UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING, SCIENCE AND MATHEMATICS

School of Ocean and Earth Science

The natural recolonisation process of the seagrass *Posidonia oceanica* (L.) Delile after the introduction of the Italo-Algerian methane pipeline in the SW Mediterranean sea

by

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ABSTRACT

FACULTY OF ENGINEERING, SCIENCE & MATHEMATICS

SCHOOL OF OCEAN AND EARTH SCIENCE

Doctor of philosophy

The natural recolonisation process of the seagrass *Posidonia oceanica* (L.) Delile after the introduction of the Italo-Algerian methane pipeline in the SW Mediterranean sea

By Giuseppe Di Carlo

This work investigates the recolonisation process and patterns of the seagrass *Posidonia oceanica* after a man-induced impact. The installation of a methane pipeline connecting Italy and Tunisia destroyed a large seagrass bed in the South Mediterranean Sea (SW Sicily, Italy). Pipes were buried and backfilled using terrestrial calcareous rubble. As a results of dumping activities, the rubble formed a mound-like seascape changing dramatically the sea bed features of the area. This research was articulated in two parts, the former involving an ecological approach to the problem where attention was focused on whether rubble mounds represent a suitable substrata for *P. oceanica* recruitment. Attention has been drawn on the role of environmental factors on such a process, and in particular how the feedback between plant recruitment, sediment dynamics and water flow is created in this new artificial environment. The results indicated how *P. oceanica* recruits via vegetative fragments which break from the meadow during storms. However, vegetative recruitment only occurs in sheltered (valley) areas. Once recruited, fragments are able to become rooted and draw on sediment nutrients providing the resources needed to grow. In valleys, once patches are well established, they create a positive feedback between plant canopy, water flow attenuation and sediment deposition. Sheltered (valley) and exposed (crests) locations on the mounds show different physical and geological characteristics. Differences in the sediment dynamics between exposed (crests) and sheltered (valleys) locations on the mound field entail differences in the amount of nutrient available to the plants for growth and photosynthesis and hence plant morphology. The latter part of this thesis was focalised on the physiological response of the plant to this new environment. Nutrient limitation and plant phenology have been considered to draw a complete picture not only on how a hard substratum might affect the recruitment of *P. oceanica* but also to assess how this species might have adapted to this new, artificial environment. Indeed, in the presence of the canopy in valleys favours particle settlement, so contributing to the formation of a sediment layer which provides the necessary nutrients for plant growth. However, the rubble topography might play an important role in seagrass resource allocation to the different biomass compartments. Although N and P content found in the valleys cannot be considered as limiting for seagrass growth plants might still experience some nutrient limitation. Thus, seagrass morphological features, biomass and production are dependent on local factors, such as substratum type and resource availability. The plasticity of *P. oceanica* modules allow the plant to adapt to a new environment. Plants living in valleys increase resource allocation to the roots to best exploit porewater nutrients. As plant on valleys need a better anchorage on the rubble they tend to allocate more resources to the below-ground organs, while above ground biomass is reduced.

This study is the first to report on the recovery of *P. oceanica* on an artificial substratum following a human impact. After a series of unsuccessful project on *Posidonia oceanica* restoration, this work indicates that *P. oceanica* is able to recover by means of vegetative growth after a large human-induced disturbance. Moreover, this thesis intends to evaluate the possibility of employ artificial substrata to favour the recovery of lost seagrass beds in the Mediterranean Sea. This would allow the restoration of damaged areas as well as minimise the effect of future marine operations that involves impacts to natural seagrass communities.

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DECLARATION OF AUTHORSHIP

I, Giuseppe Di Carlo

Declare that the thesis entitled

The natural recolonisation process of the seagrass *Posidonia oceanica* (L.) Delile after the introduction of the Italo-Algerian methane pipeline in the SW Mediterranean sea

and the work presented in it are my own. I confirm that:

- this work was done wholly or mainly in candidature for a research degree at this University;
- where part of thesis has previously been submitted for a degree of any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- none of this work has been published before submission

Signed: *Giuseppe Di Carlo*

Date: 12 September 2004

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QUOTES

“Quelli che s’innamoran di pratica senza scientia son come i nocchieri che entran in navilio senza timone o bussola, che mai han la certezza ove si vada”.

Leonardo da Vinci

"If we knew what we were doing, it wouldn’t be called research, would it?"

Albert Einstein

CHAPTER I

INTRODUCTION

1.1 SEAGRASS EVOLUTION AND BIOGEOGRAPHY

The early evolution of life on earth involved primitive organisms, which lived in an aqueous environment. The terrestrial mode of life was a much later development (about four hundred million years ago; Raven 1977) and involved many adaptations to life in air. Higher plants, once adapted to this new life style experienced greater rates of growth, as a result of faster rates of gaseous exchange. Nevertheless, angiosperms gave rise to a number of groups that returned to a completely submerged marine existence. Among these are the seagrasses. Seagrasses colonized the seas about 90 million years ago (den Hartog 1970), shortly after the appearance of angiosperms in the late Jurassic/early Cretaceous (Larkum & den Hartog 1989). By the late Cretaceous seagrasses were a well-established group, which might have colonised the sea directly from the land, and not via freshwater (den Hartog 1970). There are some 250,000 extant angiosperm species – 35,000 of them monocotyledons - so current seagrass diversity is relatively impoverished, and, in terms of species number rather than persistence in geological time, the group can be considered as unsuccessful (Larkum 1989). Early success in the exploitation of a new niche and the absence of strong competition are often cited as reasons for the lack of speciation, but past diversity may have been greater than shown by the fossil record (Larkum & den Hartog 1989). However, given the scarce fossil records available, seagrass phylogeny must be established by molecular analysis of DNA sequences (Waycott & Les 1996, Procaccini & Waycott 1998, Waycott 1998). Gene sequence techniques reported on a possible multiple origin of seagrasses. These origins include I a freshwater ancestor for the Hydrocharitaceae, an aquatic or saltmarsh ancestor for the Zosteraceae (Les et al. 1997).

Seagrasses have evolved several key adaptations for marine life including: the possession of creeping rhizomes; a reduced cuticle that lacks stomata; hydrophilous pollination, which allows submarine pollination or pollination by surface rafts of pollen as in *Zostera* spp. (Cox et al. 1992). A reduced xylem and the presence of gas filled lacunae (aerenchyma) transversed by diaphragms which prevent entry of water in damaged plants (Larkum & den Hartog 1989) are also present. Large, longitudinally extended lacunae, named as “air canals”, are a characteristic of all seagrass leaves (McRoy 1980). The role of such canals is unknown but several hypotheses have been proposed. One suggests that the main role of the aerenchyma is to deliver O_2 to the roots, allowing survival in anoxic conditions found in mudflats (Zimmerman 1997). However, Williams & Barker (1961) proposed that another role of the aerenchyma might be to reduce O_2 demand by decreasing the amount of tissue requiring oxygen.
Seagrasses are marine flowering plants and are found in shallow waters of all continents except Antarctica (Table 1.1). The name seagrass comes from their morphological resemblance with terrestrial grass species but botanically they are classified within the monocotyledonous plants. There are relatively few species of seagrasses globally (about 60) and these are grouped in 13 genera and 5 families (Short & Coles 2001). Generally, seagrasses are divided into five temperate and five tropical genera, (Green & Short 2003). However, there are many exceptions to this classification. For instance, *Cymodocea nodosa*, considered a tropical genus, is commonly

found across the Mediterranean Sea, while some species of the temperate genus *Zostera* show an opposite trend (Hemminga & Duarte 2000). The distribution of a seagrass species is identified as the geographic range over which a species occurs or an area within a location where the species is located. The highest seagrass species richness is found in the Indo-Pacific region which is considered to be the centre of the origin of seagrasses (Phillips & Meñez, 1988, Fortes 1990) (Fig. 1). The most widely distributed of all seagrasses is the eelgrass *Zostera marina* which dominates cooler temperate seas. The warmer temperate seas, on the other hand, are dominated by the genus *Posidonia* (Short & Coles 2001). The occurrence of congeneric species within the genus *Posidonia* presents some interesting aspects. A comparison between the Mediterranean species *Posidonia oceanica* and the Australian species *Posidonia angustifolia*, *Posidonia australis*, *Posidonia sinuosa* and *Posidonia ostenfeldii* show large DNA sequence divergence between the south and north temperate oceans (Waycott & Les 1996). For what concerns the Australian species, new insights on the *P. ostenfeldii* complex have been proposed thanks to modern genetic techniques. The view of the *Posidonia ostenfeldii* complex has now been accepted, stating that *P. ostenfeldii*, *P. coriacea*, *P. denhartogii*, *P. kirkmanii* and *P. robertsoniae* are a single species (Campey et al. 2000).

Figure 1.1: World seagrass distribution (from Green & Short 2003).

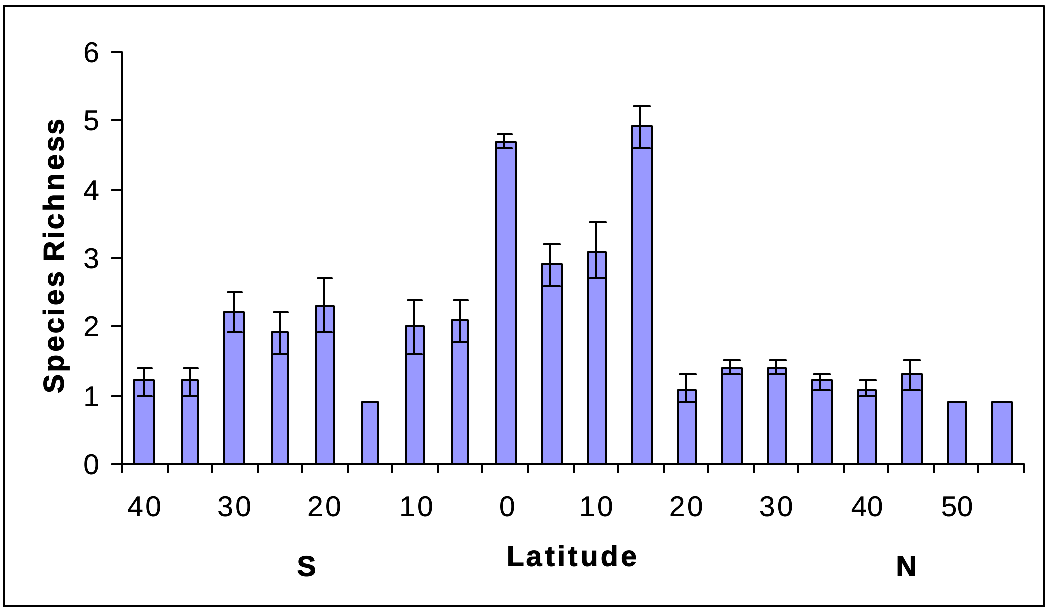


Figure 1.2: Average seagrass species richness at different latitudinal ranges (from Duarte 2000)

1.2 SEAGRASS AND THEIR ECOSYSTEM

1.2.1 Seagrass Diversity

The diversity of seagrass meadows, calculated as the Shannon-Weaver index (H) is generally close to 0, but values up to 1.56 have been reported for the Indo-Pacific region (Duarte 2000). Seagrass meadows are often monospecific, especially in temperate waters, but this is also a common pattern in tropical and subtropical regions. Thus, species diversity is lower than that found for terrestrial plant communities or communities of other organisms (Fig. 2; Hemminga & Duarte 2000). Although meadow diversity is low, seagrass environments support large communities of many species of animals (Fig. 3). Seagrass beds support three main categories of animals: (i) infauna, which are the animals living in the sediment amongst the rhizome of seagrass; (ii) epifauna, which can be divided into motile and sessile. The former group of animals is associated with the surface of the sediment or with seagrass stem and leaves. The latter group is permanently attached to the stem and leaves of seagrasses. The last group of organism associated with seagrass beds is (iii) the epibenthic fauna, which comprises large, mobile animals (fish) which are associated with these seagrass beds rather than with the single seagrass shoot. The many species of animals associated to seagrass beds can be permanent and temporary residents. In particular, fish associated with seagrass beds can be classified in four different categories (Kikuchi 1966): *permanent* and *temporary* residents and *regular* or *occasional* visitors. The high primary production of seagrasses in comparison to unvegetated patches ensures abundant supply of organic matter that represents the basic energy source for many species (Klumpp et al. 1989, Buia et al. 1992). Moreover, the structure of the meadow, and in particular the canopy, offers a large number of refugia for species at risk of predation. Indeed, a number of studies have reported how the canopy reduces the risk of predation for fish and invertebrates (Orth et al. 1984). Seagrass beds also function as nursery grounds for several commercial and non-commercial fish, crustacean and mollusc species (Bell & Harmelin-Vivien 1982). While spawning generally occurs elsewhere, juveniles move into the canopy after the pelagic larval stage and remain in the beds for several weeks (Orth et al. 1996).

In terms of genetic diversity, seagrass populations exhibit high heterogeneity. Genetic diversity is low in some species such as *Posidonia oceanica*, where a single clone can spread over many kilometres (Procaccini et al. 2001). On the other hand, microsatellite DNA analysis has proved high genetic diversity in some species (i.e. *Posidonia australis* and *Thalassia testudinum*). Population genetic studies in seagrasses have often revealed unpredictable patterns of genetic diversity, not always related to reproductive system and dispersal potential (Waycott & Les 1996, Procaccini & Mazzella 1998, Waycott 1998, Procaccini et al. 1999, Reusch et al. 2000). The main factors driving population genetic structure in seagrasses appear to be local environmental conditions and geological history. Different populations of the same species, in fact, can go from being uniclonal to being extremely genetically diverse, in relation to history of patch colonization and dynamics in different geographical areas (Reusch et al. 1999*)*. Moreover, the genetic make up of populations is determined by the balance between sexual reproduction and vegetative propagation. Meadows or patches of different size can be represented by a variable number of genotypes and the assessment of population boundaries is often difficult.

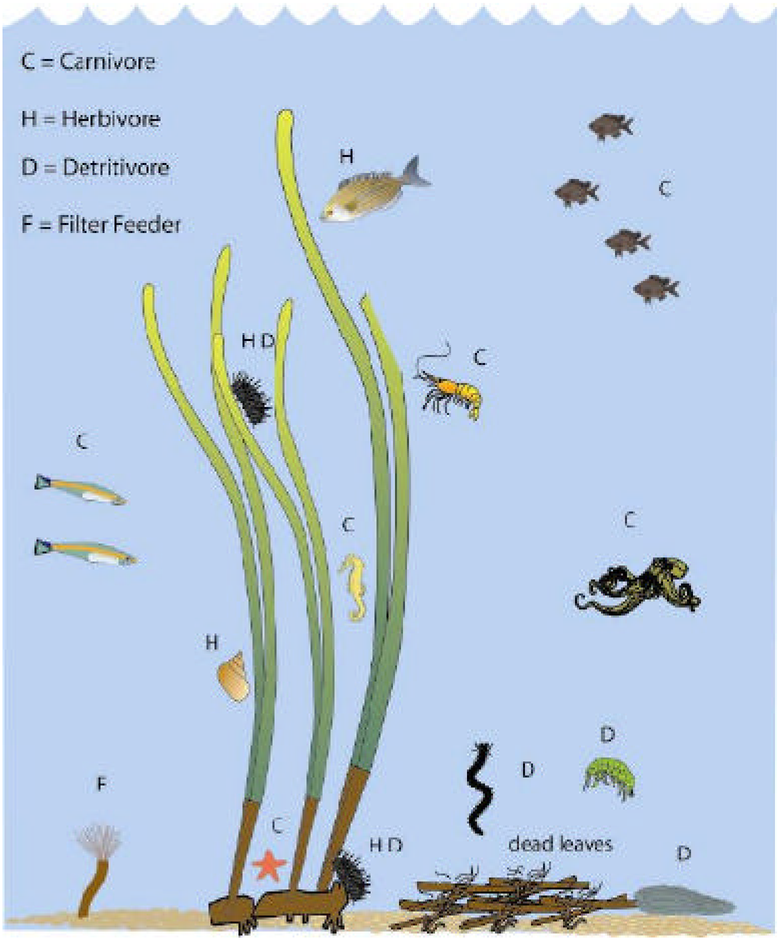


Figure 1.3: Seagrass ecosystem and their associated organisms

1.2.2 Population and Community Structure

Seagrass rhizomes form dense mats underground. The stem arises at nodes from the rhizomes and the leaves are usually flat. Leaf bases are sheathed, as are the small inflorescences, affording some protection against high salinity. The active transport of ions is undertaken by specialized transfer cells, which bear dense populations of mitochondria and an abundance of ATPases. The leaf epidermis, the main site for photosynthesis, presents cubical cells densely packed with chloroplasts, and larger mesophyll cells surround the conducting tissue and the aerenchyma (McRoy & Phillips 1980). Small roots diverge off the rhizomes, and the ability to take up nutrients via the root/rhizome system confers competitive advantage over macroalgae (Larkum 1989). All seagrass species are rhizomatous, clonal plants, occupying space through the reiteration of shoots, with their leaves and roots produced as a result of rhizome extension (Marbà & Duarte 1998, Hemminga & Duarte 2000). This asexual process appears to be the mechanism for seagrass proliferation, a mode of reproduction which is common in species like *Cymodocea serrulata* and *Posidonia oceanica* (Hemminga & Duarte 2000). Seagrass species vary about 100-fold in growth rate and lifespan, which is inversely related to size (Duarte 1991, Marbà & Duarte 1998, Hemminga & Duarte 2000).

Seagrasses form highly productive ecosystems (2.7 g DW m^{-2} per day), rivalling the most productive biological systems on earth (Duarte & Chiscano 1999). Seagrass primary production contributes about 1% of the total marine primary production (Duarte & Cebrián 1996). While most of the production of phytoplankton is used up in the marine environment, seagrass production is stored in sediments or exported to adjacent ecosystems (Duarte & Cebrián 1996). Because of the large below-ground allocation of production, the generally low use of seagrass production by herbivores (Cebrián et al. 1997), and the low decomposition rates of seagrass carbon (Harrison 1989), seagrasses store a large fraction of their substantial production, being responsible for about 15% of the carbon storage in the ocean (Pirc 1985, Duarte & Cebrián 1996). In addition, seagrasses also export on average 24% of their net production to adjacent ecosystems (Duarte & Cebrián 1996), both to the land (Ochieng & Erftemeijer 1999) and seaward (Menzies et al. 1967), acting as important trophic links with other ecosystems.

Seagrass meadows often appear as a static seascape (Robbins & Bell 2000) (Plate 1.1). However, they are subjected to intense perturbation involving the continuous replacement of shoots which maintains them in an equilibrium state.

Plate 1.1: Seagrass form continuous meadows which might appear as static seascapes.

Such equilibrium can be lost due to natural and human induced disturbance events (Hemminga & Duarte 2000). Disturbance events often reset the cyclical succession of seagrass communities altering ecosystem landscape (Campbell 2003, Whitfield 2004). Landscape ecology approaches to seagrass environments have rapidly developed and they have been applied successfully to seagrass meadows in the past (Bell et al. 1995, Irlandi et al. 1995). Perturbation can determine the pattern of seagrass landscape, introducing high heterogeneity at the large (m^2) and intermediate (cm^2) scale. Seagrass meadow structure and maintenance can be explained using patch dynamics (McRoy & Lloyd, 1981, Duarte & Sand-Jensen 1990, Marbà & Duarte 1995, Vidondo et al. 1997, Kendrick et al. 1999). When a disturbance occurs, the rate and pattern of recruitment into the “new” patch is directly related to species-specific availability of propagules (Denslow 1985, Kenworthy 2000, Kenworthy et al. 2002). Species that have seed banks, such as *Zostera marina* or *Cymodocea nodosa*, are capable of rapid recruitment into a disturbed patch if the requisite conditions apply. Species that lack a seed bank, such as *Posidonia oceanica*, show the ability to recruit through vegetative fragments which act as dispersal units through clonal growth (Marbà & Duarte 1995, Campbell 2003). However the rate of vegetative recruitment is much slower than recruitment of seedlings. As a consequence, when clonal growth is insufficient, recolonisation from seeds is required to allow the prompt recovery of damaged seagrass meadow. Seagrass reproduction is often not considered a frequent event, being dependent on seasonal (i.e. temperature, Durako et al. 1982) and latitudinal factors (i.e. irradiance). Hence, vegetative development can be considered the main mechanism for seagrass colonisation (Duarte & Sand Jensen 1990, Marbà & Duarte 1998, Rasheed 1999). Vegetative growth also regulates the rate of formation and the spatial distribution of shoots within seagrass meadows. Growth features that regulate rhizome elongation appear to be species-specific and they are scaled to plant size (Duarte 1991). At present, there is still a paucity of studies concerning seagrass colonisation processes, particularly in the Mediterranean, where there is an increasing alarm caused by seagrass decline (Duarte & Sand Jensen 1990, Orth & Moore 1983, Cambridge & McCom 1984). Moreover, the understanding of seagrass rhizome growth would provide new insights on the key factors that regulate colonisation rates as well as plant regrowth after disturbance events. The rate of patch formation has been examined for a few seagrass populations and it varies from 5 x 10^{-3} ha^{-1} yr^{-1} in a shallow coastal *Zostera marina* population to 3 x 10^{-4} ha^{-1} yr^{-1} in a *Posidonia oceanica* population. Established patches develop centrifugally with horizontal rhizomes that extend from established patches, growing faster when colonising unvegetated bare substrata (Marbà & Duarte 1998; Hemminga & Duarte 2000). A self-accelerating mechanism may increase the rate of rhizome elongation, shortening recovery time (Hemminga & Duarte 2000). The acceleration of patch growth suggests that seagrasses form a ‘mutually sheltered structure’ as they grow (Fonseca et al. 1983). This means that during patch expansion there is an increase in the flux of resources along the rhizomes from the shoots in the inner part of the patch to the rapidly growing rhizomes, referred to as ‘runners’ (Vidondo et al. 1997, Hemminga & Duarte 2000).

Although monospecific meadows are generally dominant (i.e. *P. oceanica*) in the temperate region, mixed-species meadow are frequently found in the tropics (Hemminga & Duarte 2000). The response of seagrass beds to natural and anthropogenic disturbance has provided new insights on the successional sequence within seagrass populations. Climax community species are generally large, slow growing species (i.e. *Thalassia testudinum* or *Posidonia oceanica*) while pioneer species are phenotipically smaller species with faster rhizome elongation rates such as *Cymodocea nodosa* or *Zostera marina* (Hemminga & Duarte 2000). Previous studies conducted on Mediterranean seagrass populations have highlighted an idealised successional sequence of species from initial colonisation by *Cymodocea nodosa* to a final dominance of *Posidonia oceanica* (Green & Short 2003). However, such a sequence does not appear to be always realised, and, in shallow water, the two species might co-exist (Hemminga & Duarte 2000).

In the last decade there has been increasing attention to effect of introduced species (*Caulerpa taxifolia* and *C. racemosa*) on *P. oceanica* meadows. Thus, new work is needed to give evidence on how the introduction of new invasive species might alter the proposed successional sequence for Mediterranean seagrass species.

1.3 THE ROLE OF PHYSICAL FACTORS IN SEAGRASS COMMUNITIES

1.3.1 Seagrass Habitat Requirements: an overview

Although increased attention has been focused on the sensitive nature of seagrass ecosystems and the significance of seagrass communities to coastal ecosystems, there is little published information describing the role of physical factors within seagrass meadows (van Keulen 1998). Physical factors play a major role in limiting primary production in the marine environment (Larkum 1981) and represent potential key factors in determining colonisation processes of seagrasses.

The main physico-chemical factors that potentially influence seagrass communities can be summarised as follows (Larkum 1981):

- light
- temperature
- salinity
- nutrient supply
- water motion
- sediment dynamics

The role of each of these factors in benthic marine plant communities, and in particular seagrass ecosystems, is reviewed below. It should be emphasised that none of these influences operates in isolation: any ecosystem-scale model should take into account all of these factors and the ways in which they interact to influence the subject organisms (van Keulen 1998).

1.3.2 Light

Light is generally considered the major factor controlling the distribution and abundance of seagrasses. Many studies have focused on the effect of light attenuation and availability on seagrass growth and photosynthetic characteristics. Seagrasses generally exhibit light requirements greater than that of macroalgae, requiring an underwater irradiance generally in excess of 11% of that incident on the water surface for growth. Such requirements typically set their depth limit (Dennison 1987, Duarte 1991). The penetration of light decreases steadily with depth, for this reason seagrasses are generally found in water shallower than 40 m when sufficient light is available.

The upper depth limit of seagrasses is imposed by their requirement for sufficient immersion in seawater or tolerable disturbance by waves and, in higher latitudes, ice scour (Hemminga & Duarte 2000). Although species within some genera, such as *Zostera* spp., *Phyllospadix* spp. and *Halophila* spp. can grow intertidally (den Hartog 1970, Hemminga & Duarte 2000), most seagrass species grow subtidally, and cannot tolerate long exposure to air. Some species, such as *Posidonia oceanica*, form reef structures referred to as a 'recife barrier' in shallow water. To prevent desiccation on such a recife this species forms dense continuous populations with leaves that lay flat on the surface, so retaining water.

1.3.3 Temperature

Water temperature in coastal regions is seasonally fairly constant. However, species such as *P. oceanica* living in such environment can deal with yearly fluctuation between 10 and 30 °C (Boudouresque & Meinesz 1982). However, in enclosed embayments with restricted exchange with the open sea temperature elevation might be frequent. Dense seagrass meadows have been shown to restrict water motion considerably (Fonseca et al*.* 1982, Ackerman & Okubo 1993), and temperatures within the canopy could be expected to increase beyond that of the surrounding water column temperatures during the daytime, particularly in summer when water flow is particularly reduced. It has been shown that plant metabolism is improved with moderate temperature increases, as a result of generally increased enzyme activity. Numerous studies have shown that excessively high temperatures can result in stress and impaired metabolic activity (Morris & Farrell 1971, Morris & Glover 1974). Drew (1979) noted that, while prolonged exposure to temperatures up to the plant's optimum has no negative effects on photosynthesis, even short periods above the optimum temperature will reduce photosynthetic performance significantly. Moreover, previous work has given proof of how temperature often determines the transplantation success of some species (i.e. *Posidonia oceanica*) as poor cicatrisation in rhizomes appears to be less successful at temp. above 20 °C, following artificial transplantation (Meinesz et al., 1992). Recent studies regarding the influences that temperature has on physiological rates of *Posidonia sinuosa* and *Amphibolis antarctica* from the Perth metropolitan area indicated optimum temperatures of 23° C and 18° C respectively. Both seagrasses were found to be stressed at 28° C, with reduced photosynthetic rates and shoot death observed (Walker et al*.* 1994).

1.3.4 Salinity

Seagrasses can grow in estuarine and brackish waters, but require salinity in excess of 5 to develop (Hemminga & Duarte 2000). The salinity of the water may not only affect the distribution (Orth & Moore 1984, Fletcher & Fletcher 1995) and growth (Adams & Bate 1999, Kamermans et al. 1999) of seagrasses but can also represent an environmental stressor (Zieman et al. 1999) which renders seagrasses more or less vulnerable to diseases (Burdick et al. 1993). Most seagrasses (i.e. *Zostera* spp.) can tolerate a wide range of salinity, from the area of contact with macrophytes (salinity 10psu) to extreme environments with a salinity of about 45psu. However, there are some stenohaline species, such as *Posidonia oceanica*, which do not tolerate salinity fluctuations and freshwater inputs. At low and high salinities, seagrass suffers from osmotic stress which leads from loss functionality to necrosis and death.

1.3.5 Nutrient supply

The supply of nutrients is obviously important for all plants. In general, seagrasses have a low demand for macro-nutrients and are relatively resistant to biodegradation. It has been estimated that less than 5% of total annual production of seagrasses is consumed directly (Mann 1972; Nienhuis & van Ierland 1978, Conacher et al. 1979, Kirkman & Reid 1979). The high productivity of seagrass ecosystems is therefore largely dependent on the decomposition and remineralization of nutrients derived from detritus from the seagrass community (Klug 1980). In the last two decades much effort has been put into the study of nutrient dynamics in seagrass ecosystems. Boon et al. (1986) showed that concentrations of nitrogen compounds in sediments from seagrass beds were considerably higher than in other sediments; further, he concluded that nitrogen was more likely to limit seagrass growth than phosphorus. Rates of nitrogen compound turnover were found to be very high in seagrass beds, due largely to the action of anaerobic sediment bacteria (Moriarty et al. 1985, Boon et al. 1986). Indeed, the major source of sediment nitrogen is bacterial nitrogen fixation in the rhizosphere of the plants (Patriquin & Knowles 1972). Other work carried out by Short and co-workers (Short & McRoy 1984, Short 1987), reported how seagrass can rapidly take up nitrogen from both sediments and the water-column, but concluded that the higher nutrient concentrations in the sediments made this likely to be the major source.

Further work on freshwater angiosperms by Barko and Smart (1980, 1981, 1986) showed that sediment based nitrogen and phosphorus is more significant than that from the surrounding water. The complex decomposition and remineralisation processes which occur in seagrass meadows are generally considered to adequately supply the nutrient requirements of the seagrass ecosystem, through efficient nutrient recycling systems (Fenchel 1977). Uptake of nutrients via the rhizome/root system, and the high levels of nutrient cycling within most seagrass meadows are postulated to satisfy nutrient requirements for such communities.

1.3.6 Water Motion

Water motion is recognised as a major linking factor which has effects on seagrass physiology, recruitment and sediment transport although to date it is one of the least studied (van Keulen 1998). Until the pioneering studies of Fonseca et al. (1982, 1983) the only consideration given to water motion in seagrass meadows had been with regard to sedimentation. However, in the last few years new techniques have become available which have allowed investigation of the fundamental ecological roles water flow plays in seagrass ecosystems. At present, there is paucity of data concerning the role of current velocity in seagrass recruitment (Di Carlo et al. submitted). Both seedlings and vegetative fragments most probably recruit in low flow environments (Koch 2001). However, when seagrass patches are well established they can expand into adjacent areas with higher current velocity environments (Koch 2001). This is seen when *Posidonia oceanica* grows as matte over hard substrata. In order to entangle and settle, vegetative fragments might be dependent on current speed.

Seagrasses influence the water flow around them (Ackerman & Okubo 1993, Worcester 1995, Koch & Gust 1996, Koch 2001) and in the case of extensive meadows these effects can have significant geological impacts (Scoffin 1970, Fonseca et al. 1983, Ward et al. 1984, Fonseca & Fisher 1986, Koch 2001). Water motion in marine environments exists in two major forms: ambient current flows and wave action. Such currents exist as a horizontal trend in water flow, on which wave action is superimposed. In shallow subtidal habitats, wave action can be divided into two different types: orbital waves and wind waves (van Keulen 1998). Orbital waves are produced by distant storm events whilst wind waves (short period) are directly produced by local wind conditions (Koch 2001). Wave action has a more direct impact on the ecosystem, with obvious effects on sediment transport, boundary layer processes and physical stresses. Seagrass can modify ambient current by extracting the momentum from the moving water (Madsen & Warnke 1983). The magnitude of this process is defined by the canopy density, the hydrodynamic regime of the area and by the depth of the water column (Koch 2001). Currents are slower in vegetated areas in comparison to bare substrata. The reduction of currents produces a series of advantageous effects on the meadow: 1) a reduced self shading, due to the more vertical position of the leaves (Fonseca et al. 1982); 2) lower friction velocity at the sediment surface with the vegetation, which prevents resuspension so increasing light availability (Fonseca & Fisher 1986, Fonseca 1996); 3) increased settlement of organic as well as inorganic particles, hence an higher nutrient availability in the sediment (Kenworthy et al. 1982, Fonseca 1996). Numerous workers have remarked upon the reduction of water flow by seagrass meadows. Molinier & Picard

(1952) and Ginsburg & Lowenstam (1958) were among the first to describe the phenomenon of water flow reduction and its effect on sedimentation. These workers showed that the seagrasses modified the grading of sediments. Later on, Fonseca et al. (1982, 1983) showed that the margins of seagrass meadows are areas of rapid reduction in velocity, and hence these are the major sites for sedimentation. The work of Fonseca and co-workers also reported that high current flows were directed over the canopy, greatly reducing erosion of the substratum. Subsequent studies on sediment entrainment within canopies of four different seagrasses suggest that there are significant differences between species of seagrass, with consequent implications for associated faunal distribution (Fonseca & Fisher 1986). The loss of momentum through seagrass canopies does not only reduce flow speed but also decreased wave energy (Koch 1996, Verduin & Backhaus 2000). Wave attenuation is greatest in dense seagrass meadows in shallow waters (5-15 m), where plant biomass takes up a large portion of the water column (Koch 2001). Seagrass distribution may be limited by high wave energy, even if well established meadows can sustain themselves in harsh turbulent environments (Robbins & Bell 2000, Koch 2001). The impact of waves on seagrasses can be either direct or indirect. Direct impacts of waves results in the erosion of seagrass bed edges (Clarke 1987). This leads to an alteration of the seagrass landscape (Robbins & Bell 2000). Indirect impacts include sediment resuspension, changes in sediment grain size and enhanced epiphytic growth.

1.3.7 Sediment dynamics

Although biological and chemical factors, such as epiphyte biomass and eutrophication, play a major role in altering light availability to seagrasses, there are also physical, geological and geochemical factors that affect seagrass distribution and abundance. While the former factors have received consideration in the past, the importance of the latter factors has often been underestimated (Gacia & Duarte, 2001). The vertical and horizontal distribution of seagrass is linked to the availability of a suitable substratum for growth (Hemminga & Duarte 2000). Most seagrass species growth on sandy or muddy sediments which are easily penetrated by the roots (Green & Short 2003). However, muddy sediments do not provide a firm substrate for attachment and high turbidity limits growth (Shepherd 1989). Some species (i.e. *P. oceanica*) can also colonise hard rock substrata but in order for the seagrass to become established a minimum sediment layer above the substratum is required (Di Carlo et al. submitted). A minimum of 7 cm was required for *Thalassia testudinum* establishment, while full development only occurred when the sediment layer reached a thickness of 20 to 25 cm (Zieman 1972). Seagrass roots also play an important role in stabilising sediments, reducing resuspension hence influencing sediment dynamics in littoral zones (Moriarty & Boon 1989, Koch 2001). For this reason, the loss of seagrass beds can be related to the modification of the sedimentary regimes as well as a deterioration of the quality of coastal waters (Short & Short 1984). Previous studies (Short & Short 1984, De Falco et al. 2000) have reported on the influence and dependence of seagrass meadows on the nature and dynamics of coastal sediments (Blanc 1975, Jeudy de Grissac 1984, Blanc & Jeudy de Grissac

1984, Jeudy de Grissac & Boudouresque 1985). Furthermore, the relationship between sedimentation patterns and seagrass distribution can be used to assess sedimentary processes in coastal areas and their ecological role (De Falco et al. 2000). Sediments are indeed important factors in determining the growth and distribution of seagrasses (Short 1987, Touchette & Burkholder 2000, Koch 2001). Seagrass beds are known for promoting deposition of particles and for enhancing particle retention (Gacia et al. 1999). Seagrass canopies act as a trap for suspended particles, which are then deposited onto the substratum and retained by the root-rhizome complex (Fonseca et al. 1983, Gacia et al. 1999, De Falco et al. 2000, Koch 2001). Particle resuspension may also follow seasonal trends linked to the variation in the seagrass canopy height. In areas where seagrass loss has occurred sediment erosion might follow (Gacia & Duarte 2001). Although much attention has been focused on the role of seagrass in coastal dynamics, there is still a paucity of information on the effect of seagrasses on particle deposition and resuspension. However, *Posidonia oceanica* canopies can reduce resuspension within the meadow by reducing current velocities and increasing the roughness height of the boundary layer (Dauby et al. 1995, Gacia et al. 1999, Gacia & Duarte 2001).

Reductions in current velocity and wave energy means that seagrass beds tend to accumulate organic particles (Koch 2001). Moreover, organic matter can be derived from the burial of rhizome in roots, especially in *Posidonia oceanica* beds growing on matte. The organic matter component of sediment supporting seagrass growth is generally <5% of the sediment dry weight (Barko & Smart 1981). However, higher organic matter values can be found in sediments colonised by species with larger leaves (Hemminga &

Duarte 2000). Perhaps such species survive in sediment with a high organic content because they have an enhanced oxygen production from the larger leaves and hence a higher oxygen transport to the root system (Koch 2001). Sediments with higher organic content have a high bacterial activity which produce a highly negative redox potential (Terrados et al. 1999). The redox status of the sediment plays a major role in constraining plant growth. Thus, seagrasses are found in sediments with a redox potential ranging between -100 and 200 mV with a pore water sulfide level of <100 µM in the top 10 cm of the sediment (Terrados et al. 1999). As redox potential falls below such a value the sediment may become toxic for the plants (Hemminga & Duarte 2000, Koch 2001). Sediments within seagrass beds are finer than those present in unvegetated areas (Scoffin 1970, Almasi et al. 1987). Sediment grain size might be a good indicator of a variety of physical and geochemical features of seagrass habitats. As grain size distribution becomes skewed toward the silt and clay (silt 4-63 µm; clay <4 µm), the porewater exchange with the overlaying water column decreases (Huettel & Reusch 2000). On the other hand, in coarse sediments nutrient variability may differ from that in finer sediments as there is a higher exchange of pore water with the overlaying water. Thus, nutrient availability in coarse sediment will be lower than in finer sediments (Koch 2001).

1.4 SEAGRASS AS ECOSYSTEM ENGINEERS

Many larger marine plants can significantly influence the environment around them (Gambi et al. 1990, Koch 1993, Koch & Gust 1999, van Keulen & Borowitzka 2000). Submerged aquatic vegetation (SAV) modify the substratum after settlement, alter the sediment dynamics and the hydrodynamic regime of the environment with their canopy and change the light condition of the environment. For this reason seagrasses can be seen as ecosystem engineers (Jones et al. 1994, Koch 2001).

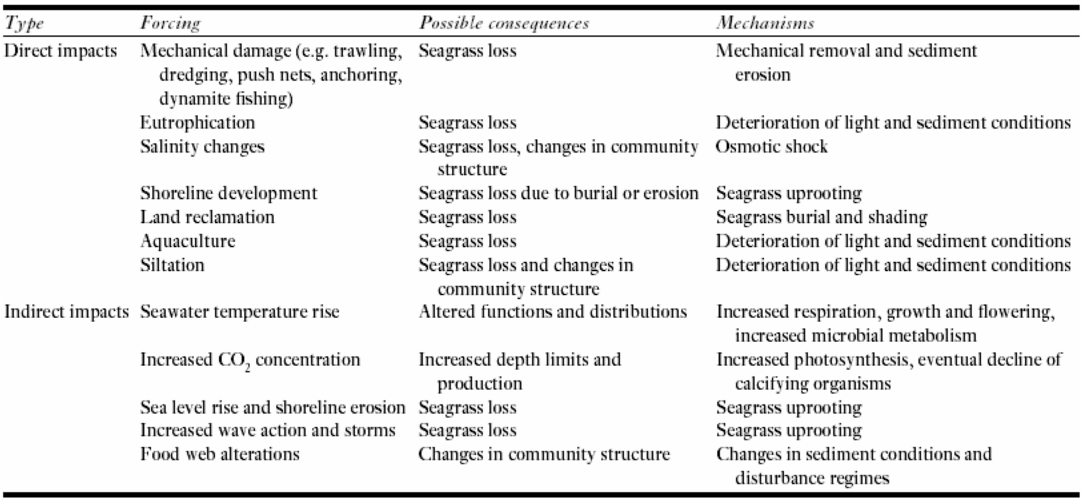
A considerable literature describes the baffling effect of seagrasses on flow through a meadow (Molinier & Picard 1952, Ginsburg & Lowenstam 1958, Logan & Cebulski 1970, Scoffin 1970, den Hartog 1971, Read 1974, Fonseca et al*.* 1982) while the influence of seagrasses on smaller scale hydrodynamics has been little studied. Recent investigations of mixing within seagrass ecosystems suggest that hydrodynamic processes are considerably reduced within the canopy (Ackerman & Okubo 1993). Experimental results to date are inconclusive as to what effect this may have on the algal epiphytes growing on seagrasses; it is proposed that the reduction in flow may be in part responsible for the restriction of algal epiphytes to the canopy of seagrass meadows, as observed by Borowitzka et al. (1990).

Different seagrass species appear to have different baffling effects on the flow regime. *Zostera marina* plants appear to be more effective at baffling flow than many other species (Fonseca & Kenworthy 1987). It would be logical to assume that seagrass morphology has a significant effect on the baffling effect of those meadows, and consequently influences the flow regime within the meadow. The variation in water flow within canopies of different density can also influence physical factors such as sediment transport, nutrient availability, light penetration and water temperature (van Keulen 1998). These factors can then impact on the species composition and distribution of epiphytes and other organisms which live within the meadow (Gambi et al. 1990, Hovel et al. 2002) The magnitude of this process is defined by the canopy density, the hydrodynamic regime of the area and by the depth of the water column. Rhizomes and roots stabilise the sediment so preventing erosion, while reduced current velocities increase sediment deposition and reduce resuspension (Duarte & Sand Jensen 1990). Thus, seagrass patches might present a positive feedback process forming a mutually sheltering structure (Thayer et al. 1984). For this reason seagrasses can be seen as ecosystem engineers (Jones et al. 1994, Koch 2001).

1.5 HUMAN THREATS TO SEAGRASSES

The health of seagrass meadows depends on water nutrient concentration (Pirc 1986) and light intensity (Dennison 1987), hence abundance is linked to water clarity (Blanc & Jeudy de Grissac 1984, Delgado et al. 1999). For this reason seagrasses are highly susceptible to anthropogenic disturbance (Pérès 1984, Shepherd et al. 1989, Short & Wyllie-Echeverria 1996). Humans impact seagrass ecosystems, both through direct proximal impacts, affecting seagrass meadows locally, and indirect impacts, which may affect seagrass meadows far away from the sources of the disturbance (Table 1.1). Proximal impacts include mechanical damage such as the construction and maintenance of infrastructures in the coastal zone, as well as effects of eutrophication, siltation, coastal engineering and aquaculture. Indirect impacts include those from global anthropogenic changes, such as global warming, sea-level rise, elevation of CO_2 levels and an increase in ultraviolet (UV) radiation, and anthropogenic impacts on marine biodiversity, such as the large-scale modification of the oceanic food web through fisheries. Indirect impacts are already becoming evident (Beer & Koch 1996).

The most unequivocal source of anthropogenic disturbance to seagrass ecosystems is physical disturbance. This susceptibility derives from multiple causes, all linked to increasing human usage of the coastal zone for transportation, recreation and food production, about 40% of the human population presently inhabit the coastal zone (Duarte 2002). Direct habitat destruction by land reclamation and port construction is a major source of disturbance to seagrass meadows (Duarte 2002), due to dredge-fill activities, which generally leads to a reduction in water transparency. The construction of new ports is associated with changes in sediment transport patterns, involving both increased erosion and sediment accumulation along the adjacent coast. Port operation often implies a reduced water transparency as well as nutrient and contaminant inputs associated with ship traffic and servicing, and dredging activities associated with port and navigation-channel maintenance. Rapid increases in seabased transport, as well as recreational boating activities have led to an increase in the number and size of ports worldwide, with a parallel increase in the combined disturbance to seagrass meadows (Duarte 2002). Fisheries activity, particularly illegal shallow trawling (Sanchez-Jerez & Ramos-Esplà 1996, Pasqualini et al*.* 1999), smaller scale activities linked to fisheries such as clam digging and use of push nets over intertidal and shallow areas and dynamite fishing (Kirkman & Kirkman 2000) also affect seagrass beds irreversibly. The exponential growth of aquaculture (Ruiz et al. 2001), has also led to impacts on seagrasses through shading and physical damage to the seagrass beds, as well as deterioration of water and sediment quality leading to seagrass loss (Delgado et al*.* 1997, 1999, Pergent et al*.* 1999, De Falco et al*.* 2000). The coastal zone also supports an increasing amount of coastal infrastructure, such as pipes and cables, the deployment and maintenance of which also entail disturbance to adjacent seagrass meadows (Duarte 2002). The development of coastal tourism, the fastest-growing industry in the world, has also led to a major transformation of the coastal zone in areas with pleasant climates. For instance, about two-thirds of the Mediterranean coastline is urbanized at the present time (Duarte 2002), with this fraction exceeding 75% in the regions with the most developed tourism industry, with harbours and ports occupying 1250 km of the European Mediterranean coastline. Urbanization of the coastline often involves destruction of dunes and sand deposits, promoting beach erosion, a major problem for beach tourism (Duarte 2002). Beach erosion, however, does not only affect the emerged beach, and is usually propagated to the submarine sand colonized by seagrass, eventually causing seagrass loss (Medina et al*.* 2001). Moreover, extraction of marine sand for beach replenishment is only economically feasible at the shallow depths inhabited by seagrasses, which are often impacted by these extraction activities (Medina et al*.* 2001). The threats coastal tourism poses to seagrasses are sometimes direct, e.g. purposeful removal of seagrass remains from beach areas to ‘improve’ beach conditions. Fortunately, there are indications that coastal tourism is attempting, at least in some areas, to embrace sustainable principles, including the maintenance of ecosystem services, such as those provided by seagrasses, and could well play a role in the future as an agent pressing for seagrass conservation.



Type	*Forcing*	*Possible consequences*	*Mechanisms*
Direct impacts	Mechanical damage (e.g. trawling, dredging, push nets, anchoring, dynamite fishing)	Seagrass loss	Mechanical removal and sediment erosion
	Eutrophication	Seagrass loss	Deterioration of light and sediment conditions
	Salinity changes	Seagrass loss, changes in community structure	Osmotic shock
	Shoreline development	Seagrass loss due to burial or erosion	Seagrass uprooting
	Land reclamation	Seagrass loss	Seagrass burial and shading
	Aquaculture	Seagrass loss	Deterioration of light and sediment conditions
	Siltation	Seagrass loss and changes in community structure	Deterioration of light and sediment conditions
Indirect impacts	Seawater temperature rise	Altered functions and distributions	Increased respiration, growth and flowering, increased microbial metabolism
	Increased CO_2 concentration	Increased depth limits and production	Increased photosynthesis, eventual decline of calcifying organisms
	Sea level rise and shoreline erosion	Seagrass loss	Seagrass uprooting
	Increased wave action and storms	Seagrass loss	Seagrass uprooting
	Food web alterations	Changes in community structure	Changes in sediment conditions and disturbance regimes

Table 1.1: Human-induced direct and indirect impacts on seagrass ecosystems (modified from Duarte 2002).

1.6 POSIDONIA OCEANICA ECOSYSTEMS

Posidonia oceanica (L.) Delile is an endemic seagrass species of the Mediterranean Sea (Den Hartog 1970). This species belongs to the subphylum Angiospermae, class Monocotyledonae, order Potamogetonales, family Posidoniaceae. *Posidonia oceanica* forms extensive meadows ranging from shallow waters to depths of about 40 m (Pérès & Picard 1964), when sufficient light is available (Den Hartog 1970, Phillips & Meinesz 1988) and is commonly found on sandy and rocky substrata with the exception of estuaries where the input of freshwater and fine sediments is high (Green & Short 2003). *Posidonia oceanica* is characterized by long persistence, slow vegetative growth, sporadic sexual reproduction and low genetic variability (Buia & Mazzella 1991, Buia et al. 1992; Mateo et al. 1997, Procaccini & Mazzella 1998). This species presents both a vegetative and a sexual reproductive strategy. The former is accomplished by clonal growth while the latter entails the production of hermaphrodite inflorescences and the formations of fruits and seeds (Mazzella et al. 1983). Sexual reproduction generally occurs at the end of the fall with fruit formation about six months later (March-April) (Mazzella et al. 1983) (Plate 1.2).

Plate 1.2: Fruit of *Posidonia oceanica*. The fruit is called ‘sea olive’ due to its morphological features (courtesy of the Stazione Zoologica di Napoli ‘A. Dorhn’).

P. oceanica fruit is generally refereed to as “sea olive” due to its phenotypic features but also because it floats on the sea surface and it is dispersed by wind currents. When the fruit is mature, it opens up and releases the seed which sinks. If favourable conditions apply, the seed germinates (Gambi et al. 1996). However, sexual reproduction in *Posidonia* is considered an odd event. Flowering occurs in October and reproduction is timed with that of congeneric species, all located around the Australian coasts (Plate 1.3). Moreover, reproduction is sporadic and dependent of several physical parameters such as temperature and possibly intraspecific competition (Giraud et al. 1979).

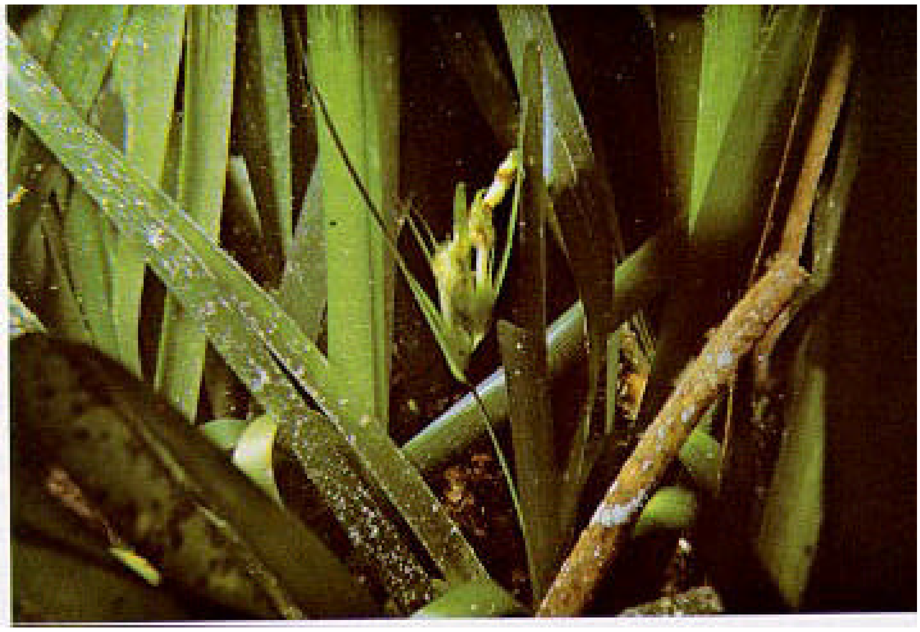


Plate 1.3: Inflorescence of *Posidonia oceanica*. Reproduction is considered a rare event and conditions affecting the process are not clear (courtesy of the Stazione Zoologica di Napoli 'A. Dorhn').

Thus, vegetative recruitment is a more common colonisation strategy. During storms, vertical rhizomes which are located at the edge of the meadow, detach from the plant and are dispersed by the action of currents and waves (Riggio 1995). Occasionally, these rhizomes get stuck in crevices, and helped by sturdy roots (Hemminga & Duarte 2000), which penetrate into the crevices effectively anchoring the plants, they settle between rocks (D'Anna et al. 2000). *P. oceanica* presents dimorphic rhizomes, consisting of two differentiated types: horizontal (plagiotropic) rhizomes, presenting large internodes, and vertical (orthotropic) rhizomes with relatively shorter internodes (fig. 1.4). Internodes are also referred to as 'leaf scars' and are the rhizome fragments between two nodes (Boudouresque et al., 1984). Horizontal rhizomes can revert into vertical rhizomes, and at the same time vertical rhizomes can branch to produce horizontal rhizome (Hemminga & Duarte 2000). Horizontal growth provides the advantage of lateral growth into new un-vegetated patches whilst vertical growth allows the plant to overcome burial and reach the light.

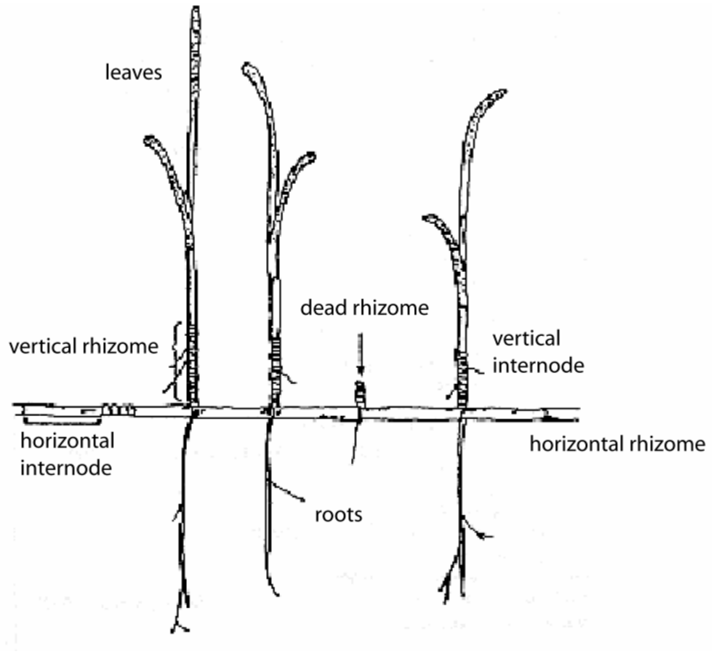


Figure 1.4: Generic diagram of the basic component of seagrass

As a consequence of vertical growth, *P. oceanica* forms a structure named “matte” created by an intertwining of roots and rhizome, to build upwards forming a strong extensive reef along the shore (fig. 1.5, plate 1.4). This structure is considered of great importance as it works as net sink of carbon and other elements as well as a breakwater, reducing beach erosion (Jeudy de Grissac & Boudouresque 1985). Such a structure can grow vertically about 1 cm yr^{-1} and its development is strictly linked to the sedimentation rate of the area (Boudouresque & Jeudy de Grissac 1983). In the Mediterranean area matte structures over 6 m thick and a thousand years old have been recorded (Molinier & Picard 1952, Green & Short 2003).

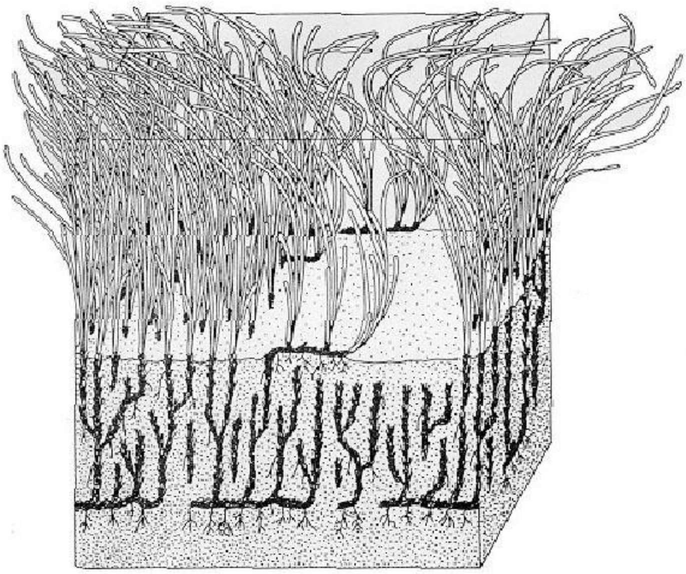


Figure 1.5: Schematic diagram of matte structure (from Boudouresque & Meinesz 1982)

Plate 1.4: Particular of a *P. oceanica* matte structure (courtesy of the Stazione Zoologica di Napoli 'A. Dorhn')

Posidonia oceanica systems are extremely rich in terms of number of associated organisms (Gambi 2002). They represent exclusive habitats for many algal and animals species some of which are considered as rare (i.e. *Hippocampus hippocampus*) or protected (i.e. *Pinna nobilis*) (Boudouresque et al. 1991). *Posidonia* beds functions as nursery grounds for the juveniles of commercially important fish and invertebrates, such as some species of the family Sparidae (i.e. *Diplodus sargus* and *Diplodus annularis*), Serranidae (i.e. *Serranus cabrilla*), Labridae (*Coris julis*) and the sea urchin *Paracentrotus lividus* (Bell & Harmelin-Vivien 1982, Green & Short 2003) (Plate 1.5).

Plate 1.5: a) the sparidae *Diplodus sargus*. One of the common fish species associated with *Posidonia oceanica* meadows; b) The labridae *Coris julis* strongly associated with *P. oceanica* meadows; c) the sea urchin *Sphaerechinus granularis*, the main grazer on *P. oceanica* meadows.

Posidonia oceanica meadows, like other seagrass ecosystems, are extremely sensitive to the moderate-to-high levels of disturbance often associated with highly human-impacted coasts. Anthropogenic changes in the hydrodynamic regime and water quality are among the numerous factors that have resulted in the fragmentation and widespread decline of seagrass meadows documented over the last two decades (Shepherd et al. 1989, Marbà et al. 1996, Short & Wyllie-Echeverria 1996, Fonseca & Bell 1998). Fishing pressure (mainly trawling) (Ardizzone & Migliuolo 1988, Sanchez-Jerez et al. 1996), nutrient loading (Pergent-Martini & Pergent 1995) and boat anchoring (Garcìa Charton et al. 1993, Francour et al. 1999) represent anthropogenic factors which lead to a slow but constant decline of *P. oceanica* beds. In addition to these impacts, there are major marine operations that significantly affect seagrass beds, such as industrial outfalls (Pergent-Martini & Pergent 1995), the exponential increase of fish farms (Ruiz & Romero 2001, Pergent et al. 1999, De Falco et al. 2000) as well as dredging and filling operations (Delgado et al. 1999, Guidetti & Fabiano 2000, Short & Coles 2001). The loss and degradation of seagrass ecosystems, and the resultant need for optimal restoration strategies, are problems of significant importance that attract the attention and efforts of both managers of coastal habitats and scientific researchers (Fonseca & Bell 1998). The goal of restoration is to re-establish impaired or lost natural resources after the removal of the original causes of the degradation (Procaccini & Piazzi 2001). For seagrasses, transplant technologies, developed as the primary restoration strategy, have resulted in variable success, depending on both the species transplanted and the techniques utilized (see Fonseca et al. 1998 for a review). In the Mediterranean Sea, a variety of transplanting techniques has been tried in an attempt to restore *P. oceanica* meadows. In various experiments, transplanting success was affected by factors such as transplant density, depth and season, and substrate type (Giaccone & Calvo 1980, Meinesz et al. 1991, 1992; Molenaar & Meinesz 1995, Molenaar et al. 1993, Piazzi et al. 1998). The variations in the phenological characteristics and the differences in the survival rate of transplanted shoots were also assessed in relation to the geographic location of the donor beds and to the distance between donor beds and transplantation sites (Meinesz et al. 1993, Piazzi et al. 1998). In both cases significant differences were found among transplants coming from different localities, independent of the distance between donor bed and transplant site. Some other factors should account for observed differences.

1.7 AIMS

At the end of the 1970s a submerged pipeline system (TRANSMED) was deployed between Cape Bon (North easternmost tip of Tunisia) and Capo Feto (south westernmost tip of Sicily) to provide Italy with Algerian methane gas. Marine operations ran for almost 3 years and included the dredging of a trench through the 30,000 ha *Posidonia oceanica* meadow at Capo Feto (Sicily). Four pipes were laid within the trench, which was backfilled with residual sediment from the excavation topped with a layer of calcarenitic rocks, which eventually eroded into gravel and pebbles. An area of approximately 70 ha of the meadow was destroyed or severely damaged as a result of the dredging technologies available at that time, which involved the use of explosives (Plate 1.6).

Plate 1.6: Seagrass patches adjacent to the construction trench. The seagrass meadow next to the construction site was affected by dredge-fill activities.

Ten years later (1992-1993) the Italian government authorized the deployment of two new pipes at Capo Feto. This second excavation enlarged the previously damaged area, even if modern techniques of excavation were used to prevent further disturbance to the adjacent meadow (Plate 1.7). The new trench was backfilled using calcareous rubble excavated from a nearby terrestrial quarry. As a result of dumping activities rubble backfill material formed a mound-like seascape. As a result, the whole impacted area formed a mosaic of different substrata, including calcarenitic pebbles, gravel and calcareous rubble as well as sand and dead matte. A monitoring was commissioned in 1993, aimed at mapping the area and assessing any regression of the *P. oceanica* coverage in the surrounding area.

Plate 1.7: Side on view of the trench. Dredge marks are clearly visible on the substratum.

Although the construction of the coastal landing at Capo Feto damaged about 150 ha of *P. oceanica* meadow (Plate 1.8), it represented a unique chance to observe the recovery of *P. oceanica* on a large scale. A four-year monitoring survey (see chapter 2) indicated a natural recovery process of *Posidonia oceanica* on the rubble mound field. This work started with the hypothesis that rubble mounds might be a suitable substratum for *P. oceanica* recruitment. The main goal of this PhD is to assess how the recruitment process of *P. oceanica* on the rubble mounds occurred and which environmental (biological and physical) factors played a primary role in such a process. The possible outcome of this research might provide new insight on the recruitment process of *P. oceanica* following disturbance. This would entail the possibility of employing artificial substrata to promote the recovery of lost seagrass beds in the Mediterranean Sea as well as minimising the effect of future marine operations that imply disturbances on natural seagrass communities.

Plate 1.8: Aerial photographs of the area of Capo Feto with the trench area visible within the seagrass meadow.

1.8 STUDY SITE

The study was conducted at Capo Feto, a natural reserve area located on the South-western coast of Sicily (SW Mediterranean, Italy) (Fig. 1.6). The study area extends Southwest from the Capo Feto coast to a depth of 30 m. Dominant winds are from the SE direction both during winter and summer storms, creating high wave energy conditions in the shallow seagrass beds. The dominant seagrass species in this area is *Posidonia oceanica* which forms one of the largest Mediterranean meadows (≈ 30.000 ha) growing on matte. The *P. oceanica* bed represents a portion of the continental shelf that characterises the Southeast coast of Sicily (Orrù et al. 1993). Geologically, the area is a wide calcareous plateau. The plateau was colonised by *P. oceanica* extending shoreward to the water-land interface (Toccaceli and Riggio, 1989). In areas with strong wave action, seagrass canopy traps sediment in the water column with its leaves.

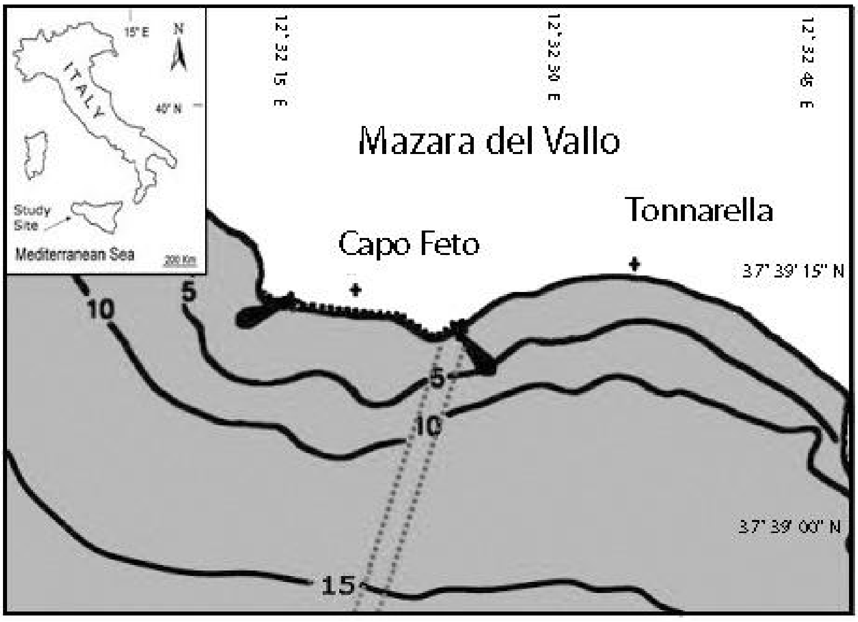


Figure 1.6: Study area at Capo Feto (impacted) and Tonnarella (control). Dotted line represents pipeline system

This process helps the “matte” to build upwards forming a strong extensive reef (*recife barrier;* Augier & Boudouresque 1970, Calvo & Fradà-Orestano 1984, Toccaceli 1990*)* along the shore (Plate 1.9). The rubble field extends from the coastline to a depth of approximately 20 m, and is 30 m wide and covers an area of about 4 ha. Due to the dumping technology used (dump barges), the rubble formed a mound-like seascape where mounds are about 1.5 ± 0.4 SD m high and 5 ± 1.1 SD m in diameter (mound crest), the distance between each mound ranges between 5 and 6 m, for a total number of about 450 mounds in the area (estimated from Side Scan Sonar data).

(Fig. 1.7, Plate 1.10, 1.11). As a reference site, sampling was carried out on the undisturbed pristine meadow stretching for miles westward from about 1 nautical mile distance from the trench located in Tonnarella (Fig. 1.6). Experimental designs adopted in this dissertation generally considered three locations on the mound field: mound crests (exposed top areas), mound sides (steep angled surfaces) and valleys between mounds (sheltered from currents).

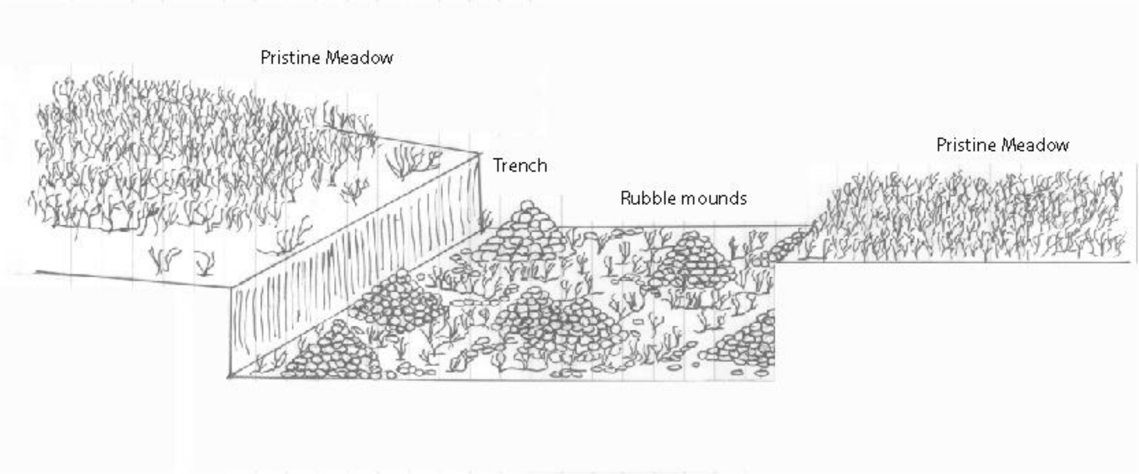


Figure 1.7: Schematic diagram of trench area with rubble mounds

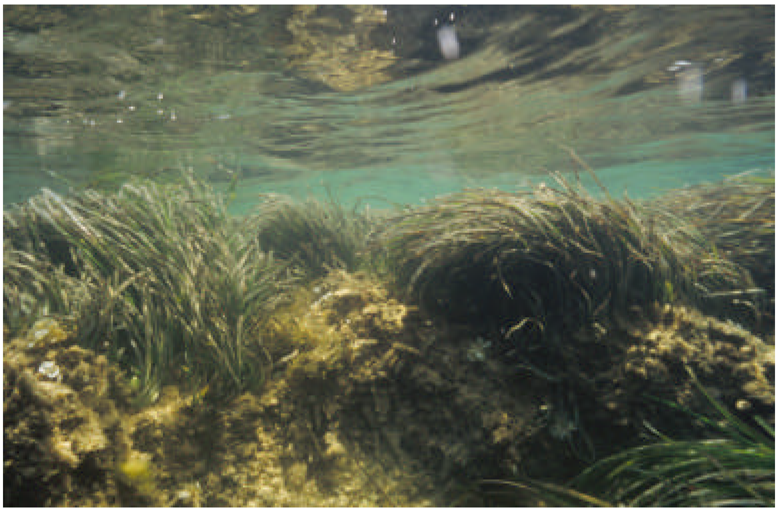


Plate 1.9: A recife barrier (barrier reef) of *P. oceanica* in the western Mediterranean area.

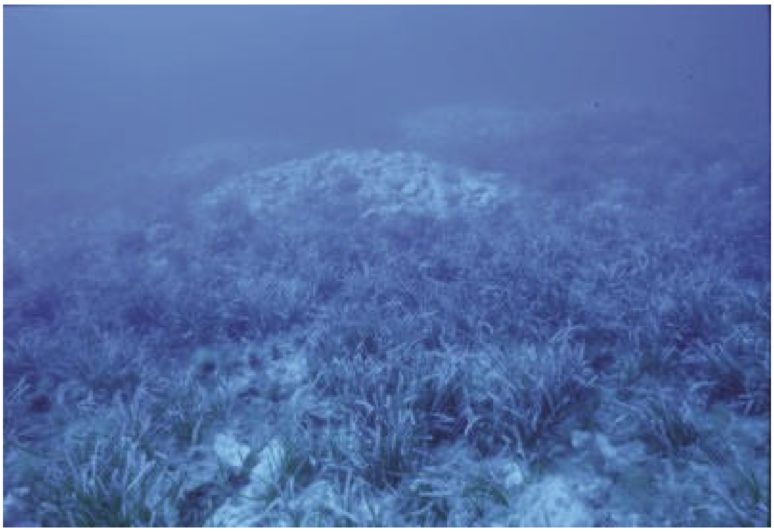


Plate 1.10: Overview of the rubble mound field at Capo Feto.

Plate 1.11: Particular of a rubble mound.

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CHAPTER II

EVALUATION OF RECOVERY DYNAMICS OF THE SEAGRASS *POSIDONIA OCEANICA* USING A REMOTELY OPERATED VEHICLE

2.1 INTRODUCTION

Worldwide, seagrasses are crucial to the structure and function of healthy coastal ecosystems (Hemminga & Duarte 2000). In the Mediterranean area, *Posidonia oceanica* is the endemic species covering about 2.5–5 million ha extending from the shore to about 40 m depth (Pergent et al. 1995). This is a slow-growing long-lived seagrass (Duarte 1991), whose vertical rhizomes may live more than 30 years (Marbà et al. 1996).

P. oceanica is considered to play an important role in coastal processes of the Mediterranean Sea. In addition, *P. oceanica* meadows form a key ecosystem component in the shallow waters of the basin, and are an important resource for fishery (e.g. Mazzella et al. 1983). Thus, their role is comparable to that of other plants in temperate and tropical seas supporting high biodiversity (e.g. Larkum et al. 1989). In shallow water areas, seagrasses are being lost at an alarming rate due to sustained impacts, such as trawl fishing, industrial and sewage outfalls, fish farms and boat anchoring (see Short and Wyllie-Echeverria 1996 for a review). These represent pulse disturbances leading to a slow but constant seagrass decline (Underwood 1994, Short and Wyllie-Echeverria 1996, Duarte 2002). Mechanical damage as well as port construction and infrastructure maintenance affect seagrass beds irreversibly. These can be considered press disturbances as they occur in single events and they do not allow seagrass recovery (Underwood 1994). Although these acute impacts damage seagrass beds, they do not affect seawater properties and they often create new environments which might allow, in the long run, seagrass recovery (Meinesz & Lefévre, 1984).

Recovery dynamics within disturbed Mediterranean seagrass communities have been documented in several studies (den Hartog 1971, Meinesz & Lefévre 1984, Guidetti & Fabiano 2000, Guidetti 2001), leading to a paradigm that describes a deterministic sequence of colonization and recovery (Hemminga & Duarte 2000) The substratum is first occupied by calcareous and rhizophytic macroalgae that may facilitate seagrass colonization through sediment stabilization, enhanced sedimentation, and by increasing nutrients in pore water (den Hartog 1971, Patriquin 1975, Zieman 1982, Williams 1990). Next, depending on the local species composition, the faster-growing seagrass *Cymodocea nodosa* continues the process of recovery from adjacent populations. In the final stages of the recovery process, the climax species *Posidonia oceanica* begins to colonize by rhizome growth from adjacent populations and, under optimum conditions, eventually displaces the other seagrass species. The degree of displacement is dependent upon environmental factors such as sediment depth, nutrient availability, and the disturbance regime, which introduces high heterogeneity at the large and intermediate scale. Seagrass meadow structure and maintenance can be explained using patch dynamics (McRoy & Lloyd, 1981, Duarte & Sand-Jensen 1990, Marbà & Duarte 1995, Vidondo et al. 1997, Kendrick et al. 1999). An heterogeneous process regulated by a synergism in clonal plants that links patch size with patch age (Vidondo et al. 1997). *Posidonia oceanica* is a large, slow-growing species with a rhizome elongation averaging around 0.07 m yr^{-1} (Caye 1980, but see Marbà & Duarte 1998) hence it forms small patches. Patch formation for *Posidonia oceanica* is reported to be

$3x10^{-4}$ ha^{-1} yr^{-1} (Meinesz & Lefevre 1984). However, the amplitude and frequency of perturbance is the main determinant of patch formation rate and the extent of spatial heterogeneity of colonising seagrasses (Duarte 1990). Changes in rhizome elongation rate may reflect density-dependent constraints as seagrass growing on bare substrata or bare sediments elongate faster than those growing in dense meadows (Patriquin 1973, Marbà & Duarte 1998). In particular, *Posidonia oceanica* is characterized by a slow growth rate and a low flowering rate (Marbà et al. 1996, Piazzi & Balestri 1997), but a self-accelerating mechanism may increase the rate of rhizome elongation, shortening recovery time scale (Hemminga & Duarte, 2000). In species that lack a seed bank, such as *Posidonia oceanica*, recruitment into the "new" patch is directly related to availability of propagules (Denslow 1985, Kenworthy 2000, 2002). Propagules, or fragments, act as dispersal units (Marbà & Duarte 1995, Campbell 2003) and recovery can occur through clonal growth (Hemminga & Duarte 2000). At Capo Feto, the large impact created by pipeline deployment between 1981 and 1993 freed a large number of *P. oceanica* fragments, which became potential colonizers of the available substrata. Thus construction of the coastal landing at Capo Feto represented a unique chance to observe a potential natural recovery of *P. oceanica* on a large scale.

In this chapter, changes in *P. oceanica* leaf areal coverage from 1993 to 1999 on the different substrata present within the trench area are reported. As *P. oceanica* is capable of colonising both sandy and rocky seabed, potential recolonisation was hypothesised over all types of substrata. In addition, as water transparency was reduced due to trenching operations (Badalamenti et al. 2004), seagrass percent cover was assumed to increase faster in shallow water than in deeper water.

2.2 MATERIALS AND METHODS

Side Scan Sonar and ROV data analysed and discussed in this chapter were collected during Pipeline Surveys and they are property of SNAMPROGETTI. I have obtained permission to use these data for the purpose of this dissertation.’

2.2.1 Site Mapping

Side scan sonar (SSS) surveys were carried out after the installation of the first (1981) and second (1993) pipelines. Data were collated from these SSS maps as well as Remotely Operated Vehicle (ROV) surveys. This produced two maps (Fig. 2.1 & 2.2) which illustrate the damage caused by pipeline construction on the seagrass meadow. Georeferentiation of the maps, using a GIS software (Manifold 5, CDA), allowed to identify four substrata: sand (0.063 and 2 mm), dead matte, calcarenitic boulders (Ø ranging between ~ 200 mm and 2000 mm) and calcareous rubble (Ø ~ 200 mm).

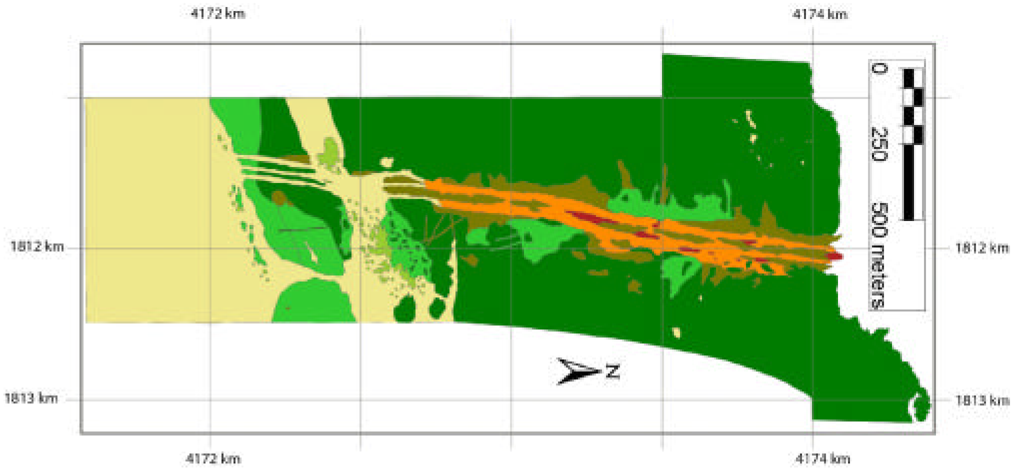

Figure 2.1: Map of the trenching area produced by mean of Side Scan Sonar showing impact caused on the meadow by construction activities in 1981

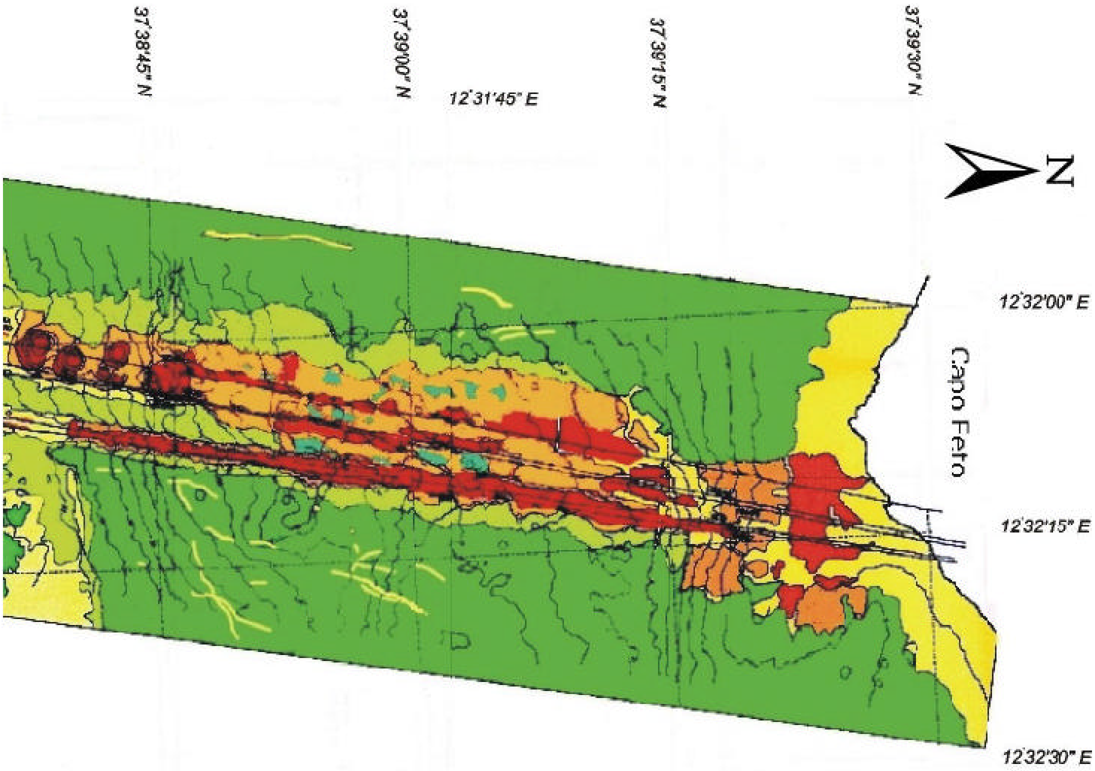

Figure 2.2: Map of the trenching area produced by mean of Side Scan Sonar showing impact caused by construction activities in 1993

2.2.2 Leaf area coverage

Trench surveys were carried out using a ROV (Hyball) (Plate 2.1). The ROV was linked to a cable that provided images to a monitor and VCR for image recording on board a research vessel. This method provided seagrass leaf area coverage data (% of seabed covered) that could not have been collected by scientific divers (Rumohr 1995). In addition, video transects avoided the problem of image separation presented by still photography, while allowing for an immediate assessment of sample quality (Malatesta et al. 1997). *P. oceanica* percent cover was assessed for each substratum using ROV transects parallel to the coastline. To allow randomization, the UTM referenced SSS maps of the study site was divided into transects (5 m wide and 600 m long), from which 70 transects were randomly chosen for each depth range: shallow (5-15 m) and in deep (16-25 m). ROV surveys were carried out immediately after backfilling operations were completed (November/December 2003) and then yearly from 1994 to 1999 always in the fall. Every year, two interspersed replicated transects were independently chosen per each combination of depth and substratum. Per each transect, six ROV video frames (4 m^2 quadrats) were chosen at random and the seagrass leaf areal coverage (% cover) was recorded.

For each frame, coordinates, ROV direction, depth and *P. oceanica* leaf area coverage were recorded. As the video camera was set at a fixed angle, video frame dimensions were calculated according to Bourgoin et al. (1985):

$$W = 2H \frac{1}{\sin \alpha} \tan \frac{\beta}{2}$$

where W is the width, H the height of the camera above the seabed, α is the camera angle from the horizontal plane and β is the lens angle. The formula provided the estimation that a single video frame or station covered an area of 4 m^2. Data were then recalculated and reported in percent cover per m^2.

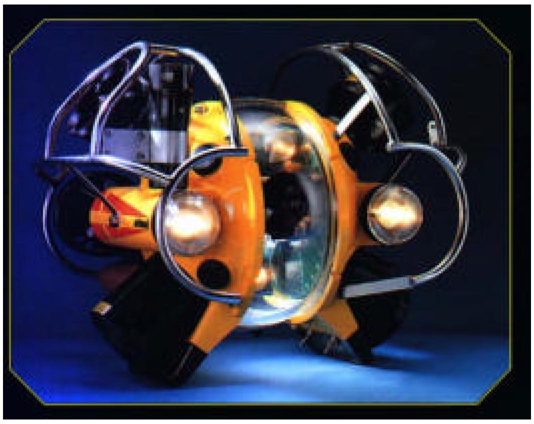

Plate 2.1: The Remotely operated vehicle ‘Hyball’ employed to collect video frames across the impacted area.

2.2.3 Data Analysis

Data sets were analysed using Analysis of Variance (ANOVA). Leaf area coverage variation between substrata was tested with a four-way ANOVA where treatment factors (and levels) were: (1) year (6 levels), (2) depth (2 levels), (3) substratum (4 levels) and (4) site (2 levels). Year, depth and substratum (fixed) were considered as fixed and orthogonal, while site was taken as random and nested in substratum.

Variations in percent cover over time on rubble were tested with a four-way ANOVA with the following design: year (6 levels), (2) depth (2 levels), location (2 levels), (3) site (2 levels). The factors year, depth and location were considered as fixed and orthogonal, site was taken as random and nested in location. Analysis of variance (ANOVA) was carried out using GMAV5 (University of Sidney, Australia) after checking the homogeneity of variance with Cochran's test (Snedecor & Cochran 1989). When significant differences were found in the ANOVA, they were compared *a posteriori* using a Student-Newman-Keuls (SNK) test (Underwood 1981, Underwood 1997).

2.3 RESULTS

2.3.1 Site Mapping

SSS maps show no recolonisation of *P. oceanica* in the damaged area between 1981 and 1993 (Fig. 2.1 & 2.2). Based on the geo-referenced SSS maps, four main substrata within the trench were identified: calcarenitic boulders covering 51% (or 9.08 ha) of the area, sand and rubble covering respectively 23% (4.21 ha) and 17% (3.06 ha) of the whole trenching area (Fig. 2.3) and dead matte accounting for 9% (or 1.68 ha) of the substrata present. The four substrata sampled covered a total area of 18.04 ha.

2.3.1 Leaf Areal Coverage

The results reported a significant variation in seagrass leaf area coverage between substrata (SNK Test, Table 2.2a). Percent cover remained equal to 0 % m^{-2} on sand, dead matte and calcarenitic boulders through all the years and at both depth ranges, whilst it increased on the rubble substratum (Fig. 2.4). No significant difference in this pattern between depths was found (Table 2.1).

The four-way ANOVA showed a significant interaction between year, depth and location (SNK test, Table 2.2). Leaf area coverage at the control site averaged at 98.5±0.1 % m^{-2} and did not vary significantly over time at both depths (SNK Test, Table 2.2c). Moreover, the pristine meadow always showed a significantly higher leaf area coverage than the rubble substratum (SNK Test, Table 2.2a). On rubble, *P. oceanica* percent cover significantly varied between 1993 and 1999 at both depth ranges (SNK

Test, Table 2.2b). Percent cover increased significantly from 0 % m^{-2} to 0.77±0.03 % m^{-2} between 1993 and 1994 in the shallow water (5-15 m). Similarly, in deep water (16-30 m), coverage increased significantly from 0 % m^{-2} to 1.22±0.07 % m^{-2} (Fig. 2.5). In 1995, *P. oceanica* percent cover in shallow water reached 2.10±1.12 % m^{-2} a value not significantly different from the average coverage recorded in the previous year. In contrast, the deep water percentage cover increased significantly between 1994 and 1995 (SNK Test, Table 2.2a). A significant increase between 1995 and 1997 was present at both depth ranges (SNK Test, Table 2.2a). In shallow water, percent cover almost doubled (3.95±0.12 % m^{-2} in deep water), whilst in the deep water range, seagrass coverage reached 4.47±1.14 % m^{-2}. Variation in coverage between 1997 and 1998 was not found to be significantly different at both depths (SNK Test, Table 2.2a). *P. oceanica* leaf area coverage in shallow water increased significantly between 1998 and 1999, when it ranged between 8.75 and 15 % m^{-2} (Fig. 2.5). At 16-30 m depth range coverage also increased significantly between 1998 and 1999, when coverage averaged 7.29±0.13 % m^{-2} (Fig. 2.5).

Differences between depths of seagrass percent cover on rubble were only found in 1999, leaf area coverage was found to be higher in the 5-15 m depth range (10.5±0.1 % m^{-2}) than in the 16-30 m depth range (7.2±0.1 % m^{-2}) (SNK test, Table 2.2c).

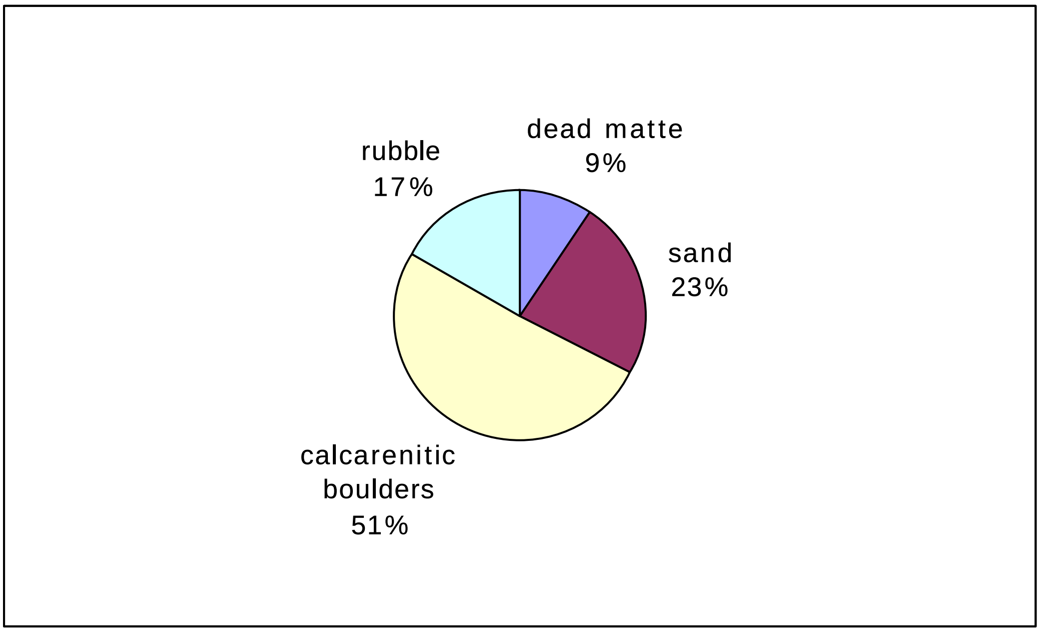

Figure 2.3: Percentage of the total area covered by each substratum

Source of Variation	Percent Cover Variation over time and substratum type			
	df	**MS**	**F**	
Year (ye)	5	64.69	111.41***	
Depth (de)	1	1.06	1.83^{ns}	
Substratum (su)	3	454.67	783.07***	
Site(yeXdeXsu)	48	0.58	1.29^{ns}	
yeXde	5	3.20	5.51***	
yeXsu	15	64.69	111.41***	
deXsu	3	1.06	1.83^{ns}	
yeXdeXsu	15	3.20	5.51***	
RES	480	0.45		
TOT	575			
Cochran's Test	C = 0.1400 (P < 0.01)			
SNK test				
Interaction YeXDeXSu				
(a) su(yeXde)	5-15 m	16-30 m		
1993	S=DM=CB>R	S=DM=CB>R		
1994	S=DM=CB>R	S=DM=CB>R		
1995	S=DM=CB>R	S=DM=CB>R		
1997	S=DM=CB>R	S=DM=CB>R		
1998	S=DM=CB>R	S=DM=CB>R		
1999	S=DM=CB>R	S=DM=CB>R		
(b) ye(deXsu)	R	S	DM	CB
5-15 m	93<94<95<97<98<99	ns	ns	ns
16-30 m	93<94<95<97<98<99	ns	ns	ns
(c) de(yeXsu)	R	S	DM	CB
1993	ns	ns	ns	ns
1994	ns	ns	ns	ns
1995	16-30<5-15	ns	ns	ns
1997	5-15<16-30	ns	ns	ns
1998	ns	ns	ns	ns
1999	16-30<5-15	ns	ns	ns

Table 2.1: Four-way ANOVA of leaf areal coverage variation from 1993 to 1999 on four different substrata at two depth ranges (5-15 and 16-30 m). R = rubble, S = sand, DM = dead matte, CB = calcarenitic boulders, ***= $p<0.001$; ** = $p<0.01$; ns = not significant; SNK = Student-Newman-Keuls test.

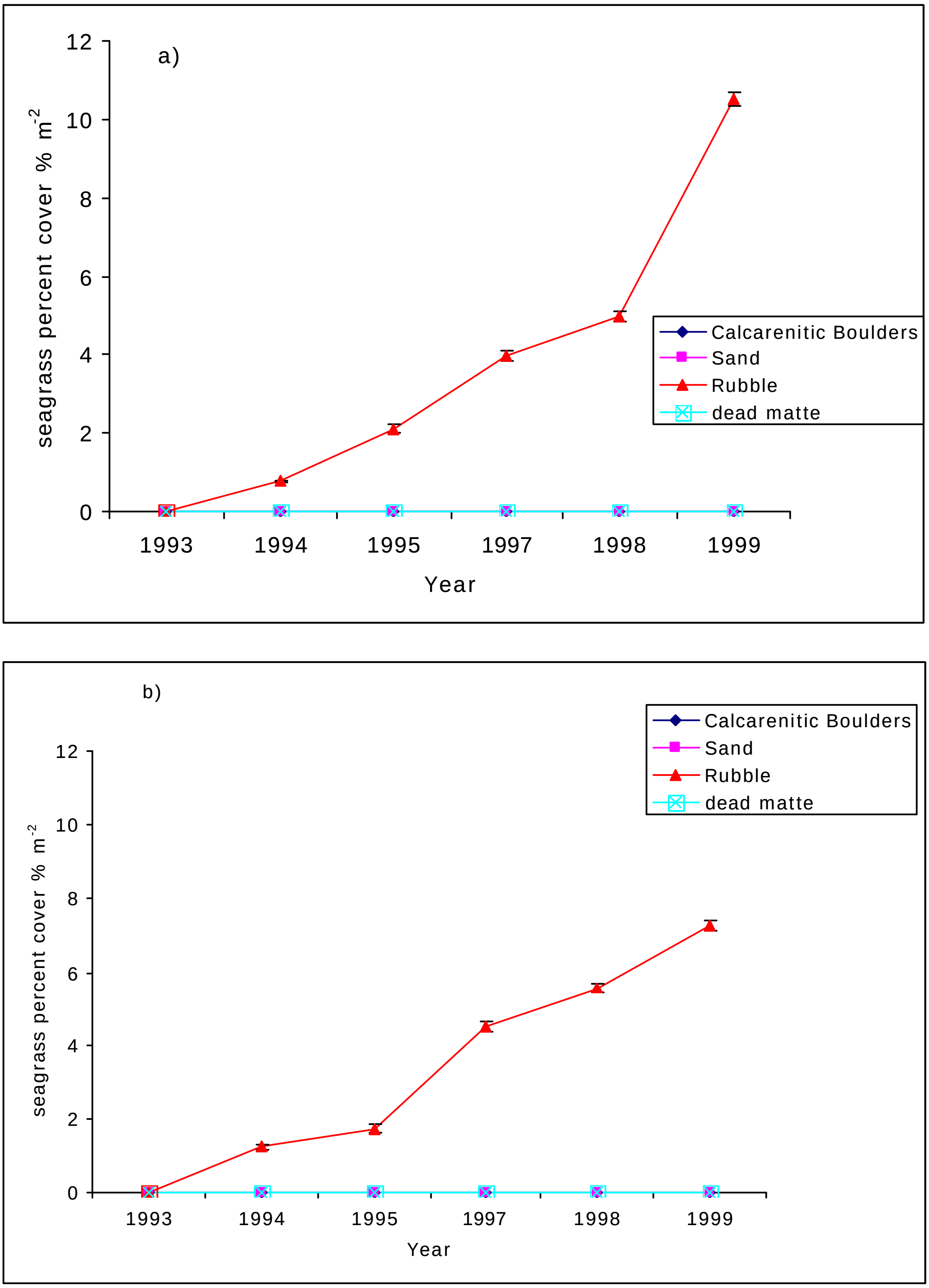

Figure 2.4: Percent Cover variation of *P. oceanica* from 1993 to 1999 over four substrata at (a) 5-15 m depth & (b) 16-30 m depth. N = 6; bars equal SE.

Source of Variation	Percent Cover Variation		
	df	**MS**	**F**
Year (ye)	5	7.24	110.05***
Depth (de)	1	0.06	2.26^{ns}
Location (lo)	1	4670.07	17851.56***
Site(lo)	2	0.26	7.19***
yeXde	5	0.20	4.11**
yeXlo	5	7.24	110.05***
yeXsi(lo)	10	0.07	1.81^{ns}
deXlo	1	0.06	2.26^{ns}
deXsi(lo)	2	0.03	0.72^{ns}
yeXdeXlo	5	0.20	4.11**
yeXdeXsi(lo)	10	0.05	1.31^{ns}
RES	240	0.04	
TOT	287		
Cochran's Test	C = 0.0938 (ns)		
SNK test			
Interaction YeXDeXLo			
(a) lo(yeXde)	5-15 m	16-30 m	
1993	R<C	R<C	
1994	R<C	R<C	
1995	R<C	R<C	
1997	R<C	R<C	
1998	R<C	R<C	
1999	R<C	R<C	
(b) ye(deXlo)	Rubble	Control	
5-15 m	93<94=95<97<98<99		
16-30 m	93<94<95<97=98<99		
(c) de(yeXlo)	Rubble	Control	
1993	ns	ns	
1994	ns	ns	
1995	ns	ns	
1997	ns	ns	
1998	ns	ns	
1999	16-30<5-15	ns	

Table 2.2: Four-way ANOVA of leaf areal coverage variation from 1993 to 1999 on rubble bed and control site at two depth ranges (5-15 and 16-30 m) . R = rubble, C = control, ***= $p<0.001$; ** = $p<0.01$; ns = not significant; SNK = Student-Newman-Keuls test.

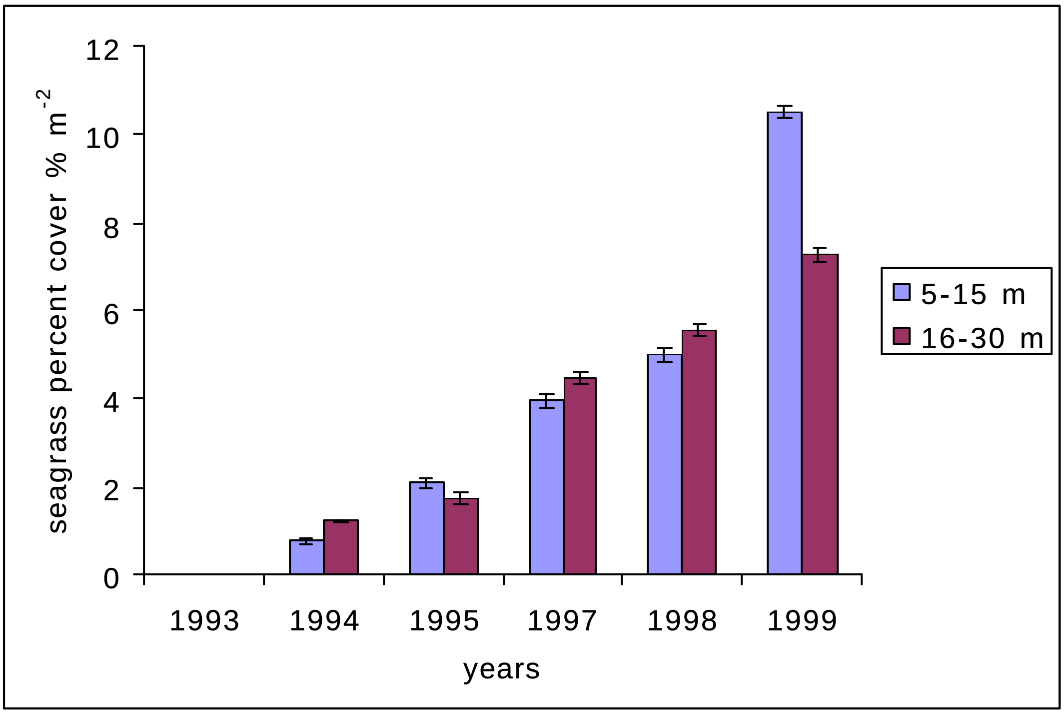

Figure 2.5: *Posidonia oceanica* percent cover changes on the rubble mounds from 1993 to 1999, both in shallow (5-15 m) and deep water (16-30 m). N = 6, bars equal SE. Control not shown as no significant variation from mean (98.5 ± 0.1 % m^{-2}) was found over time.

2.4 DISCUSSION

Several programmes have attempted to restore lost *Posidonia oceanica* beds (Cinelli 1980, Meinesz et al. 1992). Such initiatives have often considered the role of environmental parameters (i.e temperature) on the success of transplants. Moreover, attention has been focused on the percent survival success of seedlings vs vegetative fragments. However, this is the first study to report on a natural recolonisation process of *P. oceanica* by mean of vegetative fragmentation.

The results of this study indicate the importance of substratum type in the recruitment of *P. oceanica* fragments, in this case following the installation of a pipeline. Rubble mounds appear to be the only suitable substrate for recolonisation at this site. The choice of rubble as backfill material (in 1993), allowed sediments to settle and accumulate so increasing post-impact water transparency. Moreover, rubble is fairly motionless, even in harsh hydrodynamic conditions (e.g. at Capo Feto), and also does not degrade with time, like calcarenitic material. All these features lead to the conclusion that rubble provides an environment which favours settlement and survival of newly arrived plants. In contrast, sand and calcarenitic boulders might be considered as heterogeneous (composed of a mixture of minerals) and not provide the necessary stability for *P. oceanica* propagules the same way as they are unsuitable substrate for macroalgae. On rubble, *P. oceanica* leaf area coverage per m^2 increased almost linearly from 1993 to 1999. Recovery was found to be faster (10.5±0.1%) in shallow water than in the deeper areas (7.2±0.1%) possibly due to higher light availability than in the deeper areas. Previous work reported on the importance of substratum type in the colonisation process of *P. oceanica* (Molenaar & Meinesz 1995, Balestri et al. 1998). Even if *P. oceanica* is more commonly found on soft substrata (Molinier & Picard 1952), this species is also able to colonise hard substrata by clonal growth (Marbà & Duarte 1994, 1995). which suggests that it may be more suited for the colonisation of new rocky seabed areas than conventionally thought (D'Anna et al. 2000). Vegetative development is often considered to be the main mechanism of seagrass proliferation (Duarte & Sand Jensen 1990, Marbà & Duarte 1998). This mechanism for colonisation represents a way to overcome environmental changes and to adapt or colonise contrasting environments (Marbà & Duarte 1998). As stated by Marbà & Duarte (1998) changes in rhizome elongation rate may reflect density dependent constraints. Seagrass growing on bare substrata or bare sediments elongate faster than those growing in dense meadows. This represent quite well the case of *P. oceanica* at Capo Feto. As the newly available rubble substratum represents a bare patch, there is neither intraspecific nor interspecific (with *Cymodocea nodosa*) competition for space. This case study of Capo Feto could also be adopted to elucidate the mechanism of ecological succession in Mediterranean seagrass communities. The proposed successional sequence for the Mediterranean Sea proceeds from colonisation by *Cymodocea nodosa* to dominance of *P. oceanica* (den Hartog, 1970; Hemminga & Duarte 2000), considered a climax species. However, seagrass successional sequences do not represent unidirectional paths of change, as they might be reversed or short-circuited by disturbance (den Hartog 1970, Pérès 1982, Hemminga & Duarte 2000).

Seagrass present in the area of Capo Feto was partially removed due to trenching operations, but seeds and propagules remained and seawater physical-chemical properties were not altered, hence this could be considered as an autogenic secondary succession (Sousa 1979a, 1979b, Begon et al. 1996). Plant response to disturbance is believed to depend on the link between the time scale of the disturbance and the life history patterns of the species available to reoccupy the disturbed site (Sousa 1984, Duarte 1991). Disturbance is, depending on its amplitude and frequency, a source of patchiness in plant communities (Sousa 1984, Duarte 1991, Collins 1992, Cipollini 1994). Once disturbance has cleared the seabed at Capo Feto *P. oceanica* propagules found new space for colonisation. As disturbance occurred in one single event (channel dredging), the plants have been able to slowly recover in a stable environment at an increasing colonisation rate, which however appears to be driven by some physical and biological processes. As *P. oceanica* is a large, slow growing seagrass species with a rhizome elongation averaging around 0.07 m yr^{-1} (Caye, 1980, but see Marbà and Duarte, 1998), it forms small patches. It has been calculated that the patch forming rate for *P. oceanica* is $3x10^{-4}$ ha^{-1} yr^{-1} (Meinesz and Lefévre, 1984).

In conclusion, the substratum and sediment stability appear to play a major role in seagrass recolonisation. Careful consideration should be taken when attempting the re-establishment of an area in order to favour seagrass recruitment after a human-induced disturbance. Rubble intermixed with finer particles seem to meet the necessary requirements for *P. oceanica* vegetative recruitment. Even if *P. oceanica* is a large, slow growing species with recovery times that generally exceed a century (Meinesz and Lefevre, 1984), a self-accelerating mechanism may increase the rate of rhizome elongation, shortening the time scale for recovery. This recovery is linked to the formation of patches, allowing the plant to colonise new available space in a short period of time. The heterogeneous pattern found in the colonisation process of *P. oceanica* probably reflects the heterogeneity of the environment, revealing a link between hydrodynamic activity and landscape features of the seagrass bed (Fonseca & Bell 1998, Kendrick et al. 1999). Finally, the postulated successional sequence for Mediterranean seagrasses (*Cymodocea* → *Posidonia*, Hemminga & Duarte 2000) does not appear to have happened at Capo Feto, and it should be understood that such sequences generally represent an idealised progression in the absence of disturbance.

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CHAPTER III

COLONIZATION PROCESS OF VEGETATIVE FRAGMENTS OF *POSIDONIA OCEANICA* ON RUBBLE MOUNDS.

3.1 INTRODUCTION

Seagrasses are capable of colonizing both unconsolidated (soft) and consolidated (hard) substrata and tend to expand into natural or disturbed unvegetated areas (Marbà & Duarte 1998, Kenworthy et al. 2002). When a disturbance occurs, the rate and pattern of seagrass recruitment into the “new” patch is directly related to the availability of propagules (Denslow, 1985, Kenworthy et al. 2002). Species that have a seed bank, such as *Halophila decipiens* and *Cymodocea nodosa* (Rasheed 1999, Campbell 2003), are capable of rapid recruitment and establishment in a disturbed area if conditions for growth are suitable. In contrast, previous work (Rasheed 1999, 2004) reported on the ability of species that lack a seed bank, such as P*osidonia australis*, to recruit through vegetative fragments which act as dispersal units (Campbell 2003). However, direct evidence are still lacking. Once fragments are recruited, seagrass recovery can occur through clonal growth (Hemminga & Duarte 2000). Seagrass recruitment after disturbance may not be only a function of the availability of fragments but also of the type of substratum available for colonisation. Substratum is considered to be an essential factor for plant growth and settlement (Koch 2001, Long & Thom 2001) and it has been found to affect the outcome of *P. oceanica* transplantation experiments (Moleenar & Meinesz 1995).

Posidonia oceanica, the dominant seagrass species in the Mediterranean Sea (Gacia & Duarte 2001), is in widespread decline (Marbà et al. 1996) due to the exploitation of coastal waters, in particular by fisheries, aquaculture and tourism (Delgado 1999, Francour et al. 1999, Ruiz et al. 2001). *Posidonia* meadows are now a national and international conservation priority (Green & Short 2003). Restoration initiatives have included both seed culture (Piazzi & Balestri 1997, Balestri et al. 1998)and the utilisation of vegetative recruits from donor beds (Moleenar & Meinesz 1995). However, the rate of success of *P. oceanica* transplantation programs is, to date, fairly low (Balestri et al. 1998). In addition, there is no evidence in the literature of natural recovery of *P. oceanica* following disturbance, with the exception of recovery after bomb blasting (Meinesz & Lefèvre 1984).

In recent years there has been an increase in the development and construction of coastal infrastructures, such as the installation of pipelines (for oil and gas) (Duarte 2002). Such acute impacts entail the removal of seagrass meadows and they modify the characteristics of the substratum and sedimentary processes. However, dredging operations do not alter seawater properties in the long term and the infill may provide new environments which might allow for partial or full seagrass recovery.

At Capo Feto, (SW Sicily, Italy) a monospecific *P. oceanica* meadow was severely damaged by a dredge and fill operation during the construction of a gas pipeline in 1993. A large portion (≈70 ha) of the seagrass meadow was mechanically removed in the process. After deployment, the pipeline trench was backfilled with calcareous rubble. Although *P. oceanica* is a large, slow-growing species with recovery times that generally exceed a century (Meinesz & Lefèvre, 1984), in the previous chapter I reported on how the large availability of fragments from the adjacent meadow favoured natural recolonisation by *P. oceanica* in a rubble substratum in a matter of 9 years, whilst no recovery was observed on other unconsolidated substrata (sand and gravel). D'Anna et al. (2000)

suggested how fragments of *P. oceanica* may detach from the meadow and drift with bottom currents, becoming lodged and between small rubble at the bottom of artificial reefs (Riggio 1995). This *P. oceanica* recruitment process might be also linked to hydrodynamics and sedimentary requirements. For other large-bodied species it has been suggested that these requirements be currents less than 10 cm s^{-1}, but possibly as high as 100cm s^{-1} (Koch 2001, Dierssen et al. 2003), and at least 7 cm of unconsolidated sediment above bedrock (Zieman 1972).

This chapter reports on the natural recruitment patterns of vegetative fragments of *P. oceanica* on a artificial rubble substratum. I hypothesised that, once recruited on rubble, fragments are not dislodged and that the successful survival of recruited fragments depends on appropriate conditions of water flow, sediment deposition and sediment thickness they produce a stable seagrass establishment on the rubble field.

3.2 MATERIALS AND METHODS

3.2.1 Study Site

The locations identified for the experimental design of this study were: rubble mound crests (exposed top areas) (Plate 3.1), mound sides (steep angled surfaces) and valleys between mounds (sheltered from currents) and one or two reference locations chosen on the undisturbed meadow stretching westward about 500 m distance from the trench (Plate 3.2).

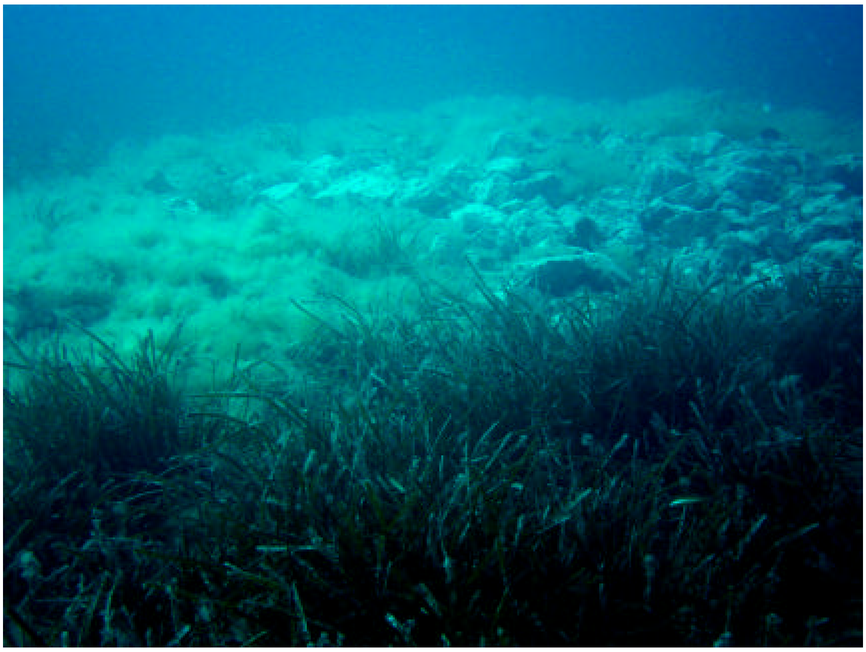

Plate 3.1: Close-up photo of a mound crest

Plate 3.2: Close up photo of a mound valley

3.2.2 Seagrass Shoot Density and Percent Cover

Posidonia oceanica shoot density on different locations of the mounds (valleys, sides and crests) at 5, 10, 15 m depth was recorded using 0.16 m^2 quadrates. Samples were always collected in the same month (June) over a three year period (2001 to 2003). Three sites were sampled for each depth with 6 replicate quadrates each. Two control stations at each depth were chosen randomly on the undisturbed pristine meadow growing on matte in Tonnarella (see Fig. 1.6). Each control station included three sites with 6 replicates each and was sampled at the same time as the mounds. A similar experimental design was adopted for the assessment of *P. oceanica* percentage cover over different locations of the mounds at 5, 10, 15 m depth. Sampling was carried out over a 3-year period at the same time as shoot density. Percentage cover was estimated using 4 m^2 PVC quadrates (Plate 3.3). Instead of six replicates, four randomly chosen replicates were taken. Two control stations were taken as reference on the undisturbed bed in the locality of Tonnarella.

Plate 3.3: PVC quadrates used to assess seagrass percent cover changes at different depths and locations.

3.2.3 Persistence of propagules over time

To test whether vegetative fragments were dislodged by water motion after entanglement, recruited *P. oceanica* fragments were marked. Fragment recounts were carried out every six months, from June 2001 to June 2003.

More than 20 sites were selected for each of the three locations in the rubble field (crest, side and valley) and two reference locations both at 5 and 10 m (±2 m SD) depth. At each site, six fragments were marked by superimposing a 4 m^2 grid (divided into 100 squares) and by extracting random numbers. Every six months, a survey re-counted fragments present on 3 randomly selected sites per each location.

3.2.4 Water Flow

Although several criticisms have been made regarding the application of gypsum devices in the quantification of current speed (Porter et al. 2000, Koch & Verduin 2001), this method can be used to estimate flow intensity in natural environments (Porter et al. 2000). However, a series of checks and calibrations are needed (see Porter et al. 2000 for a review).

Gypsum spheres (diameter 3 cm) were prepared using Plaster of Paris following the method described in Muus (1968) and Doty (1971). The gypsum was mixed with deionised water in a ratio of 2:1 as detailed in Gambi et al. (1989). Dissolution rate was measured at 5 and 10 m (±2 m SD) depth. For each depth, 6 balls were randomly placed in each location (crest, side, valley and reference sites) approximately 20 cm above the substratum (Plate 3.5). Plaster balls were left in the field for 24h as established by preliminary trials. As weather conditions did not allow winter sampling, this measure of water motion was only recorded monthly over a period of six months (May to October 2003). After retrieval, plaster balls were dried and reweighed and dissolution rates established. In order to calibrate the plaster balls, a calibration coefficient was determined using a set of six plaster balls placed in a 500 l seawater aquarium filled with still-water at the same temperature and salinity as the study site. In order to reduce the high variability of still-water calibrations (Thompson & Glenn

1994), a large volume of water was used and gypsum devices were kept at mid water level. The diffusion index factor (DF) was determined by using the equation described in Doty (1971).

Although criticisms have been made regarding the application of gypsum devices in the quantification of water flow (Porter et al. 2000, Koch & Verduin 2001), this method can be used to estimate relative flow intensity if a series of checks and calibrations are conducted (see Porter et al. 2000 for a review).

Plate 3.4: Plaster balls were prepared by inserting the plaster mixed with water into ice cube moulds.

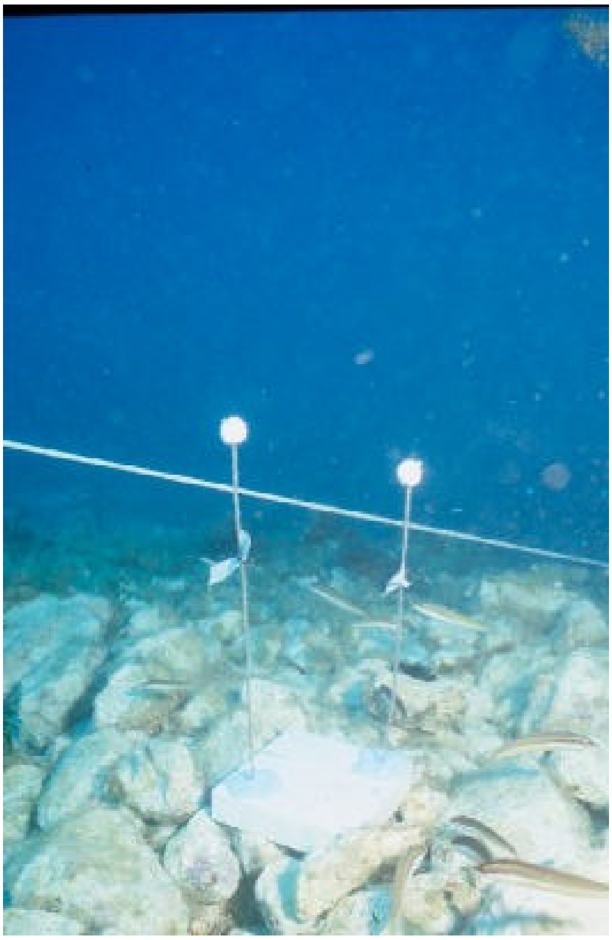

Plate 3.5: Plaster balls were deployed and left for a 24h period in the water.

3.2.5 Sediment thickness

Rubble mound sediment thickness was measured by inserting a thin metric calibrated metal rod (Ø 3 mm) into the sediment until it hit the seabed. Sediment depth was measured in July 2003 at three depths (5± 2, 10± 2 and 15 ± 2 SD m). Within each depth, 3 sites were randomly chosen applying the same randomisation technique as for shoot density. At each site, six independent measurements were taken at each location (crests, sides and valleys). A comparison with a reference site was not possible as *P. oceanica* grows on matte, which does not allow for the accurate quantification of sediment depth.

3.2.6 Data analysis

Data sets were analysed using analysis of variance (ANOVA). Shoot density data and percent cover were tested using a four-way ANOVA with the following design: year (3 levels: 2001, 2002 and 2003), depth (3 levels: 5, 10 and 15 m), location (5 levels: crest, side, valley and controls 1, 2) and site (3 levels). The first three factors were considered as fixed and orthogonal, while the sites where taken as random and nested (in the interaction between location and depth). Persistence of fragments over time was analysed with a three-way ANOVA where factors were: time (5 levels: 0, 6, 12, 18, 24 month), factor 2 was depth (2 levels: 5 and 10 m) and factor 3 was location (5 levels: crest, side, valley and controls 1, 2). All factors were taken as fixed and orthogonal. Water motion data were analysed using a three-way ANOVA, where factor 1 was time (6 levels: May, June, July, August, September, October), factor 2 was depth (2 levels: 5 and 10 m) and factor 3 was location (4 level: crest, side, valley and control). Data from only one control were included in the statistical analysis as gypsum devices at the other control location were lost in the field. All factors were taken as fixed and orthogonal. Finally, sediment thickness data were analysed using a three-way ANOVA, where factors were: depth (3 levels: 5, 10 and 15 m) location (3 levels: crest, side and valley) and site (3 levels). The first two factors were considered as fixed and orthogonal, while sites were considered random and nested (in the interaction between location and depth). Homogeneity of variance was checked using Cochran's test (Snedecor & Cochran 1989). Data were transformed using Ln(X+1) where necessary (Underwood 1997). When significant differences were found in the ANOVA, they were compared *a posteriori* using a Student-Newman-Keuls (SNK) test (Underwood 1981, 1997).

3.3 RESULTS

3.3.1 Seagrass Shoot Density and Percent Cover

Posidonia oceanica shoot density was higher at the pristine meadow where values remained constant over the years (control 1 and control 2 respectively averaged at 428.37±63.6 and 429.5±62.3 shoot m^{-2}) than at the rubble mound field (93.1±4.6 shoot m^{-2}) (Fig. 3.1). In the rubble mound field, shoot density was higher in the valleys (283.8±10.1 shoot m^{-2}) than in the two other locations (crest = 7.5±0.8 and side = 50.2±2.9 shoot m^{-2}). A depth gradient, with higher densities in shallow water (5 m), was present in control sites and valleys but not on rubble sides and crests (Fig. 3.1).

The four-way ANOVA showed a significant interaction between year, depth and location (Table 3.1). Shoot density significantly varied between locations at all depths over the three year sampling period (SNK test, Table 3.1a). From June 2001 to June 2003, shoot density in valleys was always significantly higher than on mound sides and crests (SNK test, Table 3.1a). At 5 and 15 m depth no significant differences were reported between crests and sides and between valleys and controls. On the other hand, at 10 m depth, crests had significantly lower density values than sides, which in turn had lower values than valleys and controls (SNK test, Table 3.1a).

A depth gradient in shoot density was only evident at valleys and controls, with density values lower at 15 m than at the other two depths (SNK test, Table 3.1b). Crests showed similar values at all depths and sides presented only one significant difference in 2001 where shoot density was higher at 10 m than at 5 and 15 m depth. Shoot density increased over the years in valleys (SNK test, Table 3.1c). In 2001, mean shoot density for valleys reached a value of 169±2.5 shoots m^{-2} (Fig. 3.1a), a value similar to that of the following year (170.1±2.2 shoots m^{-2}) (Fig. 3.1b). However, in 2003, a significant higher density was observed with an average value of 329±27.6 shoots m^{-2} (Fig. 3.1c). Such a trend was constant at all depths. The high heterogeneity of the environment is confirmed by a significant difference in density between sites (Table 3.1).

P. oceanica percent cover showed a similar trend to shoot density. Constant values over time were recorded for control sites (control 1 = 89±2.9% and control 2 = 90±2.9%) which were higher than the average value on rubble mounds (16.5±2.6%, Fig. 3.2). On rubble mounds percent cover decreased from valleys (37.8±6%) to crests (4±0.6%) with intermediate values found on sides (7.4±1.2%) (Fig. 3.2). A non significant difference was found between depths (Fig. 3.2). The four-way ANOVA reported a significant interaction between year, depth and location (Table 3.2). Percent cover varied significantly among locations at all depths from June 2001 to June 2003. Comparatively higher values were found in valleys than on crests and sides (SNK test, Table 3.2a). At 5 m depth, no significant difference was present between crests and sides in the last sampling event (SNK test, Table 3.2a). At 10 m depth the same finding was reported both in 2001 and 2003 (SNK test, Table 3.2a). A depth gradient was only evident between crests and valleys (SNK test, Table 3.2b). On crests, higher values were found in 2001 at 10 m depth (5.8±1.8%) than at 5 and 15 m where averages were respectively 2.6±0.9 and 2.6±0.3 % (Fig. 3.2a). The following year (2002) a depth gradient was still present on crests with decreasing percent cover from 5 to 15 m depth (SNK test, Table 3.2b). At this location, values for June 2003 were found to be higher at 5 and 10 m (respectively 10.1±0.5 and 6.8±0.2%) (Fig. 3.2c). Finally, valleys showed a significant difference in depth in 2001 with higher values at 10 m depth than at 15 m and 5 m (SNK test, Table 3.2b). Percent cover varied significantly between years on the rubble bed while it remained constant at control sites. Seagrass coverage significantly increased from 2001 to 2003 even if a high heterogeneity was found in these data as reported in Table 3.2c.

Table 3.1: Four-way ANOVA of *Posidonia oceanica* shoot density on rubble mounds at 5, 10 and 15 m depth at different locations (crests, sides, valleys, controls) over a 3-year period (2001, 2002, 2003). Ye = year, De = depth, Lo = location, Si = site.*** = p<0.001; ** = p<0.01; ns = not significant; SNK = Student-Newman-Keuls test; C = crest, S = side, V = valley, CT = Control....15

Source of variation	*Posidonia oceanica* shoot density		
	DF	**MS**	**F**
Location (lo)	4	6545019.6	692.49***
Year (ye)	2	120173.0	7.65**
Depth (de)	2	1086416.6	59.74***
Site(de)	6	18186.8	5.1***
loXye	8	120850.2	9.94***
loXde	8	163967.0	17.35***
loXsi(de)	24	9451.4	2.65***
yeXde	4	9247.5	$0.59^{\text{n.s.}}$
yeXsi(de)	12	15715.6	4.41***
loXyeXde	16	23559.5	1.94***
loXyeXsi(de)	48	12152.3	3.41***
RES	675	3566.4	
TOT	809		
Cochran's Test	C = 0.0358 (n.s.)		
SNK Test			
Location	CREST<SIDE<VALLEY<CTRL1=CTRL 2		
Year	2001 = 2002 < 2003		
Depth	15 < 10 = 5		
		Location	
Year			
2001	CREST=SIDE<VALLEY<CTRL1=CTRL 2		
2002	CREST=SIDE<VALLEY<CTRL1=CTRL 2		
2003	CREST=SIDE<VALLEY<CTRL1=CTRL 2		
Depth			
5	CREST=SIDE<VALLEY<CTRL1=CTRL 2		
10	CREST<SIDE<VALLEY<CTRL1=CTRL 2		
15	CREST=SIDE<VALLEY<CTRL1=CTRL 2		

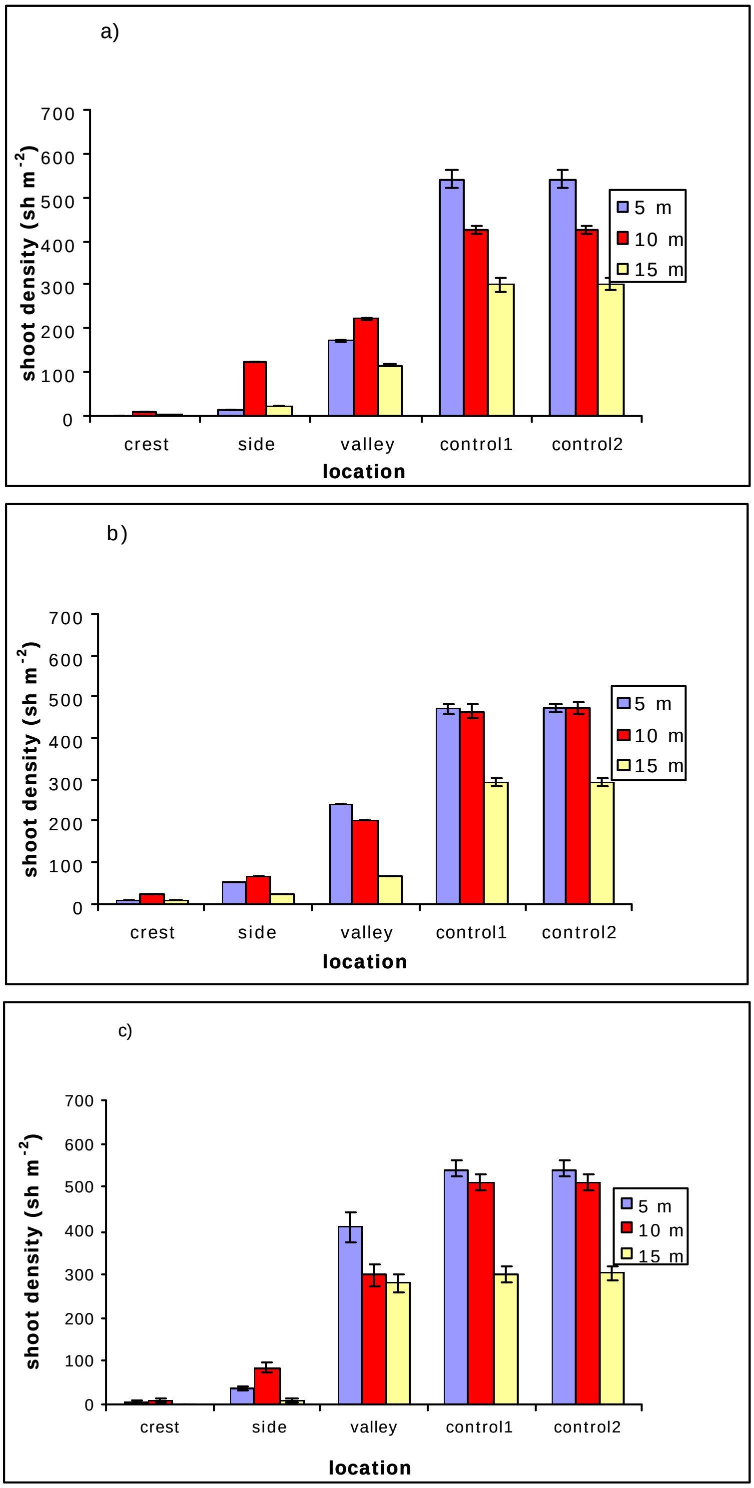

Figure 3.1: *Posidonia oceanica* shoot density at different locations on rubble mounds (crests, sides and valleys) and controls (control 1 and 2) at different depths (5, 10, 15 m) over a three year period (a = 2001; b = 2002; c= 2003, n = 18; bars = SE).

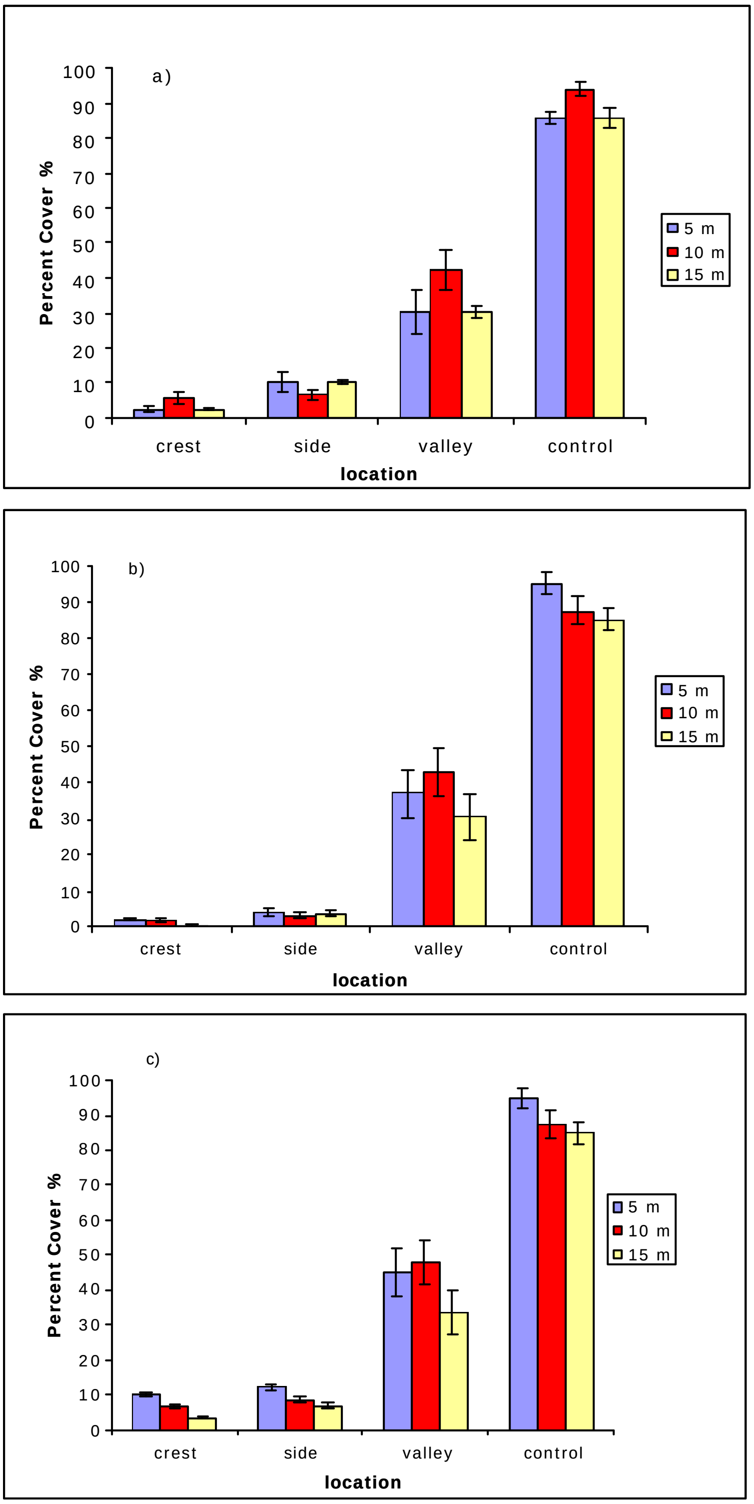

Figure 3.2: *Posidonia oceanica* percent cover at different locations on rubble mounds (crests, sides and valleys and control) and depths (5, 10, 15 m) over a three year period (a = 2001; b = 2002; c= 2003, n = 18; bars = SE).

Table 3.2: Four-way ANOVA of *Posidonia oceanica* percent cover on rubble mounds at 5, 10 and 15 m depth at different locations (crests, sides, valleys, controls) over a 3-year period (2001, 2002, 2003). Ye = year, De = depth, Lo = location, Si = site.*** = p<0.001; ** = p=0.01; ns = not significant; SNK = Student-Newman-Keuls test; C = crest, S = side, V = valley, CT = Control.

Source of variation	*Posidonia oceanica* percent cover		
	df	**MS**	**F**
Year	2	11.2	45.74***
Depth	2	1.7	2.85^{ns}
Location	4	248.5	410.82***
Site(DexLo)	30	0.6	3.1***
YexLo	4	3.0	12.28***
DexLo	8	3.9	15.71***
YexDe	60	0.2	1.26^{ns}
YeXSi(DexLo)	8	1.1	1.76^{ns}
YexDexLo	16	0.6	2.26**
Residuals	405	0.2	
Total	539		
Cochran's test	C = 0.04 (ns)		
SNK test			
Interaction YexDexLo			
(a) lo (yeXde)	2001	2002	2003
5	C<S<V<CT1=CT2	C<S<V<CT1=CT2	C=S<V<CT1<CT2
10	C=S<V<CT1<CT2	C<S<V<CT1=CT2	C=S<V<CT1<CT2
15	C<S<V<CT1=CT2	C<S<V<CT1=CT2	C<S<V<CT1=CT2
(b) de(loXye)	2001	2002	2003
Crest	15=5<10	15<10<5	15<10=5
Side	5=10=15	5=10=15	5=10=15
Valley	5<15<10	5=10=15	5=10=15
CTRL1	5=10=15	5=10=15	5=10=15
CTRL2	5=10=15	5=10=15	5=10=15
(c) ye (loXde)	5	10	15
Crest	2001<2002<2003	2002<2001<2003	2002<2001<2003
Side	2001=2002<2003	2002<2001=2003	2002<2001=2003
Valley	2001<2002=2003	2001=2002=2003	2001=2002=2003
Control1	2001=2002=2003	2001=2002=2003	2001=2002=2003
Control2	2001=2002=2003	2001=2002=2003	2001=2002=2003

3.3.2 Persistence of propagules over time

The percentage of recounted seagrass fragments was found to decrease over time on both crests and sides but not in valleys and controls (Table 3.3). Control locations as well as valleys had the highest number of non-dislodged propagules over time, followed by sides and lastly by crests. This pattern was consistent at both 5 and 10 m depth (Table 3.3). A significant difference in the interaction between time and location was found (Table 3.4). On crests, percentage of fragments recounted was stable for the first six months (June to November 2001) while it decreased from 100 to 83±10% in the following 6 months (November 2001 to June 2002) (SNK test, Table 3.4a). Within a year, such percentage had lowered to 72±19% to reach values of 61±19% after a two year period (SNK test, Table 3.3a). On sides, a similar pattern was recorded. After six months the percentage had lowered to 94±10% to further decrease during the following six months (83±10% recounted fragments). After two years the number of fragments recounted on sides equalled 72±19% (SNK test, Table 3.4a). The percentage of fragments recounted in the valleys and controls at six months intervals remained stable (100%) (SNK test, Table 3.4a).

The SNK test revealed that for the first 12 months after the tagging experiment, there was no significant difference in the percentage of recounted fragments at different locations. After 12 months, the number of recounted fragments on crests became significantly lower in comparison to all the other locations (Table 3.3b). As time increased to 18 months, a significant decrease of recounted fragment was found from crests to sides and from sides to valleys and controls. This pattern remained constant until the end of the manipulation experiment (24 months) (SNK test, Table 3.3b).

Table 3.3: Persistence of fragments reporting percentage of *Posidonia oceanica* fragments found every six months on crests and valleys (mean±SE)

5 m					
	June	**November**	**June**	**November**	**June**
CREST	100±0	100±0	83±10	72±19	61±9
SIDE	100±0	100±0	94±10	83±10	72±19
VALLEY	100±0	100±0	100±0	100±0	100±0
10 m					
	June	**November**	**June**	**November**	**June**
CREST	100±0	100±0	83±10	83±10	61±9
SIDE	100±0	94±10	94±10	89±17	67±16
VALLEY	100±0	100±0	100±0	100±0	100±0

Table 3.4: Three-way ANOVA of *Posidonia oceanica* of percentage of recounted fragments over a 24 month period on rubble and control sites at 5 and 10 a m depth at different locations (crests, sides, valleys, control). Ti = time, De = depth, Lo = location. *** = $p<0.001$; ns = not significant; SNK = Student-Newman-Keuls test; C = crest, S = side, V = valley, CT = Control.

Source of variation	Percentage of recounted fragments		
	df	**MS**	**F**
Time (ti)	4	965.7	22.7***
Depth (de)	1	1.9	0.04^{ns}
Location (lo)	4	1627.8	38.2***
tiXde	4	25.0	0.6^{ns}
tiXlo	16	389.4	9.1***
deXlo	4	11.1	0.3^{ns}
tiXdeXlo	16	11.1	0.3^{ns}
Residuals	100	42.6	
Total	149		
Cochran's Test	C = 0.13 (ns)		
SNK test			
Interaction TiXLo			
(a) Ti(Lo)			
Crest	24<18=12<6=0		
Side	24<18<12=6=0		
Valley	24=18=12=6=0		
Control1	24=18=12=6=0		
Control2	24=18=12=6=0		
(b) Lo(Ti)			
0	ns		
6	ns		
12	C<S=V=CT1=CT2		
18	C<S<V=CT1=CT2		
24	C<S<V=CT1=CT3		

3.3.3 Water flow

Plaster balls always registered significantly lower values of dissolution at the reference location where canopy height was higher than at other sites, with an exception in June when control and valleys reported similar values (SNK tests, Table 3.5 a). The three-way ANOVA reported a clear pattern for gypsum dissolution rate on rubble mounds. Dissolution rate decreased from crests (highest water flow) to sides and then to valleys (lowest water flow) at both 5 and 10 m depth (SNK test, Table 3.5c and Fig. 3.3). However, this trend was not constant over time. In May, June, August and October water motion was highest on crests with decreasing values from side to valleys (SNK test, Table 3.5a and Fig. 3.3). In July and September no significant difference was found between valleys and sides. The three-way ANOVA also demonstrated that dissolution rate was consistently less at 10 m than at 5 m depth (SNK test, Table 3.5b). This implied that more hydrodynamically active conditions generally occurred in shallow areas (wind driven waves) and tended to diminish with increasing depth (Fig. 3.3). This trend was held for all the locations studied.

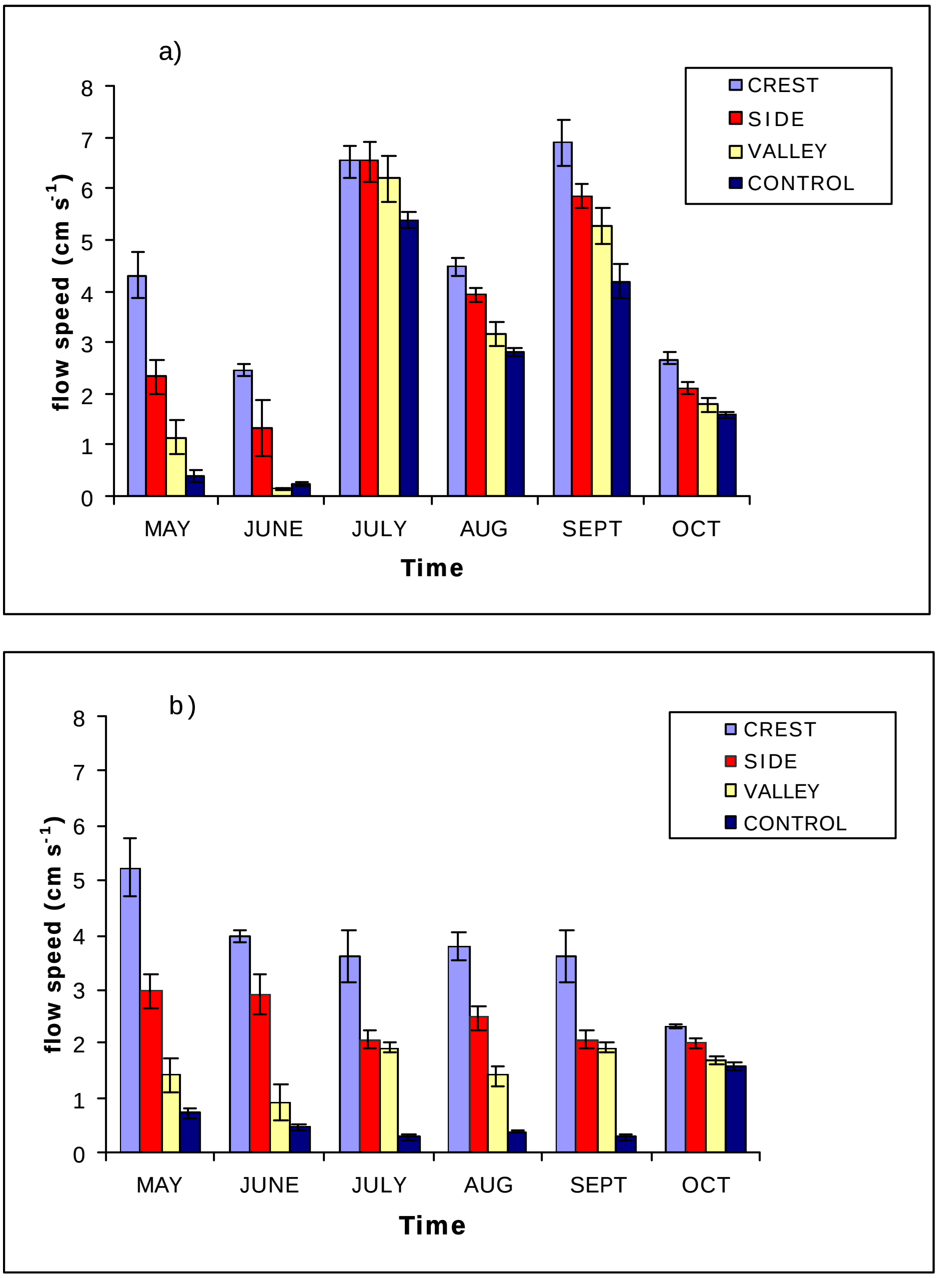

Figure 3.3: Flow speed data recorded over a six month period (May 2003 to October 2003) at different locations on rubble mounds (crests, sides and valleys) and in the undisturbed control site (a = 5 m depth; b = 10 m depth; n = 6; bars = SE).

Table 3.5: Three-way ANOVA of flow speed measured over a six month period at 5 and 10 m depth at different locations (crest, side, valley, control). Ti = time, De = depth, Lo = location. *** = p<0.001; ns = not significant; SNK = Student-Newman-Keuls test; C = crest, S = side, V = valley, CT = Control.

Source of variation	Water motion		
	df	**MS**	**F**
Time	5	48.0	130.8***
Depth	1	127.6	347.6***
Location	3	90.9	247.7***
TixDe	5	57.3	156.0***
TixLo	15	3.4	9.3***
DexLo	3	3.1	8.3***
TixDexLo	15	0.6	1.5 ns
Residuals	240	0.4	
Total	287		
Cochran's test	C = 0.09 (ns)		
SNK test			
Interaction TixLo			
(a) Lo(Ti)			
May	CT<V<S<C		
June	CT=V<S<C		
July	CT<V=S<C		
August	CT<V<S<C		
September	CT<V =S<C		
October	CT<V<S<C		
Interaction DexLo			
(b) De(Lo)			
Control	10 < 5		
Crest	10 < 5		
Side	10 < 5		
Valley	10 < 5		
(c) Lo(De)			
5	CT<V<S<C		
10	CT<V<S<C		

3.3.4 Sediment Thickness

Sediment thickness was lower on mound crests (1.14±0.3 cm) than on side (3.0±0.6 cm) and valleys (9.8±0.9 cm). Moreover, a depth gradient appeared to be present with a decreasing sediment thickness with increasing depth (5 to 15 m) (Fig. 3.4). At 5 m depth, mean sediment thickness at the crests was 0.3±0.6 cm (Fig. 3.45). This value was found to be higher at 10 m depth (3.1±1.5 cm), whilst virtually no sediment was present in crest crevices at 15 m depth (Fig. 3.4). On sides values average at 2.2±0.4 cm at 5 m, while at deeper depths they ranged between 4.02±0.3 and 2.9± 0.8 cm (at 10 m and 15 m, respectively). In valleys, a thicker layer of sediment was found at 5 m with an average depth of 11.9±1.9 cm. At 10 m, the value decreased to 9.8±0.4 cm. Sediment thickness decreased further at 15 m with a mean value of 7.4±0.7 cm (Fig. 3.4).

A significant difference was found in the interaction between depth and location, and between sites (Table 3.6). The statistical tests show a significant gradient of sediment thickness, with decreasing values from valleys to crest at 5 and 15 m depth. In contrast, at 10 m depth, values for crests and valleys were not different but they were significantly lower than those found in valleys (SNK test, Table 3.6a). Finally, sediment thickness on the mound sides did not differ between depths, whilst values for valleys were significantly higher at 10 and 15 min than valleys, and values for crests were also significantly different at 10 and 5 m (SNK, table 3.6b).

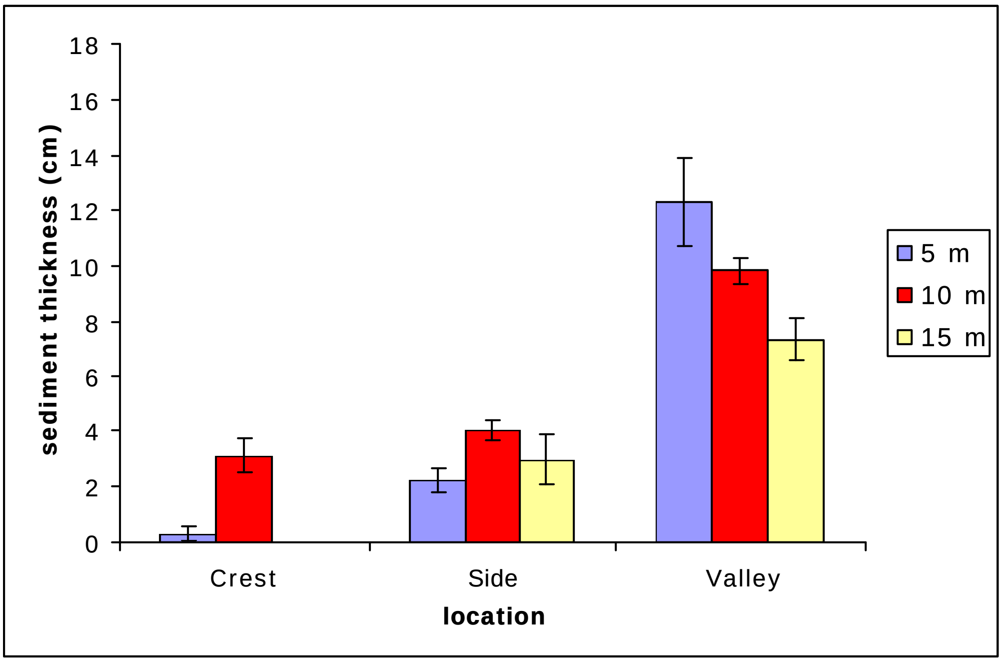

Figure 3.4: Sediment thickness at different locations on the rubble mounds (CR = Crest; SD = Side; VAL = Valley) and depths (5, 10, 15 m; n = 18; bars = SE).

Table 3.6: Three-way ANOVA on sediment thickness data on rubble mounds at 5, 10 and 15 m at different locations (crests, sides, valleys). De = depth, Lo = location, Si = site.*** = p<0.001; ns = not significant; SNK = Student-Newman-Keuls test. C = crest, S = side, V = valley.

Source of variation	Sediment depth data		
	df	**MS**	**F**
Depth	2	5.6	24.4***
Location	2	41.4	179.7***
Site(DexLo)	18	0.4	2.8***
DexLo	4	4.0	17.6***
Residuals	135	0.1	
Total	161		
Transform:	Ln(X+1)		
Cochran's test	C = 0.12 (ns)		
SNK test			
Interaction DexLo			
(a) Lo(De)			
5 m	C<S<V		
10 m	C=S<V		
15 m	C<S<V		
(b) De(Lo)			
Crest	15=5<10		
Side	5=10=15		
Valley	15=10<5		

3.4 DISCUSSION

The recruitment of *Posidonia oceanica* vegetative fragments occurred over the whole rubble field at Capo Feto. Rubble, used to backfill the construction trench at Capo Feto, offered a large number of crevices that favoured the entanglement and anchorage of vegetative fragments. Rubble mounds are fairly motionless even in harsh hydrodynamic conditions, and calcareous rubble does not degrade with time. All these features provided a secure and stable environment for the settlement of newly arrived fragments.

Persistence and shoot density data were found to differ between locations. Crests and side showed very low density values with no increase over time. However, as percentage of re-counted fragments on crests and sides decreased over time, while density remained constant, it can be inferred that on these locations there is a slow but continuous recruitment of fragments, which are eventually dislodged. In valleys, a stable persistence of fragments and a significant increase in shoot density suggest a continuous recruitment over time, but also the appearance of new shoots from previous recruits. Moreover, fragment recruitment in the valley may be facilitated by the dislodgment of recruits from crests and sides which can be deposited in valleys.

The increase in shoot density in valleys between rubble mounds is considered to be the result of a combination of physical (hydrodynamical and sedimentological) processes. Relatively strong water flow (possibly a combination of currents and waves) can lead to fragment dislodgement on crests and sides of rubble mounds. This could be especially severe during winter months when storms generate large waves that penetrate relatively deep into the water column. Plaster ball dissolution rates obtained during the summer and input into the equation proposed by Bailey-Brock (1979) suggest that water flow intensity at crests and sides reached a maximum value of 7 cm s^{-1}, a flow unlikely to dislodge healthy shoots within a well established canopy (currents in seagrass habitats have been measured to be as high as 100 cm s^{-1}; Fonseca et al. 1983, Dierssen et al. 2003). However, these relatively slow flows may indeed dislodge loosely entangled fragments of *P. oceanica*. This remains to be confirmed. Water flows obtained compare well with data recorded by Granata et al. (2001) during relatively calm conditions using a current meter (average of 6 cm s^{-1}). These authors also observed current speeds of 17 cm s^{-1} in a *P. oceanica* habitat in Spain during the beginning of a storm event. Therefore, in healthy *Posidonia* beds currents stronger than 17 cm s^{-1} are expected to occur on a regular basis.

Previous work (Eckman et al. 1989, Gacia et al. 1999) stated that sediment resuspension was greatest where canopy height was at its minimum. Total deposited sediment in the rubble field reached a maximum value of 200 g DW m^{-2} y^{-1}. A similar value for sediment deposition was reported by Gacia & Duarte (2001) for a Spanish *P. oceanica* meadow. Mound valleys had significantly higher depositional rates than crests. This would suggest that low sediment resuspension in valleys is likely to be the result of roots and rhizomes holding the sediment in place. At the same time, it could be inferred that valleys represent a physical refuge from wave and current turbulence, as the depression acts as a good trap for sediments. Sediment particles entrapped in the rubble crests and sides might drain down into the valleys between the mounds. As a result, *P.*

oceanica fragments entangled in crests and sides might not survive due to the lack of sediment, which might prevent fragments from rooting, hence facilitating dislodgment and transport to other locations. A minimum sediment layer of 7 cm was required for *T. testudinum* establishment in Florida (USA) while full development only occurred when the sediment layer reached a thickness of 20 to 25 cm (Zieman 1972). On the crests and sides of Capo Feto rubble mounds, the sediment layer never reached thicknesses greater than 4 cm (1.6 in average) suggesting that: 1) after recruitment, a thick sediment layer is needed to support the development of *P. oceanica* canopies, 2) waves and/or currents may be resuspending sediment particles (especially in the shallow areas), and 3) valleys act as sediment traps via reduction of water flow hence facilitating sediment accumulation at the bottom of the mounds.

Seagrass patch development in valleys between rubble mounds seems to be possible due to relatively quiescent conditions (water movement of 2 cm s^{-1}) and suitable substratum for anchoring and resource acquisition (12 cm sediment thickness). Water flow was more quiescent in valleys than at crests and sides, but not as quiescent as in well established *P. oceanica* beds in a nearby control area. Even so, *P. oceanica* fragments were recruited in the valleys and developed into seagrass patches. Once recruited, fragments were able to become rooted and presumably draw on sediment nutrients providing the resources needed to grow. The 12 cm of sediment found between rubble in the valleys may still be limiting to *P. oceanica* as suggested by higher seagrass densities observed in the control areas (˜600 shoots m^{-2}) versus in valleys (˜300 shoots m^{-2}). Perhaps the 20 to 25 cm sediment requirement established for *T. testudinum* (Zieman

1972) also applies to *P. oceanica*. However, it could be further hypothesised that long recovery times are required for *P. oceanica* growing in valleys to reach ambient density. An increased in the number of shoots is likely to lead to further sediment accumulation (see Duarte & Sand Jensen 1990, Marbà & Duarte 1998, Granata et al. 2001) between the rubble and, therefore, further seagrass expansion. Consequently, it can be suggested that, once the valleys have become vegetated, the seagrass patches may expand upwards along the sides of the rubble mounds and ultimately reach the crests.

In summary, this is the first study to report on *P. oceanica* vegetative recruitment to an artificial rubble substratum after a disturbance event. Based on the findings described, it is concluded that rubble mounds can be considered a suitable substratum for *P. oceanica* recruitment and establishment, after a human-induced impact.

Although *P. oceanica* is a slow-growing species, it shows a vegetative recruitment mechanism that represents a way to overcome environmental disturbance and to colonise physically impacted environments such as rubble mounds. Although rubble mounds may not appear suitable as a seagrass substrate (sterile environment with minimal nutrient availability and relatively strong water flow), the quiescent conditions within rubble mounds and in valleys between mounds led to seagrass fragment entanglement and sediment deposition. The sediment between rocks then apparently provided the resources necessary to sustain seagrass patches which, once established, may start a positive feedback of current attenuation, sediment accumulation and seagrass patch expansion (e.g. Fonseca et al. 1983, Thayer et al. 1984). Perhaps this principle of natural recruitment of seagrass fragments in areas to be restored can also be applied to other species.

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CHAPTER IV

SEDIMENT-NUTRIENT INTERACTIONS IN THE RUBBLE ENVIRONMENT

4.1 INTRODUCTION

Seagrass meadows are believed to stabilise sediments (Koch 2001) and promote sediment deposition (Short & Short 1984, De Falco et al. 2000). Thus the loss of seagrass patches often results in an enhancement of sediment resuspension, erosion (Terrados & Duarte 2000) and transport (Hine et al. 1987). Sediment deposition and retention within seagrass patches is achieved by the leaves reducing the ambient water flow by extracting the momentum from the moving water (Gacia & Duarte 2001, Koch 2001), which also results in an increase in light availability. As a result, there is a complex but clear feedback between seagrasses and the physical habitat they colonize (Koch & Verduin, 2001). Previous work (Short & Short 1984) reported on how seagrass leaf canopy can trap suspended materials (Almasi et al. 1987, Marbà & Duarte 1995) and the hypothesis that seagrass meadows enhance sedimentation has been supported by *in situ* assessment of suspended matter (Fonseca & Fisher 1986). However, there is still a general dearth of information on sediment deposition and direct measurements of resuspension in seagrass beds (Koch 1999). Recent work by Gacia & Duarte (2001) has tested the hypothesis that particle deposition rates are higher within *Posidonia oceanica* canopies compared to unvegetated substrata and that sediment resuspension is more significant in bare areas than within the seagrass meadows. *P. oceanica* slows current velocity (Terrados & Duarte 2000, De Falco et al. 2000), especially at the edge of the meadow, increasing the roughness height of the benthic boundary layer, hence enhancing particle trapping (Gacia & Duarte 2001). Sediment accumulation is an important factor in determining the growth and distribution of seagrasses (Zieman 1972).

At Capo Feto, vegetative fragments of the endemic seagrass *Posidonia oceanica* recruited in the voids between the rubble used to backfill a pipeline trench. As described in chapter 3, recruited fragments only settled and survive in areas referred to as valleys. On the other hand, fragments found on crests tended to be dislodged over time. Once recruited fragments tend to retain the accumulated sediment hence a thicker sediment layer was present. On the other hand, sediment particles on crests and sides was thought to be resuspended or to drain to the bottom of the mound with the result that no sediment layer is found. In valleys, once patches are well established, a positive feedback between the canopy, water flow attenuation and sediment deposition was expected. Thus, seagrass fragments would generally be able to root and take up nutrients needed for growth from the sediment (Fourqurean et al. 1992). Sediments provide the necessary nutrients for plant growth (Hemminga et al. 1991, Alcoverro et al. 1995) and sediment grain size is considered a good indicator of physical and geochemical characteristics in seagrass habitats (Erftemeijer & Koch 2001). In chapter 3 attention was focused on the importance of a minimum sediment thickness for seagrass colonisation and growth (Zieman 1972). However, sediment composition is also a major factor affecting seagrass growth (Huettel & Gust 1992, Erftemeijer & Middelburg 1993). Sediment grain size does not generally limit seagrass growth *per se*, however it involves a series of geochemical and physical processes that play a primary role in seagrass growth (Huettel & Rush 2000, De Falco et al. 2000). Sediments present in seagrass beds are finer than those found in unvegetated areas (Scoffin, 1970, Almasi et al., 1987, Koch 2001). As grain size distribution becomes skewed towards the silt and clay, the porewater exchange with the overlaying water column decreases (Huettel & Rush 2000, Koch 2001), which can lead to an increased nutrient concentration (Kenworthy et al. 1982). On the other hand, in coarse sand environment the exchange of pore water with the overlaying water column will be higher than in finer sediments (Erftemeijer & Middelburg 1993) hence seagrass might experience low nutrient availability (Huettel & Rush 2000). Nutrient availability in the sediment and in pore water are the main nitrogen (N) and phosphorus (P) sources for plants (Barko & Smart 1981, Fourqurean et al. 1992), to photosynthesise and build tissue (Touchette & Burkholder, 2000, McGlathery et al. 2001). Although seagrasses might take up nutrients from the leaves, leaf uptake is considered to be of secondary importance since roots penetrate the more nutrient-rich medium of the sediment (Fourqurean et al. 1992). Thus, it is generally assumed that the nutrient reservoir of the sediment is the primary source for submerged aquatic vegetation. Porewater nutrients concentration are typically one to several orders of magnitude higher than those in the water column (McGlathery et al. 2001), however this nutrients pool can be depleted rapidly by plant uptake and in this situation leaf uptake would become an important N and P source (McGlathery et al. 2001). Nutrient-limited growth does appear to be a common phenomenon (Short et al. 1985, 1990, Bulthuis et al. 1992, Murray et al. 1992, Agawin et al. 1996) and often nutrient availability is controlled locally by land inputs, sediment dynamics and type (Hemminga et al., 1991). A previous study conducted by Short (1987) assessed the importance of geochemical characteristics of the sediment in the study of nutrient limitation of seagrass growth. Short (1987) concluded that seagrass occurring in terrigenous environments are often nitrogen limited whilst those species living in carbonate sediments generally experience P limitation. In certain cases, seagrass can be co-limited by N and P (Touchette & Burkholder, 2000). Nutrient availability generally follows a seasonal trend with higher nutrient availability in the warm season (Touchette & Burkholder 2000). Higher NH_4^+ in both the water column and the pore water is available on a diel basis (Touchette & Burkholder 2000), mainly provided by cyanobacteria and eubacterial nitrogen fixation (Capone et al. 1979). The majority of the nitrogen (N_i) supply for seagrasses occurs through leaf absorption (Terrados & Williams, 1997), even if the roots can take up N_i sources in the sediment in form of amino acids and urea (Touchette & Burkholder 2000). On the other hand, most P_i (phosphorus) absorption occurs through the root system (Brix & Lyngby 1985). As P is highly biologically active, it presents a short residence time (Day et al. 1989) and it can be consumed steadily by the plant. Alcoverro et al. (1995) demonstrated a strong seasonality of *P. oceanica* growth with an early summer maximum and an autumnal minimum (Ott 1980, Romero 1989, Alcoverro et al. 1995). The same study (Alcoverro et al. 1995) highlighted a strong relationship between the solar cycle and nutrient availability, with a nutrient depletion following fast growth rates (McGlathery et al. 2001). Consequently, local factors can play a key role in shallow seagrass environments, determining seasonal growth patterns where light availability is high (Alcoverro et al. 1995). Pore water geochemistry and sediment dynamics can be considered part of the positive feedback between seagrasses, water flow and sediment deposition. As waves and currents enhance solute transfer between sediments and the overlaying water column (Koch 1999), the hydrodynamic conditions and porewater geochemistry may be interpreted as interdependent factors regulating plant growth and morphology (Huettel & Rush 2000). Moreover, the reduction of current velocity by the canopy might minimise porewater flux through the sediment, which can result in an accumulation of nutrients in the sediments (Erftemeijer & Koch 2001). Thus, N and P availability is enhanced (Kenworthy et al. 1982).

In this section the sedimentation rate at different locations is assessed (rubble mound crests and valleys, rubble field water column, control and control bed water column) on the rubble field and the control site. A comparatively similar total depositional rate over both environments (rubble field and pristine meadow) is hypothesised. Moreover, seasonal changes in sediment nutrient availability for seagrass growth are evaluated. To achieve this sediment origin, porewater nutrient concentration and nutrient content of *P. oceanica* tissue were assessed.

4.2 MATERIAL AND METHODS

4.2.1 Total sediment deposition

Total sediment deposition was monitored every two months on the rubble field and a reference site over a period of one year (May 2002 till March 2003) using small sediment traps. Traps consisted of 16 cm (diameter) funnels (30 cm in depth) attached to 100 ml plastic bottles (Plate 4.1).

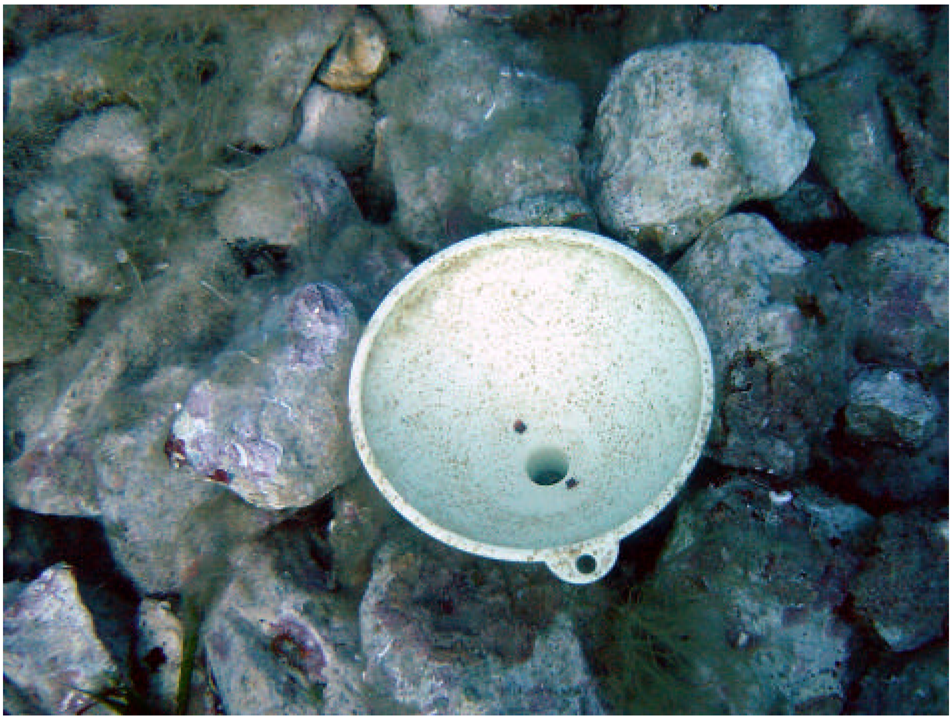


Plate 4.1: Total sediment deposition was monitored using small sediment traps. Traps consisted of funnels attached to plastic bottles.

To measure total deposition rate traps were deployed on crests and valleys of the rubble field and within the matte in the control site at the same level as the seabed (the funnel top was at the same level as the seafloor). To assess water column sediment particle fluxes at each site, similar traps were positioned at three different heights in the water column (1, 3, 5 m from the surface) mounted on a structure assembled by SCUBA divers and held by buoys. Since the variance between different heights on the water column was not significantly different (ANOVA, $p>0.05$), all water column sediment traps were used as replicate units. In all traps were deployed at 6 different locations (crest, valley, rubble bed water column, control, control water column) at a depth range between 5 and 10 m. For each location, 8 traps were deployed. However, some traps were lost as a result of winter and summer storms, hence four replicates per location was utilised to run the statistical test. Prior to deployment traps were filled with subsurface seawater and covered with caps. Caps were removed a few minutes after positioning to avoid the collection of resuspended sediments during manipulation.

In the laboratory, traps were inspected for active swimmers and other organic material (i.e. decapods limbs) which were removed if present (Michaels et al., 1990). Trap content was filtered through 25 mm pre-weighted GF/F filters and stored in an oven at 60 °C for 48 h before weighing.

4.2.2 Sediment grain size

Sediment grain size can be considered a good indicator of several physical and geochemical characteristics of seagrass habitats. For this reason, sediment samples were collected in August 2003 both on the rubble field (vegetated and unvegetated patches) and on the control meadow (seagrass growing on matte).

For each location 3 randomly chosen samples were collected at 5, 10 and 15 m depth. Sediments were collected by SCUBA diving using plastic bags closed as tightly as possible using rubber stoppers to reduce air content.

Samples were then transported to the laboratory in a cool box, out of sunlight. Sediments were processed within 10 days from collection.

Samples were homogenised by mixing in a container and divided into two sub-samples (100 g wet weight each). For a sub-sample a wet weight and dry weight was obtained by weighting and drying the sample at 60 °C for 24h. The other sub-sample was treated with H_2O_2 (6%) within a 1 l beaker, diluted with MilliQ water to remove sea salt and left it to decant overnight. Samples were then weighed and dried at 100 °C and divided into sediment fractions by dry sieving. For this analysis, 18 sieves with a mesh comprised between $-4.0\,\phi$ and $4.0\,\phi$ (16 mm and 63 μm) were adopted. For sediment grain size classification the Krumbein (1934) size class was adopted. This sediment size classification is a Log_2 transposition of Udden-Wentworth (1922) sediment size distribution.

The "GradiStat" software for Windows was used to estimate the distribution of sediment particles in relationship to their diameter. This package allows the operator to check sample weight, to control the sieve shaker and the electronic balance and to calculate the distribution of particles for each sieved sediment fraction.

4.2.3 Sediment porewater

Sediment porewater nutrient concentrations are generally higher than those in the water column and often represent the primary source of nutrients for most rooted aquatic plants. Thus, total oxidisable N and P in sediment porewater was determined both in the rubble field and the control seagrass bed. Sediment porewater was sampled in June 2003 at four locations (crests, valleys, control 1, control 2). Water samples were also taken from the rubble field water column and the control bed water column to be considered as blanks. For each location, six random replicate samples were collected. Porewater samples were withdrawn by a 100 ml syringe. The first 10 ml were discarded while 25 ml were retained and filtered through 0.5 µm Millipore filters. The filtrate was kept on ice in the dark until arrival at the laboratory where they were stored at – 80 °C for later analysis. N and P concentration were then analysed using a scalar 1000 auto-analyser (SAN SYSTEM) (Plate 4.2).

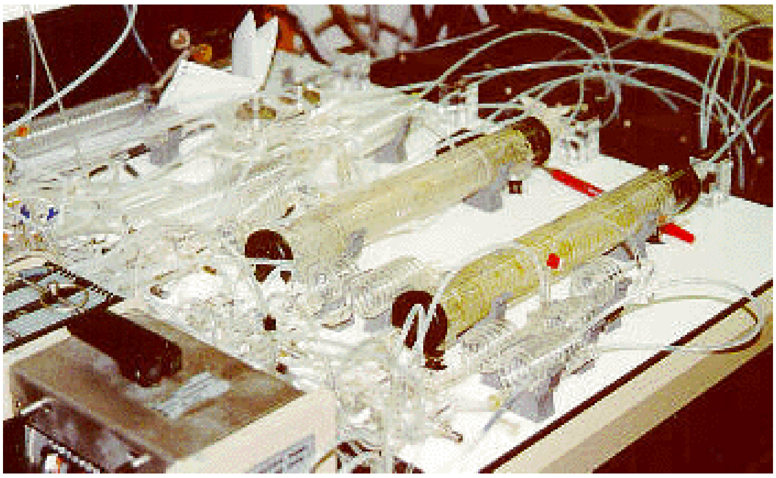


Plate 4.2: The auto analyser SAN SYSTEM can run both N and P samples at the same time.

4.2.4 Seagrass elemental analysis

Nutrient content was analysed in *P. oceanica* leaves, rhizomes and roots. Sampling was carried out in October 2002 and May 2003 to evaluate temporal variations. Six random and independent shoots, rhizomes and root samples were collected at 10 m depth at different locations on the rubble mounds (crest, side, valley) and at one reference site on the pristine meadow at Tonnarella. Seagrass leaves were divided by age (A = adult, I = intermediate, J = juvenile, Giraud 1977) and green leaf tissue was separated from the non-photosynthetic tissue in the sample. The leaves were gently scraped using a razor blade and washed in flowing tap water to remove epibionts and sediment that had adhered to the leaves. Washed samples were then weighted and placed in an oven at 60 °C for 48 h. Rhizome and root samples were also rinsed in tap water and dried using the same procedures. Dried samples were re-weighed and dry weight was expressed as g DW (dry weight). For determination of elemental content of *P. oceanica* leaves, rhizomes and roots, samples were first homogenised by milling them to a fine powder. Carbon and nitrogen content was then determined for duplicate samples by oxidation in a Carlo Erba Model 1500 CHN analyser (Plate 4.3).

Plate 4.3: The Carlo Erba CHN analyser was used to assess the C+N content of *Posidonia oceanica* tissue.

For total P determination a modified technique from Solorzano & Sharp (1980), also described in Fourqurean & Zieman (1992), was adopted.

Samples (17 to 21 mg) were weighed into tared 20 ml glass vials and 0.2 ml of 0.17 M $MgSO_4$ and 1 ml H_2O deionised water (DIW) water were added to each vial. The vials were dried in an oven at 70 ºC overnight, uncapped. Dry vials containing samples were ashed at 500 ºC for 4 hours. After cooling, 5 ml of 0.2 N HCl was added to each vial, the vials were capped and heated up in an oven at 80 ºC for 30 minutes. The contents of each vial was then diluted with 10 ml of DIW water and allowed to stand overnight to let ash settle. Phosphate (P) concentration of the solution in the vials was then determined colorimetrically using an auto sipper spectrophotometer (HITACHI U-2000). P concentration was converted, where necessary, to nutrient weight as percent of tissue dry weight (% DW), the unit most commonly used in the literature. It was found that the method adopted for total oxidisable P determination yielded 55-80% of the reported content of standards (*Ulva lactuca* and *P. oceanica* root tissue). C:N, C:P and N:P ratios were computed as a molar ratio as described in several previous studies (Pirc & Wollenweber 1988, Duarte 1990, Fourqurean & Zieman 1992)

4.2.5 Data analysis

Sedimentation rate and sediment porewater data were analysed using a two-way analysis of variance (ANOVA). For sedimentation rate, the following design was adopted: time (6 levels: May, July, September, November, January, March) and location (5 levels: Crest, Valley, Rubble Field Water Column, Control, Control Water Column), where both factors were taken as fixed and orthogonal. Sediment porewater design included two factors, location (5 levels: Crest, Valley, Control 1, Control 2, Water

Column) and site (2 levels: Site 1, Site 2). The first factor was considered fixed and orthogonal whilst sites were taken as random and orthogonal. Homogeneity of variance was checked using Cochran's test (Snedecor & Cochran 1989). When significant differences were found in the ANOVA, they were compared *a posteriori* using a Student-Newman-Keuls (SNK) test (Underwood 1981, 1997).

CNP tissue analysis was run using a multifactorial multivariate design with three factors (time, location and plant tissue) all fixed and orthogonal. The analysis included three variables (C, N, P). Analysis was carried out using the FORTRAN Programmes XMATRIX AND DISTLM (Anderson 2003a,b). Moreover a Canonical Analysis of Principal coordinates (CAP; Anderson 2003c) was run on the CNP tissue data set. Although NP-MANOVA and CAP test a similar multivariate hypothesis for a linear model, the former test does not take into account the correlation structure among the variables. CAP provides a constrained ordination diagram hence finding the axis (or axes) in the principal coordinate space that is best at discriminating among the *a priori* groups (Anderson 2003c).

4.3 RESULTS

4.3.1 Sedimentation rate

Total sediment deposition showed a clear trend with lower values in the control pristine seagrass bed than on the rubble field. In the control area, deposited sediments averaged at 1.2±0.1 g DW m^{-2} with values ranging between 4.2±0.3 g DW m^{-2} recorded in July to 0.05±0.02 g DW m^{-2} in January (Fig. 4.1). The amount of deposited sediment in water column traps at this location averaged 0.4±0.05 g DW m^{-2}. In the rubble mound field, values of total deposited sediments were higher in valleys (average 3.4±0.3 g DW m^{-2}) than on crests (2.6±0.2 g DW m^{-2}) and on the water column (0.5±0.03 g DW m^{-2}) (Fig. 4.1). A temporal trend was recorded with lower total depositional rates found during winter months (0.03±0.1 g DW m^{-2} in average) and higher rates in May (0.6±0.2 g DW m^{-2}) and July (2.1±0.3 g DW m^{-2}).

The two-way ANOVA showed a significant interaction between time and location (Table 4.1). The amount of deposited sediments at the different locations varied significantly between the six sampling periods (SNK Test, Table 4.1). On crests, total sediment deposition was always significantly higher in May and July (6.0±0.4 g DW m^{-2}), than in November, January and March (average 1.2±0.1 g DW m^{-2}), which in turn presented higher depositional rates than September (SNK test, Table 4.1a). Traps set in valleys showed a slightly different trend. In valleys a significant difference was found between sampling periods, with average depositional values higher in July (7.9±0.9 g DW m^{-2}) than in May (6.4±0.5 g DW m^{-2}) and November (2.0±0.5 g DW m^{-2}) (Fig. 4.1). Significantly lower values were found in March and January with smallest values found in September (0.7±0.09 g DW m^{-2}) (SNK Test, Table 4.1a). Values recorded in the pristine seagrass meadow showed a significant difference lowest depositional rates recorded in July (4.2±0.3 g DW m^{-2}) and May (1.6±0.04 g DW m^{-2}) (SNK Test, Table 4.1a). Significantly lower values were found in March and November which showed similar values (0.7±0.06 g DW m^{-2}). Depositional rates were found to be lowest in January and September which did not significantly differ from each other (SNK Test, Table 4.1a). No difference was found between sediments accumulated in the water column between the rubble mound field and the pristine seagrass bed (Fig. 4.1). At both locations, trapped material was significantly higher in July (2.4±0.1 g DW m^{-2}) with intermediate values in May (0.5±0.06 g DW m^{-2}) and significantly lower values in September, November, January and March (average 0.04±0.01 g DW m^{-2}) (SNK Test, Table 4.1a).

A posteriori comparisons in table 4.1 give details on the differences among locations within each sampling period (SNK Test, Table 4.1b). The results show a similar trend in May and July with significantly higher depositional rates present in valleys. Significantly lower values were found on crests, with depositional rates that further decreased in the control bed (SNK Test, Table 4.1b). In September a non-significant difference was found among all locations. In the next sampling period (November), values were still significantly higher in valleys, but no significant difference was found between crests and control (SNK Test, Table 4.1b). In January, total sediment deposited in the undisturbed meadow did not differ from the trapped material in the water column. A significant difference was found between crests and valleys, where the latter locations presented higher values. In the last sampling time, no significant difference was found between crests and valleys. However, values in the rubble field were significantly higher than in the control site (SNK Test, Table 4.1b).

Figure 4.1: Total deposited sediment at Capo Feto on the rubble mound field (crests and valleys) and in the *P. oceanica* pristine meadow (control). Depositional rates are compared to particles trapped in the water column. Error bars represent ± SE (N = 4).

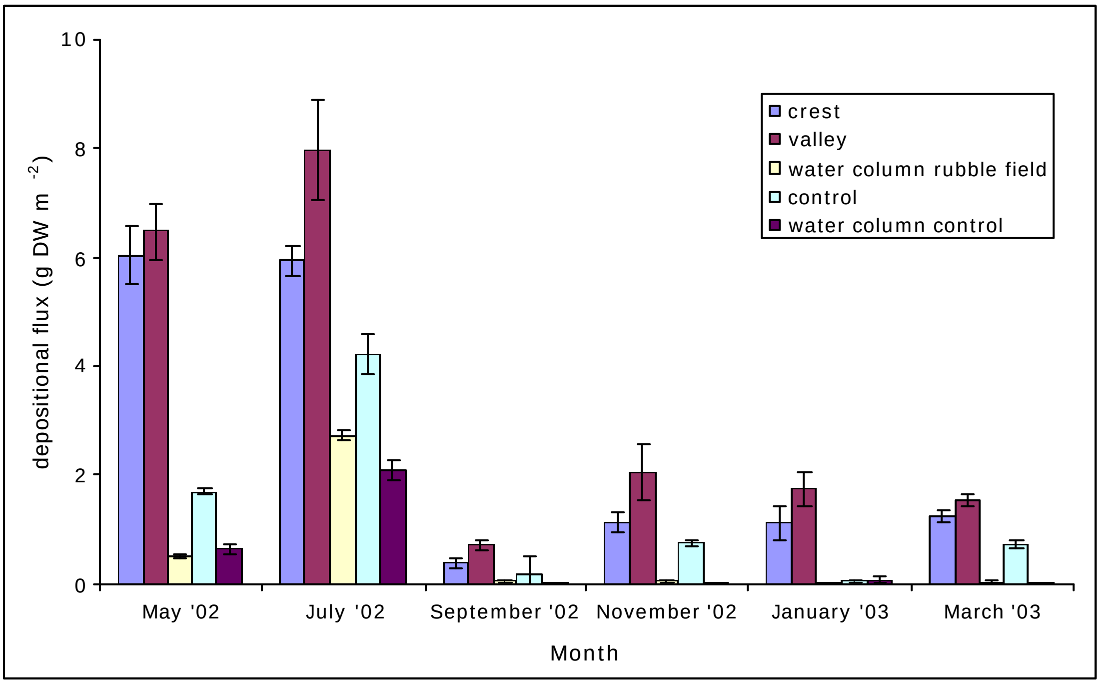


Table 4.1: Two-way ANOVA of total deposited sediment on rubble mounds at different locations (crests, valleys) and a control *P. oceanica* bed over a year period (2003). Ti = time Lo = location.*** = p<0.001; ns = not significant; SNK = Student-Newman-Keuls test; C = crest, V = valley, CT = Control, RW = rubble mound water column, CW = control water column; S = September, N = November, J = January, MC = March, JL = July, M = May.

Source of variation	Total depositional flux		
	df	**MS**	**F**
Time (ti)	5	4.2	424.4***
Location (lo)	4	3.5	350.7***
tiXlo	20	0.2	22.2***
Residuals	90	0.0	
Total	119		
Cochran's Test	C = 0.138 (ns)		
Transform	Sqrt(X+1)		
SNK test			
Interaction TiXLo			
(a) Ti(Lo)			
Crest	S<N=J=MC<JL=M		
Valley	S<J=MC<N<M<JL		
Rubble wat col	S=N=J=MC<M<JL		
Control	S=J<N=MC<M<JL		
Control wat col	S=N=J=MC<M<JL		
(b) Lo(Ti)			
May	RW=CW<CT<C<V		
July	RW=CW<CT<C<V		
September	ns		
November	RW=CW<C=CT<V		
January	RW=CT=CW<C<V		
March	RW=CW<CT<C=V		

4.3.2 Sediment grain size

Sediment grain size analysis did not show a difference among locations (rubble vs control) and between unvegetated and vegetated patches. This could be due to the few number of samples available which provided a higher variability. However, all samples indicated a strong sand component (average 98.7±0.2%, Table 4.2) with a small silt fraction. Moreover, the clay component resulted to be virtually absent.

Although all sediments are mainly composed by sand and gravelly sand (Fig. 4.2b,c) samples collected in valleys indicate a higher silt component than unvegetated patches at all depths (Table 4.2). The trench area is a result of a mosaic of sediments (see Chapter II) due to dredge-fill operations. This could have affected the grain size distribution of sediments within the rubble field which reported a polymodal distribution (Fig. 4.2a). Thus, the variable hydrodynamic regime of the area leads to the presence of poorly sorted or moderately sorted sediment particles within the rubble environment.

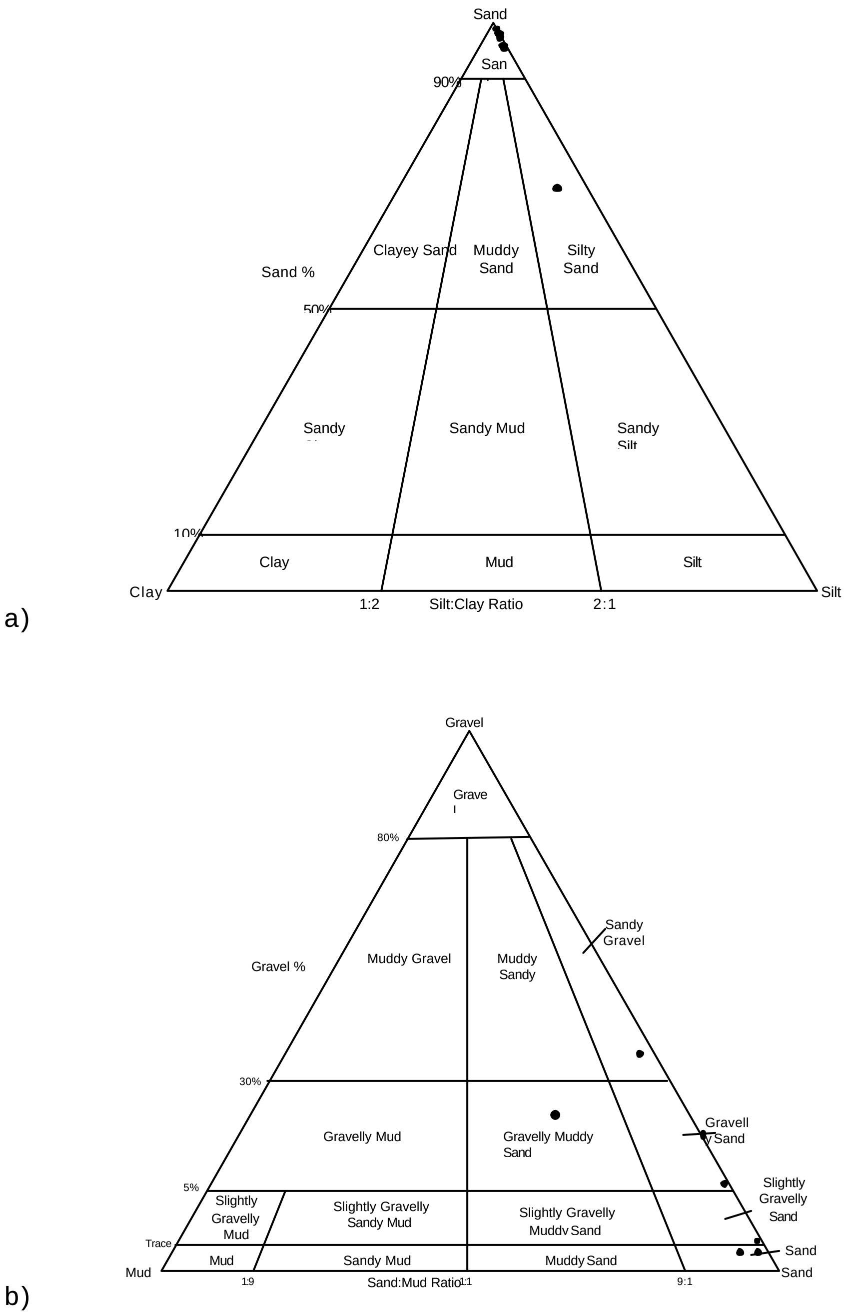


Figure 4.2: Shepard's diagrams with the distribution of samples (a) with a large component of silt sand; (b) sediment sample show a large sand component and are distributed along the gravel/sand axis.

Table 4.2 : Sediment grain size results. All sediment samples collected report a large sand component with a small silt component. Clay is virtually absent.

	Sand %	**Silt %**	**Clay %**
5M-control	99.82	0.18	0.00
5M-rubble mounds	99.59	0.41	0.00
5M-unvegetated	100.00	0.00	0.00
10M-control	99.82	0.18	0.00
10M-rubble mounds	99.58	0.42	0.00
10M-unvegetated	93.83	6.17	0.00
15M-control	99.84	0.16	0.00
15M-rubble mounds	94.01	5.99	0.00
15M-unvegetated	99.82	0.18	0.00

4.3.3 Sediment porewater

Total nitrogen concentration in porewater averaged at 3.04±0.2 µmol l^{-1} in valleys while on crests it ranged between 1.96 and 0.34 µmol l^{-1} (Fig. 4.3). The average N concentration found in the control (undisturbed) bed was 1.83±0.46 µmol l^{-1} while the water column concentration used as a blank was 0.23±0.05 µmol l^{-1} (Fig. 4.3). The one-way ANOVA highlighted a significant difference between locations (Table 4.3a). SNK test showed that the significantly higher values found in valleys accounted for the statistical difference (SNK Test, Table 4.3a). No significant difference was found between any of the other locations (SNK Test, Table 4.3a).

Total inorganic phosphorus concentration in sediment porewater showed a similar trend to that of nitrogen. A significant difference was found between locations, with significantly higher P values in valleys than in all the other locations (which did not differ among each other) (Table 4.3b). P concentration in valleys averaged at 1.98±0.37 µmol l^{-1}, whilst crests had a lower P concentration, 1.17±0.4 µmol l^{-1} (Fig. 4.3). P values in the reference matte sediment porewater had a mean value of 1.3±0.1µmol l^{-1}, while water column concentration had the lowest P value (0.43±0.1 µmol l^{-1}) (Fig. 4.3).

Figure 4.3: Sediment porewater nutrient (N and P) concentration on the rubble mound field both on crests and valleys. Values for controls sites are also shown. Differences between nutrient concentration in the sediment and in the water column are highlighted. Error bars represent

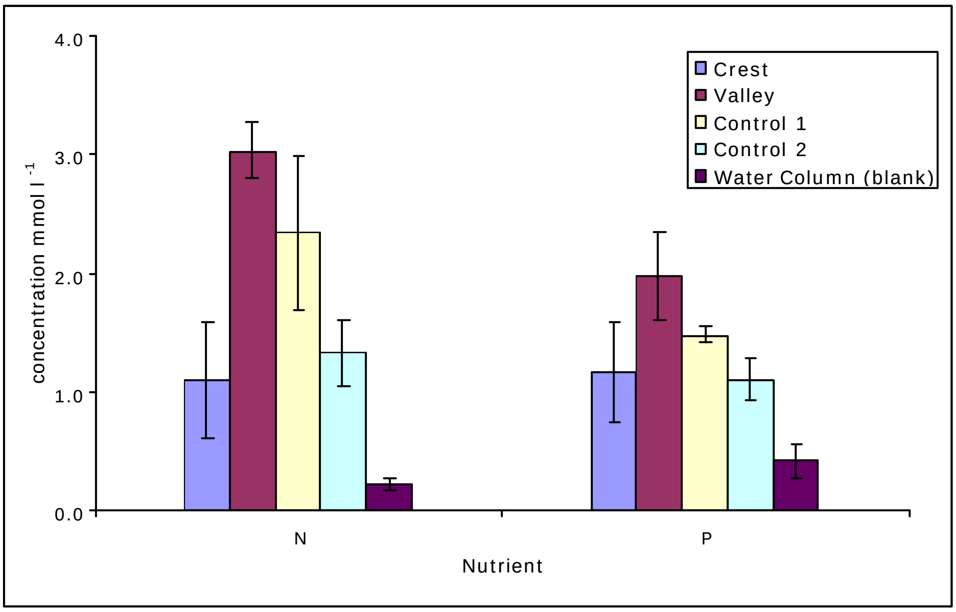


Table 4.3: One-way ANOVA of nutrient (N and P) concentration in sediment porewater on rubble mounds at different locations (crests, valleys) and a control site. *** = p<0.001; ns = not significant; SNK = Student-Newman-Keuls test; C = crest, V = valley, CT1 = Control, CT2 = Control2, WT = water column.

Source of variation	N conc. in pore water		
	df	**MS**	**F**
Location (lo)	4	6.3	10.75***
RES	25	0.6	
TOT	29		
Cochran's Test	C = 0.4868 (ns)		
SNK test			
(a) Location	C=CT1=CT2=WT<V		

Source of variation	P conc. in pore water		
	df	**MS**	**F**
Location (lo)	4	1.9	4.54***
RES	25	0.4	
TOT	29		
Cochran's Test	C = 0.4724 (ns)		
SNK test			
(b) Location	C=CT1=CT2=WT=V		

4.3.4 Seagrass nutrient content

Average (± SE) nutrient content (as % DW) in *Posidonia oceanica* leaves was 46.2±0.2% carbon, 0.9±0.04% nitrogen and 0.7±0.05% phosphorus, with a consistent variation around these average values (Fig. 4.4 & 4.5). Average nutrient content translated into a median C:N:P ratio of 3157:61:1, a consistently higher ratio than those previously reported for seagrasses (Duarte 1990), macrophytes (Atkinson & Smith 1984) and marine seston (Redfield et al 1963). The C:N and C:P ratio indicated that the nitrogen (N) and phosphorus (P) content was low, with a strong N limitation as reflected by the N:P ratio (Table 4.5), although N levels increased over winter.

NP-MANOVA and CAP analysis showed a seasonal variation of CNP concentration in *P. oceanica* tissue with higher value in the Spring and lower value in the Autumn (Table 4.4; Fig. 4.6). NP-MANOVA results reported a significant interaction among the three factors tested (Table 4). Tissue carbon content did not vary significantly between October and May on crest, valley and control, where values in the Autumn averaged 46.3±0.1 % DW, whilst Spring values gave an average of 40.1±0.3 % DW (Fig. 4.4 & 4.5). No significant difference in C % DW was found between leaves and rhizomes over time, however carbon content in the roots on crests decreased significantly from October to May (respectively 42.9±0.6 and 37.3±0.8 % DW). N and P content varied synchronously over time showing how seagrass tissue on crests, valleys and control always presented lower levels in October than in May. This pattern remained constant for leaves, rhizomes and roots. The only exception was found in juveniles leaves which show a high P content in October in the control site (2.3±0.007 % DW).

Nutrient content also showed a significant variation among locations over time and for each plant tissue examined. In the Autumn, carbon content of plant tissue living on crest, valley and control did not vary significantly (Fig. 4.4a). At the same time, N concentration was found not to differ significantly between adult leaves (A) on crests and valleys which however had significantly lower value than A leaves in the control site. This trend was consistent also for intermediate (I) and juvenile (J) leaves, whilst no difference was recorded among locations in rhizome and root N content. Autumn values for N were always significantly higher in control samples than on valleys with minimum values on crests for all leaf ages and rhizomes (Fig. 4.4b). No significant difference was found for what concerns the roots. A similar trend to that of N can be observed for P where crests and valleys do not differ significantly in leaf tissue in October but they are significantly smaller than values at the control site (Fig. 4.4c). No significant difference was found for rhizomes and roots among locations. In samples collected in May, there were no significant differences in P concentration among locations in leaf tissue (A and I leaves). Rhizomes however, showed a significantly decreasing P content from control to crest samples with minimum values present in plants living in valleys (Fig. 4.5c). Seagrass fragment lack a root structure hence values for the root system could not be retrieved. Values for control and valleys were found to be similar.

CAP analysis highlighted how nutrient content decreases from the younger leaves (I and J) to adult leaves, and from adults leaves to the rhizomes with smallest CNP concentrations found in the roots (Fig. 4.6 a,b). Carbon concentration showed no variation among leaves and rhizomes at all locations and time of the year (average 47.1±0.03 % DW). However, in the Autumn significantly lower values were found for the roots both on crest, valley and control (42.9±0.6 % DW). An opposite trend was recorded in the N content of tissue collected in October. No significant differences were found between leaf and rhizome (average 0.1±0.01 % DW) on crests, valleys and control, whilst roots had significantly higher N values (0.62±0.04 % DW). N concentration of plants living on crests showed a significant difference among the different plant tissue. Lowest values were found in the roots, with increasing concentrations through the rhizomes, adult and juveniles leaves to reach a peak in intermediate leaves. On valleys and control the same trend was recorded, however intermediate and juvenile leaves did not differ significantly. Juvenile leaves on crests and valleys appeared to have the highest P content in the Autumn, while no significant difference was found between adult and intermediate leaves which in turn had higher P concentration than roots and rhizomes (Fig. 4.4c). In the undisturbed bed, a significant difference was also present between adult (0.6±0.02 % DW) and intermediate leaves (1.03±0.02 % DW). In May a very similar pattern was found, with significantly higher P content in intermediate than adult leaves at all locations (Fig. 4.5c). However, a distinct difference was present on crests and control between rhizomes and roots, where the former had relatively higher P contents. Finally, in the control bed, no significant difference was found in the P concentration level between adult leaves and rhizomes.

Figure 4.4: Average concentration of (a) carbon, (b) nitrogen and (c) phosphorus (as % of dry weight) in the Autumn (October). Differences among rubble mounds crests, valleys and a control site are reported as well as variations between A, I & J leaves, rhizomes and roots. Error bars represent ± SE (N = 6).

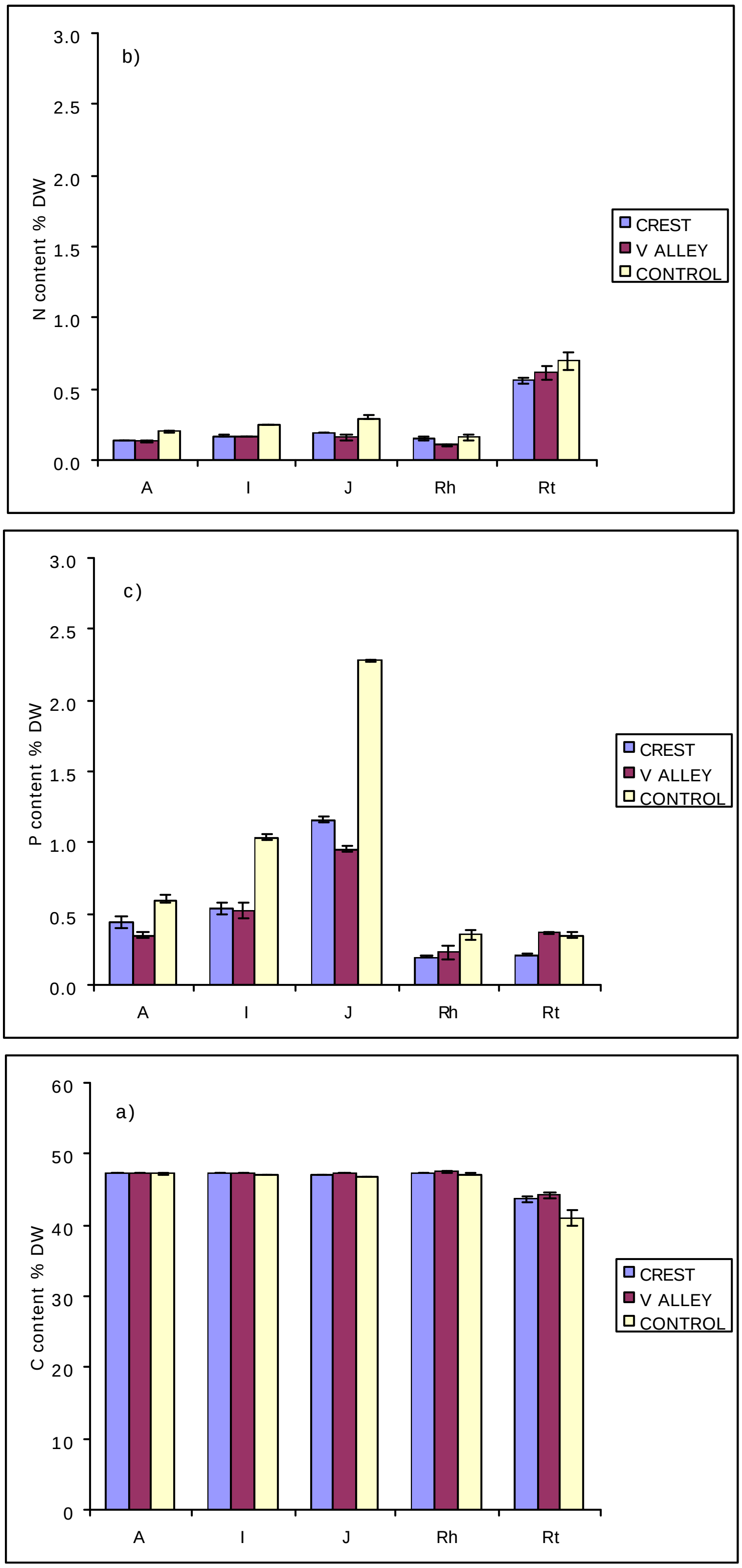


Figure 4.5: Average concentration of (a) carbon, (b) nitrogen and (c) phosphorus (as % of dry weight) in the spring (May). Differences among rubble mounds crests, valleys and a control site are reported as well as variations between A, I & J leaves, rhizomes and roots. Error bars represent ± SE (N = 6).

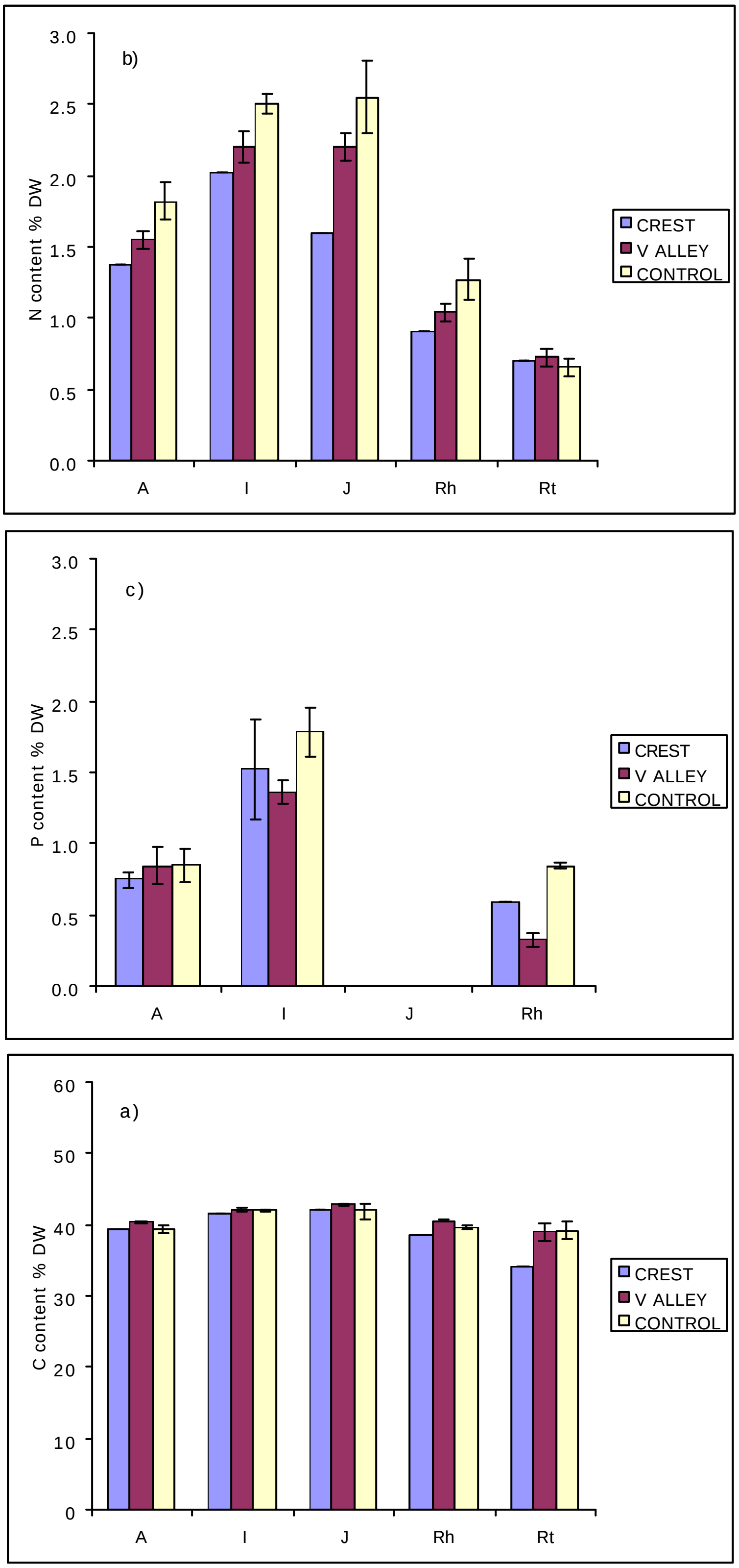
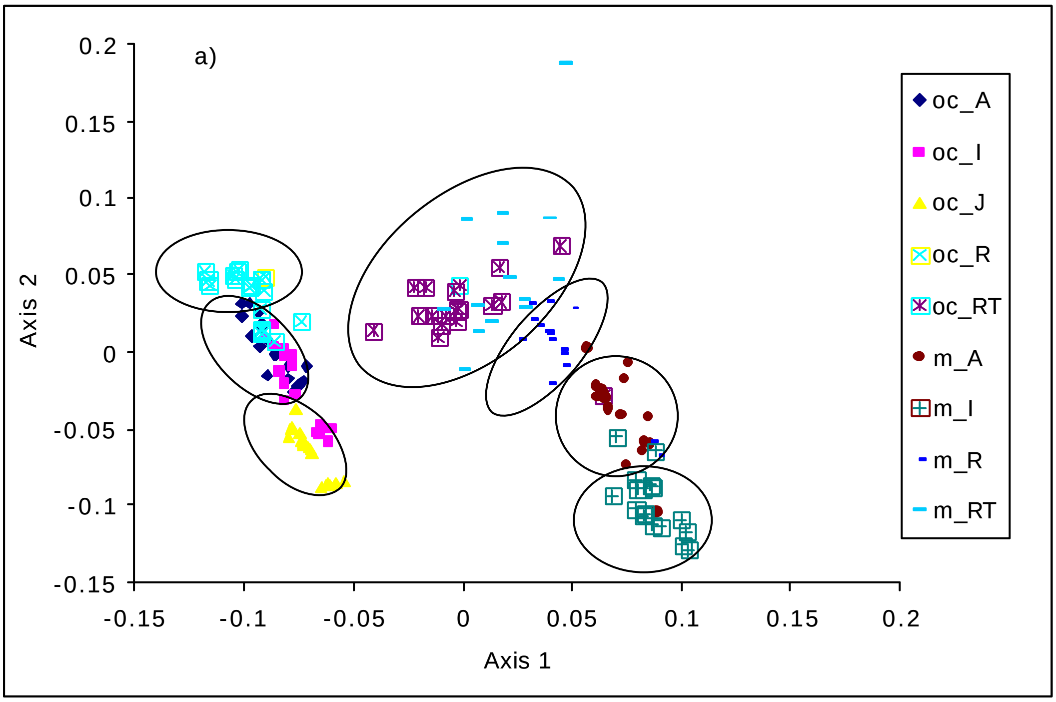


Figure 4.6: Constrained ordination of differences in nutrient content (C, N and P) among 9 groups including time (October and May) and plant tissue (A, I and J leaves, rhizomes and roots). The plot of the first two canonical axes produced by CAP shows apparent differences between October and May and an decreasing nutrient content from leaves to rhizomes and then to roots; oc = October, m = May, R = rhizome, RT = root.



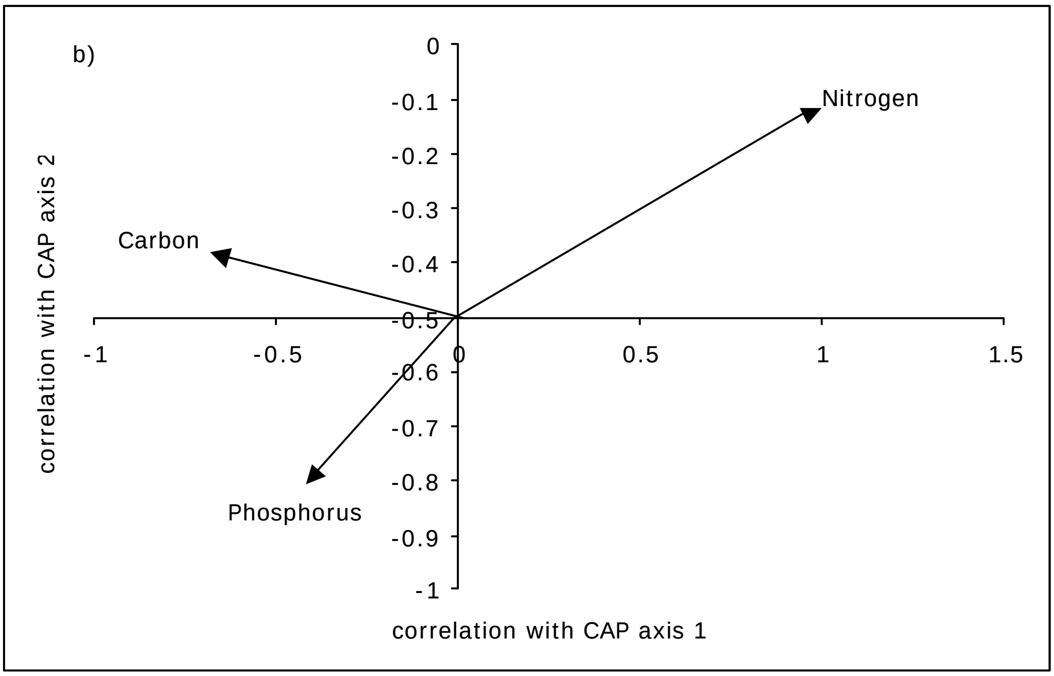


Table 4.4: NP-MANOVA results of nutrient content (C, N, P) in leaves, rhizomes and roots of plants living on rubble mounds and the pristine *P. oceanica* meadow. Ti = time, Lo = location, Pp = plant tissue.

Source	df	EMS	Perm.units	SS	MS	F	P(perm)	P(MC)
Time	1	RES	180	48.74	48.74	2624.69	0.001	0.001
location	2	RES	180	1.57	0.78	42.25	0.001	0.001
plant part	4	RES	180	13.64	3.41	183.68	0.001	0.001
TiXLo	2	RES	180	0.52	0.26	14.09	0.001	0.001
TiXPp	4	RES	180	18.71	4.68	251.89	0.001	0.001
LoXPp	8	RES	180	1.09	0.14	7.36	0.001	0.001
TiXLoXPp	8	RES	180	1.22	0.15	8.22	0.001	0.001
RES	150			2.79	0.02		0.001	0.001
TOT	179			88.29				

Data transformed to fourth root

No standardisation

Analysis based on Gower dissimilarity

Unrestricted permutation of raw data

Integer used as seed = 5

No. of permutations used = 999

Table 4.5: C:N, C:P and N:P yearly average values in *P. oceanica* leaves, roots and rhizomes on mound crests and valleys and on the control site.

C:N			
	leaf	rhizome	root
Crest	79	37	48
Valley	72	39	50
Control	56	31	61
C:P			
Crest	2253	6212	3894
Valley	2758	6219	3998
Control	1230	4033	4149
N:P			
Crest	28	186	56
Valley	38	198	66
Control	22	102	83

4.5 DISCUSSION

Total sediment depositional rates showed a temporal trend with low depositional rates through the winter while spring and early summer where characterized by a high sediment deposition. Although, storm events commonly occur from November to June, sediment data indicate that episodic events involving rough seas might have been stronger from early spring to summer. Particle concentration in the water column varied between the rubble field and the pristine *Posidonia* bed, indicating that resuspension on the rubble mound field is significantly higher. This confirms the capacity of *P. oceanica* to stabilise sediments as it slows current velocity and increases the roughness height of the bottom boundary layer (Gacia et al. 1999), potentially increasing particle trapping (Gacia et al. 1999). A further difference was detected between valleys and crests, where the former presented significantly higher depositional rates suggesting that sediment resuspension in such areas is prevented by roots and rhizomes which hold in place sediment and stabilise the substratum. On crests the absence of the canopy favours sediment resuspension into the water column. Previous work (Eckman et al. 1989, Gacia et al. 1999) stated how resuspension was greatest where canopy height was at its minimum. Low depositional rates in the control meadow are probably attributable to reduced turbulence within the *P. oceanica* canopy which buffer resuspension, as previously reported by Gacia & Duarte (2001). Total deposited sediment at Capo Feto in the rubble field reached a maximum value of 200 g DW m^{-2} y^{-1}, a value that is one order of magnitude smaller than those found in seagrass beds growing on sandy environments (Gacia & Duarte 2001 and references therein). However, deposition values presented in this study are in accordance with values reported by Gacia & Duarte (2001) for a Spanish *P. oceanica* meadow. Thus, it can be inferred that depositional rates do not represent a limiting factor for *P. oceanica* growth.

Differences in total deposited sediments reflect differences in porewater nutrient concentrations among locations. Valleys showed the highest porewater nutrient concentration of all locations. This is probably due to the thicker sediment layer present. Previous work has given evidence of how dense seagrass roots and rhizomes are linked to elevated nutrient concentrations in the porewater (Erftemeijer & Middelburg 1993, McGlathery et al. 2001). This can be attributed to organic matter decomposition in the sediment (Short et al. 1985; Erftemeijer & Middelburg 1993; Jensen et al. 1998). However, results from this study show N and P concentrations in porewater to be comparatively low, compared to previous published data of seagrass beds in terrigenous sediments (Fourqurean et al. 1992). In particular, N concentration in pore water sediment was found to be an order of magnitude lower than those expected in seagrass environments. In an earlier study, Duarte (1990) investigated growth requirements of *P. oceanica* and indicated an average of 1.8 % DW nitrogen to suffice for balanced seagrass growth. This value was then confirmed by a study on several Spanish seagrass populations by Alcoverro et al. (1995), where average N concentration in plant leaves ranged between 1.75 and 2.4 % DW. At Capo Feto, leaf nitrogen concentration varied from 0.4 in the Autumn to 2.5 % DW in the Spring. This finding suggests N is available for plant growth in the spring, while N content in the leaves is depleted through the Summer due to the high growth rates and leaf senescence (Stapel & Hemminga 1997). However, the significant differences among locations indicated a diverse N availability between the rubble environment and the pristine bed. In the rubble field, plants living in valleys showed an average leaf N content of 1.9% in the Spring, a value that cannot be considered as limiting for plant growth (Duarte 1990). On the other hand, plants living on crests showed a lower leaf N content (Spring average 1.6%) hence N availability might not suffice for plant demand. In the undisturbed pristine bed, N leaf content appears to be well above the average N requirement for plant growth indicated by Duarte (1990). Differences in N content between plants living on crests and valleys can be attributed to rubble topography and the presence/absence of a sediment layer above the substratum. Although seagrasses may obtain nutrients from the water column by leaf uptake (Fourqurean et al. 1992, McGlathery et al. 2001) and that there may be considerable resorption of nutrients before leaf senescence (Hemminga et al. 1991), in valleys and in the control site the primary source of nutrient uptake for *P. oceanica* is likely to be in the root system, as indicated by the higher nutrient concentration in sediment porewater than in the water column. On the other hand, plants living on crests have to rely exclusively on leaf nutrient uptake from the water column. N limitation in the rubble environment is in accordance with data from Fourqurean et al. (2001) and Smith (1984) who concluded that seagrasses in temperate marine ecosystems have higher P for growth, but lack N. This concept also applied to Mediterranean populations as proven by Pirc and Wollenweber (1988) on a *Posidonia oceanica* study in Ischia (Gulf of Naples). These authors described how the possibility of N storage in the rhizomes seems to be limited in temperate species, this suggests that plant demand for nutrients are high as stocks are quickly depleted by the roots (Touchette & Burkholder 2000).

The results indicated a synchronous increase in seagrass tissue of N and P during Winter, with possible maximum concentration in late Winter early Spring. From Spring to late Summer, values may drop to minimum levels due to the high seagrass growth rates during this season and leaf senescence. Moreover, Pirc & Wollenweber (1988) reported on higher N concentration in young and intermediate leaves than in old leaves (Pirc 1985). The Canonical analysis of principal coordinates confirms this finding and highlights a decreasing N-concentration from younger leaves to older leaves. Nutrient limitation for Mediterranean seagrasses has been proposed in the past (Pérès & Romero 1992), and it can be linked to the solar cycle (Alcoverro et al. 1995). Indeed, the solar cycle controls water temperature and light availability hence controlling local factors such as nutrient availability (Alcoverro et al. 1995). While N and P contents in seagrass tissue varied over time, C concentration showed little variation through the year with a lower seagrass C content in the spring at all locations. This confirms comments by Hellblom et al. (2001), suggesting that carbon limited seagrass growth is rare, due to the high carbon availability in seawater. Moreover, *P. oceanica* can store carbon resources in its roots and rhizomes (Pirc 1985), hence substantial growth rates during the winter can be supported at the expense of starch accumulation (Pirc 1985, Alcoverro et al. 1995).

The ratio between nitrogen and phosphorus content to carbon content can also provide information about seagrass nutritional status.

Spatial and temporal variations in C:N:P ratios have been previously adopted as indicators of seagrass nutrient content, especially N (Fourqurean et al. 1992, Touchette & Burkholder 2000). Duarte (1990) described how high C:N and C:P rations reflect nitrogen and phosphorus limitation in plant tissue. This implies that C:N and C:P ratios should shift from high to low as nutrients become more available for plant growth (Duarte 1990). At Capo Feto, C:N in the leaves was found to be higher in the rubble field (76) than in the control bed (56). A limitation in nitrogen availability and substratum characteristics might explain the high root biomass, as earlier studies have proven how root biomass allocation increases with decreasing nutrient availability (Terrados et al. 1999). Additionally, the C:P ratio was also higher in the rubble field than at the control seagrass meadow with an average value of 2506 in the leaves and higher values in the rhizome and root. This finding does not compare well with values suggested in other papers addressing seagrass nutrient ratio (Duarte 1990, Fourqurean et al. 1992, Touchette & Burkholder 2000). The higher C:P ratio in rhizomes and roots would suggest that the P allocation is greater in the leaves than in the rhizomes (Terrados et al. 1999). In his review of seagrass nutrient content, Duarte (1990) suggested a median C:N:P ratio of 474:24:1, a value lower than those previously indicated for marine macrophytes (Atkinson & Smith 1984). However, in this study I found a higher C:N:P ratio of 3157:61:1 representing high nutrient depletion in the rubble mound environment. In contrast, *P. oceanica* growing on matte on the pristine meadow presented a lower C:N:P ratio than the rubble environment. This finding indicates a higher nutrient availability for seagrass growth within the matte structure

In summary, the results presented here show how the presence of the canopy in valleys favours particle settlement, so contributing to the formation of a sediment layer which provides the necessary nutrients for plant growth. However, the rubble topography might play an important role in seagrass resource allocation to the different biomass compartments. Although N and P content found in the valleys cannot be considered as limiting for seagrass growth (Duarte 1990), plants might still experience some nutrient limitation. In contrast, sediment deposited onto mound crests is resuspended as seagrass shoots are mostly absent. This suggests that the plants living on crests are resource-limited. Total deposited sediment varied between locations leading to differences in porewater nutrient concentration. Higher N and P were found in interstitial water in the valleys and their concentrations seem to meet plant demand. Consequently, the primary source of nutrient for *P. oceanica* patches growing in valleys and controls appear to be porewater. On crests, patches have to rely exclusively on leaf uptake from the water column hence they experience a limited nutrient availability. This would also explain the limited resources found in the roots.

The work presented in this chapter confirms the findings of Pirc & Wollenweber (1988), Duarte (1990), Fourqurean et al. (1992), Alcoverro et al. (1995) and Touchette & Burkholder (2000) that N rather than P is limiting to seagrasses in temperate zones. N-limitation in *P. oceanica* is in accordance with the results of this study, which pointed out high C:N and C:P ratios. Nutrient limitation might be related to substratum type (rubble) where decomposition and sediment availability are low. Thus, nutrient-limited rocky seabeds might determine reduced growth and peculiar morphological features of *Posidonia oceanica*.

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CHAPTER V

STRUCTURAL AND MORPHOLOGICAL FEATURES OF *P. OCEANICA* IN THE RUBBLE MOUND ENVIRONMENT

5.1 INTRODUCTION

Seagrass communities play two primary roles in coastal ecosystems: (1) a trophic role, through their direct contribution to ecosystem primary production (Zieman 1982, Thayer et al. 1984) and (2) a structural role (Kenworthy et al. 1988), as they provide settlement surfaces for epiphytic organisms (Borum & Wium-Andersen 1980). Thus, they sustain a highly diverse biota (Kenworthy et al. 1988, Duarte 1989). These two roles are coupled mainly because of the small consumption of seagrass tissue, resulting in the accumulation of biomass, which might determine seagrass structural importance (Thayer et al. 1975, Duarte 1989). *Posidonia oceanica* (L.) Delile is, together with some other Australian *Posidonia* species (i.e. *P. australis*), one of the most productive marine phanerogams (Sand Jensen 1975, Mc Roy & McMillan 1977, Pergent et al. 1994, Duarte 1989). *P. oceanica*, with its leaf epiphytes, plays a major role in benthic primary production in the Mediterranean Sea (Romero 1989, Pergent et al. 1994, Pergent et al. 1997). A small amount of such production is consumed on the plant by macro-herbivores [(i.e. the sea urchin *Paracentrotus lividus (*Bulteel et al. 1992)] and fish [i.e. *Sarpa Salpa* (Harmelin-Vivien & Francour 1992) (Plate 5.1)]. However, most of the production goes into litter when leaves are shed (Pergent et al. 1994). Although the importance of *Posidonia oceanica* in Mediterranean ecosystems is now largely recognised (Romero et al. 1992, Hemminga & Duarte 2000, Green & Short 2003), anthropogenic pressure is causing a wide spread decline of Mediterranean coastal habitats, which is leading to a deterioration of water quality and shoreline erosion (Hemminga & Duarte 2000, Green & Short 2003). Evaluation of such degradation is complex due to the paucity of long term records on water quality and sediment dynamics for this region. As a consequence, there is an increasing interest in assessing a valid biological indicator of marine environmental quality (Pergent et al., 1995, Marbà & Duarte 1997, Guidetti 2001).

Plate 5.1: The seagrass grazer *Sarpa salpa*.

Posidonia oceanica is the dominant seagrass species in the Mediterranean sea and ranks among the longest lived seagrasses (Duarte 1991), with vertical rhizomes living more than 30 years and clones living over millennia (Guidetti 2001). Such longevity renders this species an appropriate indicator of environmental changes (Marbà et al., 1996, Guidetti and Fabiano, 2000). Changes in distribution patterns, homogeneity of cover, abundance at the lower limits of its depth distribution, density, partitioning of the total biomass in the different plant components (leaves, rhizomes and roots) and dynamic features, primary production, rhizome growth, demography of seagrass communities have been widely used to obtain an integrated response to disturbances (Neundorder & Kemp 1993, Pergent et al., 1995; Marbà and Duarte, 1994;

Marbà et al., 1996; Short and Wyllie-Echeverria, 1996; Guidetti and Fabiano, 2000). Furthermore, changes in annual leaf production are reported to largely reflect variations in the characteristics of the water column (Dawes and Tomasko, 1988; Neundorder and Kemp, 1993). However, *P. oceanica* is not only exposed to alterations in environmental conditions according to water depth gradients or biogeographical process, but also to considerable variation in topography, resource availability (i.e. nutrients) as well as an extensive range of biological and physical disturbance (Duarte 1991, Marbà et al. 1994, Robbins & Bell 1994, Balestri 2003).

Due to the plasticity of seagrass rhizome growth modules, and in particular of those of *P. oceanica*, seagrasses have the potential to adapt their form and function to the immediate environment (Marbà & Duarte 1998). Thus, it can be expected that *P. oceanica* will adapt in accordance to the spatial scale of environmental heterogeneity (c.f. van Tussenbroek 1995, Hemminga & Duarte 2000). Such patterns have been previously reported for terrestrial plants, with special emphasis on how land plants allocate their biomass to different components in accordance to resource availability (Lieth 1974, Lechowitcz 1984). Such work has inspired seagrass researchers to assess how seagrasses allocate their biomass in nutrient depleted environments or in physically disturbed areas. Balestri et al. (2003) assessed variation of *P. oceanica* biomass in relationship to large scale habitat changes such as differences in substratum type and sediment characteristics. At Capo Feto, *P. oceanica* living on the rubble environment appears to be nutrient limited as concluded in chapter 4. Nutrient limitation appears to be mainly due to substratum type (rubble) where decomposition rate and sediment availabity are low. Thus, nutrient limited rocky seabed might determine *P. oceanica* growth patterns and resource allocation to the below and above ground biomass (Hillman et al. 1989, Duarte et al. 1998). Previous work by Duarte & Chiscano (1999) indicated how seagrass biomass and production were kept below their potential maximum by resource limitation or by physical disturbance. In addition, several other authors (Short 1983, Agawin et al. 1996, Udy & Dennison 1997) report that as nutrient availability in the sediment increases, allocation of resources is shifted towards above ground biomass and rhizome growth, with least amount of resources distributed to the roots (Terrados et al. 1999). Although Duarte & Chiscano (1999) reported a roughly equal allocation of biomass to above- and below-ground components, below-ground biomass often dominates the total plant biomass of seagrass communities (below ground to above-ground biomass ratio >1; Duarte et al. 1998). However, studies on seagrass biomass have often neglected below-ground biomass and production, and only recently has the importance of this compartment been examined and its distribution related to sediment properties (Duarte et al 1998, Duarte & Chiscano 1999). At present, no studies are available on *P. oceanica* above- and below-ground biomass and production in relationship to artificial substrata and seascape heterogeneity.

This chapter reports on *P. oceanica* morphological variations between the rubble field and a pristine meadow growing on matte at two depths. These two environments (rubble vs matte) present a different type of substratum, hence involving a different utilisation of the root system. It was hypothesised that a difference partitioning of the total biomass in the different plant components and leaf production between the rubble field and the pristine meadow would be seen. Differences in phenological features of *P. oceanica* as well as biomass and production within the rubble field, crests-sides vs valleys, due to differences in sediment and resources availability between such locations were also predicted.

5.2 MATERIAL AND METHODS

5.2.1 Plant Phenology

Phenological parameters were measured to compare the difference between plants living on the rubble field and plants living on the pristine bed. Samples were collected in June 2001 at three depths (5, 10 and 15 m) on mound crests, sides and valleys and control site chosen in the nearby location of Tonnarella. At each location, 20 randomly chosen shoots were collected (Plate 5.2). Since *Posidonia oceanica* is a clonal plant, samples were taken at about 5-10 m distance from each other in order to obtain independent replicates. Samples were kept cool until arrival in the laboratory where they were frozen until processing.

Plate 5.2: Shoot collection for phenological analysis in the pristine meadow.

Once defrosted, shoots were rinsed using tap water and leaves were stripped from each shoot in distichous order of insertion and epiphytes were removed by scraping each leaf using a razor blade. The leaves were then separated into the various categories described by Giraud (1979): adult leaves (A; length greater than 50 mm with sheath), intermediate leaves (I; length greater than 50 mm without sheath) and juvenile leaves (J; length less than 50 mm without sheath). For each leaf, total length and width was measured as well as sheath and the brown tissue. The percentage of leaves that had lost their apex due to herbivore grazing was also recorded. Such measurements allowed calculation of the following phenological parameters:

- Mean number of leaves (A, I, J) per shoot;
- Leaf total length and width;
- Mean leaf surface for A, I and J;
- Mean leaf photosynthetic surface for A, I and J;
- Mean leaf area index (LAI) for A, I and J.
- Mean canopy height

Leaves were then weighed and dried to constant weight at 60 °C. Once dried, leaves were re-weighed for comparison between wet weight (WW) and dry weight (DW).

5.2.2 Biomass Partitioning

Biomass was sampled by removing clods within quadrats 40x40 cm at 5 and 10 m depth on the mounds (crests, sides and valleys) and in a control site. For each location, 3 random sites were chosen, within which six independent replicates were collected. The above ground portion was subdivided into leaf blades, bases and brown tissue. The below ground biomass was divided into dead and living rhizomes, dead and living roots. Samples were rinsed to remove detritus and sediment. To determine the dry weight (DW) of each sample, the various biomass compartments were dried to constant weight at 60 °C.

5.2.3 Leaf Production

Production was estimated using the Zieman method (1974). Six stations were chosen randomly in mound valleys and six in the control bed in June 2003 at 10 m depth. Stations were set up using 1 m^2 quadrats within which shoot density was recorded (Plate 5.3).

Plate 5.3: Quadrates set up for the production assessment using the Zieman method (1972).

Each shoot within the quadrate was punched by pushing a hypodermic syringe needle into the leaf bundle 3 cm above the ligula. These leaves were marked at the same time by holding the entire leaf bundle and pushing the needle through the leaves. After all the shoots within the quadrat were marked, a surveying flag was placed into the sediment next to the quadrat so that it could be relocated. Shoots were generally collected after allowing a sufficient period of time interval for new leaf growth, a period of time that needs to exceed one plastochrone interval (Short & Duarte 2001). In this experiment, marked shoots were collected after 68 days (July 2003). The plants were harvested and individual replicates were placed in bags and transported in cool dark conditions to the laboratory for analysis. For morphology measurements, 2 shoots from each sample were arbitrarily selected. The leaves from each of these shoots were cut with a razor blade at the leaf sheath/leaf interface (ligula). Each leaf was then arranged in age-order. The base-mark length is the distance from the razor blade cut (original leaf marking position) to the base-mark (syringe needle hole).

As the leaves grow, this base-mark will be located further from the original marking location (leaf sheath/leaf interface). Leaves that emerge past the base-mark position after the original marking will not have a base-mark. These innermost unmarked leaves are considered "new leaves (n)". After recording the length, width, and base-mark length for each of the leaves on the two shoots, leaves were cut again at the position of the needle hole. The cut leaves were separated into 2 groups. New leaves and the rectangular growth segments (the leaf portions between the original marking position and the needle hole) are one group labelled as "growth".

The other group comprises those leaf segments from the base-mark (needle hole) to the leaf tip. This second group is called the "old standing crop" (OSC). The 2 groups (growth and OSC) were packaged into numbered pre- weighed aluminium foil tare envelopes, their tare weights having been recorded. These packaged leaf segments were dried in a drying oven at a temperature of 60 °C. When the samples were completely dry, the gross dry weights were recorded. Net leaf weights of "growth" and "OSC" leaf segments were determined by subtracting the foil tare weights from the gross dry weights.

From these measurements, the following parameters were calculated:

- Areal leaf biomass (standing crop), g cm^{-2} = (net "growth" + net "OSC")/ area of quadrat;
- Leaf productivity per shoot, g $shoot^{-1}$ day^{-1} = net "growth"/(N. of shoots X growth period);
- Areal leaf productivity, g cm^{-2} day^{-1} = net "growth"/(area of quadrat X growth period);
- Mass-specific leaf productivity, g g^{-1} day^{-1} = net "growth"/[(net "growth" + net "OSC") X (growth period)];
- Plastochrone interval, days = (total number of marked shoots/number of newly initiated leaves) X growth period.

5.2.4 Data Analysis

Phenological variables were analysed using the FORTRAN Programme NP-MANOVA (Anderson 2003a). The experimental design included two factors and 18 variables (number of leaves (A, I, J) per shoot; Leaf mean total length and width for A, I, J leaves; leaf surface for A, I and J leaves; leaf photosynthetic surface for A, I and J leaves; leaf area index (LAI) for A, I and J leaves).

Factor one was depth (3 levels) and factor two was location (4 levels). Both factors were considered fixed and orthogonal. After pair wise comparisons, a CAP (canonical analysis of principal coordinates; Anderson 2003b) and an MDS (multidimensional scaling) plot were used to highlight trends in the distribution of data. The MDS plot was built using the multivariate PRIMER package (Clarke & Warwick 1994).

A multivariate NP-ANOVA was applied to the biomass data set with the following design: factor 1, depth, 2 levels, fixed and orthogonal; factor 2, location, 4 levels, fixed and orthogonal; factor 3, site, 3 levels, random and nested in location. The analysis tested differences among 3 variables: leaf, root and rhizome biomass. Analysis was carried out using the statistical packages XMATRIX AND DISTLM (Anderson 2003c,d). Moreover, constrained CAP, and an unconstrained, PCA (Principal Coordinates Analysis), ordinations were used to plot data distribution in relationship to location and depth. Whenever necessary all data sets were opportunely transformed (Underwood, 1997).

Differences in leaf production between valley and control were tested with a one-way ANOVA with the factor location (2 levels) fixed and orthogonal. Homogeneity of variance was checked using Cochran’s test (Snedecor & Cochran 1989). When significant differences were found in the ANOVA, they were compared *a posteriori*, using a Student-Newman-Keuls (SNK) test (Underwood 1981, 1997).

5.3 RESULTS

5.3.1 Plant Phenology

The results indicated a distinct pattern with highest values of leaf length, width and leaf surface found in the control and decreasing as a trend through valleys, sides and crests. Both the constrained (CAP) and unconstrained (MDS) ordination methods showed how values for all variables for the control site were distinct from the rubble field (Fig. 5.1 & 5.2). Confidence in this pattern is supported by the low MDS stress values (Fig. 5.2).

The mean number of leaves per shoot did not vary significantly (Table 5.1, NP-MANOVA test) between the side, valley and control samples (range between 5 and 6 leaves per shoot), but a significantly lower number of leaves per shoot was recorded on crests (4±0.5). This trend was consistent at all depths (5, 10 and 15 m), which did not differ significantly (Table 5.1). The mean number of leaves per shoot belonging to different age classes differed significantly among locations and depths (Table 5.1). At 5 m depth, the mean number of A, I and J leaves for the control site and the valleys were found to be similar while they were significantly higher than values for the side and crests (CREST=SIDE<VALLEY=CONTROL; Fig. 5.3a). At 10 m depth, the mean number of I leaves was found to be significantly higher on crests and valleys than on control and side. In contrary, average values for A and J leaves were reported to be significantly higher on control and valleys than crests and sides (Fig. 5.3b). At 15 m depth, average number of I leaves was significantly higher on the rubble field (CREST=SIDE=VALLEY) than on the pristine meadow. However, at the control site the mean number of

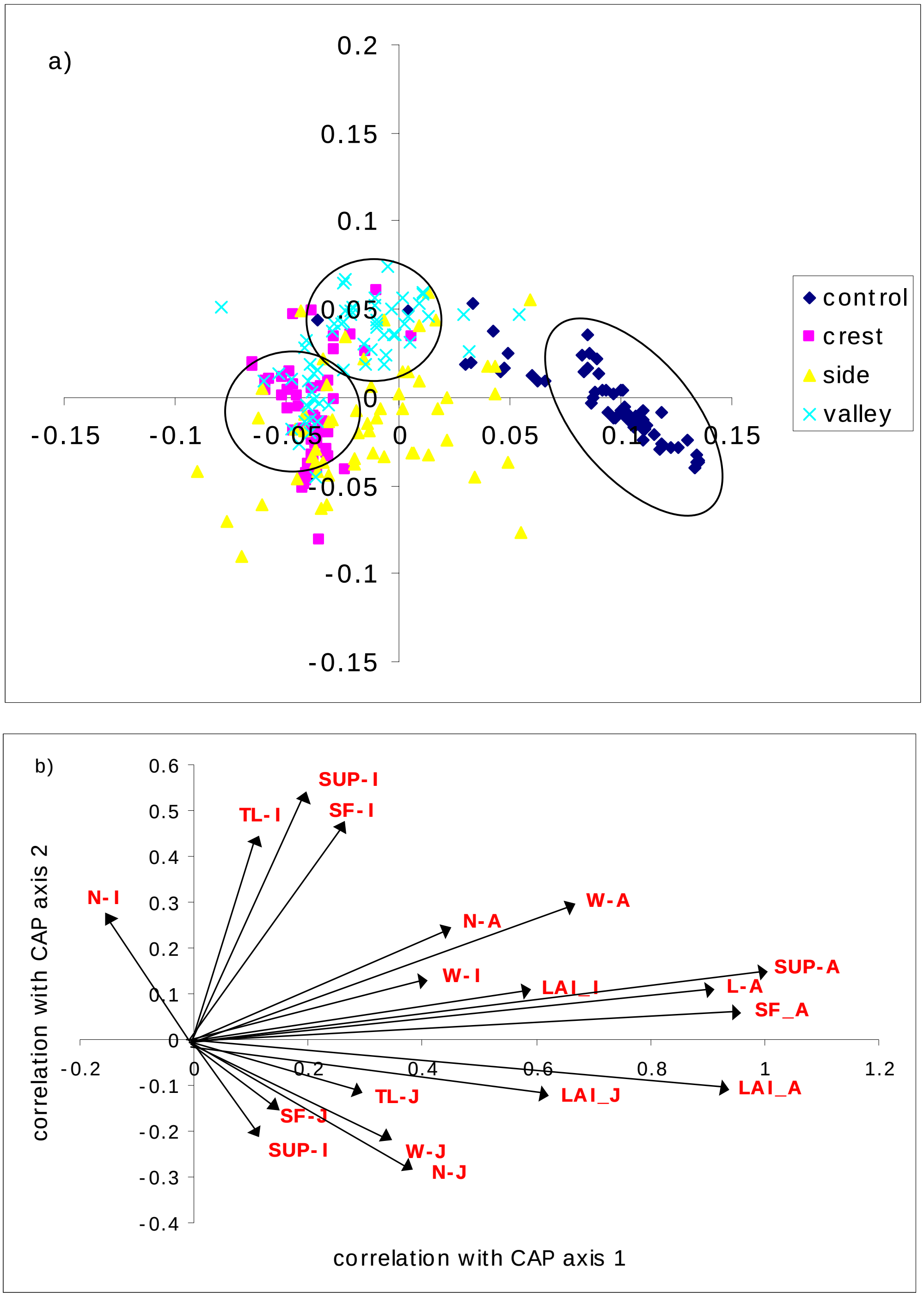
A and J leaves per shoot was significantly higher than in crests, sides and valleys which all presented similar values (Fig. 5.3c).

Canopy height was found to be higher in the pristine meadow than on the rubble field. Maximum leaf length in pristine meadow ranged between 54.2 cm and 69.4 cm, whilst rubble field values ranged between 10.9 and 29.4 cm. Multivariate analysis of variance showed highly significant differences in the lengths of different leaf age classes of *P. oceanica* at different locations (Table 5.1) and depths. At 5 m depth, A and I leaf length did not differ significantly between locations in the rubble field, but were significantly lower than the control site (Fig. 5.4a). The same trend was present at 10 m depth where the pristine bed showed significantly higher values of A, I and J leaf length than the rubble field, within which locations did not vary significantly (Fig. 5.4b). At 15 m depth, a significantly higher A and I leaf length was recorded between valleys crests and sides (Fig. 5.4c). Moreover, average A and I leaf length in valleys appeared to be significantly lower than values found at the control site. No significant difference was found for J leaf length at 5 and 15 m depth at all locations.

The shoot surface area (for all leaf age classes) was found to be highest at the control location than in the rubble field. At 5, 10 and 15 m depth, average shoot surface area for A and I leaves on crests and sides did not vary significantly (Table 5.1), even if they had significantly lower averages than valleys (Fig. 5.5). No significant difference in shoot surface area for juvenile leaves was found between depths at all locations.

Leaf area index (LAI) showed considerable differences between locations within the rubble mound field and between the rubble field and the control bed. LAI is a parameter correlated to shoot surface area and shoot density, hence it reflects differences not only in leaf length and width but also in seagrass shoot density. The MDS plot (Fig. 5.2) points out how values for the control are distanced from values recorded in the rubble field. Most of the difference between the two environments is due to differences in the leaf area index. Mean total LAI (average for all depths) was higher in the pristine bed (3.4±0.3 m^2m^{-2}) than in the rubble mound field (0.11±0.01 m^2m^{-2}). Valleys showed significantly lower values than the average LAI for all leaf age classes in the control site (Table 5.1). However, valleys showed significantly higher LAI values than crests and sides for all leaf age classes with values ranging between 2.4 x 10^{-4} for J leaves at 10 m depth to 0.96 for A leaves at 5 m depth (Fig. 5.6). This pattern was consistent at all depths.

A decreasing trend for phenological parameters between 5 m depth to 15 m depth and shoot density was hypothesised. However, as indicated in chapter 3, the large variability within the rubble mound field does not allow the detection of a clear trend. The NP-MANOVA detected no significant difference between 5 and 15 m depth, which in turn were found to be significantly higher than at 10 m depth.



N-A,I,J = Number of leaves (A, I, J); TL-A,I,J = Total leaf length (A, I, J)
W-A,I,J = Leaf Width (A, I, J); SUP-A,I,J = leaf surface (A, I, J)
SF-A,I,J = Leaf Photosynthetic surface (A, I, J); LAI-A,I,J = Leaf area index (A, I, J)

Figure 5.1: (a) Constrained CAP ordination including 18 variables on plant phenology at different locations (control, crest, side, valley). The plot of the first two canonical axes produced by CAP shows differences among the rubble field and the control bed as well as differences among locations within the rubble environment. (b) Correlations of several original variables with the two CAP axes of Fig 1a.

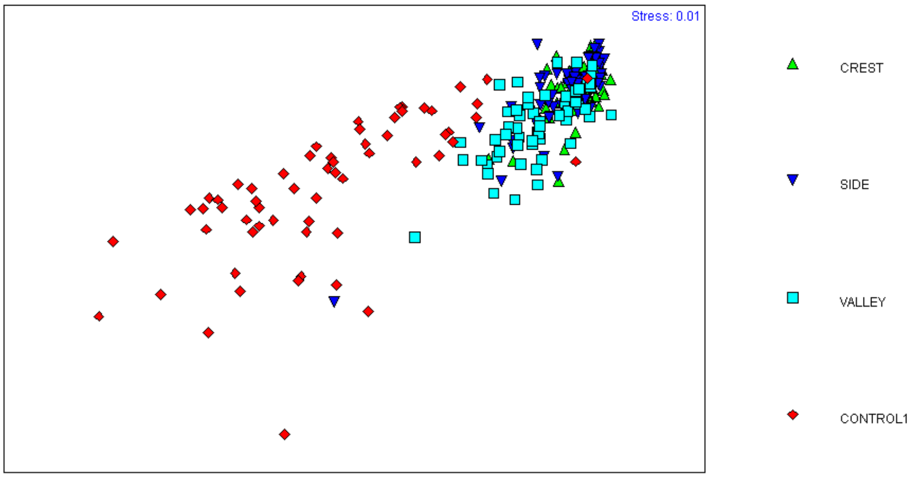


Figure 5.2: MDS ordination model of the different locations (crest, side, valley, control) for the 18 structural variables analysed.

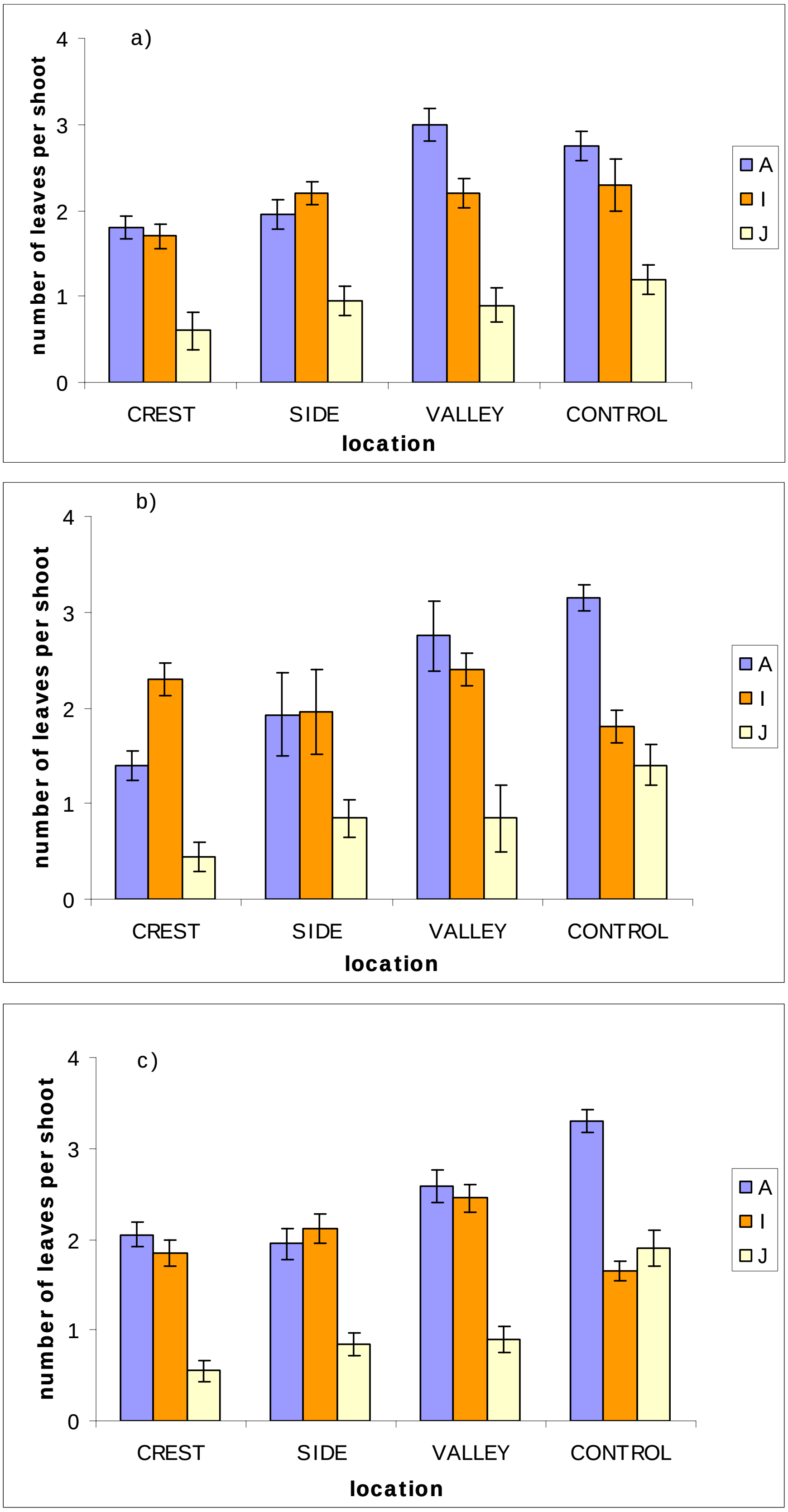


Figure 5.3: Variation in the number of different age classes of leaves (A, I, J) at different locations (crest, side, valley and control) at (a) 5, (b) 10 and (c) 15 m depth. N= 20; Bars indicate standard errors.

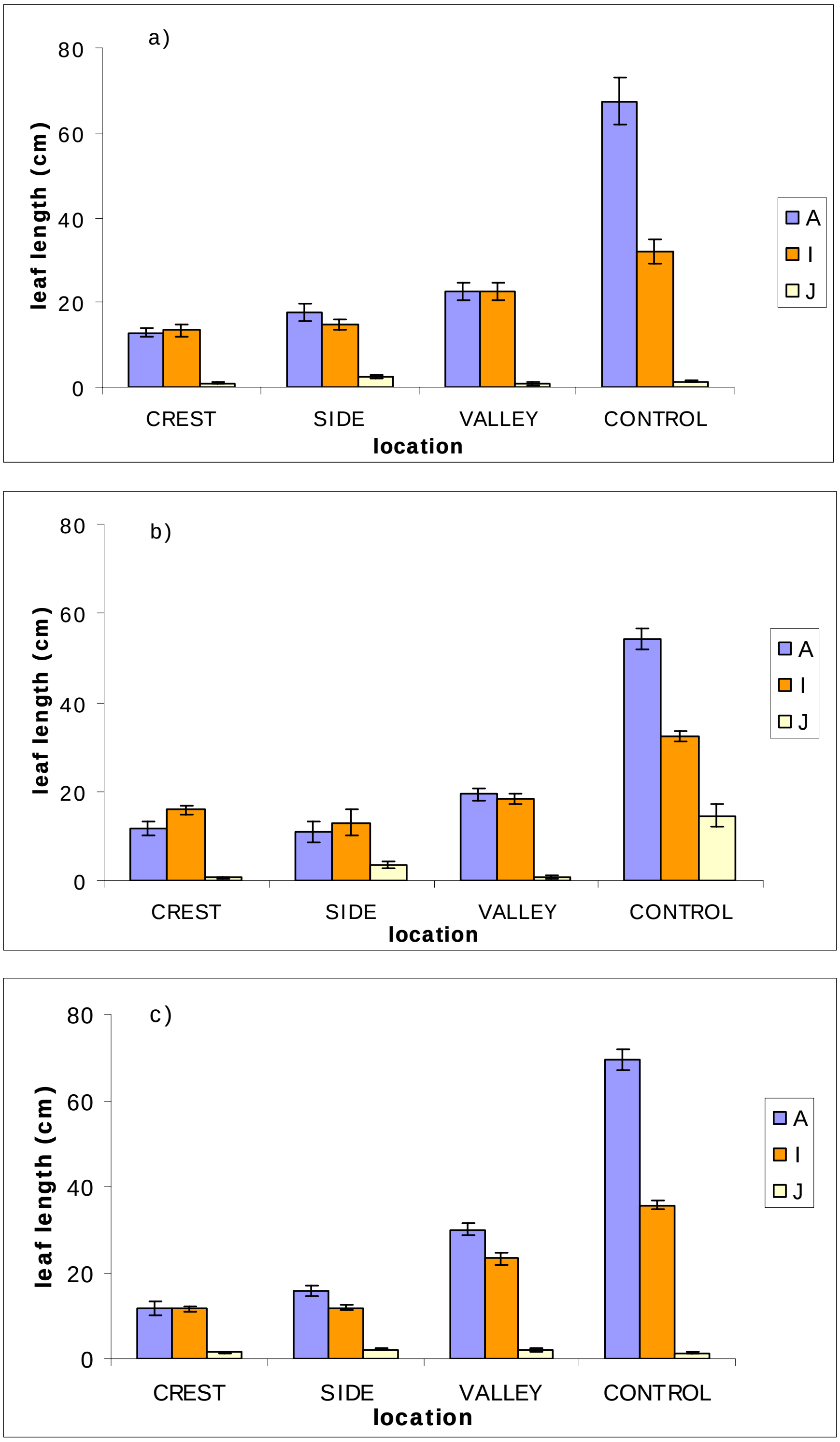


Figure 5.4: Variation of leaf length of different age classes (A, I, J) of *P. oceanica* at different locations (crest, side, valley and control) at (a) 5, (b) 10 and (c) 15 m depth. N= 20; Bars indicate standard errors.

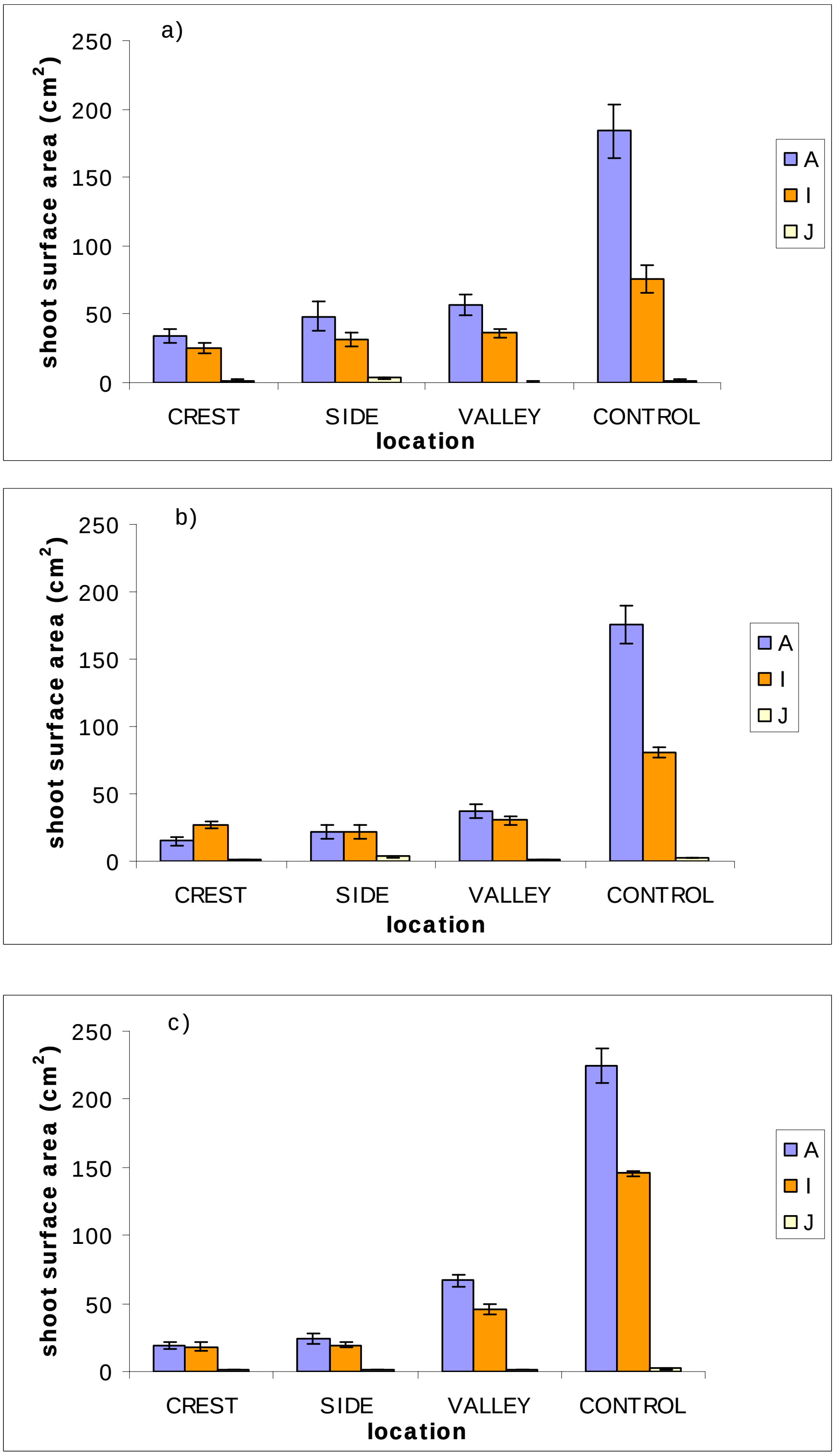


Figure 5.5: Variation of leaf surface per shoot of different age classes (A, I, J) of *P. oceanica* at different locations (crest, side, valley and control) at (a) 5, (b) 10 and (c) 15 m depth. N= 20; Bars indicate standard errors.

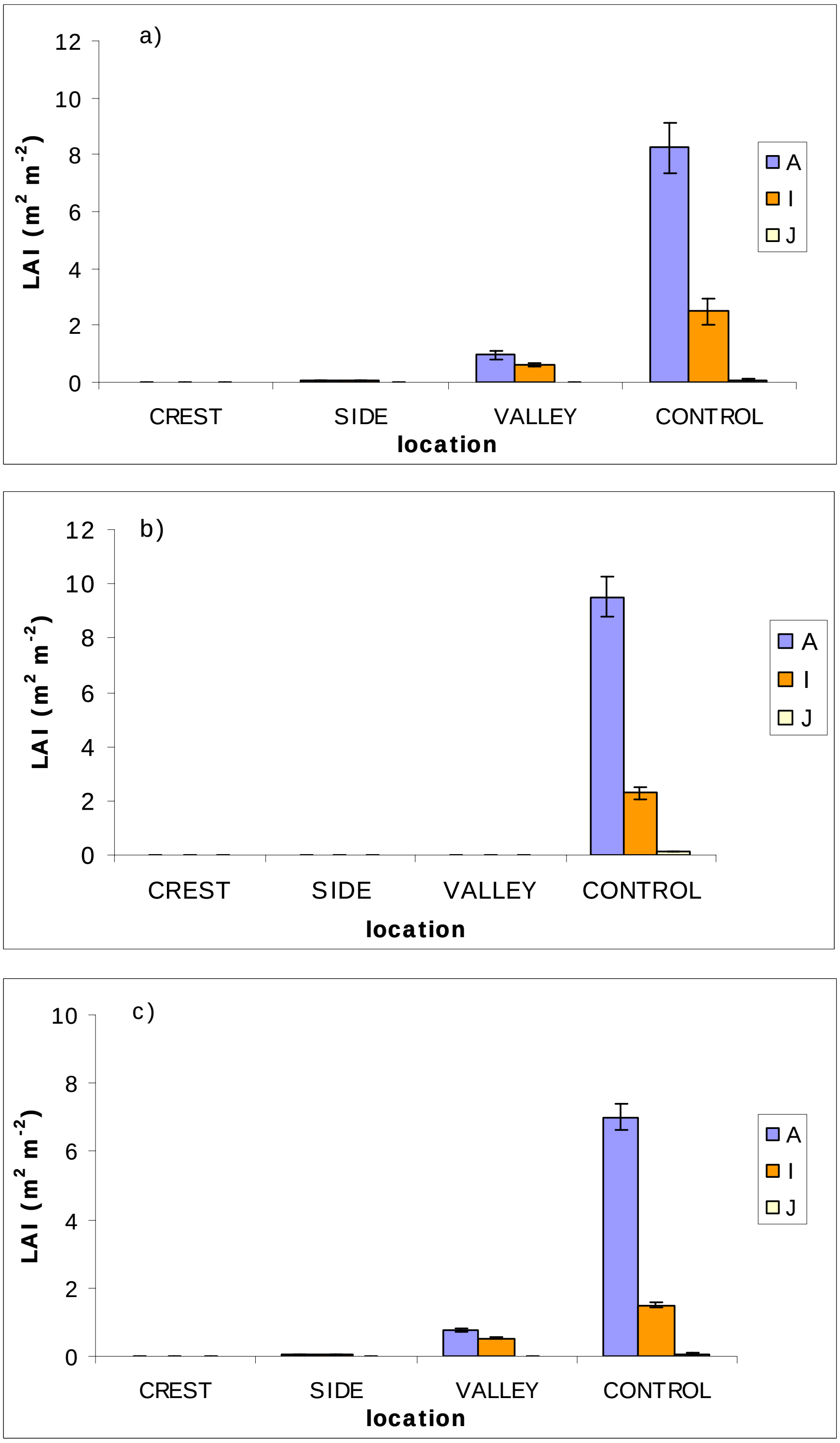


Figure 5.6: Leaf area index, LAI, per age classes (A, I, J) of *P. oceanica* at different locations (crest, side, valley and control) at (a) 5, (b) 10 and (c) 15 m depth. N= 20; Bars indicate standard errors.

Table 5.1: NP-MANOVA results of phenological characteristics of plants living on rubble mounds (crest, side and valley) and the pristine *P. oceanica* meadow. The analysis included 18 variables (number of leaves (A, I, J) per shoot; Leaf total length and width for A, I, J leaves; leaf surface for A, I and J leaves; leaf photosynthetic surface for A, I and J leaves; leaf area index (LAI) for A, I and J leaves)

Source	df	EMS	Perm.units	SS	MS	F-pseudo	P.perm
Depth (De)	2	RES	228	7962.8	3981.4	6.3299	0.0002
Location (Lo)	3	RES	228	147375	49125.1	78.1026	0.0002
DeXLo	6	RES	228	13896.1	2316.01	3.6822	0.0002
RES	228			143408	628.981		
TOT	239			312642			
No transformation			Integer used as seed = 5				
No standardisation			No. of permutations used = 4999				
Permutation of raw data			Analysis based on Bray-Curtis Dissimilarities				

5.3.2 Biomass Partitioning

The relative contribution of the above- and below-ground compartments varied among locations within the rubble field and between the rubble field and the control site.

At the control site, above- and below ground compartments were balanced in terms of biomass allocation, with average values of 56.7 and 43.3 % at 5 m and 46.1 and 42.5 % at 10 m (Fig. 5.7). In contrary, on the rubble field, allocation of biomass was found to be skewed towards the below-ground biomass (averages of 98.2 and 95.0 % respectively at 5 and 10 m depth), with little biomass found in the above-ground compartment (averages of 1.8 and 5.0 %) (Fig. 5.7).

CAP and PCA analysis show values of biomass for the pristine bed grouped together in a distinct cluster separate from that of the rubble field (Fig. 5.8). CAP ordination also indicated a distribution trend of locations within the rubble field (crests, side and valleys) along the Y axis (Fig. 5.8). The NP-MANOVA test reported significant difference between locations (Table 5.2). The results indicate a significantly higher overall biomass on the pristine meadow than in valleys. In turn, valleys showed significantly higher values than side and crests (Fig. 5.9). Moreover, biomass partitioning into the different compartment was found to differ for each location (Table 5.2). On the undisturbed meadow at 5 m depth, there was a significantly higher allocation of biomass to the rhizome (1343.7±281.6 g m^{-2}) than to the leaves (1036.5±112.2 g m^{-2}) which in turn was significantly higher than root biomass (14.7±2.5) (Fig. 5.9). A similar pattern was found at 10 m depth. At 5 m in the valleys, seagrass had significantly higher rhizome biomass (1519.2±407.3 g m^{-2}), with smaller values found for root biomass (194.4±96.7 g m^{-2}) and least biomass allocated to the leaves (27.5±2.3 g m^{-2}) (Fig. 5.9a). A similar biomass partitioning was found in the location 'side', where significantly higher values were reported for rhizome biomass at 5 m depth (436.3±88.4 g m^{-2}), root biomass averaged at 36.4±9.8 g m-2 while leaf had once again the smallest biomass allocation ranging between 2.5 and 26.2 g m^{-2}) (Fig. 5.9a). Crests presented a different biomass partitioning. A significantly higher biomass was allocated to the rhizome (245.3±33.3 g m^{-2}), however leaf biomass was significantly higher than root biomass, which respectively averaged at 4.1±1.0 and 1.9±1.4 g m^{-2} at 5 m depth (Fig. 5.9a). Although the trends described above were consistent at both depths (5 and 10 m) (Fig. 5.9), a significant difference between depths was detected in the statistical analysis (Table 5.2). A significantly higher total biomass was found at 5 m depth than at 10 m depth. This was a constant trend at all locations.

Table 5.2: NP-MANOVA results of biomass partitioning in *Posidonia oceanica* on rubble mounds (crest, side and valley) and the pristine *P. oceanica* meadow. Differences among the different plant compartments (leaf, rhizome, root) were tested.

Source	df	EMS	Perm.units	SS	MS	F-pseudo	P.perm	P.MC
Depth (De)	1	DeXSite(Lo)	6	84.50	84.50	45.07	0.0002	0.0002
Location (Lo)	3	Site(Lo)	12	964.90	321.63	171.54	0.0002	0.0002
Site(Lo)	16	RES	144	30.00	1.88	1.26	0.1594	0.1544
DeXLo	3	DeXSite(Lo)	6	10.97	3.66	1.95	0.1014	0.0932
DeXSite(Lo)	16	RES	144	30.00	1.88	1.26	0.1594	0.1544
RES	123			182.50	1.48			
TOT	143			1261.90				

Data were transformed to ln(x+1)
No standardisation
Analysis based on Euclidean distances
Integer used as seed = 5
Permutation of raw data
No. of permutations used = 4999

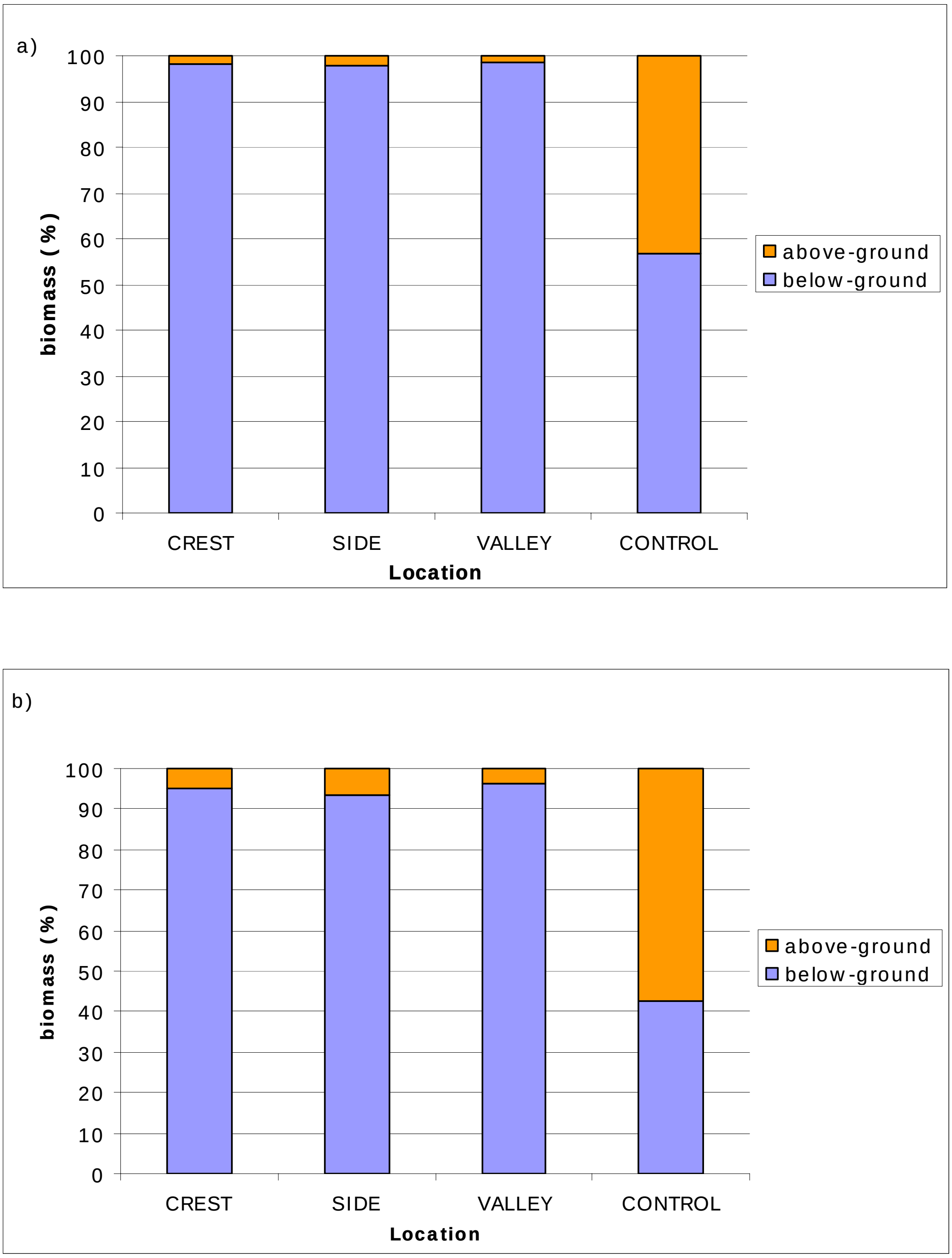


Figure 5.7: Percent contribution of the above- and below-ground compartments of *P. oceanica* at different locations (crest, side, valley and control) and at (a) 5 m and (b) 10 m depth. N = 6, bars equal standard error.

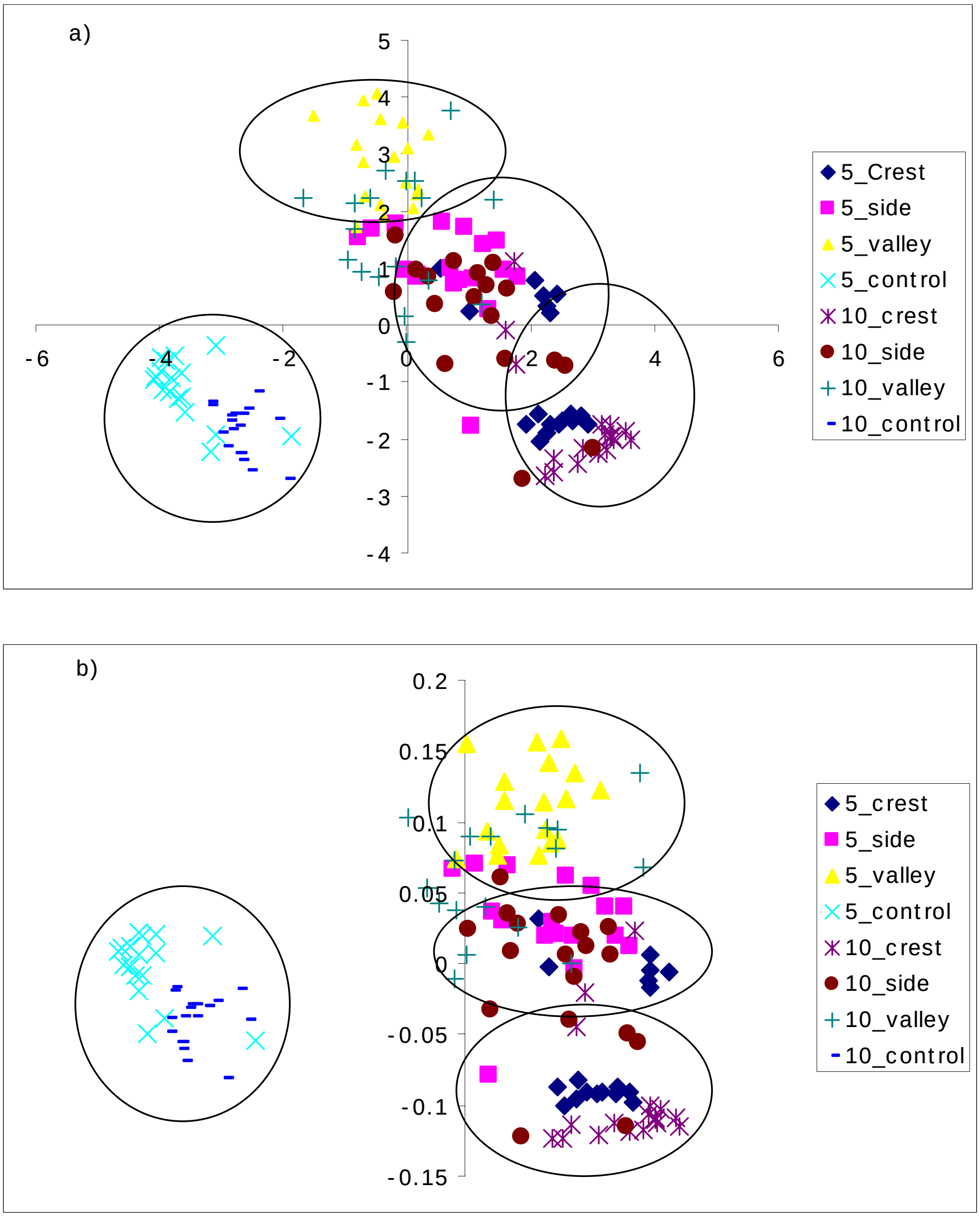


Figure 5.8: Visual comparison of the method used to reduce dimensions in (a) an unconstrained and (b) a constrained ordination procedure. Both ordination plots included 18 variables on plant phenology at different locations (control, crest, side, valley) and depths.

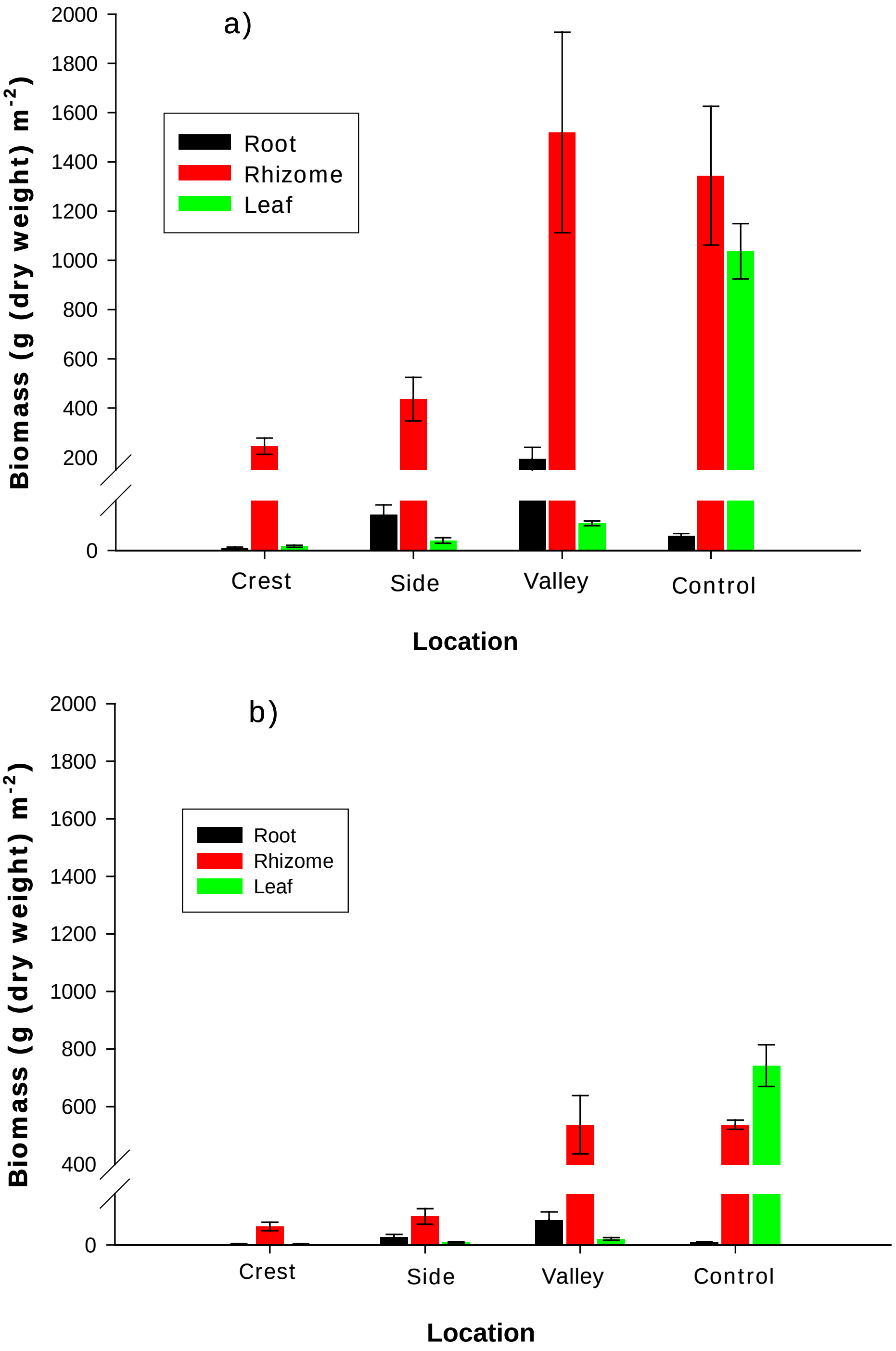


Figure 5.9: Variation in biomass allocation to different plant compartments at different locations (crest, side, valley and control) and at (a) 5 m and (b) 10 m depth. N = 6, bars equal standard error.

5.3.3 Leaf Production

The production of new leaves differed between the control site and the valleys. In the pristine bed, old standing crop (OSC) was 61.7% with a percentage of new leaves of 38.2% (Fig. 5.10). In contrast, in the valleys a higher OSC was found with an average value of 74.6%, but a smaller % of leaves produced over the total mass production was recorded at this location (25.3%) (Fig. 5.10). The one-way ANOVA showed significant differences in the leaf standing crop (LSC), mass-specific leaf production and the plastochrone index (PI) between the valley and the control area in Tonnarella (Table 5.3). LSC was found to be significantly higher at the control site (71.6±19.5 g cm^{-2}) than the average value for valley areas (38.9±10.6 g cm^{-2}) (Table 5.4). The same pattern was recorded for mass-specific leaf production, where in the control seagrass bed the average value was 4.2±0.2 mg g^{-1} d^{-1}, a significantly higher value than that present in the rubble mound valleys (6.27±0.6 mg g^{-1} d^{-1}) (Table 5.3, SNK test; Table 5.4). Leaf plastochrone interval (P_L) provides an estimate of the time of new leaves production over time. In this study, P_L was significantly higher in the control bed (average 56.7±17.5 days) than in the valleys (average 45.3±35.1) (Table 5.4). No significant difference was found between control and valleys in leaf productivity per shoot and areal leaf productivity (Table 5.3).

Table 5.3: One-way ANOVA of *P. oceanica* leaf production and biomass at different locations (control, valleys) over a growth period of 68 days. C = control, V = valley, = $p<0.001$; ns = not significant; SNK = Student-Newman-Keuls test.

Source of variation	Seagrass production		
	df	**MS**	**F**
		Areal leaf biomass	
LOCATION	1	3217.6875	13.07**
RES	10	246.2542	
TOT	11		
Cochran's Test	C = 0.7718 (ns)		
SNK test	C>V		
		Leaf productivity per shoot	
LOCATION	1	0	0.56^{ns}
RES	10	0	
TOT	11		
Cochran's Test	C = 0.6439 (ns)		
		Mass-specific leaf productivity	
LOCATION	1	5.85E-06	30.6**
RES	10	1.91E-07	
TOT	11		
Cochran's Test	C = 0.8725 (ns)		
SNK test	C>V		
		Areal leaf productivity	
LOCATION	1	0.0118	1.69^{ns}
RES	10	0.007	
TOT	11		
Cochran's Test	C = 0.5539 (ns)		
		Plastochron interval	
LOCATION	1	10308240.33	13.82**
RES	10	745905.1653	
TOT	11		
Cochran's Test	C = 0.6014 (ns)		
SNK test	C>V		

Table 5.4: Foliar primary productivity and biomass for *P. oceanica* estimated with the Zieman (1974) method. Productivity was assessed both on the control pristine meadow and valley areas within the rubble field in June-July 2003.

Sample	Areal leaf biomass (g cm^{-2})	Leaf productivity per shoot (mg sh^{-1} d^{-1})	Mass-specific leaf productivity (mg g^{-1} d^{-1})	Areal leaf productivity (g cm^{-2} d^{-1})	Plastochron interval (days)
C1	67	4.534	4.061	0.272	34
C2	110.4	11.380	4.329	0.478	68
C3	59.7	3.130	4.089	0.244	34
C4	70	5.603	3.969	0.286	68
C5	58	4.787	4.209	0.244	68
C6	64.9	4.184	4.577	0.297	68
MEAN (±SD)	**71.6±19.5**	**5.6±2.9**	**4.2±0.2**	**0.30±0.1**	**56.7±17.5**
V1	35.5	8.680	6.000	0.356	68
V2	34.4	2.981	6.412	0.221	0
V3	33.8	4.412	5.743	0.194	0
V4	54.5	4.369	5.532	0.301	68
V5	26.3	2.545	5.032	0.132	68
V6	49	3.930	4.892	0.240	68
MEAN	**38.9±10.6**	**4.48±2.2**	**6.27±0.6**	**0.24±0.1**	**45.3±35.1**

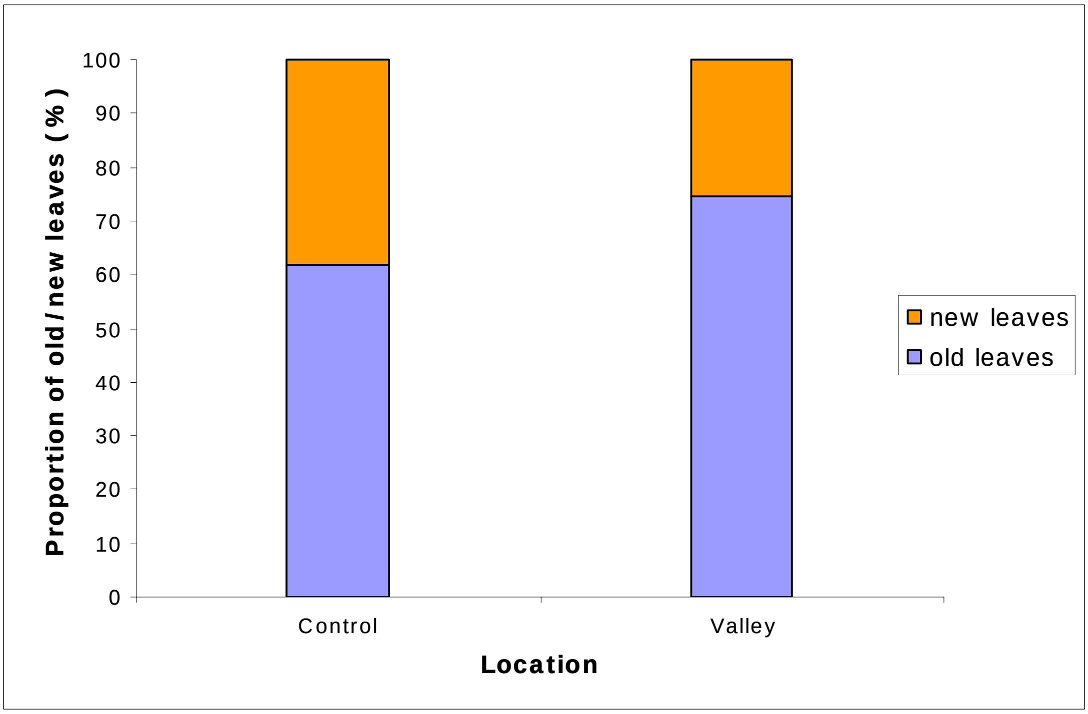


Figure 5.10: Foliar primary production and biomass for *P. oceanica* estimated with the Zieman (1974) method. Production was assessed both on the control pristine meadow and valley areas within the rubble field in June-July 2003.

5.4 DISCUSSION

Structural parameters (number of leaves per shoot, leaf length, leaf surface area and leaf area index) of seagrass patches living in the rubble environment were found to be different from those of plants living on reference bed settled on the matte. *P. oceanica* in the rubble field showed reduced leaf features and a smaller canopy height compared to that of the matte. Leaf area index (LAI), a parameter correlated to shoot density and leaf surface, is a good indicator of phenological difference among meadows. At Capo Feto, average LAI values in the rubble field were over one order of magnitude lower than those in the pristine meadow. This suggests a possible substratum effect (rubble vs matte). Previous work reported that variations within seagrass meadows reflect topographic complexity and in particular how substratum type and sediment characteristics determine morphological and growth variables (Marbà & Duarte 1995, Duarte et al. 1998, Fonseca & Bell 1998, Townsend & Fonseca 1998). Phenological diversities were also found between plants living on crests and in valleys. In chapter 4, emphasis was given to assessing habitat differences between locations on the mounds. Thus, variations in seagrass structural parameters between mound locations (crest, side, valley) can be related to the sediment characteristics and to the different hydrodynamic regimes of the two microhabitats (crest vs valleys). Plants living on crests lack a sediment layer which provides the necessary nutrients for plant growth (Plate 5.4). This means that nutrient uptake only occurs through the leaves and plant demand is not fulfilled (Fourqurean et al. 1992). On the other hand, *P. oceanica* patches in valleys create a feedback mechanism which entraps sediment particles and increases the depth of the sediment layer. As a consequence, there is a higher nutrient availability for root uptake in sediment porewater (Fourqurean et al. 1992, McGlathery et al. 2001). These conclusions are supported by differences in biomass allocation to the various compartments (leaf, rhizome, root) between plants living on crests, valleys and the control meadow. Biomass values found in the pristine meadow show how above- and below-ground biomass components are balanced in terms of g DW m^{-2}.

Plate 5.4: Plants living on crest lack a sediment layer which provides the necessary nutrients for plant growth.

This indicates no resource limitations exist on the matte. This finding is in agreement with what discussed by Duarte & Chiscano (1999) in their seagrass biomass reassessment. Duarte & Chiscano (1999) reported an equal biomass distribution between the above- and below-ground components in large seagrass species (i.e. *Posidonia*). In particular, Duarte

& Chiscano (1999) reported how *P. oceanica* develops high above-ground biomass (around 500 g DW m^{-2} or higher) and that the below-ground biomass developed by this species exceeds that of other seagrasses (about 1000 g DW m^{-2} or higher). Values of above- and below-ground biomass found in this study on the pristine meadow confirm these results as both above and below ground compartments average over a 1000 g DW m^{-2}. In the same article Duarte & Chiscano (1999) observed how seagrass biomass and production are kept below their potential by resource limitation. This comment is applicable to plants living in the rubble environment. On crests most of the biomass is allocated to the rhizomes while root and leaf weight present low amount. Fragments living at this location on the mound experience nutrient limitation hence biomass allocation to leaves and roots is virtually absent (Plate 5.5). In addition, the action of herbivores (mainly sea urchins), present in large aggregation on crests (Di Carlo, pers observ), might affect further leaf growth. Plants living in valleys presented a different biomass distribution in the various compartments (Plate 5.6). At this location, plants studied allocated more resources to root growth than other populations studied in previous work (Duarte & Chiscano 1999). This may reflect the need for better anchorage in the rubble environment. Marbà et al. (1994) found higher resources allocated to the roots in a hurricane swept area in Caribbean seagrass species. Thus, physical disturbance and substratum type play a main role in root development in seagrass species (Duarte et al. 1998, Balestri et al. 2003). Moreover, Guidetti et al. (2002) suggested that below-ground organs exert a structural role such as mechanically anchoring the plants to the substratum, accounting for the stability and persistence of seagrass patches. It can also be inferred that plants living in valleys allocate more resources to root growth to increase nutrient uptake. As a sediment layer is present in these regions, nutrient uptake occurs primarily through the roots (Bulthuis & Woelkerling 1981, Fourqurean et al. 1992, Touchette & Burkholder 2000, Gras et al. 2003). Several studies have highlighted how seagrass communities allocate proportionally more biomass to the roots in nutrient limited environments (c.f. Perez et al. 1994). The sizable production and turnover of seagrass roots suggests that considerable carbon and oxygen must be allocated to support root metabolism, since the carbon taken up by the roots generally exceeds that needed for growth (Duarte et al. 1998). Temporal patterns in biomass allocation and production resemble patterns for many terrestrial plants (Lieth 1974). In land plants, fine roots are important components of biomass and production (Gross et al 1993, Hendricks et al. 1993) and vary in accordance to several environmental variables (Lechowitcz 1984).

Plate 5.5: On crests biomass allocation to leaves and roots is virtually absent.

Plate 5.6: Plant on valleys need a better anchorage on the rubble hence they tend to allocate more resources to the below-ground organs, while above ground biomass is reduced.

Biomass distribution into the different plant compartments is strongly correlated to plant production and in particular to the production of new leaves (Duarte 1991). The results presented in this chapter point out how plants living in valleys have a lower leaf production per shoot and lower areal leaf production than plants in the control location. As resource allocation to the roots increases, production of new tissue is reduced especially in nutrient limited environment. In valleys, shoots also showed a shorted plastochrone interval (P_L) than control site shoots. P_L reflects the 'biological age of the plant' (Lamoreaux et al. 1978, Duarte 1991, Short & Coles 2001) and leaf plastochrone interval provides an estimate of new leaves produced over time. Duarte (1991) discussed a strong relationship between the plastochrone interval and turnover rates (P/B: production/biomass yr^{-1}). This can be confirmed by this study as a positive correlation between turnover rate and P_L was found in plants living in valleys. This finding suggests a habitat-dependence of *P. oceanica* in terms of biomass and production (Duarte 1991). In valleys, P_L was found to be shorter implying a faster leaf turnover rate. Thus, a shift of resources from the rhizomes to the leaf can be assumed. On the other hand, leaves in the control bed have an average P_L value of 56.7±17.5, similar values were reported for *P. oceanica* by Marbà et al. (1996) and Short & Coles (2001). This implies higher resource allocation to the rhizome, which exerts a carbon storage mechanism (Pirc 1985). Thus, *P. oceanica* is able, unlike other temperate seagrasses (Sand-Jensen 1975), to support substantial growth rates during the winter at the expense of large starch accumulation over the Summer and the Autumn (Pirc 1985, Alcoverro et al. 1995).

In conclusion, the results provided here indicate how seagrass morphological features, biomass and production are dependent on local factors, such as substratum type and resource availability. The plasticity of *P. oceanica* modules allow the plant to adapt to a new environment (Marbà & Duarte 1998). In particular, plants living on mound crests lack sediment as reported in previous chapter. This can account for the lack of resources hence the low biomass allocated to all plant compartments. In contrast, the sediment present in valleys provides the necessary nutrient for plant growth. However, nitrogen and phosphorus concentration at this location might not be meeting full plant demand. Thus, plants living in valleys increase resource allocation to the roots to best exploit porewater nutrients. Furthermore, a different biomass allocation was found between plants living in valleys and those living in the pristine bed. These findings are probably due to bottom topography. As plant on valleys need a better anchorage on the rubble they tend to allocate more resources to the below-ground organs, while above ground biomass is reduced. On the other hand, seagrass in the control meadow experienced high nutrient availabity as well as the stability of a structure such the matte. As a consequence, above-ground biomass tends to increase faster than below ground components (Duarte & Chiscano 1999). These results confirms those of previous work (Alcoverro et al. 1997, Balestri et al. 2003) stating a strong relationship between large scale variation in seagrass bed factors such as physical disturbance, topographic complexity and nutrient availability.

5.5 LITERATURE CITED

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CHAPTER VI

HOW CAN WE REPRESENT THE PROCESS?

6.1 INTRODUCTION

It is well documented that global environmental changes are ongoing. However, the cumulative ecological consequences of these changes and the alteration made to ecological systems are as yet unknown. Global environmental changes are mainly due to the industrial and agricultural development of the human society and to population growth which is overexploiting natural resources (Costanza et al. 1997). The world’s population is expected to double within this century which will lead to a depletion of natural capital stock. Predictions of the future have reported how natural capital and ecosystems will become more stressed and scarce hence their value will increase (Costanza et al. 1997). Changes in the quality of several ecosystems can have a direct impact on human welfare (Costanza et al. 1997). In order to protect and manage ecosystem services in a sustainable way, scientists should increase public awareness and find a common language with policy makers. This would mean that both scientists and resource managers could work on regulatory frameworks to mitigate impacts and increase effective enforcement of regulations. Scientists and managers are always faced with uncertainty in decisions regarding ecosystem management. As pointed out by Vitousek (1997) for global environmental change issues, scientists know with certainty that changes are occurring and that they are human-caused. What scientists cannot do is always predict the particular consequences of a given human activity on the environment.

The possible effects of global environmental changes, including climate change, on terrestrial plant communities have already received considerable attention. In contrast, relatively little work has been published describing such effects on aquatic plant communities, including seagrasses (Duarte 1999). Seagrass are widely recognised as key ecosystems which play a main role in the marine environment (Moriarty & Boon 1989, Duarte 2002; Green & Short 2003). The loss of key ecosystems entails a loss in biodiversity hence a loss of ecosystem function. In recent decades some researchers have addressed the importance of seagrass communities with the aim of increasing public awareness (Duarte 2000). This effort was directly linked to the numerous conservation and restoration programs dedicated to seagrasses (Wyllie-Echevarria et al. 1994). The critical role that seagrasses play in many coastal environments, coupled with their extensive losses, have created widespread support for their conservation and restoration (Fonseca et al. 1996). Meanwhile, numerous policy changes have occurred at the state and local level over the last ten years to support a ‘no-net-loss of habitat’ philosophy (Redmond 2000). Therefore, an information based system of judging the value of seagrass ecosystems has emerged over the last decade addressing the question on how seagrasses should be protected. There is now an increasing need to implement an effective strategy for conservation. This requires the development of techniques to reliably forecast impacts of specific disturbances on seagrass beds. A detailed forecast of seagrass loss was attempted by Duarte (1999) who reported on the need to predict the time course of seagrass decline and recovery following disturbance. In addition, effort should be put in assessing the habitat requirements of seagrasses in order to create a powerful tool for both scientists and coastal managers. To this end conceptual diagrams were developed by IAN (Integration and Application Network, University of

Maryland Center for Environmental Science) staff to enhance science communication and to explain complex ecological processes by the use of minimal graphical skills. In this chapter, I adopted conceptual diagrams to explain rather than represent the essential attributes of a disturbance/recovery process on a *P. oceanica* meadow in the SW Mediterranean sea. The utilisation of conceptual models has recently increased as they represent a useful communication tool which can reach both the scientific and policy making audience as well as the general public. Thus, conceptual models can be adopted by managers and biologists to discuss environmental related issues. The following discussion of the seven conceptual models details the major features and processes within the seagrass meadow at Capo Feto. Reference is made throughout the text to the figure legend (Fig. 6.1), so icons for process are clearly identified. Icons are kept constant throughout the models. Full reference is given where possible to justify the processes and features summarised in the models (Carruthers et al. 2002).

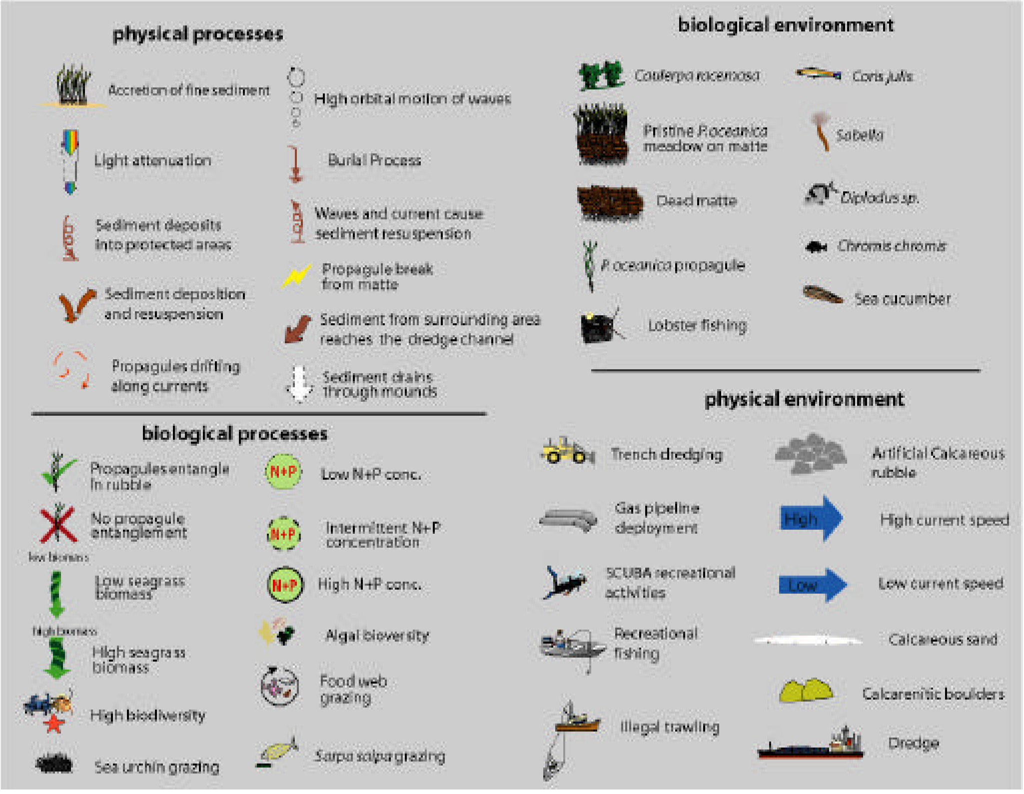

Figure 6.1: Legend for the icons used in seagrass conceptual models

6.2 POSIDONIA OCEANICA ECOSYSTEMS

Posidonia oceanica (L.) Delile is the dominant endemic seagrass in the Mediterranean basin, covering about 2.5–5 million ha extending from the shore to about 40 m depth (Pergent et al. 1995). *P. oceanica* presents long-lived modules, which are also thick and tend to decompose very slowly. The persistence of seagrass material, leads to the slow eventual burial of the associated carbon in the sediments. This species is able to form reefs (Molinier & Picard 1952); robust extensive reefs along the shoreline (fig. 6.2). This reef structure is considered of great importance as it stabilise coastal sediments with a consequent maintenance and protection of beaches from erosion (Blanc & Jeudy de Grissac 1984, Jeudy de Grissac & Boudouresque 1985). *P. oceanica* is also considered to play an important role in the coastal geomorphology of the Mediterranean Sea (De Falco et al. 2000). Several studies have shown the influence of seagrass meadows on the nature and dynamics of sediments in coastal areas. Seagrass beds are known to promote deposition of particles, and loss of seagrass meadows is often followed by sediment erosion (Hine et al. 1987). Seagrass ecosystems in the western Mediterranean are extremely rich in terms of associated plant and animal species as well as representing important nursery grounds for the juvenile of many commercially important species of fishes and invertebrates (Fig. 6.2).

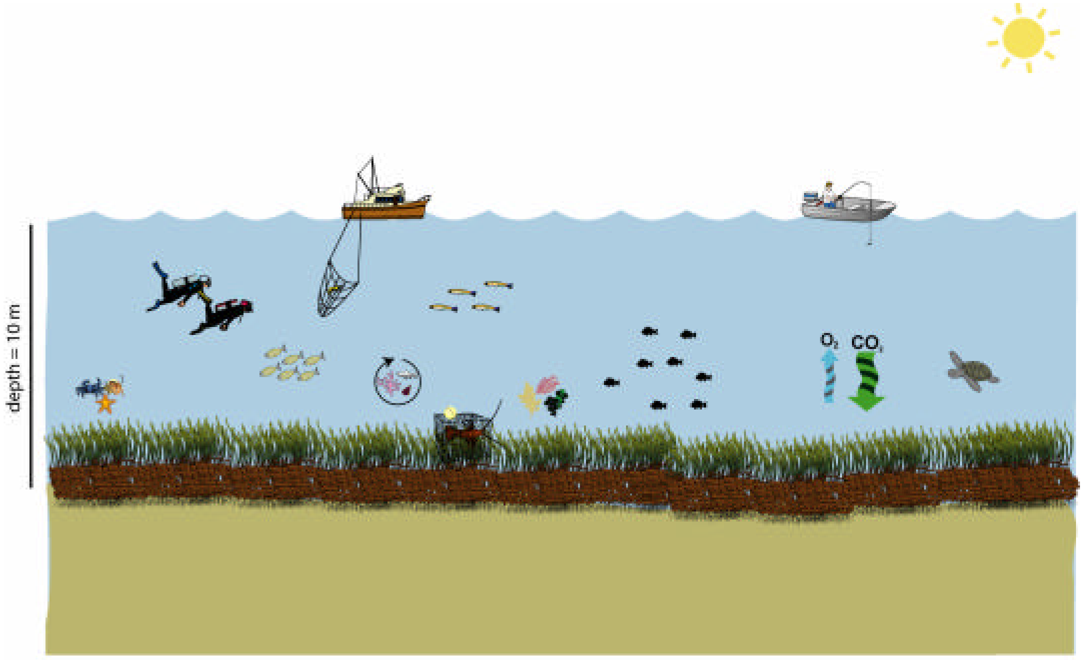

Figure 6.2: The seagrass meadow at Capo Feto before the construction of the pipeline in 1981 and 1993. Seagrass meadows are important ecosystems for many recreational and commercial activities.

6.3 MECHANICAL IMPACT: PIPELINE DEPLOYMENT

The first part of the Italo-Algerian pipeline system was built at the end of the 1970s. Mechanical operations included the dredging of a trench through the *Posidonia oceanica* meadow at Capo Feto (SW Sicily, Italy). The whole impacted area (old and new) formed a mosaic of different substrata, including calcarenitic boulders, rubbles, as well as sand and dead matte (Fig 6.3). An area of approximately 150 ha of seagrass was cleared for pipe deployment. In addition, construction work (i.e. bomb blasting) increased the amount of sediment resuspension in the water column which steadily decreased water transparency. The large load of resuspended sediment deposited on the adjacent meadow buried the seagrass in the proximity of the trench. As *P. oceanica* is a slow growing species, vertical growth cannot compensate for high burial rates. Hence, when the equilibrium between vertical growth and sediment deposition is skewed toward the latter process, the seagrass can suffocate and die off (Fig. 6.3).

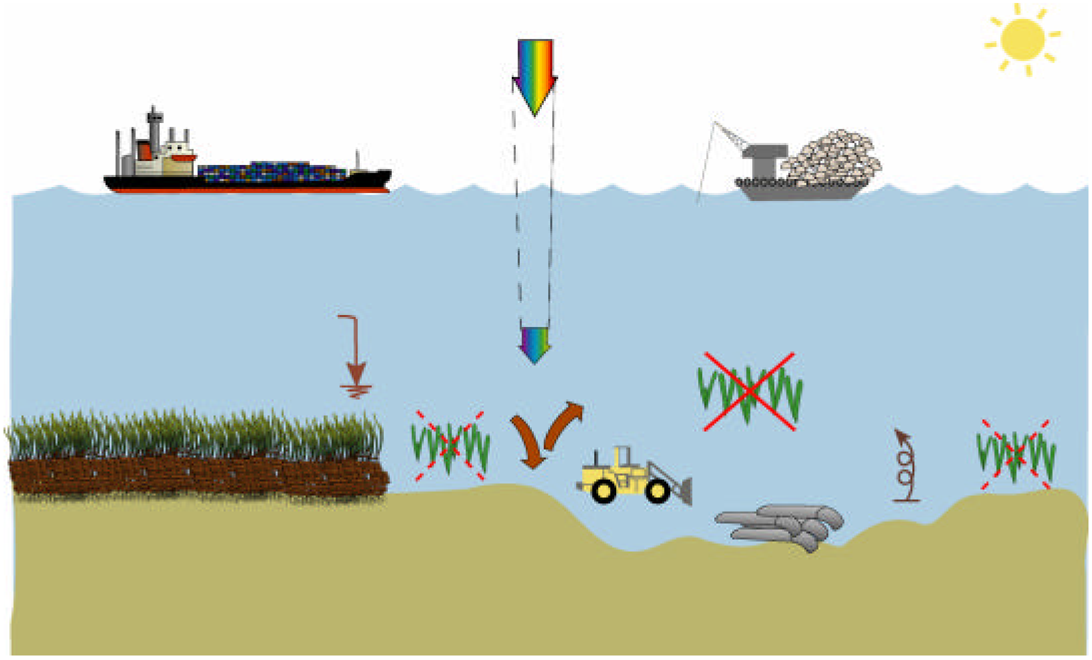

Figure 6.3: Dredging of the meadow to deploy gas pipeline and consequent seagrass loss. Construction activities enhance sedimentation in the adjacent seagrass beds causing rhizome burial.

6.4 THE NATURAL RECOVERY PROCESS

Both mechanical and physical disturbance can affect seagrass beds irreversibly. Although recovery times for *P. oceanica* in bare patches are faster (Hemminga & Duarte 2000), full recovery after bomb blasting for this species are reported to be around 3 x 10^{-4} patches ha^{-1} yr^{-1}.

Although *Posidonia oceanica* is reported to grow mainly on sandy bottoms, it is commonly found on rocky seabed in several areas of the western Mediterranean Sea. Sexual reproduction is considered to be sporadic and colonisation of new areas often occurs through the recruitment of vegetative fragments. During storm events, fragments detach from the meadow and they drift along with bottom currents. Eventually they might entangle in crevices on suitable substrata. At Capo Feto, fragments of *P. oceanica* recruited into rubble crevices whilst no recolonisation was present on sand or calcareous boulders. The spreading of the invasive green algal species, *C. racemosa*, prevented seagrass fragments becoming entangled in the dead matte. *C. racemosa* rhizoids form a dense structure which prevents *P. oceanica* fragments penetrating the matte.

Vegetative recruitment of seagrass fragments on rubble mounds at Capo Feto is dependent on propagule availability from the adjacent meadow. During storm events, seagrass fragments, located at the edge of the matte, detach from the meadow and are dispersed by the action of currents and waves. Such fragments are collected within the pipeline trench as this acts as a sediment and propagule trap (fig 6.4). *P. oceanica* fragments entangle in crevices or settle between rubble helped by sturdy roots which effectively provide anchorage (D'Anna et al. 2000, Hemminga & Duarte 2000).

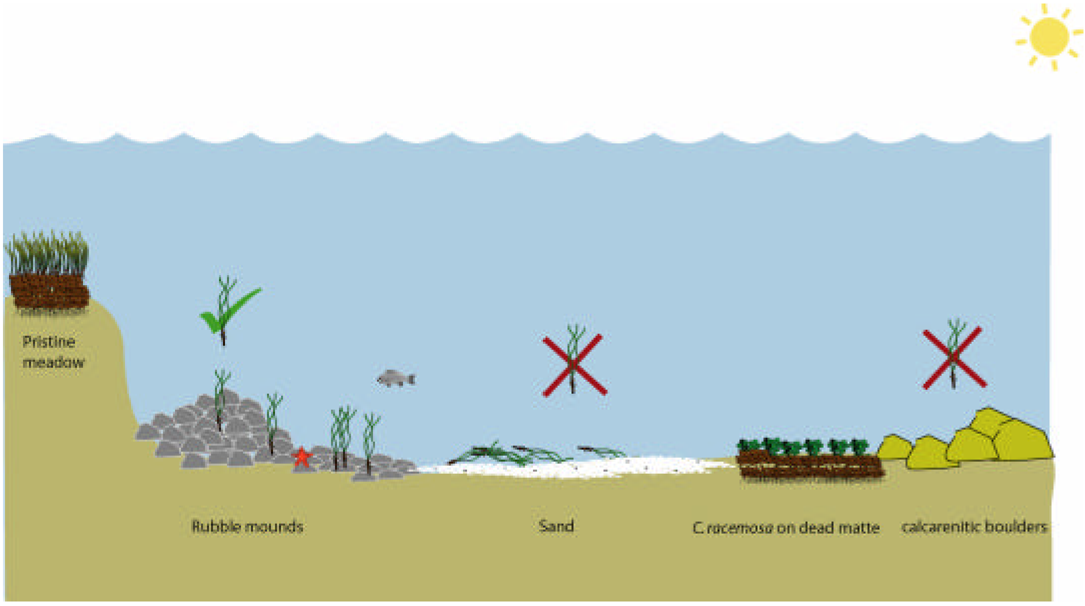

Figure 6.4: due to the utilisation of different backfilling material the trench is formed by a mosaic of different substrata. However, only rubble allowed recruitment of vegetative fragments of *P. oceanica*.

6.5 THE ROLE OF PHYSICAL FACTORS

Although fragment entanglement occurs at all locations on the rubble mounds (crests, sides and valleys), patch formation is only observed in valleys. This pattern is most likely driven both by physical and geological processes. The quiescent hydrodynamic conditions within rubble mounds and in valleys between mounds led to sediment deposition and seagrass fragment entanglement (Fig 6.5). The sediment between rocks provides the resources necessary to sustain seagrass patches which, once established, may start a positive feedback between plant canopy, water flow attenuation and sediment deposition (e.g. Fonseca et al. 1983, Thayer et al. 1984) (Fig. 6.6).

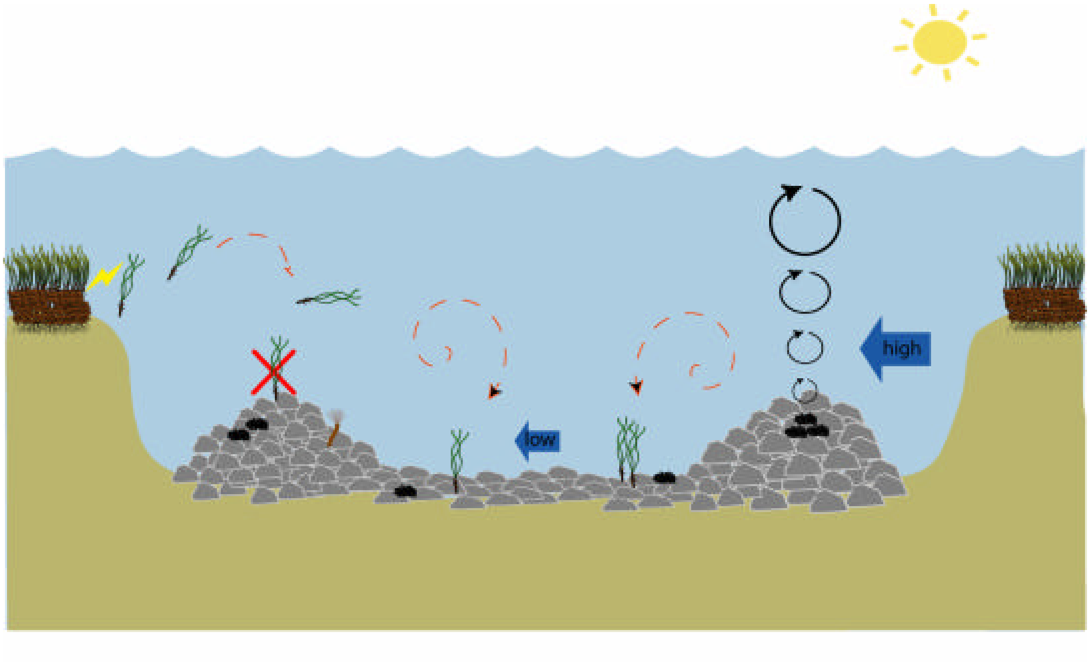

Figure 6.5: During storms fragments break from the meadow and flow along bottom currents. Quiescent hydrodynamic conditions favour the settlement of fragments into the rubble.

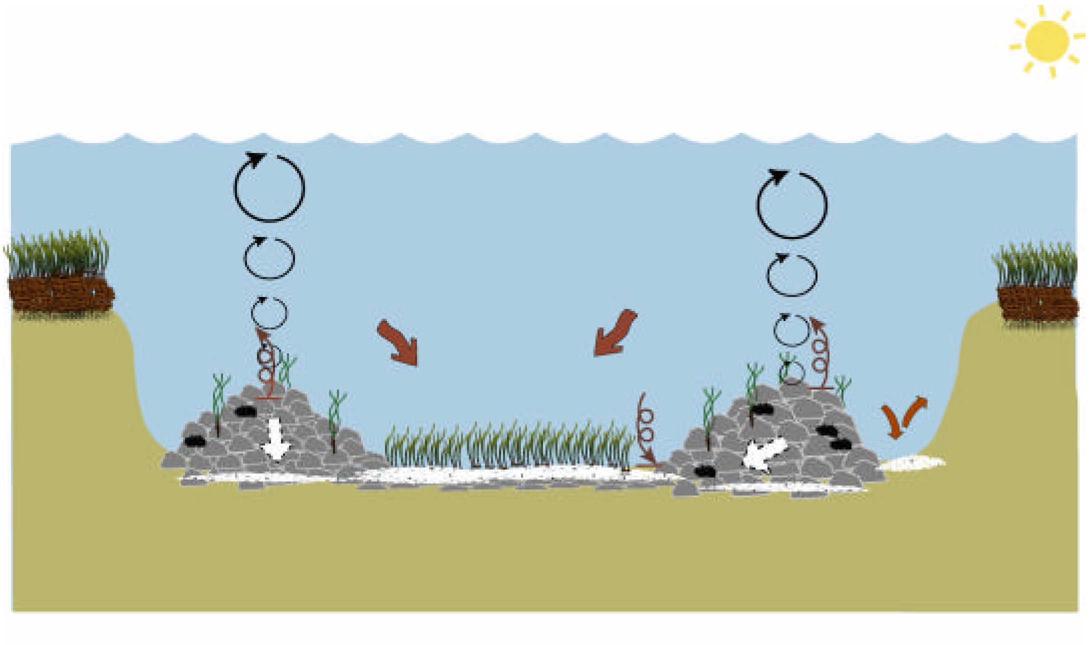

Figure 6.6: once the fragments are established, seagrass canopy favours particle settlement. The formation of a sediment layer provides necessary nutrients for plant growth.

6.6 LINK BETWEEN PHYSICAL AND BIOLOGICAL FACTORS

Seagrass patches in valley areas contribute to particle settlement hence to the formation of a sediment layer, which provide the necessary nutrients for plant growth. On the other hand, deposited sediment on mound crests is resuspended as fragments do not develop a rhizome/root system. This indicates that plants living on crests have limited resources for growth and biomass build up. A thicker sediment layer in valleys entails a higher porewater nutrient concentration (Fig. 6.7). Higher N and P are found in interstitial water, confirming that nutrients in these locations can meet plant nutrient demand. Consequently, porewater can be considered the primary nutrient source for *P. oceanica* patches growing in valleys and controls, where nutrients are taken up by the roots. On crests, patches have to rely exclusively on leaf uptake from the water column hence they experience a limited nutrient availabity.

The plasticity of *P. oceanica* modules allow the plant to adapt to a new environment (Marbà & Duarte 1998). The lack of a sediment layer in mound crests can account for the lack of resources hence for the low biomass allocated to all plant compartments. In contrast, the thicker sediment layer present in valleys provides the necessary nutrient for plant growth. Thus, plants living on valleys increase resource allocation to the roots to best exploit porewater nutrients (Fig. 6.7). As plants in valleys need a better anchorage on the rubble they tend to allocate more resources to the below-ground organs, while above ground biomass is reduced. On the other hand, seagrass plants in the control meadow experience high nutrient availabity as well as the stability of a structure such the matte, hence above-ground biomass tends to increase faster than below ground organs (Duarte & Chiscano 1999). Biomass variations on the largest scale might reflect differences in seagrass biomass and production due to the type of habitats (rubble vs matte) and sediment characteristics. On a smaller scale, factors such as disturbance, topographic complexity and nutrient availability might operate to modify *P. oceanica* phenological and growth features (Alcoverro et al. 1995, Balestri et al. 2003)

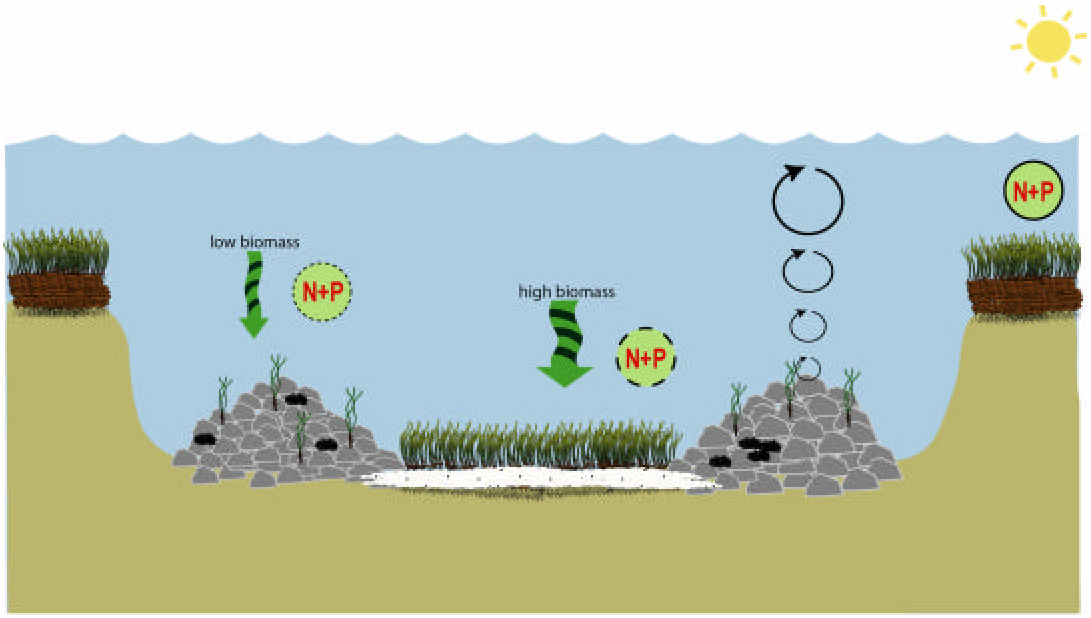

Figure 6.7: a higher biomass is found in the valleys as plant uptake nutrients from the porewater. A higher root biomass is found in the valleys as plants need better anchorage on the rubble.

6.7 C0NCLUSIONS: A NEW HABITAT

This work represent a unique case of *Posidonia oceanica* recovery after a mechanical impact. Previous human driven restoration actions have reported no growth or a low percent of fragments survival . Moreover, *P. oceanica* is a slow growing species with a mean patch expansion rate of $3x10^{-4}$ ha^{-1} yr^{-1} (Meinesz & Lefévre 1984). Growth rates of individual seagrass rhizomes, measured at the scale of shoots (cm), may also not be extrapolated to estimate the growth dynamics of seagrass patches or meadows. For example, patch growth of *C. nodosa* in the Mediterranean accelerated with increased patch size and age (Duarte and Sand-Jensen, 1990; Marbà and Duarte, 1995; Vidondo et al., 1997). The causes of accelerated growth were thought to be increased growth rates of the shoot population, combined with increased fluxes of resources from middle to edge of patches as the perimeter decreased with increased area of patches (Vidondo et al., 1997). Although pipeline deployment entailed a major disturbance to the adjacent meadow and a large portion of seagrass was removed, rubble mounds represent a valid backfilling material to favour *P. oceanica* resettlement (Fig. 6.8). This process is clearly linked to the high availability of propagules in the area.

In conclusion, this work is the first to report on a natural recolonisation process of *P. oceanica* on a hard substratum after a human-induced impact. The outcome of this research might be considered on a large scale to implement an effective restoration plan for *P. oceanica*, given that natural physical, chemical and biological conditions are restored in the environment. Conceptual diagrams can be applied to ecological, and in particular to seagrass research, to describe and explain process within seagrass ecosystems. This tool can be adopted to design and monitor effectively restoration activities both by researchers and environmental managers.

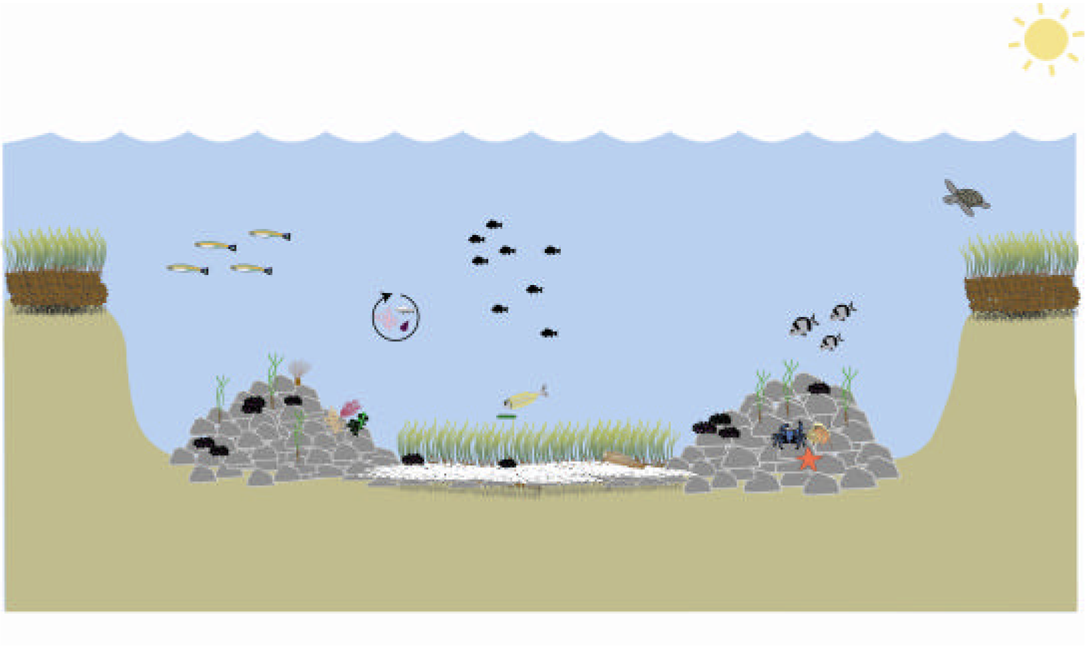

Figure 6.8: *Posidonia oceanica* coverage is slowly increasing in the rubble field and eventually the seagrass patches may expand upwards along the sides and ultimately reach the crests. Rubble mounds represent a new seagrass habitat on a hard substratum.

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CHAPTER VII

CONCLUSIONS

7.1 CONCLUSIONS

Seagrass restoration has received increasing attention in the last few years because of the ongoing global environmental changes (Costanza et al. 1997), and destruction of meadows as a consequence of human disturbance (Short & Wyllie-Echeverria 1996). In addition, there is a fast growing economic interest in Mediterranean coastal areas linked to the tourism industry and the development of new infrastructure, such as hotels and marinas (Duarte 2002). These are built along the coast to fulfil human needs for oil, fuel and water (Duarte 2002).

The effects of global environmental change can impact marine ecosystems at the small or large scale (Short & Neckles 1999). In turn, these global changes will modify the impacts of local human activities (Costanza et al. 1997), mostly in a summative fashion. The loss of seagrass ecosystems, through a blend of global and local effects, entails a decrease in biodiversity hence a loss of ecosystem function, such as the loss of seagrass nursery habitats for fish (Bell & Harmelin 1982) leading to a depletion of fish stocks (Costanza et al. 1997). The critical role that seagrasses play in many coastal environments (Hemminga & Duarte 2000, Green & Short 2003) coupled with their extensive losses, have created widespread support for their conservation and restoration (Fonseca et al. 1998). Many countries have now introduced strict environmental policies, which regulate activities likely to cause damage or alteration to marine coastal habitats (Redmond 2000). In 1964, Pérès & Picard hypothesised that if the rate of *Posidonia oceanica* decline had remained constant, this species would have become endangered. Indeed, this seagrass species is now protected under the EU regulations and several restoration initiatives have been promoted to reimplant lost *P. oceanica* ecosystems (Green & Short 2003). Such initiatives included both seed culture and the utilisation of vegetative recruits from donor beds (Piazzi et al. 1996, Piazzi & Balestri 1997, Orth et al. 1999). However, the rate of success of *P. oceanica* transplantation programs is up to date fairly low. Such failure has been attributed both to substratum choice as well as environmental conditions (i.e. seasonality, water temperature) (Meinesz et al. 1992, Molenaar & Meinesz 1992).

Substratum is considered to play a primary role in the recruitment process of *P. oceanica* (Moleenar & Meinesz 1995, Balestri et al. 1998). Even if *P. oceanica* is thought to prefer soft substrata (Molinier & Picard 1952), this large growing species is able to colonise hard substrata by the recruitment of vegetative fragment (Marbà & Duarte 1994, 1995). During storms, vertical rhizomes located at the edge of the meadow, detach from the mother plant and they are dispersed by the action of currents and waves. When suitable substratum conditions are encountered, vegetative fragments entangle in crevices. In this study, how a suitable substratum can support seagrass recruitment via vegetative fragments was assessed. The rubble material used to backfill the construction trench at Capo Feto was and is fairly motionless, even in harsh hydrodynamic conditions, and it also has not degraded with time, like calcarenitic material would. All these features lead to the conclusion that rubble provide a secure and stable environment for the settlement and the survival of newly arrived fragments. The settlement process of *P. oceanica* is generally favoured by sturdy roots (Hemminga & Duarte 2000), which penetrate into the crevices effectively anchoring the plants (D’Anna et al. 2000). Vegetative development is often considered to be the main mechanism of seagrass proliferation (Duarte & Sand Jensen 1990, Marbà & Duarte 1998). This mechanism for colonisation represents a way to overcome environmental changes and to adapt or colonise contrasting environments (Marbà & Duarte 1998).

The recruitment process on rubble mounds is probably linked to the high availability of fragments that detach from the surrounding meadow. The seagrass meadow adjacent to the construction trench at Capo Feto is one of the largest present in the Mediterranean sea with a total coverage of over 30,000ha. This entails that during storm events a large number of fragments detaches from the meadow and it is dispersed in nearby areas. New studies have lately highlighted a high dispersal mechanism for *P. oceanica*, which results in clones being spread across vast areas (i.e. 100m, Procaccini pers. comm.). The patchy distribution of fragments over the rubble area studied can be thought of as a plant response to disturbance. Success of such a response is believed to depend on the relationship between the time scale of the disturbance and the life history patterns of the species available to reoccupy the disturbed site (Sousa 1984, Duarte 1991). Furthermore, disturbance is, depending on its amplitude and frequency, a source of patchiness in plant communities (Sousa 1984, Duarte 1991, Collins 1992, Cipollini 1994). Although fragment entanglement appeared to occur at all locations (crests, sides and valleys) at the study site, patch formation was only recorded in valleys. This appears to be the result of a combination of physical and geological processes. Seagrass patch development in the valleys seems possible because of the relatively quiescent hydrodynamic conditions (2 cm s^{-1}) and to the availability of resources that support plant growth which were present in the sediment layer (12 cm sediment thickness). The recruitment may occur via direct deposition of fragments in the relatively quiescent areas (i.e. a natural trapping mechanism) and/or via the accumulation of fragments previously entangled in crests and sides but subsequently dislodged by currents and waves leading to their deposition in the valleys.

Once a significant shoot density has become established in the valleys, a positive feedback between seagrasses, water flow attenuation and sediment deposition is expected (e.g. Fonseca et al. 1983, Thayer et al. 1984). This process is likely to lead to further sediment accumulation (see Duarte & Sand Jensen 1990, Marbà & Duarte 1998, Granata et al. 2001) between the rubble and, therefore, further seagrass expansion. Consequently, it can be hypothesized that, once the valleys have become vegetated, the seagrass patches may expand upwards along the sides and ultimately reach the crests. Although *Posidonia oceanica* is a large, slow growing seagrass species with a rhizome elongation averaging around 0.07m yr^{-1} (Caye 1980, but see Marbà & Duarte 1998), as stated by Marbà & Duarte (1998) changes in rhizome elongation rate may reflect density dependent constraints. Previous work by Meinesz & Lefèvre (1984) calculated a patch formation rate of $3x10^{-4}$ ha^{-1} yr^{-1} for this Mediterranean seagrass species. However, seagrass growing on bare substrata or bare sediments elongate faster than those growing in dense meadows. In the rubble field, newly available mounds can be thought as bare patches, with the absence of both intraspecific and interspecific (with *Cymodocea nodosa*) competition for space. Although there is a large lack of knowledge on interspecific processes concerning *P. oceanica* and *C. nodosa*, the proposed successional sequence for the Mediterranean Sea proceeds from colonisation by *C. nodosa* to dominance of *P. oceanica* (den Hartog 1970, Hemminga & Duarte 2000), considered to be a climax species. However, seagrass successional sequences do not represent unidirectional paths of change, as they might revert or be short-circuited by disturbance (Hemminga & Duarte 2000). Furthermore, Sousa (1979a) states that the tolerance and inhibition models (Begon et al. 1996) assume that any species, including those which usually appear later, can colonise at the beginning. Thus, the absence of competition coupled with substratum availability may account for the acceleration of patch growth (an increase of percent cover of 42.5±10.02% m^{-2} over a seven year time period) at the study site, a trend never reported before for this species. Seagrass expansion in valleys was and is supported by the deposition and accumulation of sediment particles, which provide the necessary nutrients for plant growth. Total deposited sediment in valleys reached a maximum value of 200g DW m^{-2} y^{-1}. This number is in accordance with previous work by Gacia & Duarte (2001) where values for a Spanish *P. oceanica* meadow ranged between 1.5 and 500 g DW m^{-2} y^{-1}. Gacia & Duarte (2001) stated the importance of total sediment deposition for rhizome growth and increase in primary production. Early work by Zieman (1972) suggested at least 7 cm of sediment above bedrock as one of the main seagrass habitat requirements. The presence of such a thick sediment layer at the study site was reflected by differences in porewater nutrient concentrations between locations. Valleys showed the highest porewater nutrient concentration of all locations (crest and side). This was probably due to the presence of a thicker sediment layer. Previous work has given evidence of how dense seagrass roots and rhizomes are linked to elevated nutrient concentrations in the porewater (Erftemeijer & Middelburg 1993, McGlathery et al. 2001). Thus, as sediment deposited onto mound crests was resuspended, fragments living on crests were resource-limited. This means that fragments living on crests had to rely exclusively on leaf nutrient uptake from the water column. On the other hand, fragments settling in valleys were able to take up nutrients from the porewater via the root system at a faster rate. Such results are confirmed by the N and P concentrations present in interstitial water in the valleys which seems to meet plant requirements. However, nutrient concentrations in plant tissue and in particular in the root system appeared to be fairly low. A limitation in nitrogen availability and the substratum characteristics might well explain the high root biomass, as shown by Terrados et al. (1999) reporting how root biomass allocation increases with low nutrient availability. Thus, nutrient-limited rocky seabeds might determine growth patterns and morphological features of *Posidonia oceanica*. Previous work by Duarte & Chiscano (1999) reported how seagrass biomass and production are kept below their potential by resource limitation and/or heavy losses of nutrients caused by physical disturbance. Duarte & Chiscano (1999) also described an equal biomass distribution between the above- and below-ground components in large seagrass species. However, at Capo Feto, recruits living in valleys were found to present a different biomass distribution, with more resources allocated to root growth than to above-ground biomass. This may reflect the need for better anchorage in the rubble environment than in the environment studied by Duarte &

Chiscano (1999). Marbà et al. (1994) found higher root biomass compared to above-ground biomass in a hurricane swept area in the Caribbean seagrass species *Thalassia testudinum*. Thus, physical disturbance and substratum type play a main role in the development of the root system in seagrass species (Duarte et al. 1998, Balestri et al. 2003). Moreover, Guidetti et al. (2002) suggested that below-ground organs exert a structural role such as mechanically anchoring the plants to the substratum, accounting for the stability and persistence of seagrass patches. This pattern gives evidence for the plasticity of *P. oceanica* modules, allowing the plant to adapt to a new environment (Marbà & Duarte 1998).

In conclusion, substratum can be considered as a main factor determining seagrass recruitment patterns as well as seagrass physiological and morphological features. Disturbance events should be considered as a source of patchiness and they influence patterns in seagrass landscape. Although rubble might not seem, at a first glance, a suitable substratum for seagrass recruitment it has provided the necessary support for the entanglement of vegetative fragment of *Posidonia oceanica* at Capo Feto. Once established, fragments may contribute to an attenuation of currents hence increasing sediment deposition. This suggests that patch size will increase over time and eventually they might expand horizontally reaching the crests.

The current paradigm states that *P. oceanica* recruitment following disturbance mainly occurs via seed distribution, survival and germination, whist not taking into account the important role of substratum. This study gives evidence to reject such long held beliefs as the system observed at Capo Feto highlights the importance of vegetative fragments interacting with the substratum as a primary factor for clonal growth. Moreover, this is the first study to establish that *P. oceanica* fragments are negatively buoyant and that they drift with bottom currents, providing an ability for dispersal and recruitment where suitable conditions apply. The role of the substratum does not only affect the recruitment process of *P. oceanica*, but also it influences physiological processes the plants undergo once settled. Such processes involve a different allocation of resources which in turn have an effect on the plant morphology and production.

In 1993, the construction of the gas pipeline system TRASMED removed about 70 ha of seagrass. Within the time frame of 10 years *P. oceanica* has recruited and increased its shoot density up to an average of 300 shoot m^{-2}. This has only been possible due to the presence of rubble in the area. This project has provided new insights into seagrass restoration and mitigation. Perhaps rubble deposition could be applied on a larger scale to mitigate future impacts on *P. oceanica* communities, by reducing sediment particle resuspension and by providing a substratum for seagrass fragments anchorage which would favour clonal growth on the margin of existing meadows. Moreover, where seagrass restoration is required to replace lost meadow habitats (such as that caused by coastal developments of hotels and marinas) rubble deposition could be used to provide a stable substratum in the initial phase of meadow regeneration in the Mediterranean Sea.

7.2 FUTURE WORK

Future work should be focused on the dispersal mechanism of seagrass recruits. Moreover, I believe that a population genetic approach should also be considered to assess the genetic diversity of the clones and the potential of ramets and fragments to disperse across meadow. Recent work by Procaccini et al. (2004) suggests a new view of *P. oceanica* genetic diversity suggesting a high genetic variability within *P. oceanica* meadow. This could have implications for the successful restoration and management of Mediterranean seagrass beds.

In addition, a model could be developed for artificial seagrass habitats, such as rubble mounds, coupling recruitment patterns with environmental variables. This would allow a better understanding of how sedimentary processes as well as currents and waves affect the recruitment and dispersal of seagrass fragments. The model would provide the necessary knowledge and expertise to facilitate restoration and mitigation actions, which could be specifically ‘tuned’ for each kind of human and environmental disturbance. Moreover, the model could include a community ecology approach, hence assessing the role of herbivores on seagrass fragments survival as well as how the animal assemblages (both invertebrates and fish) change and/or adapt to habitat alteration.

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