits of the functioning of the *Posidonia oceanica* seagrass ecosystem. *Biol Mar Medit*, 13(4), 109-113.
- Boudouresque, C. F., Van Klaveren, M. C., & Van Klaveren, P. (1996). Proposal for a list of threatened or endangered marine and brackish species (plants, invertebrates, fish, turtles and mammals) for inclusion in appendices I, II and III of the Bern Convention. *Council of Europe, Document S/TPVS96/TPVS48E, 96A*, 1-138.
- Buia, M. C., Gambi, M. C., & Dappiano, M. (2003). I sistemi a fanerogame marine. *Manuale di metodologie di campionamento e studio del bentos marino mediterraneo. Biol. Mar. Mediterr*, 10, 145-198.
- Buia, M. C., Gambi, M. C., & Dappiano, M. (2004). Seagrass systems. *Biologia Marina Mediterranea*, 10(suppl), 133-183.
- Cabaço, S., Apostolaki, E. T., García-Marín, P., Gruber, R., Hernandez, I., Martínez-Crego, B., ... & Romero, J. (2013). Effects of nutrient enrichment on seagrass population dynamics: evidence and synthesis from the biomass–density relationships. *Journal of Ecology*, 101(6), 1552-1562.
- Cabaço, S., Santos, R., & Duarte, C. M. (2008). The impact of sediment burial and erosion on seagrasses: a review. *Estuarine, Coastal and Shelf Science*, 79(3), 354-366.
- Cabioch, J., Floc'h, J. Y., Boudouresque, C. F., Meinesz, A., & Verlaque, M. (1995). *Guía de las algas de los mares de Europa: Atlántico y Mediterráneo*.
- Calvo, J. C. C., & Valdes, C. E. (1995). *El ecosistema marino mediterráneo: guía de su flora y fauna*. Juan Carlos Calvin.
- Cebrian, J., & Duarte, C. M. (2001). Detrital stocks and dynamics of the seagrass *Posidonia oceanica* (L.) Delile in the Spanish Mediterranean. *Aquatic botany*, 70(4), 295-309.

- Ceccherelli, G., Campo, D., & Milazzo, M. (2007). Short-term response of the slow growing seagrass *Posidonia oceanica* to simulated anchor impact. *Marine Environmental Research*, 63(4), 341-349.
- Ceccherelli, G., Piazzini, L., & Cinelli, F. (2000). Response of the non-indigenous *Caulerpa racemosa* (Forsskål) J. Agardh to the native seagrass *Posidonia oceanica* (L.) Delile: effect of density of shoots and orientation of edges of meadows. *Journal of Experimental Marine Biology and Ecology*, 243(2), 227-240.
- Conde Poyales, F. (1989). Ficogeografía del mar de Alborán en el contexto del Mediterráneo occidental. In *Anales del Jardín Botánico de Madrid* (Vol. 46, pp. 21-26). Rueda.
- Connolly, R. M., Hindell, J. S., & Gorman, D. (2005). Seagrass and epiphytic algae support nutrition of a fisheries species, *Sillago schomburgkii*, in adjacent intertidal habitats. *Marine Ecology Progress Series*, 286, 69-79.
- Crouzet, A., Boudouresque, C. F., Meinesz, A., & Pergent, G. (1983). Evidence of the annual character of cyclic changes of *Posidonia oceanica* scale thickness (erect rhizomes). *Rapp. Commiss. int. Mer Médit*, 28(3), 113-114.
- Cubasch, U., Meehl, G. A., Boer, G. J., Stouffer, R. J., Dix, M., Noda, A., ... & Yap, K. S. (2001). Projections of future climate change. , in: JT Houghton, Y. Ding, DJ Griggs, M. Noguer, PJ Van der Linden, X. Dai, K. Maskell, and CA Johnson (eds.): *Climate Change 2001: The Scientific Basis: Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel*, 526-582.
- Den Hartog, C. (1970). *The Sea Grasses of the World*, vol. 1.
- Devlin, M., Best, M., & Haynes, D. (2007). Implementation of the Water Framework Directive in European marine waters. *Marine Pollution Bulletin*, (Spec. Issue 1-6).
- Dimech, M., Borg, J. A., & Schembri, P. J. (2002). Changes in the structure of a *Posidonia oceanica* meadow and in the diversity of associated decapod, mollusc and echinoderm assemblages, resulting from inputs of waste from a marine fish farm (Malta, Central Mediterranean). *Bulletin of Marine Science*, 71(3), 1309-1321.
- Duarte, C. M., & Chiscano, C. L. (1999). Seagrass biomass and production: a reassessment. *Aquatic botany*, 65(1), 159-174.
- Elkalay, K., Frangoulis, C., Skliris, N., Goffart, A., Gobert, S., Lepoint, G., & Hecq, J. H. (2003). A model of the seasonal dynamics of biomass and production of the seagrass *Posidonia oceanica* in the Bay of Calvi (Northwestern Mediterranean). *Ecological modelling*, 167(1), 1-18.

- Elzinga, C. L., Salzer, D. W., Willoughby, J. W., & James, P. Gibbs. 2001. Monitoring plant and animal populations.
- Fernández-Torquemada, Y., Díaz-Valdés, M., Colilla, F., Luna, B., Sánchez-Lizaso, J. L., & Ramos-Esplá, A. A. (2008). Descriptors from *Posidonia oceanica* (L.) Delile meadows in coastal waters of Valencia, Spain, in the context of the EU Water Framework Directive. *ICES Journal of Marine Science*, 65(8), 1492-1497.
- Folk, R. L., & Ward, W. C. (1957). Brazos River bar: a study in the significance of grain size parameters. *Journal of Sedimentary Research*, 27(1).
- Francour, P., Ganteaume, A., & Poulain, M. (1999). Effects of boat anchoring in *Posidonia oceanica* seagrass beds in the Port-Cros National Park(north-western Mediterranean Sea). *Aquatic conservation: marine and freshwater ecosystems*, 9(4), 391-400.
- Fredj, G., Bellan-Santini, D., & Meinardi, M. (1992). État des connaissances sur la faune marine méditerranéenne. *Bulletin de l'Institut océanographique*, 133-145.
- Gacia, E., & Duarte, C. M. (2001). Sediment retention by a Mediterranean *Posidonia oceanica* meadow: the balance between deposition and resuspension. *Estuarine, coastal and shelf science*, 52(4), 505-514.
- Gacia, E., & Duarte, C. M. (2001). Sediment retention by a Mediterranean *Posidonia oceanica* meadow: the balance between deposition and resuspension. *Estuarine, coastal and shelf science*, 52(4), 505-514.
- García March, J. R., Garcia Martinez, M., Trigos Santos, S., Tena Medialdea, J., Torres Gavilla, J., & Chirivella Martorell, J. (2015). Valoración ecológica y pesquera del medio marino que rodea al peñón de ifach con el fin de crear, si procede, una reserva Marina de interés pesquero.
- Gilly, W. F., Beman, J. M., Litvin, S. Y., & Robison, B. H. (2013). Oceanographic and biological effects of shoaling of the oxygen minimum zone. *Annual review of marine science*, 5, 393-420.
- Giorgi, J., & Thelin, I. (1983). Phénologie, biomasse et production primaire de *Posidonia oceanica* (feuilles et épiphytes) dans la baie de Port-Cros. *Lab. Ecol. Benthos, Fac. Sci. Luminy, Univ. Aix-Marseille ii et parc nation. Port-cros, france*, 126.
- Giovannetti, E., Lasagna, R., Montefalcone, M., Bianchi, C. N., Albertelli, G., & Morri, C. (2008). Inconsistent responses to substratum nature in *Posidonia oceanica* meadows: An integration through complexity levels?. *Chemistry and Ecology*, 24(S1), 83-91.

- Giraud, G., Boudouresque, C. F., Cinelli, F., Fresi, E., & Mazzella, L. (1979). Observations sur l'herbier de *Posidonia oceanica* (L.) Delile autour de l'île d'Ischia (Italie). *Plant Biosystem*, 113(4), 261-274.
- Gobert, S., Sartoretto, S., Rico-Raimondino, V., Andral, B., Chery, A., Lejeune, P., & Boissery, P. (2009). Assessment of the ecological status of Mediterranean French coastal waters as required by the Water Framework Directive using the *Posidonia oceanica* Rapid Easy Index: PREI. *Marine Pollution Bulletin*, 58(11), 1727-1733.
- González-Correa, J. M., Sempere, J. T. B., Sánchez-Jerez, P., & Valle, C. (2007). *Posidonia oceanica* meadows are not declining globally. Analysis of population dynamics in marine protected areas of the Mediterranean Sea. *Marine Ecology Progress Series*, 336, 111-119.
- Guillén, J. E., Lizaso, J. L. S., Jiménez, S., Martínez, J., Codina, A., Montero, M., ... & Zubcoff, J. J. (2013). Evolution of *Posidonia oceanica* seagrass meadows and its implications for management. *Journal of sea research*, 83, 65-71.
- Hemminga, M. A., & Duarte, C. M. (2000). *Seagrass ecology*. Cambridge University Press.
- Krause-Jensen, D., Quaresma, A. L., Cunha, A. H., & Greve, T. M. (2004). How are seagrass distribution and abundance monitored. *European seagrasses: An introduction to monitoring and management*, 45-53.
- Kuo, J., & Den Hartog, C. (2001). Seagrass taxonomy and identification key. *Global seagrass research methods*, 33, 31-58.
- Leoni, V., Pasqualini, V., Pergent-Martini, C., Vela, A., & Pergent, G. (2006). Morphological responses of *Posidonia oceanica* to experimental nutrient enrichment of the canopy water. *Journal of Experimental Marine Biology and Ecology*, 339(1), 1-14.
- Marbà, N., Alvarez, E., Vivas, S., Barrajon, A., Moreno, D., Remon, J. M., De la Rosa, J., Leon, D., Penalver, P., Diaz-Armela, E., & Mendoza, R. M.. (2015) Estudio demográfico de *Posidonia oceanica*. "Proyecto LIFE09NAT/ES/000534. Conservación de las praderas de *posidonia oceanica* en el mediterráneo andaluz".
- Marbà, N., Duarte, C. M., Cebrián, J., Gallegos, M. E., Olesen, B., & Sand-Jensen, K. (1996). Growth and population dynamics of *Posidonia oceanica* on the Spanish Mediterranean coast: elucidating seagrass decline. *Marine Ecology Progress Series*, 203-213.

- Mayot, N., Boudouresque, C. F., & Leriche, A. (2005). Unexpected response of the seagrass *Posidonia oceanica* to a warm-water episode in the North Western Mediterranean Sea. *Comptes Rendus Biologies*, 328(3), 291-296.
- Mazzella, L. (1992). Plant-animal trophic relationships in the *Posidonia oceanica* ecosystem of the Mediterranean Sea. *Plant-animal Interactions in the Marine Benthos: Systematics Association*, 46, 165-187.
- Meinesz, A., & Laurent, R. (1978). Cartographie et état de la limite inferieure de l'herbier de *Posidonia oceanica* dans les Alpes-maritimes (France)—Campagne Poseïdon 1976—. *Botanica marina*, 21(8), 513-526.
- Montefalcone, M. (2009). Ecosystem health assessment using the Mediterranean seagrass *Posidonia oceanica*: a review. *Ecological indicators*, 9(4), 595-604.
- Montefalcone, M., Chiantore, M., Lanzone, A., Morri, C., Albertelli, G., & Bianchi, C. N. (2008). BACI design reveals the decline of the seagrass *Posidonia oceanica* induced by anchoring. *Marine Pollution Bulletin*, 56(9), 1637-1645.
- Montefalcone, M., Lasagna, R., Bianchi, C. N., Morri, C., & Albertelli, G. (2006). Anchoring damage on *Posidonia oceanica* meadow cover: a case study in Prelo Cove (Ligurian Sea, NW Mediterranean). *Chemistry and Ecology*, 22(sup1), S207-S217.
- Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G., & Worm, B. (2011). How many species are there on Earth and in the ocean?. *PLoS biology*, 9(8), e1001127.
- Occhipinti-Ambrogi, A. (2007). Global change and marine communities: alien species and climate change. *Marine pollution bulletin*, 55(7), 342-352.
- Panayotidis, P., Boudouresque, C. F., & Marcot-Coqueugniot, J. (1981). Microstructure de l'herbier de *Posidonia oceanica* (Linnaeus) Delile. Microstructure of *Posidonia oceanica* (Linnaeus) beds. *Botanica marina*, 24(3), 115-124.
- Pergent, G., & Pergent-Martini, C. (1990). Some applications of lepidochronological analysis in the seagrass *Posidonia oceanica*. *Botanica marina*, 33(4), 299-310.
- Pergent, G., & Pergent-Martini, C. (1991). Leaf renewal cycle and primary production of *Posidonia oceanica* in the bay of Lacco Ameno (Ischia, Italy) using lepidochronological analysis. *Aquatic Botany*, 42(1), 49-66.
- Pergent, G., Rico-Raimondino, V., & Pergent-Martini, C. (1997). Fate of primary production in *Posidonia oceanica* meadows of the Mediterranean. *Aquatic Botany*, 59(3), 307-321.

- Pergent-Martini, C., & Pergent, G. (2000). Marine phanerogams as a tool in the evaluation of marine trace-metal contamination: an example from the Mediterranean. *International Journal of Environment and Pollution*, 13(1-6), 126-147.
- Pergent-Martini, C., Leoni, V., Pasqualini, V., Ardizzone, G. D., Balestri, E., Bedini, R., ... & Boumaza, S. (2005). Descriptors of Posidoniaoceanica meadows: Use and application. *Ecological Indicators*, 5(3), 213-230.
- Picard, J., & Molinier, R. (1952). *Recherches sur les herbiers de Phanérogames marines du littoral méditerranéen français*. Masson.
- Platini, F. (2000). La protection des habitats aux herbiers en Méditerranée. *Report. PNUE, PAM, CAR/ASP*.
- Por, F. D. (2012). *Lessepsian migration: the influx of Red Sea biota into the Mediterranean by way of the Suez Canal* (Vol. 23). Springer Science & Business Media.
- Procaccini, G., Alberte, R. S., & Mazzella, L. (1996). Genetic structure of the seagrass Posidonia oceanica in the Western Mediterranean: ecological implications. *Marine Ecology Progress Series*, 153-160.
- Raniello, R., & Procaccini, G. (2002). Ancient DNA in the seagrass Posidonia oceanica. *Marine Ecology Progress Series*, 227, 269-273.
- Roca, G., Romero, J., Columbu, S., Farina, S., Pagès, J. F., Gera, A., ... & Alcoverro, T. (2014). Detecting the impacts of harbour construction on a seagrass habitat and its subsequent recovery. *Ecological Indicators*, 45, 9-17.
- Romero, J. (1989). Primary production of Posidonia oceanica beds in the Medas Islands (Girona, NE Spain). In *International workshop on Posidonia oceanica beds* (Vol. 2, pp. 85-91). GIS Posidonie Marseille.
- Romero, J. (2004). Las praderas de Fanerógamas marinas. La producción primaria y su destino. Características de los restos de la planta. *Praderas y bosques marinos de Andalucía. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla*, 74-81.
- Romero, J., Martínez-Crego, B., Alcoverro, T., & Pérez, M. (2007). A multivariate index based on the seagrass Posidonia oceanica (POMI) to assess ecological status of coastal waters under the water framework directive (WFD). *Marine Pollution Bulletin*, 55(1), 196-204.
- Romero, J., Pergent, G., Pergent-Martini, C., Mateo, M. A., & Regnier, C. (1992). The detritic compartment in a Posidonia oceanica meadow: litter features, decomposition rates, and mineral stocks. *Marine Ecology*, 13(1), 69-83.

- Ruiz, J. M., & Romero, J. (2001). Effects of in situ experimental shading on the Mediterranean seagrass *Posidonia oceanica*. *Marine Ecology Progress Series*, 215, 107-120.
- Ruiz, J. M., & Romero, J. (2003). Effects of disturbances caused by coastal constructions on spatial structure, growth dynamics and photosynthesis of the seagrass *Posidonia oceanica*. *Marine Pollution Bulletin*, 46(12), 1523-1533.
- Ruiz, J. M., Barberá, C., Marín-Guirao, L., García, R., Bernardeau, J., & Sandoval, J. M. (2012). Las praderas de *Posidonia* en Murcia. Red de seguimiento y voluntariado ambiental. IEO Institutional Digital Repository.
- Salat, J., & Pascual, J. (2002, April). The oceanographic and meteorological station at L'Estartit (NW Mediterranean). In Tracking long-term hydrological change in the Mediterranean Sea Mediterranean Science Commission Ciesm Workshop Series, nu (Vol. 16, pp. 29-32).
- Sandulli, R., Bianchi, C. N., Cocito, S., Morri, C., Peirano, A., & Sgorbini, S. (1998). An experience of 'balisage' in monitoring the effects of the Haven oil spill on some Ligurian *Posidonia oceanica* meadows. *Oebalia*. Taranto, 24, 3-15.
- Short, F. T., & Coles, R. G. (Eds.). (2001). *Global seagrass research methods* (Vol. 33). Elsevier.
- Short, F. T., & Wyllie-Echeverria, S. (1996). Natural and human-induced disturbance of seagrasses. *Environmental conservation*, 23(1), 17-27.
- Wellman, C. H., Osterloff, P. L., & Mohiuddin, U. (2003). Fragments of the earliest land plants. *Nature*, 425(6955), 282-285.
- Y Royo, C. L., Casazza, G., Pergent-Martini, C., & Pergent, G. (2010). A biotic index using the seagrass *Posidonia oceanica* (BiPo), to evaluate ecological status of coastal waters. *Ecological Indicators*, 10(2), 380-389.