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Proceedings of the Mediterranean Seagrass Workshop 2006

Mediterranean
Seagrass
Workshop

May 29 – June 4 2006, Marsascala, Malta

Maria Cristina Gambi
Joseph A. Borg
Maria Cristina Buia
Giuseppe Di Carlo
Christine Pergent-Martini
Gerard Pergent
Gabriele Procaccini

(Editors)

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FOREWORD

The Mediterranean Seagrass Workshop 2006 was convened in response to the need to promote a periodic event that would host scientists interested in Mediterranean seagrasses, and international scientists who are involved in projects that are focused on the Mediterranean marine environment, to discuss current knowledge and present the findings of their latest research.

The concept of holding an international meeting originated during the International Seagrass Biology Workshop (ISBW6) held in 2004 in Queensland, Australia. In particular, one of the goals of ISBW6 was to identify key ecological issues and environmental trends within a number of geographical regions. This stimulated the idea of taking such topic to a higher level; the Mediterranean scale.

The Mediterranean Sea is a rare and vulnerable ecoregion, one of the planet's biodiversity hot spots, where many of the species present are endemic (around 20%). The Mediterranean Sea also has a millenarian history of human use of its coasts. However, the current exponential increase of human pressure on the coastal zone for living space, transportation, recreation and food production is expected to have dramatic long-term impacts on the Mediterranean marine environment. Being located in shallow coastal areas close to human settlement, seagrasses are bearing the brunt of disturbance from such anthropogenic activities, with the result that degradation and loss of seagrass habitats is widespread in the whole Mediterranean Sea. Thus, there is great concern that the functions which seagrasses have performed in the Mediterranean marine ecosystem will be weakened or, in some places, lost altogether.

Within the Mediterranean Sea, previous Workshops addressed aspects of the biology of *Posidonia oceanica* meadows (International Workshops on *Posidonia oceanica* beds in 1983 and 1985), as well as of world seagrasses in general (ISBW4 held in Corsica in 2000). Thus, the main goal for MSW 2006 was to focus primarily on all species present in the Mediterranean basin, but considering also those adjacent geographic areas where Mediterranean species are also present (e.g., Atlantic coast of Europe, including Canary islands).

Discussions on the scientific aspects of MSW 2006 were directed by the organizing Committee (Fig. 1) to promote closer links with seagrass biologists from North Africa and the eastern Mediterranean, given that information on seagrasses from these regions is still largely insufficient or lacking. Therefore, efforts were initiated to identify potential sponsors to help participation of scientists from these regions. Thanks to funding kindly received from the Mediterranean Action Plan (MAP) of the United Nations Environment Programme (UNEP), the Joint Research Centre (JRC) and the Institute for Environment and Sustainability (IES) of the European Commission (EC), 4 young scientists from four North African and East Mediterranean countries (Algeria, Tunisia, Egypt and Turkey) were able to attend the workshop and present papers on their work.

The organizing Committee identified three main general themes for the Workshop which allowed inclusion of the majority of aspects on seagrass research:

- Seagrass Biology and Ecology;
- Ecosystem Services of Seagrass beds;
- Monitoring, Management and Restoration of Seagrass Habitats.

In addition, these themes were integrated with specific discussion topics, addressed through Round Table sessions held as a relevant part of the Workshop:

- Use of seagrasses as bioindicators of the ecological status of coastal waters, in relation to implementation of the Water Framework Directive (WFD);
- New methodologies of ecosystem approach to seagrasses;
- Conservation of Mediterranean aquatic vegetation.

To ensure easy access to participants from Mediterranean countries and neighboring regions, it was decided to hold the MSW 2006 in Malta, located at the centre of the Mediterranean basin. Having joined the European Union in 2004, Malta is now the southernmost EU member state and is considered by many as a stepping stone between Europe and North Africa. Marine research has always been a priority for the Maltese scientific community, but there has been considerable interest in seagrass biology over the past 15 years. The Department of Biology at the University of Malta, kindly accepted to host the meeting, and the necessary organization procedures were activated, including the setting up of a web site (http://events.um.edu.mt/msw2006/). The Corinthia Jerma Palace Hotel, located in Marsascala, on the southeastern coast of mainland Malta, was chosen as the Workshop venue.

A total of 105 scientists comprising 47 students (from graduate to PhD level) and 58 non-student participants from 17 countries (see table of participants below) attended the Workshop.

Table 1. Number of MSW attendants according to country

Country	**Number of Participants**
Algeria	1
Belgium	3
Croatia	2
Egypt	2
France	9
Greece	1
Israel	1
Italy	45
Malta	8
The Netherlands	2
Portugal	4
Slovenia	1
Spain	15
Tunisia	4
Turkey	1
United Kingdom	4
United States of America	2

Overall, about 100 contributions (oral communications and posters) were presented, 60 of which are included in these Proceedings, including the outcome of two of the three Round Table Sessions.

The Workshop attendance was very heterogeneous, in terms of topics presented, age classes and countries represented. This, as well as the high number of students, made the Workshop particularly successful, with strong integration among people and across participating countries.

The meeting proceeded smoothly and attendance during all sessions (including the Round Table sessions) was very good. The weather was pleasant throughout the workshop, but because of strong Mistral winds, the planned boat trip and dives in the Malta-Comino Channel and Cirkewwa to visit deep-water seagrass beds, were cancelled. Marsascala, the small seaside resort where the hotel that served as venue for MSW 2006 was located, provided an ideal setting with its pretty seaside promenade, good restaurants and other essential commodities.

Fig. 1 - Members of the organizing Committee of the Mediterranean Seagrass Workshop 2006 at the closing ceremony in Malta. From left to right: Maria Cristina Gambi, Gabriele Procaccini, Maria Cristina Buia, Christine Pergent-Martini, Gerard Pergent, Giuseppe Di Carlo, Joseph A. Borg (the local conveyner).

Mediterranean Seagrass Association

The MSW 2006 provided the platform for launching the Mediterranean Seagrass Association. This will be achieved by modification of the existing Association "Seagrass 2000", which was set up for the organization of ISBW4, held in Corsica in 2000. The idea of promoting the Association was raised during the

MSW 2006 and it was proposed to the audience and acclaimed. The Association will be in place by the first quarter of 2007. The Association has set a series of priority activities: increase awareness of seagrass issues by promoting and activating a number of initiatives, provide support for the organization of future MSW and local meetings, produce and maintain a web page for literature, facilitate exchange of data and information, and render support for conservation, monitoring, and educational issues linked to Mediterranean seagrass ecosystems.

The Association will liaise with the WSA (World Seagrass Association, www.worldseagrass.org), acting as an independent regional Association. The liaison with the WSA will result in joint newsletters and web site, containing data and literature for exchange among the scientific community. Moreover, the WSA and the MSA will strive to organize joint meetings (ISBW and MSW) to reach a larger audience inside and outside the seagrass research field.

Finally, we would like to thanks all MSW participants, as well all Authors of the papers included in these Proceedings. A special thank is due to all members of the Scientific Committee who gave their precious help in revising the papers, and to Prof. Giulio Relini (Genoa, Italy), Director of “Biologia Marina Mediterranea” and the President and the Directive of the Italian Society of Marine Biology (SIBM), who allowed to use the official Journal of the Society to host the Proceedings. Last but not least, we wish to thank the President of the Stazione Zoologica “A. Dohrn” of Naples, Prof. Giorgio Bernardi, for financial support in publishing the present volume, in the frame of the long tradition and history of the publications of the Institute.

Maria Cristina Gambi
Joseph A. Borg
Maria Cristina Buia
Giuseppe Di Carlo
Christine Pergent-Martini
Gerard Pergent
Gabriele Procaccini
(Editors)

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Giuseppe Di Carlo
IAMC-CNR Laboratorio di Ecologia Marina
Palermo, Italy

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Department of Biology (Host)
University of Malta, Malta

Maria Cristina Buia
Maria Cristina Gambi
Gabriele Procaccini
Stazione Zoologica 'Anton Dohrn'
Laboratorio di Ecologia del Benthos
Napoli, Italy

Gérard Pergent
Christine Pergent-Martini
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Université de Corse, France

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Javier Romero
University of Barcelona, Spain

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Institute of the Republic of Slovenia
for Nature Conservation, Regional Unit
Piran, Slovenia

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Stazione Zoologica “Anton Dohrn”, Naples

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Malta Environment and Planning Authority (MEPA)

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SECTION 1

SEAGRASS BIOLOGY AND ECOLOGY

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S. Beer, L. Axelson, M. Björk

Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel.

MODES OF PHOTOSYNTHETIC BICARBONATE UTILISATION IN SEAGRASSES, AND THEIR POSSIBLE ROLES IN ADAPTATIONS TO SPECIFIC HABITATS

Abstract

Bicarbonate (HCO_3^-) utilisation can in seagrasses be facilitated by (a) the activity of extracellularly acting carbonic anhydrase (CAe), which catalyses the "dehydration" of HCO_3^- to CO_2 within the diffusion boundary layer (DBL) of the photosynthesising epidermal cells, followed by CO_2 uptake, and/or (b) proton (H^+) extrusion, which facilitates the "dehydration" to CO_2 within the DBL [or possibly drives direct uptake of HCO_3^- into the cells via HCO_3^-/H^+ symport (or HCO_3^-/OH^- antiport)]. As a third means (c), CAe and H^+ extrusion act together so as to efficiently form CO_2 within acidic zones. Thus, seagrasses can be classified into different "modes" of HCO_3^- utilisation depending on the relative contribution of each such means [modes (a)-(c)]. Most tropical seagrasses use HCO_3^- according to mode (c). This may confer to them the ability to grow in dense stands, or in tidal pools, where pH may be high (>8.8) during active photosynthesis. So far, Halophila ovalis *is the only tropical species in which normal photosynthesis can be supported solely by mode (b), restricting it to monospecific pools of pH<8.6 in the intertidal. The temperate species* Zostera marina *shows two alternative/complementary modes of HCO_3^- use, (a) and (b), while* Ruppia cirrhosa *features mode (c). Mediterranean seagrasses show CAe activity, and we propose a simple protocol by which it would be possible to determine also their modes of HCO_3^- utilisation.*

Key-words: *Seagrass, Photosynthesis, Bicarbonate Utilisation, Adaptation.*

Introduction

Seagrasses, like all marine macrophytes, utilise the in seawater abundant bicarbonate ions (HCO_3^-) as a source of inorganic carbon for their photosynthetic needs (reviewed in Beer, 1996). While this has been known for some 30 years, it has recently been realised that, like for macroalgae, the "mode" of HCO_3^- use varies between species. Thus, the utilisation of HCO_3^- can in seagrasses be divided principally into three main modes [(a)-(c), Beer *et al.*, 2002a]. In mode (a), HCO_3^- is converted ("dehydrated"), mainly via extracellularly acting carbonic anhydrase (CAe), to CO_2 within the diffusion boundary layer (DBL, including the cell wall) of the photosynthesising epidermal cells, followed by CO_2 uptake. Mode (b) features proton (H^+) extrusion into acid zones within the DBL, resulting in HCO_3^- "dehydration" and CO_2 uptake, or, possibly HCO_3^-/H^+ symport (or HCO_3^-/OH^- antiport) into the cells. In mode (c), HCO_3^- is "dehydrated" to CO_2 with the aid of CAe acting in acidic DBL zones formed by H^+ extrusion, followed by CO_2 uptake. The different modes of HCO_3^- utilisation can be determined by measuring photosynthetic rates in the presence of either a CAe inhibitor or an H^+ scavenger, or both (see Fig. 1).

Most tropical seagrasses utilise HCO_3^- according to mode (c) (Uku *et al.*, 2005). This may confer to them the ability to grow in dense stands, or in tidal pools, where the pH is high during the time of active photosynthesis. So far, *Halophila ovalis* is the only tropical seagrass that can express full photosynthetic rates based on HCO_3^- utilisation solely according to mode (b), and it was found that this seagrass cannot photosynthesise at high pH values (>8.6) e.g. such as created in intertidal pools by other seagrasses featuring mode (c); therefore, it was suggested, this species is restricted to mono-specific pools in which the pH it generates is <8.6 (Beer *et al.*, 2006). One tropical species, *Cymodocea serrulata*, could support its photosynthesis according to HCO_3^- utilisation mode (a) (Uku *et al.*, 2005). Among the temperate seagrasses, *Zostera marina* may use modes (a) or (b) (Hellblom *et al.*, 2001) while *Ruppia cirrhosa* features mode (c) (Hellblom and Axelsson, 2003). Regarding Mediterranean seagrasses, both *Cymodocea nodosa* and *Posidonia oceanica* have been reported to show CAe activity (Invers *et al.,* 1999).

We propose here a way in which the mode of HCO_3^- utilisation could be determined for any seagrass, and speculate on the possible adaptive, or limiting, features that different modes of HCO_3^- utilisation could confer to seagrasses growing in various habitats.

Materials and methods

The different modes of HCO_3^- utilisation can principally be determined by measuring photosynthetic rates as a function of pH and, especially, the presence or absence of inhibitors of either CAe, extruded H^+ or both. Photosynthetic rates in seagrasses have classically been measured as O_2 evolution, but can also be measured by pulse amplitude modulated (PAM) fluorometry. The classical inhibitor of CAe is acetazolamide (AZ), which hardly penetrates cell membranes, and, thus, acts at extra-cellular level. Any proton buffer will dissipate low pH DBL zones by neutralising extruded H^+, and we have usually used TRIS buffer for this purpose. The different HCO_3^- utilisation modes are depicted in Fig. 1, as are the expected photosynthetic responses to the inhibitors; for details on the O_2 evolution measurements and inhibitor concentrations see e.g. Hellblom *et al.* (2001) and Uku

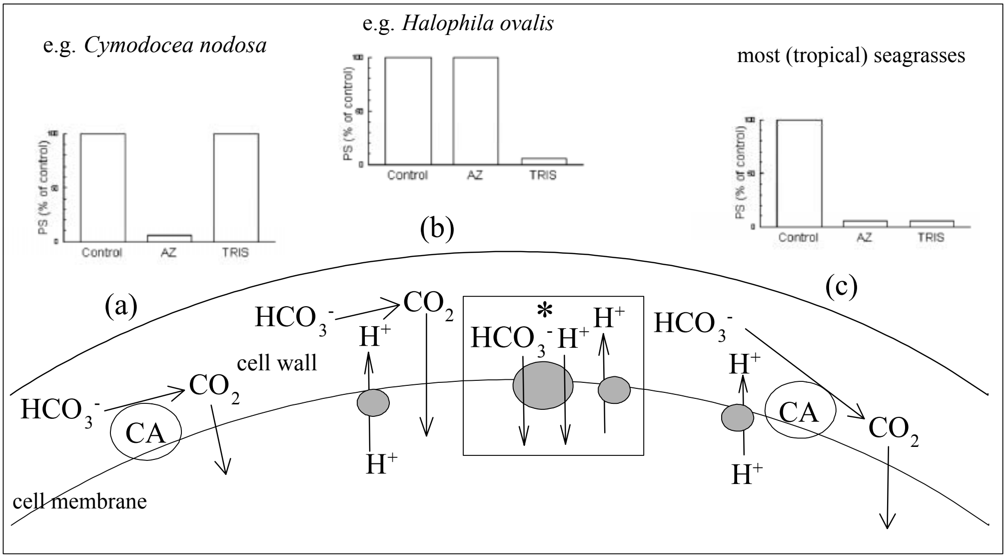

Fig. 1 - The different modes of HCO_3^- utilisation in seagrasses (see text for details). AZ, acetazolamide; TRIS, Tris buffer; CA, externally acting carbonic anhydrase (CAe in the text). From Beer *et al.* (2002a). *There is little direct evidence for this system.

et al. (2004); for details on the use of PAM fluorometry in seagrasses, see e.g. Beer *et al.* (2002b); for *in situ* PAM fluorometric measurements see Schwarz *et al.* (2000). In some cases, photosynthetic measurements were done also at high pH: At pH values close to 9, the CO_2 equilibration concentrations within the DBL would be too low to account for any significant influx of CO_2 to support photosynthesis, and means (a) would not be operative.

Results

Using the principal approach depicted in Fig. 1, i.e. measuring photosynthetic rates in the presence and absence of AZ or TRIS, and, in some cases, also at high pH values, some seagrasses have been categorised with respect to their photosynthetic modes of HCO_3^- utilisation (Table 1).

Tab. 1 - Main modes of HCO_3^- utilisation for various seagrasses as based on photosynthetic rate measurements in the presence of AZ, a proton buffer and, in some cases, various pH values (*, as determined mainly by AZ additions).

Species		mode of HCO_3^- utilisation	Reference
Cymodocea serrulata		a	Uku *et al.* (2004)
Halophila ovalis		b	“
Halodule wrightii		c	“
Cymodocea rotundata		c	“
Thalassia hemprichii		c	“
Thalassodendron ciliatum		c	“
Syringodium isoetifolium		c	“
Enhalus acoroides		c	“
Posidonia australis		a*	James and Larkum (1996)
Zostera marina	pH 8.1	a+b	Hellblom *et al.* (2001)
“	pH 8.6	b	“
Ruppia cirrhosa		c	Hellblom and Axelsson (2003)
Posidonia oceanica		a?*	Invers *et al.* (1999)
Cymodocea nodosa		a?*	

Discussion

Most seagrasses investigated so far show HCO_3^- utilisation mode (c). This seems to be the most “advanced” means in that it combines the catalysis of HCO_3^- to CO_2 conversion with DBL acidification in acidic zones, where the low pH *per se* is also conducive to HCO_3^- “dehydration” and where inorganic carbon equilibrium CO_2 concentrations would be high and, thus, conducive to efficient inward diffusion of CO_2. Another “broad” adaptation strategy within one species may be found in *Zostera marina*’s possibility to switch between modes (a) and (b) (Hellblom *et al.*, 2001).

While it may be interesting to elucidate the modes of HCO_3^- utilisation *per se* in seagrasses, it may be even more challenging to ascribe the different modes ecological significances. For seagrasses, there has only been one report linking their mode of HCO_3^- utilisation with adaptations to specific environments: Beer *et al.*

(2006) found that within the tropical intertidal pools formed in Zanzibar as the tides recede, *Halophila ovalis*, which can support full photosynthetic rates based solely on mechanism (b), always grows monospecifically, i.e. never together with e.g. *Cymodocea rotundata* and/or *Thalassia hemprichii* which feature mode (c) and grow in other pools in which they create higher pH values. Thus, it seems that mode (b), which may rely mainly on the outwards pumping of H^+, is sensitive to high pH values. While mode (c) also features an H^+ pump, it also relies on CAe activity in the acidic zones, and can thus survive at higher pH values (provided that the latter are localised to isolated cell wall "pockets"). The pH sensitivity of these three otherwise (in deeper waters) co-occurring species was verified by pH drift experiments in which *Halophila ovalis* could raise the pH of its surrounding water to 8.5 before photosynthesis ceased, while the other two species could raise the pH to 8.8 and 9.2, and similar pH values were also found in the actual pools where these seagrasses grew. These adaptabilities based on mode of HCO_3^- utilisation are somewhat analogous to those found in *Ulva* and *Enteromorpha* (now *Ulva*). In *Ulva intestinalis*, a mode of direct HCO_3^- uptake, *via* an anion exchange protein located at the cell membrane (exchanging an inwards flux of HCO_3^- with and outwards flux of OH^-), confers to it the ability to photosynthesise in extremely high pH waters (>10) such as generated by its photosynthesis in rockpools, and this prevents other algae, featuring modes of HCO_3^- utilisation that are more sensitive to high pH, from growing there (Björk *et al.,* 2004). However, no such HCO_3^- /OH^- exchange mechanism has yet been found for seagrasses.

In seagrass species that are not always exposed to high light and high temperature, it is interesting to note that *Zostera marina* featured mainly mode (a) at a normal seawater pH (ca. 8.1), but could also feature mode (b) under conditions of higher pH (8.6) (Hellblom *et al.*, 2001). Such higher pH values may be found in dense seagrass stands during conditions of high temperature and irradiance.

While the Mediterranean seagrasses *Cymodocea nodosa* and *Posidonia oceanica* feature CAe activity (Invers *et al*., 1999), their exact modes of HCO_3^- utilisation according to Fig. 1 have not yet been determined. Since it is implied here that the different modes of HCO_3^- utilisation may be differently sensitive to pH, and that the co-occurring species *Zostera noltii* is less pH sensitive (Invers *et al*., 1997), it may be of both academic and ecological interest to investigate the modes of HCO_3^- utilisation in these Mediterranean species too.

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S. Acunto, F. Rende, ,R. Cozza, A.M. Innocenti

Dipartimento di Biologia, Unità di Biologia Marina ed Ecologia
Università di Pisa – via A. Volta, 6 – 56126 Pisa, Italy

STATE OF HEALTH OF *POSIDONIA OCEANICA* MEADOWS ALONG THE NORTHERN TYRRHENIAN COAST OF CALABRIA, ITALY

Abstract

In the framework of the MEMOBIOMAR project, the distribution and conditions of Posidonia oceanica *meadows along the northern Tyrrhenian coast of Calabria (Italy) were studied. Aim of the work was the assessment of the structure of the meadows, the phenology of the plant and the distribution and abundance of epiphytes. Moreover, the hypothesis of differences among meadows subjected to different levels of human disturbance was investigated. Our results suggest that human disturbance influenced some of the structural and morphological variables analysed. Epiphytes have high variability in distribution and abundances, particularly at the smallest spatial scale considered (i.e. among leaves or rhizomes) and among areas (tens of meters apart). Among the leaf epiphytes, only the bryozoan* Electra posidoniae *appears to be directly influenced by disturbance, while, among the epiphytes of rhizomes, similar results were observed for the red algae* Peyssonnelia *spp. and the foraminiferan* Miniacina miniacea*.*

Key-words*:* Posidonia oceanica*, structure, phenology, epiphytes, bioindicator.*

Introduction

Posidonia oceanica (L.) Delile meadows are considered good indicators of the state of health of coastal environment for their sensitivity to human induced disturbances (Pergent *et al.*, 1995; Kirkman, 1996; Pergent-Martini *et al.*, 1999; Guidetti, 2001). In particular, enhanced turbidity and consequent reduction of water transparency, are considered the most common causes of *P. oceanica* meadows regression. In the framework of the MEMOBIOMAR project[1] we studied the distribution and conditions of *P. oceanica* meadows along the northern Tyrrhenian coast off Calabria (Italy). The aim of the study was the assessment of the meadows structure, plant morphology, distribution and abundance of epiphytes. Moreover, the hypothesis of differences among meadows subjected to different levels of human disturbance was investigated. Disturbance was represented by high levels of sedimentation and wastewater coming from urban settlements.

Material and methods

Posidonia oceanica samples were collected along the Calabria Tyrrhenian Coast between Praia a Mare (Cosenza, Italy 39°54'28.83"N – 15°46'28.38"E) and Scalea

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[1] Italian "Ministero dell'Università e della Ricerca Scientifica e Tecnologica. Piani di potenziamento della rete scientifica e tecnologica; Cluster 10: Ambiente marino".

(Cosenza, 39°48’45.71”N – 15°47’53.74”E). The study was carried on through a hierarchical sampling design that enable comparisons at spatial scales ranging from metres (areas) to kilometres (locations). Locations correspond to stretches of coast 150-200 meters long, affected by different levels of disturbance: D = Disturbed location; C1 = Control 1 location; C2 = Control 2 location. Within each location, three sites (a, b, c) were randomly chosen. During May 2002 within each site, the density of shoots was estimated (using quadrats of 0.25 m^2) and samples of 15 shoots of *P. oceanica* were randomly collected by SCUBA diving at 10 m depth, in three random areas 25 m^2 wide.

We studied variables at three different levels: a) meadow: shoots density, Leaf Area Index; b) plant: number, length and width of leaves; c) epiphytic community: percentage cover of species, groups of species or morphological groups over the outer side of the old leaves and rhizomes (Piazzi *et al.*, 2004). The analyses on the algal epiphytes of *P. oceanica* were carried out for the most abundant groups: *Peyssonnelia* spp., encrusting coralline, corticated-terete, filamentous and foliose algae (Piazzi *et al.*, 2004).

Data of morphological variables and percentage covers of epiphytes were analysed by univariate techniques (ANOVA).

Results

In the disturbed location both the density, ranging from 303±13.6 to 405±42.4 (shoots/m^2), and the Leaf Area Index, ranging from 3.9±0.2 to 11±1.1 (m^2/m^2), were lower than in the control locations where the density varied from 331±25.5 to 576±25 (shoots/m^2) and the L.A.I. from 4.6±0.5 to 15.3±0.7 (m^2/m^2).

The differences in the number of leaves of *P. oceanica* were always significant for the factor “Area” and, only in the case of the old leaves, for the factor “Site”. Anyway, the observed variability seems to be independent from human disturbance (Tab. 1). The analyses on the leaves length were significant for all the factors considered with the exception of the factor “Disturbance” (Tab. 2).

Tab. 1 - ANOVA on the mean number of leaves/shoot (old and intermediate) of *Posidonia oceanica*.* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant. Significant values are in bold.

		Old leaves		**Intermediate leaves**	
Source of variability	**df**	**MS**	***F***	**MS**	***F***
Disturbance	2	0.417	0.14	0.941	0.96
Site (Disturbance)	6	3.032	**3.13***	0.975	1.80
Area (Disturbance x Site)	18	0.968	**1.79***	0.541	**1.71***
Residual	378	0.540		0.316	
Cochran’s C Test		$C = 0.13$; $P < 0.01$		$C = 0.06$ ns	
Trasformation		none		none	

Tab. 2 - ANOVA on the mean length of leaves/shoot (old and intermediate) of *Posidonia oceanica*.* = p < 0.05; ** = p < 0.01; *** = p< 0.001; ns = not significant. Significant values are in bold.

		Old leaves		**Intermediate leaves**	
Source of variability	**df**	**MS**	***F***	**MS**	***F***
Disturbance	2	2071.368	0.19	4385.235	0.49
Site (Disturbance)	6	10659.053	**3.68***	9002.460	**3.03***
Area (Disturbance x Site)	18	2895.882	**9.89*****	2967.920	**9.64*****
Shoot (Disturbance x Site x Area)	378	292.9567	**1.21***	307.860	**1.67*****
Residual old	810	242.387			
Residual intermediate	405			184.069	
Cochran's C Test		*C* = 0.01 ns		*C* = 0.05 ns	
Trasformation		none		none	

However, the graphical analysis showed that leaves of *P. oceanica* were longer in control sites rather than in the disturbed one (Fig. 1). The analyses on the leaf width gave similar results, but the observed variability appeared independent from human disturbance.

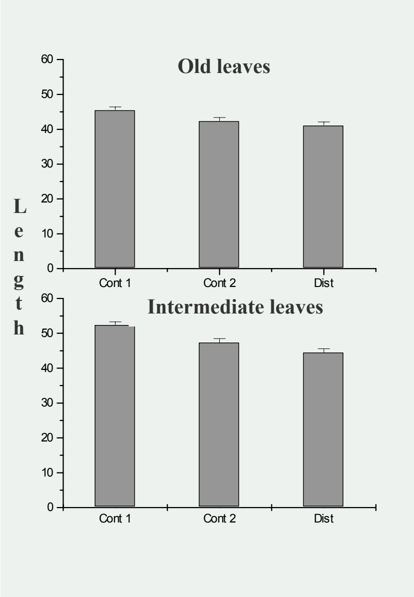

Fig. 1 - Mean length of the leaves (old and intermediate) of *Posidonia oceanica* in each sampling location. 2 Bars indicate SE (old leaves n = 405; intermediate leaves n = 270). Cont 1 = Control 1 location; Cont 2 = Control 2 location; Dist = Disturbed location.

Epiphytes of the leaves: All the analyses detected significant differences among Leaves and Areas. Moreover, the filamentous algae showed significant differences among shoots, while the encrusting coralline among sites. The observed variability never appeared dependent from human disturbance.

Among sessile invertebrates, hydrozoans (6 taxa) and bryozoans (12 taxa) were the most represented among the total 21 taxa observed on the leaves of *P. oceanica*. Analyses on percentage covers demonstrated high variability mainly at the smallest spatial scales. Again, the observed variability never appeared dependent from human disturbance.

The analyses conducted on the most abundant bryozoans demonstrated patterns of variability similar to those observed for the hydrozoans. However, the percentage covers of *Electra posidoniae* Gautier were higher in the Control 2 sites than in the Disturbed sites suggesting that the distribution and abundance of this species is dependent from disturbance (Fig. 2). The other invertebrates analyzed were foraminiferans and the colonial ascidian *Botrillus schlosseri* (Pallas). In both cases the analyses were significant for the factors "Leaf" and "Shoot". Moreover, the foraminiferans showed variability also among sites (Fig. 2).

Epiphytes of the rhizomes: All the analyses were significant for the factors "Rhizome". While the filamentous algae showed significant differences also among areas, all the other taxa, with the exception of the corticated-terete algae, showed variability among sites. Percentage covers of *Peyssonnelia* spp. resulted lower in Control 2 sites than in the disturbed sites (Fig. 2).

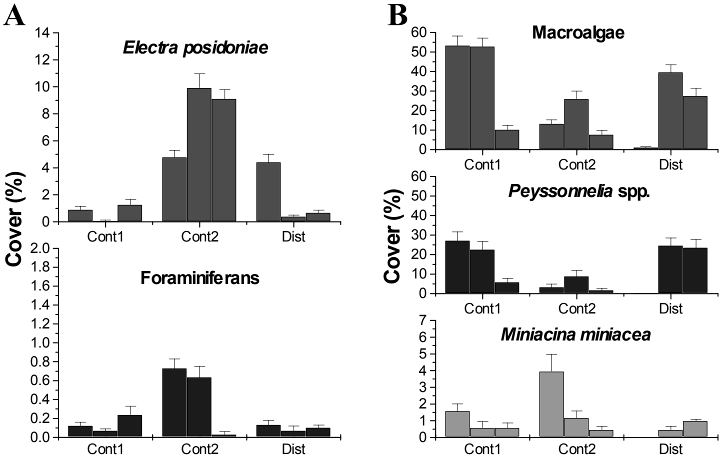

Fig. 2 - A: Mean percentage cover of some epiphytes of *Posidonia oceanica* leaves at each sampling site. Error bars indicate SE (n = 300). B: Mean percentage cover of some epiphytes of *Posidonia oceanica* rhyzomes at each sampling site. Error bars indicate SE (n = 75). Cont 1 = Control 1 sites; Cont 2 = Control 2 sites; Dist = Disturbed sites.

Among sessile invertebrates bryozoans (10 taxa) were the most represented on a total of 16 taxa of invertebrates observed. The analyses conducted on the most abundant species demonstrated high variability at small spatial scale, but the distribution and abundance of all the species of bryozoans analyzed never appeared dependent from the factor "Disturbance".

The other invertebrates analysed were hydrozoans, sponges and the foraminiferan *Miniacina miniacea* (Pallas). All these analyses were significant for the factor "Rhizome". Percentage covers of the hydrozoans were variable among areas and those of *M. miniacea* among sites. Moreover, the graphical analysis showed a lower abundance of *M. miniacea* in the disturbed than in the control locations (Fig. 2).

Discussion

Our results suggest that human disturbance influenced some of the structural and morphological variables analyzed: both density of shoots and Leaf Area Index are lower in the disturbed than in the control locations. According to the classification proposed by Pergent *et al.* (1995) modified by Buia *et al.* (2004), the values of density observed in the disturbed location configure a "disturbed meadow"; in this meadow, the L.A.I. is far from the value expected for the spring season at this depth and the graphical analyses shows that the length of leaves is shorter than in the control meadows.

Epiphytes of the leaves show distributions and abundances highly variable, particularly at the smallest considered spatial scale (i.e. among leaves), very often among areas (tens of meters apart) and sometimes also at the scale of hundred of meters (i.e. among sites).

Among all the taxa analysed, only the bryozoan *E. posidoniae* appears to be directly influenced by disturbance, showing percentage covers lower in the disturbed than in the Control 2 site.

Epiphytes of the rhizomes show percentage covers highly variable both at the smallest (i.e. among rhizomes) and at the largest spatial scale considered (i.e. among sites hundred of meters apart). Among macroalgae, *Peyssonnelia* spp. was the most abundant taxon. Its pattern of distribution, showing lower abundances in the Control 2 sites, is probably due to the strong sciaphilous *habitus* of *Peyssonnelia* spp. that doesn't suffer for the reduction of water transparency occurring in the disturbed sites. Among other epibionts, only the foraminiferan *Miniacina miniacea* resulted influenced by disturbance, showing percentage covers lower in the disturbed than in the control sites.

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S. Aliani[1], G.P. Gasparini[1], C. Micheli[2], A. Molcard[3], A. Peirano[4]

[1]CNR ISMAR – Forte di S. Teresa, 19036 - Pozzuolo di Lerici, La Spezia, Italy
[2]ENEA Casaccia - Roma, Italy
[3]LSEET - Université de Toulon, France
[4]ENEA S.Teresa - La Spezia , Italy

CAN SOUTHERN MEADOWS OF THE MEDITERRANEAN SEAGRASS *POSIDONIA OCEANICA* (L.) DELILE SUPPLY NORTHERN ONES? A MULTIDISCIPLINARY APPROACH IN THE LIGURIAN SEA

Abstract

Three rare fruiting events of Posidonia oceanica *in the Ligurian Sea in 1992, 1993 and 2004 were analysed to investigate the possibility that floating fruits coming from southern meadows of Corsica and Livorno (Tuscany) can supply northern meadows. Meteorological and oceanographic data are compared to data on stranded fruits to identify possibile oceanographic forcings in fruits transport and distribution.*

Key-words: *seagrasses,* Posidonia oceanica*, fruiting, circulation, Ligurian Sea*.

Introduction

The completion of the sexual cycle of the seagrass *Posidonia oceanica* (L.) Delile, from flowering to dissemination is a rare event in the Ligurian Sea (Balestri, 2004).

Since 1990 the cycle had been monitored in the *P. oceanica* meadow of Monterosso al Mare, included in the Marine Protected Area of “Cinque Terre” (Ligurian Sea, North-Western Mediterranean) weekly checking the beach for stranded fruits (Stoppelli and Peirano, 1996; Peirano *et al.*, 2000).

Fruits have been first recorded in May 1993 and 1994 when a massive fruiting event occurred along all the Mediterranean coasts (Diaz-Almela *et al.*, 2006). In 1994 floating fruits had been recorded along all the Ligurian coast from spring to the end of July (Stoppelli and Peirano, 1996). In 2004 the fruiting in the Monterosso meadow was particularly intense in abundance, spatial and temporal distribution with stranded, fruits observed until the 5th of July.

In this paper we aim to assess whether fruits or seeds coming from the Tyrrhenian basin can be carried northward by the currents and whether changes in the oceanographic contrains due to climatic variability can affect the dispersion of fruits from the warm Tyrrhenian basin to the colder Ligurian Sea.

The major large scale feature of the water dynamic in the surface layers of the Ligurian Sea is a cyclonic circulation active all year round, more intense in winter than in summer. Southern waters filling the Ligurian Sea occur in two main currents running along each side of northern Corsica. The West Corsica Current running along the western side of Corsica and the warm and salty Tyrrhenian current passing trough the Corsica Channel between Capraia and Corsica (Astraldi and Gasparini, 1992). The two waters merge to the north of Corsica and they flow together along the Ligurian coast toward the the Provençal area. The meadow of Monterosso al Mare is about 80 km from the nearest, southern upstream meadow (Livorno, Tuscany). Observations collected by visual census on the meadow and on stranded fruits are compared with meteorological data (wind speed and direction, surface water and air temperature) and with the result of a matemathical model of transport.

Material and methods

The flowering and fruiting had been surveyed since 1991 both by diving observations and collecting fruits cast ashore at Monterosso (Ligurian Sea, North-Western Mediterranean).

In 2004 visual sightings and sampling were conducted along the Tuscany shores and up to 100 km offshore during oceanographic cruises. Collected inflorescences and fruits were measured and grouped into the four size classes of Boudouresque and Thelin (1985). Sub samples were put in floating bottles filled with seawater to test their floating duration in days.

Data on sea surface and air tempearture, wind and atmospheric pressure had been extracted from COADS database, data on fluxes in the Corsica Channel are from the CNR ISMAR SP database (Astraldi and Gasparini, 1992). To study the probability of dispersion of floating fruits the Lagrangian model by Falco *et al.* (2000), simulating the motion of independent particles in a turbulent flow was used and implemented. The release of a high number of particles (fruits) was simulated from two dispersal sites, Corsica Channel and Livorno. For the mean flow we used the output of a general circulation model applied to the Mediterranean Sea (Demirov and Pinardi, 2002) forced by a perpetual (monthly mean) surface forcing for a 7-year period, and averaged in a unique climatological surface mean current field. The model has a 12 km grid resolution. The computational domain where we applied the Lagrangian model extends between 8.5°E-12.5°E in longitude and between 41.25°N-44.5°N in latitude.

Results

Since the last major fruiting event in 1992-93 sparse and rare flowers/fruits were recorded at Monterosso in September-December 1994, 1997, 1998, 1999 but the fruiting cycle had never been completed.

In 2004 fruiting was intense all over the Mediterranean (Diaz-Almela *et al.*, 2006) and diving surveys showed that at Monterosso the fruiting involved the whole meadow from 6 to 18 m depth. However, it wasn't recorded the massive, prolonged transportation of floating fruits observed in 1994.

Hundreds of fruits had been casted ashore in front of the Monterosso meadow since the end of March 2004 expecially following a period of stormy weather (March, 19-23). Most of inflorescences in Monterosso (from 50 to 95%) were bearing two and in some case three fruits and were quite different in size according to Boudouresque and Thelin classification. Some epibiont (likely *Lepas* spp. juv.) were found attached to the base of the fruits of some inflorescences and the lenght of the barnacle was between 3 and 5 mm. On the 24th of March fruits were also collected along the Tuscany shores respectevely at 5 and 11 nautical miles from Monterosso.

In May 2004 the fruits collected randomly from the Monterosso meadow had the same size of the fruits collected on the beach and no epibionts were found on them.

Stranding on beach continued at Monterosso since the 8th of June and a second maximum was after another period of storms (5-8 May). However no floating fruits were observed offshore nor fruits had been collected along the Tuscany coast.

A low percentage (1.7 %) of stranded fruits sunk after 9 days in a container with seawater. At least 12 days were necessary to the majority of fruits altough they showed signs of corruption and loss of floatation.

The analysis of the fluxes across Corsica channel from 1992 to 2005 showed that in the period 1992-1996 less water came in the Ligurian Sea from the Tyrrhenian Sea, i.e. the West Corsica Current prevailed, while the opposite happened in 1996-2005 (Fig. 1).

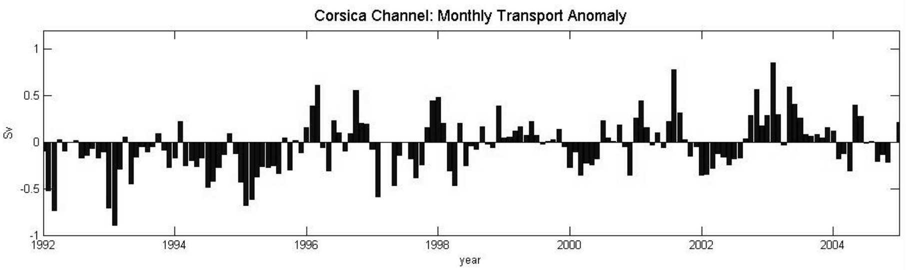

Fig. 1 - Anomalies of monthly transport in Corsica Channel in Sverdrup ($10^6 m^3 sec^{-1}$) for the period 1992-2005.

This is in agreement with meteorological data which stressed the existence of some important negative anomalies in summer sea surface and air temperature in 1993-1994 (Fig. 2), with summer waters colder than the average of the decade. The reverse was observed in 2003. Also wind speed anomalies showed positive peaks in intensity in 1992, 1993 and the opposite in 2003-2004.

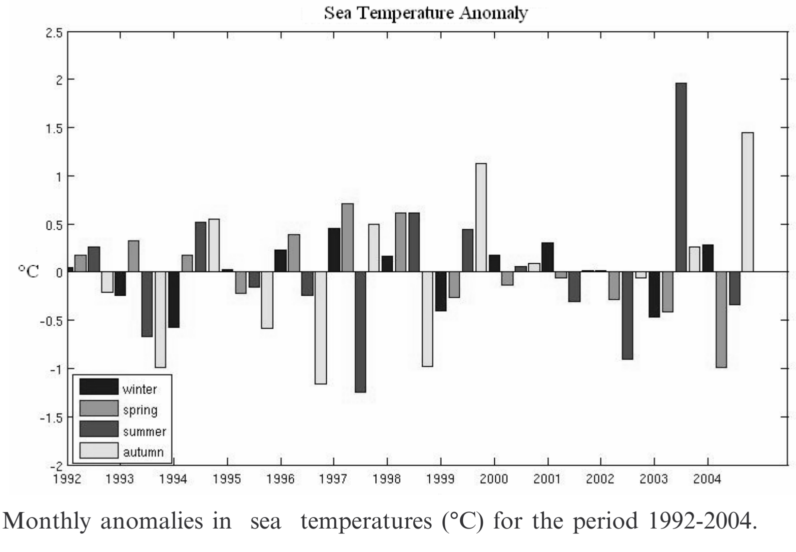

Fig. 2 - Monthly anomalies in sea temperatures (°C) for the period 1992-2004.

In the numerical experiment 1000 particles were released in the Corsica channel (42.5°N, 9.625°E) corresponding to the main flow of the Tyrrhenian current (years 1996-2005). The model showed that the percentage of particles that are able to reach the Ligurian Coast (all the particles that are crossing the 44°N parallel are considered landed in the Ligurian Coast) were very low (3% in 30 days). When particles were released from the closest meadow of Livorno the percentage of particles reaching Liguria not exceeded 10%. These results and the metereological analysis are in agreement with the low transportation of floating fruits in 2004. Hence, it could be hypothesised that the observed, massive fruit transportation observed in 1994 could be related to the prevailing and strongest West Corsica current.

Conclusions

The stranding of inflorescences along Tuscany coasts evidenced the chance for fruits to be transported by the wind and waves alongshore and towards the North. Moreover, the measured length of the lepads (5 mm) found on fruits at Monterosso is consistent with one-two weeks of floating. This period is in agreement with the floating period of fruits (Buia and Mazzella 1991) and the results of our floating test (9 days).

The intensities of the Tyrrhenian (7-14 km day^{-1}) and Corsica currents (10-12 km day^{-1}) may suggest a possible connection between meadows. The metereological data analysis at basin scale and the model simulations showed that only extreme events and anomalies, like those recorded in 1992-1993, may enhance the probability in transporting *Posidonia* seeds over long distance.

Further studies are in progress to assess the role of strong winds and other meteorological events in controlling dispersal patterns; furthermore, stranded fruits collected on the beaches are reared in aquaria for genetic comparison between stranded samples and original populations.

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I. Blanch[1], E. Dattolo[2], G. Procaccini[2], R. Haroun[1]

[1]BIOGES, Department of Biology, Campus Tafira, University of Las Palmas Gran Canaria,
35017 Las Palmas, Canary Islads, Spain
[2]Stazione Zoologica 'A.Dohrn', Laboratorio di Ecologia del benthos, Punta S. Pietro 80077 Ischia (Napoli) - Italy

PRELIMINARY ANALYSIS OF THE INFLUENCE OF GEOGRAPHIC DISTRIBUTION AND DEPTH ON THE GENETIC STRUCTURE OF *CYMODOCEA NODOSA* MEADOWS IN THE CANARY ISLANDS

Abstract

Meadows formed by the seagrass Cymodocea nodosa *(Ucria) Ascherson are the dominant vegetated communities in shallow soft bottoms throughout the Canary Islands (Central East Atlantic Ocean). The Canarian Archipelago represents the western, and almost the southern distributional limit of the species. Due to the hydrodynamic conditions, C. nodosa is only present on the protected southern coasts of the islands. Meadows are usually distributed over a wide depth range where they show clear variations of structural and morphometric attributes (bottom coverage, shoot density and mean length of leaves).*

The aim of this project was to assess the genetic structure of C. nodosa *in the Canarian Archipelago at different hierarchical, spatial scales. In particular we aimed to investigate the existence of genetic patterns at four different levels: between stands collected at different depths in the same locality, between different localities sampled in the same island, among stands collected at the same depths in different islands, and between meadows in the Archipelago and in the Mediterranean Sea. To achieve this goal, we performed a hierarchical random sampling design, collecting samples in seven islands, in two locations within each island, in two sites within each location and in two depths within each site. A total of 1260 samples were sampled and genotyped for seven microsatellite loci already used to analyse Mediterranean populations. The data analysis have shown no strong genetic differentiation between stands growing at different depths. This could be due to the fact that in the Atlantic Ocean the thermocline appears not before than 50 m.*

Overall genetic diversity across the Archipelago is not very high in comparison with Mediterranean populations. Main gene flow follows a east-west direction showing that Fuerteventura, the closest Island to the African coasts and the one with the highest allelic richness, represents a bridge between the Mediterranean and the Canarian populations.

***Key-words**:* Cymodocea nodosa*, microsatellites, gene flow, genetic structure.*

Introduction

The Canary Islands (27°38 - 29°25'N, 18°10' - 13°25'W) represent the western and almost southern distributional limit of the seagrass species *Cymodocea nodosa* (Ucria) Ascherson. Populations of *Cymodocea nodosa* are generally located along the southeast coast of the islands. Meadows of this species, locally known as "Sebadales", are distributed along the sheltered soft bottoms of the archipelago forming a patchy mosaic from 0 to 40 m in depth. Clonal diversity and structure of seagrass meadows is determined by the influence of currents, sediment quality and water turbidity (Ellshand and Rose, 1987; Calem and Piece, 1992). However, changes in genetic variability of *C. nodosa* meadows, in relation to differences in environmental conditions, have never been studied in the Canary Islands. The aim of this project was to investigate whether the bathymetric gradient in the species distribution represents a possible source of genetic divergence between populations. In addition, we compared allelic richness between Canarian and Mediterranean populations and analyzed genetic structure within the Archipelago.

Materials and methods

A hierarchical sampling design was chosen with the following scheme: seven islands; two locations within each island separated by >1 km; two depths within each location divided into shallow (<5 m) and deep (>12 m); two sites within each depth separated by >100 m; and three patches within each site separated by >10 m. Ten leaves were collected from each seagrass patch and dried in silica gel.

Seven milligrams of dried tissue from each individual sample were ground in a Mixer Mill MM300 (QIAGEN). Subsequent DNA extraction was carried out using the KIT NUCLEOSPIN MULTI-96 PLANT (Macherey-Nagel).

Seven polymorphic microsatellite loci (Ruggiero et al. 2004) were used to obtain multilocus individual genotypes. PCR conditions are given in Ruggiero *et al.* (2004). Fragments were separated and analyzed on a CEQ 2000XL DNA Analysis system (Beckman Coulter).

Genotypes were analyzed with the software GIMLET (Valière, 2002). FCA was performed with the software GENETIX (Belkhyr *et al.,* 1996-2002; website: www.univmontp2/-genetix/genetix/intro.htm). Geneclass (Cornuet *et al.*, 1999) was used for the determination of the probability of migration of *Cymodocea nodosa* individuals among the different islands. Fstat ver.2.9.3.2 (Goudet, 1995) was used to calculate pairwise Fst values.

Results

Our analysis showed a clear differentiation between the Mediterranean and the Canarian Archipelago populations (Fig. 1). Individuals from the Fuerteventura meadows are in an intermediate position between the two main clusters. Not clear structure is detectable within the Archipelago through the AFC analysis also when the analysis is performed only on the Canary Island samples (data not shown). Gene flow exists within the archipelago. Nevertheless, allelic richness is heterogeneous among the different Islands, with higher values in the easternmost ones (Table 1). Eastern populations also represent the main origin of gene flow.

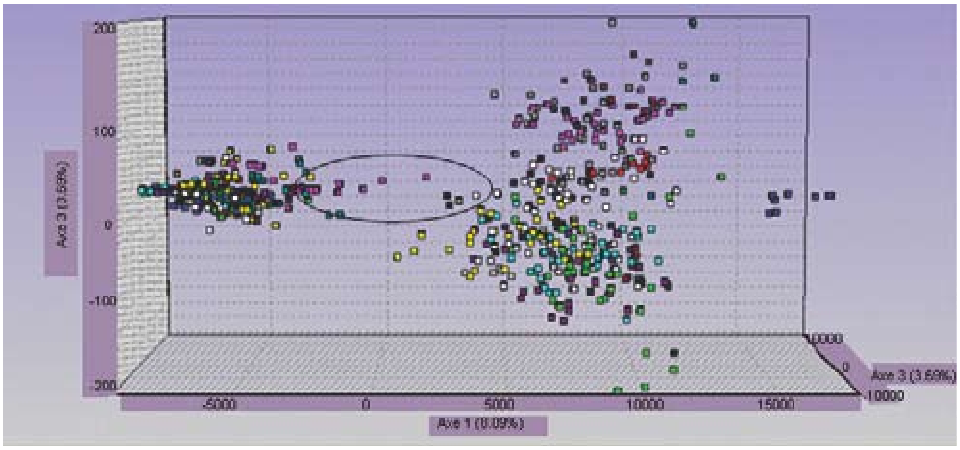

Fig. 1 - AFC analysis of populations belonging to the Canary Islands, clustered on the left, and the Mediterranean basin, on the right. Samples from the Fuerteventura Island are circled in the middle.

Assessment of migration patterns performed with assignment methods, in fact, revealed that the main migration pattern follows a East-West corridor from Chinijo to Fuerteventura and to Gran Canaria. Populations to which a selected individual was assigned with the highest probability value were defined as donor. El Hierro Island does not exchange migrants with the other populations.

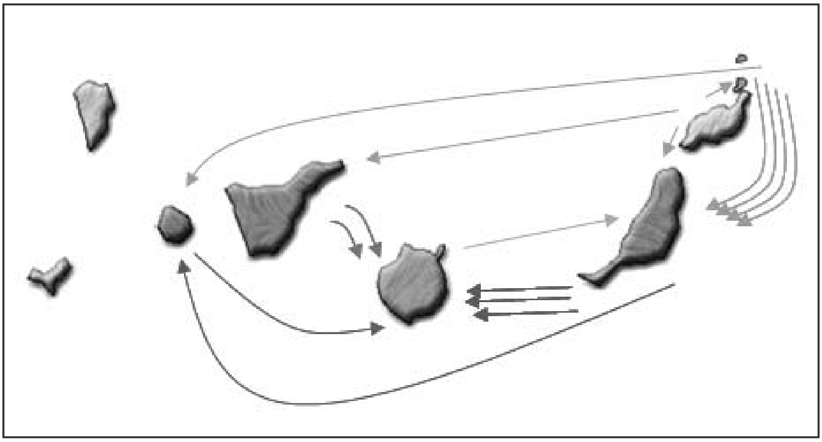

Fig. 2 - Migration pattern has been obtained with GeneClass using a Bayesian method for Assignment of Migrants. Each arrow represents a single migrant. For population code see Table 1.

Stands growing at different depths within the same population showed no difference in genotypic richness (G/N) (data not shown). Heterozygosity values were also comparable, with no univocal indications of excess *v*s deficiency of heterozygosity (Table 1).

Values of θ between stands were low but always statistically significant. This suggests a low degree of distinction along the bathymetric gradient (Table 1).

Conclusions

The present investigation represents a preliminary study carried out on a reduced set of samples of the total sampling performed on the Canarian Archipelago. Preliminary results suggest the following: the Canarian and Mediterranean populations of *Cymodocea nodosa* are genetically distinct and Fuerteventura represents a link between the two areas. Within the Canarian Archipelago, there is population differentiation, although a clear structure is not evident.

In addition, the analysis of the flow of migrants suggests that genotypes have entered the Archipelago by East, as suggested by a main south/south-west direction of gene flow. This result agrees with the fact that easternmost part of the Archipelago is the nearest to the African coast, suggesting an entrance of genotypes from there to the western Islands (see also Alberto *et al.,* 2006).

Tab. 1 - Estimates of allelic richness (A), expected heterozygosity (He), observed heterozygosity (Ho) and θ between stands collected at different depth in the same population. Asterisks represent significant values (P<0.001).

Island	Population/ depth	A	He	Ho	θ
Gran Canaria	GC1 S	1.28	0.29	0.20	0.04*
	GC1 D	1.25	0.26	0.21	
	GC2 S	1.19	0.24	0.23	0.117*
	GC2 D	1.23	0.23	0.12	
Fuerteventura	FT1 S	1.33	0.36	0.28	0.2*
	FT1 D	1.38	0.39	0.50	
	FT2 S	1.27	0.29	0.23	0.08*
	FT2 D	1.29	0.30	0.31	
Lanzarote	LZ1 S	1.33	0.34	0.44	0.1*
	LZ1 D	1.35	0.37	0.51	
	LZ2 S	1.30	0.31	0.36	-
Tenerife	TF1 S	1.18	0.16	0.21	-
	TF1 D	1.26	0.25	0.26	
	TF2 S	1.19	0.23	0.20	0.03*
	TF2 D	1.29	0.14	0.28	
Chinijo	CH1 S	1.31	0.31	0.39	0.11*
	CH1 D	1.34	0.31	0.36	
La Gomera	G1 S	1.20	0.22	0.16	0.1*
	G1 D	1.20	0.25	0.28	
El Hierro	H1 D	1.28	0.27	0.50	-

No strong differences have been found between stands collected at different depths. This could be due to the fact that in the Atlantic Ocean the thermocline appears not before than 50m, determining that environmental conditions are almost homogeneous at the considered depths.

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S. Cabaço, A. Alexandre, R. Santos

Marine Plant Ecology Research Group, CCMAR - Centre of Marine Sciences, Universidade do Algarve,
Gambelas, 8005-139 Faro, Portugal

SURVIVAL AND GROWTH OF THE SEAGRASS *ZOSTERA NOLTII* IN DIFFERENT CULTURE MEDIA

Abstract

The survival, growth and production of the seagrass Zostera noltii *were assessed in three different culture media (sand, agar and seawater). Plants composed of 3 modules were placed in acrylic containers (n = 24 per treatment) and assigned into 3 replicate aquaria, kept in a growth chamber. Plant survival and growth were determined after 30 days and at the end of the experiment (60 days). Plant survival was high (> 80%) for the three culture media tested, during the experimental period. No significant differences were found in shoot production, internode production or rhizome elongation rates among the different culture media, even though all parameters decreased significantly with time. Plants in agar and sediment media (81% and 83%, respectively) showed higher branching during the first 30 days of experiment than plants cultured only in seawater (67%), but no significant differences were detected. No significant effects of the culture media or incubation time were found for the elongation of branches, and branch internode production. However, branch production rate decreased significantly with time. These results showed that a simple seawater medium can be used in laboratory microcosm experiments, particularly for short-term experiments (up to 30 days).*

Key-words**:** *Culture media,* Zostera noltii*, survival, growth, branching*

Introduction

Zostera noltii is a small seagrass species occurring along the intertidal and subtidal areas of estuaries and shallow coastal lagoons, from Northern Europe to North-West Africa, and in the Mediterranean Sea (den Hartog, 1970). Its small size represents a big challenge for plant-specific fieldwork, especially in studies, which require a meticulous handling of the plants. Particular technical hitches arise when there is the need for tagging individual plants in the field, since rhizomes are too fragile to be tagged, often being broken or damaged. The difficulty in using tagging techniques limits the investigation of the demographic parameters of this species such as growth, mortality and recruitment. Moreover, the dense and delicate system of rhizomes and roots within the muddy sediments, which frequently characterize *Zostera noltii* meadows, constitutes another major drawback while assessing the plant's belowground fraction. However, unlike many other seagrasses, its small size represents an enormous advantage for laboratory microcosm experiments, especially in terms of lab space facilities.

Preparing culture media for laboratory microcosm experiments involves, most of the time, vast logistic and great human effort, such as bringing natural sediments to the laboratory. A simple alternative is to grow the plants in seawater, but this does not seem quite representative of natural conditions, as seagrasses are rooted plants.

This study aimed to assess the survival, growth and production of *Zostera noltii* plants in three different culture media, namely natural sediment, agar and seawater. Leaf chlorophyll content was determined as a physiological indicator of the plant's condition.

Materials and methods

Zostera noltii plants composed of 3 modules (i.e. 1 apical shoot plus 2 shoots and respective internodes and roots) were collected in the Ria Formosa lagoon (southern Portugal) in August 2005 and immediately transported to the laboratory in seawater. Three different culture media were prepared: a monophasic medium of seawater (35 PSU), and two biphasic media, one consisting of natural sandy sediments (with 0.5% ± 0.2 of organic matter content) and the other consisting of an agar-solidified layer (2% agar w/v with seawater as solvent). Each culture medium (treatment) was independently applied to 72 plants, each placed in an acrylic container (8.5 cm diameter). Twenty-four containers of each culture medium were randomly assigned into 3 replicate 30 l aquaria. The aquaria were kept in aerated seawater within a growth chamber, with a light intensity of 150 µmol quanta m^{-2} s^{-1} and a 14 h day: 10 h night photoperiod, at field temperature (22 ºC during day and 18 ºC during night) and salinity (35 PSU) conditions. In order to avoid confounding the nutrients effect with the culture media tested, the same natural seawater (5.01 µM NH_4^+, 2.01 µM $NO_3^- + NO_2^-$, and 0.15 PO_4^{3-}) was used for the three treatments and aquaria. Four plants from each treatment and from each aquarium were randomly harvested without replacement after 30 days, and at the 60th day of experiment. Plant survival was determined and the number of new shoots and internodes produced was counted to estimate the shoot and internode production rates (n. d^{-1}). The length of newly developed rhizome was measured to calculate rhizome elongation rate (mm d^{-1}). Plant branching was observed and the number and length of the new branches, and the number of their internodes was recorded to estimate branch production, branch elongation, and branch internode production rates, respectively.

Chlorophyll content of leaves was determined as a physiological indicator of the plant's condition. Plant leaves from each treatment in each aquarium were pooled (n = 3, 20-30 mg of FW each), and the leaf tips and sheaths discarded. Chlorophyll of the intermediate parts of the leaves was extracted overnight in N, N-Dimethyl-

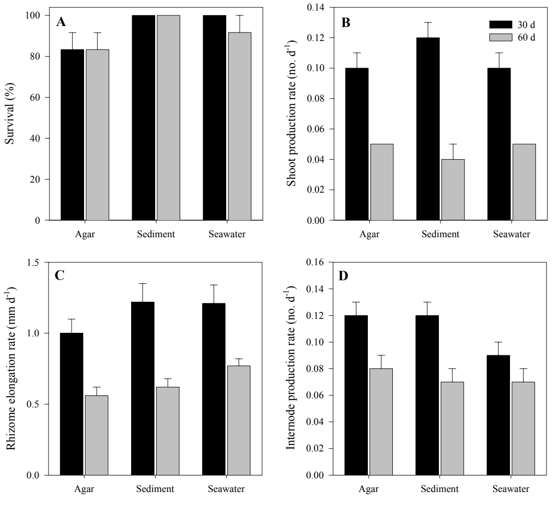

Fig. 1 - *Zostera noltii* survival and growth under three different culture media. (A) Survival, (B) shoot production rate, (C) rhizome elongation rate, and (D) internode production rate of plants during the experiment (mean ± SE).

formamide (DMF) and determined spectrophotometrically using the equations of Wellburn (1994). Differences in data sets were investigated using two-way ANOVA ($p < 0.05$) with treatment and time as main effects (Sokal and Rohlf, 1995).

Results

Survival of *Zostera noltii* was high (> 80%) in the three experimental culture media. In sandy sediment medium plant survival was 100%, along the time of the experiment (Fig. 1A). No significant differences were found in shoot production, internode production or rhizome elongation rates among the culture media tested (Table 1). However, these rates decreased significantly with time (Fig. 1).

Tab. 1 - Statistical results and significance of ANOVA (F) for the effects of culture medium and incubation time on *Zostera noltii*. (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, ns: not significant.

Plant variables	Medium	Time	Medium × Time
Survival	F = 1.50 ^{ns}	F = 0.50 ^{ns}	F = 3.50 ^{ns}
Rhizome elongation rate	F = 2.31 ^{ns}	F = 40.60 ***	F = 0.43 ^{ns}
Shoot production rate	F = 0.31 ^{ns}	F = 151.36 ***	F = 3.86 *
Internode production rate	F = 2.40 ^{ns}	F = 21.01 ***	F = 1.66 ^{ns}
Branching	F = 0.46 ^{ns}	F = 0.89 ^{ns}	F = 1.38 ^{ns}
Branch production rate	F = 0.001 ^{ns}	F = 9.00 **	F = 1.92 ^{ns}
Branch elongation rate	F = 0.56 ^{ns}	F = 1.44 ^{ns}	F = 0.05 ^{ns}
Branch internode production rate	F = 0.46 ^{ns}	F = 0.86 ^{ns}	F = 0.46 ^{ns}
Chlorophyll *a*+*b*	F = 1.90 ^{ns}	F = 7.98 *	F = 0.69 ^{ns}
Chlorophyll *a*/*b* ratio	F = 3.60 ^{ns}	F = 1.63 ^{ns}	F = 0.85 ^{ns}

Plant branching did not vary significantly among the different culture media or along the time of experiment (Table 1), even though plants cultured in agar presented a lower branching after 60 days (Fig. 2A). As well, no significant effects

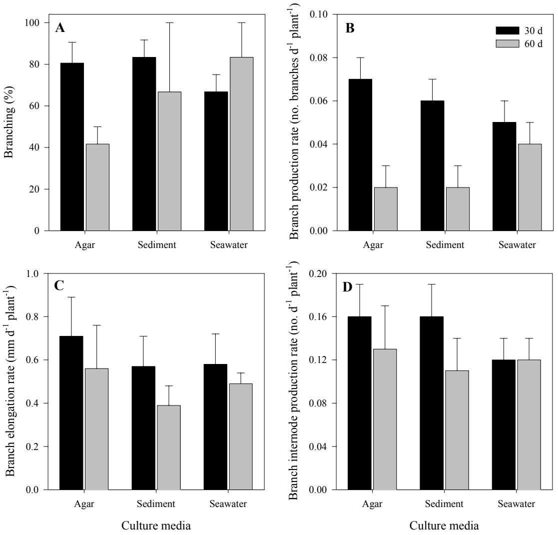

Fig. 2 - *Zostera noltii* branching and branch growth under three different culture media. (A) Branching, (B) branch production rate, (C) branch elongation rate, and (D) branch internode production rate of plants during the experiment (mean ± SE).

of the culture media or time (Table 1) were found on the elongation of branches, and internode production of branches (Fig. 2). However, a significant effect of time was observed in branch production rate (Table 1). The elongation of the branches in each plant (Fig. 2C) was lower than the elongation of the main rhizome (Fig. 1C), particularly after 30 days, which emphasises the relative importance of belowground plant growth through the rhizome main axis.

The total chlorophyll content (Chl *a* + Chl *b*, Fig. 3A) of *Zostera noltii* leaves did not vary significantly among culture media, but did decrease significantly with the time of experiment (Table 1). The decrease of chlorophyll content was proportional between Chl *a* and Chl *b*, because the ratio Chl *a*/Chl *b* remained constant between the two sampling moments (Fig. 3B). This ratio ranged between 2 and 3, and was not significantly different among culture media or time (Table 1).

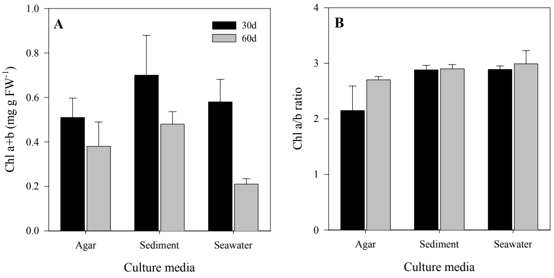

Fig. 3 - *Zostera noltii* chlorophyll content under three different culture media. (A) Chlorophyll *a*+*b* content, and (B) Chlorophyll *a*/*b* ratio of leaves during the experiment (mean ± SE).

Discussion

The results obtained show that *Zostera noltii* plants can grow and survive for 60 days in three different culture media, with a survival range between 80% and 100%. Albeit the high plant survival percentage in the different culture media, plants analysed at the 60th day of experiment showed reduced vigour (e.g. narrower and lighter-green leaves) compared to those analysed after 30 days. The significant decreases in plant growth observed along the time of experiment confirm these observations. Furthermore, the leaf chlorophyll content also decreased significantly along the experimental period, as an indication of the decline of the plants' physiological condition (Fig. 3). The artificial light intensity (150 μmol quanta m^{-2} s^{-1}) inside the plant-growth chamber during the experiment was much lower than the natural light intensity received by the intertidal plants, when emerged in a sunny day (ca.1500 μmol quanta m^{-2} s^{-1}, J. Silva pers. comm.). This lower light intensity may account for the significant decreases observed in plant growth, production and leaf chlorophyll content, from the 30th to the 60th day of experiment. In the field, leaf chlorophyll content of *Zostera noltii* ranged between 1.3 to 3.6 mg g^{-1}DW (Silva, 2004), whereas the leaf chlorophyll content of the experimental plants ranged between 0.05 to 0.18 mg g^{-1}DW (0.2 to 0.7 mg g^{-1}FW, Fig. 3A). Changes in plant physiology and decreases in plant growth have been reported for *Zostera noltii* (Peralta *et al.*, 2002; Brun *et al.*, 2003) and other seagrass species (e.g., Kraemer and Hanisak, 2000), when plants are exposed to low light levels. The decreases in plant growth, production and leaf chlorophyll content observed in this study suggest that microcosm experiments with the seagrass *Zostera noltii* under low light should not be extended for long periods (> 30 days), since changes in plant performance related to the time span of the experiment may confound the effects to be tested.

The lack of significant differences in the growth of *Zostera noltii* plants, under the different culture media tested suggests that a simple seawater medium can be used for laboratory microcosm experiments. This simple medium represents an advantage over other media (e.g. biphasic media), by simplifying the experimental set-up. These results constitute a base study for future laboratory experiments on the effect of controlled variables on the species growth, morphology and physiology.

To our knowledge, there are no studies on the effect of different culture media on seagrasses or submerged aquatic vegetation. The few studies related to this subject have tested different culture media from the chemical perspective, in particular the plant nutritional requirements or the toxic effects of nutrients or other chemical compounds in aquatic plants (Bird *et al*., 1996; Moore and Wetzel, 2000; Ösbay, 2001; Peralta *et al*., 2003), whereas the present study focused on the physical media for plant growth.

In conclusion, this study showed that a simple seawater medium can be used in laboratory microcosm experiments with *Zostera noltii*, particularly for short-term experiments (up to 30 days), since there were no differences in plant survival and growth cultured under three different media, namely natural sandy sediment, agar and seawater.

Acknowledgements

We thank J. Silva for clarifying the methodology for the chlorophyll content analysis.

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L. Castellano[1], M. Capriolo[1], G. Gnone[1], M. Montefalcone[2], M. Matassini[2]

[1] Acquario di Genova, Area Porto Antico, Ponte Spinola, 16128 Genova, Italy
[2] DipTeRis, Università di Genova, Italy.

EX SITU GERMINATION AND GROWTH OF *POSIDONIA OCEANICA* L. DELILE (MONOCOTYLEDONAE, POSIDONIACEAE) SEEDS

Abstract

A 21 months experience of germination and growth of Posidonia oceanica *(L.) Delile (Monocotyledonae, Posidoniaceae) seeds is reported. The results suggest that the conditions of culture we adopted are suitable for the species examined. Thanks to this experiment it will be possible to work out a technical protocol to cultivate this species ex situ in order to transplant the seedling in damaged meadows, once the cause of damage has been removed.*

***Key words*:** Posidonia oceanica, *seeds, flowering, sea grass, Mediterranean Sea, transplantation.*

Introduction

For many years scientists have been looking for new techniques of transplantation of seagrasses in order to restore natural meadows. Good results have been obtained by transplanting cuttings collected from the wild (Meinesz *et al*., 1993; Molenaar *et al*., 1993) in areas where the causes of damage were removed; limitations of these techniques are the environmental impact of cuttings collection and their slow growth.

Flowering is an irregular phenomenon that can be probably related to pluriannual cycles, but the latest lepidochronological studies (Pergent *et al*., 1989) prove to be less uncommon than usually thought. Moreover the production of seeds can be very successful, as it happened in spring 2004.

Transplantation of seedlings as an alternative restoration method received little attention but seems to be a valuable system in order to avoid environmental impact of cuttings and preserve the natural genetic diversity in restored population (Caye and Meinesz, 1989; Balestri *et al*., 1998)

The aim of this work is to define the first guide lines for a protocol to obtain good numbers of seedlings from *ex situ* seeds that can be transplanted to restore damaged areas.

Materials and methods

In fall 2003-2004 a significant flowering of *Posidonia oceanica* beds occurred in the Mediterranean Sea so that the following spring seeds could be collected, transported, measured and sowed in the tanks of the Aquarium of Genova where they germinated and grew.

The size of the first tank (T1) was 30 x 70 x 80 (cm) with a capacity of 100 l equipped with a wave generator, two neon 36 W Fluora, and a plastic mesh to keep the seeds in a fixed and known position.

After 3 months the young plants were transferred into small pots with a limestone and perlon substrate in order to facilitate fixation of roots. After 6 months the young plants were transferred into a bigger and deeper (60 cm) tank (T2) illuminated by 2 neon 36W Fluora.

At the age of 12 months the seedlings were transferred into a larger tank (T3) with a strong water movement, with light provided by a HQI 150 W, and at the age of 18 months they were transferred into a new tank (T4) of the same size to the second one, with light provided by 2 neon 36 W Fluora. At the age of 21 months the young plants were all transplanted into the exhibit tank, with a capacity of 3300 l and light provided by 2 HQI 400W. All the tanks were equipped with an adequate LSS (Life Support System). The photoperiod was of 12 hours. The water parameters were controlled twice a week and kept under optimal values as reported in Tab. 1

Tab. 1 - Parameters of the water quality

Temperature	16-18 °C
Salinity	36-37.5 ‰
pH	8-8,2
N-NH$_4$	<0.08 mg/l
N-NO$_2$	<0.15 mg/l
NO$_3^{2-}$	<18 mg/l
PO$_4^{3-}$	<1.5 mg/l

In order to measure the seeds, plastic callipers were used while holding the seeds in the water; a plastic ruler was used to measure the length of leaves, roots and rhizomes while holding the young plants out of the water for a few minutes.

Results

The mortality of the seeds and plants after 1 month was 20%. The mortality after 3 months and from 6 to 21 months was of 50%.

We observed that all the seedlings showed continuous growth producing at least one leaf per month.

The average longest leaf reached a length of 28.6 cm at the age of 15 months. (Fig. 1)

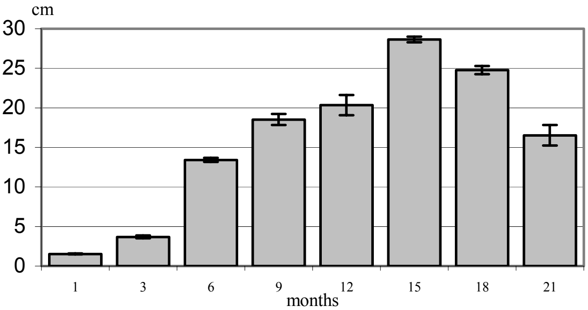


Fig. 1 - Longest leaf length. Values are means ± SE ($n_{1\ month}$= 96, $n_{3\ months}$= 78, $n_{6\text{-}21\ months}$ = 17)

The average number of leaves increased until 3 months of age then it remained more or less constant (6 leaves/shoot) (Fig. 2).

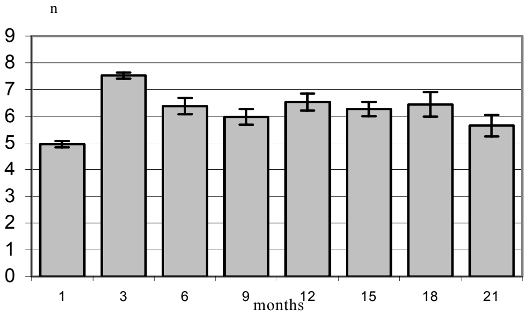
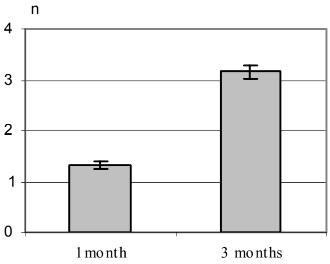


Fig. 2 - Average number of leaves per seedling. Values are means ± SE ($n_{1\ month} = 96$, $n_{3\ months} = 78$, $n_{6\text{-}21\ months} = 17$)

The number of secondary roots and the length of the primary roots, measured until the seedlings were planted into pots, doubled in 3 months Fig. 3 and Fig. 4.



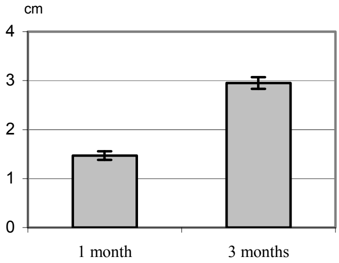


Fig. 3 - Number of secondary roots. Values are means ± SE ($n_{1\ month} = 96$, $n_{3\ months} = 78$)

Fig. 4 - Length of primary root. Values are means ± SE ($n_{1\ month} = 96$, $n_{3\ months} = 78$)

Rhizomes appeared and could be measured after 16 months and their growth was slow and constant (Fig. 5).

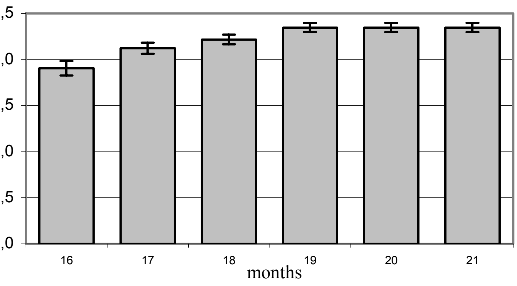


Fig. 5. Rhizome length. Values are means ± SE (n= 17)

The average life span of the leaves of each plant varied from 91 to 228 days.

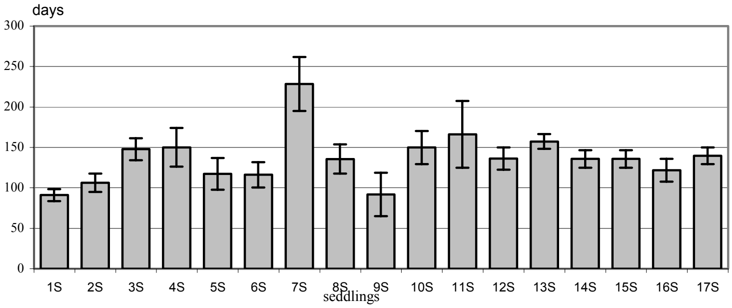


Fig. 6 - Average life span of the leaves. Values are means ± SE (n=5-6)

Discussion and conclusion

The cause of mortality in the first month can be due to the health status of the seeds when collected while after 6 months it was probably due to an unknown disease that killed the meristema. Handling of the plants can probably be another cause of mortality.

Romero (1989) observed a seasonal growth of leaves, in adult wild plants, while our seedlings had a continuous growth. The average longest leaf was a bit longer than in Piazzi *et al.* (1999) in natural seedlings of the same age. Comparing the number of leaves, Caye and Meinesz (1989) observed an average number of 8 after 42 days in artificial conditions while Piazzi *et al.* (1999) observed 6.4 leaves in natural seedlings. The number of secondary roots and primary root length are comparable with the values in Bedini *et al*. (1997). The rhizomes of our seedlings were smaller (2.4 cm) than in natural seedlings of Piazzi *et al.* (1999) (3.4 cm) at the same age.

Average life span reached 228 days in seedling S7, while in adult plants long-lived leaves reached 300 days.

All these observations, along with the good survival rates recorded, suggest that the conditions of culture we adopted are suitable for the species examined. Water quality, light and water movement are surely very important and must be kept under control to obtain long living seedlings that are strong enough to be transplanted in the wild. Much can be done to preserve them from diseases and to enrich culture media with phyto-stimulators if necessary.

The next steps in this research could be a massive culture of seedlings that can be transplanted in different sites at different ages and on different substrata to find the best conditions for good survival. Balestri *et al*. (1998) indicate, in a pilot experience, the dead matte as best substratum for transplanted seedlings. Piazzi *et al*. (1999) observed that natural seedlings survive better if fixed on dead matte at the depth of 10 m and that the critical period for survival of the juvenile shoots seems to be limited to their first year of life.

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B. Celebi, A. Cemal Gucu, M. Ok, S. Sakinan, E. Akoglu

Institute of Marine Sciences, Middle East Technical University, Mersin 33731, Turkey

HYDROGRAPHIC INDICATIONS TO UNDERSTAND THE ABSENCE OF *POSIDONIA OCEANICA* IN THE LEVANT SEA (EASTERN MEDITERRANEAN)

Abstract

In the present study the three hydrological factors turbidity, salinity and temperature which may be possible reasons of the absence of Posidonia oceanica *in Levant Sea were investigated. The temperature has come out as the most remarkable factor between the two sides of the* Posidonia oceanica *boundary in the northeastern Mediterranean Sea. The observations suggested that the 28.4 ºC is a limit for the growth of the species.*

***Key-words*:** Posidonia oceanica*, Levant Sea, temperature, salinity, turbidity*

Introduction

As an endemic seagrass species to the Mediterranean *Posidonia oceanica* (L.) Delile can be found in the littoral zones of the Mediterranean and Marmara Seas (Green and Short, 2003). There is little information about the distribution of *P. oceanica* in the eastern Mediterranean.

Along the eastern Mediterranean coast of Turkey there are only two records of the species, which are located in the bays of Iskenderun (Tutin, *et al.* 1964) and Mersin-Akkuyu (Cirik, 1991). However, recent field studies revealed that there are no meadows present in these given areas and the meadows end with a sharp border in the eastern Levant Sea, at 36º 09' N, 33º 26' E (Gucu and Gucu, 2002).

In this study, temperature which controls the geographical distribution of the meadows, and light penetration which determines the limits of its vertical distribution (Borum *et al.*, 2004), were compared to evaluate their significance in the distribution of the plant. The salinity, which is apparently an important factor limiting *P. oceanica* growth (Fernández-Torquemada and Sánchez-Lizaso, 2005) was also included in the evaluations.

Material and methods

The southern coast of Turkey, between Kizilliman/Mersin and Cevlik/Hatay were surveyed in the study. The 50 meter depth contour was taken as the lower depth limit for all the measurements. The eastern most boundary of the *P. oceanica* at 36º 09' N, 33º 26' E, which fell within the study area provided an opportunity for comparative measurements. The surveys took place inside the boundary, to the west, where there were healthy meadows, and outside this boundary, to the east, where meadows were absent.

Turbidity and Salinity measurements:

Turbidity was measured and quantified using a Secchi disk between 2000 and 2005 at 35 stations to the east and to the west of the *P. oceanica* boundary.

PAR/Irradiance profiles were measured at 69 stations for the calculation of light penetration coefficients in the study area, between 2004 and 2005. Salinity profiles during the warmest periods (August) of the years 1990 and 1991 were used for the comparisons of salinity ranges on both sides of the boundary.

Temperature measurements:

Two types of temperature measurements were made; 1) warmest seasonal water column temperature of the previous years (1990 - 1991), and 2), continuous water temperature in the warmest period of 2005 at several fixed depths.

Temperature profiles were measured at 219 stations and using these profiles a general temperature limit for the growth of *P. oceanica* in the Levant Sea was estimated.

For continuous water temperature recordings 10 HOBO Pendent Temperature Data Loggers were deployed during the warmest period of the year 2005, from 12th of August to 11th of September. They were set to record seawater temperatures with 10 min intervals, at 5 different stations, at 2 different depths; St-1 was set as the reference station on a meadow located at 8.3 nm west of the *P. oceanica* boundary; St-2 was set on the meadow forming the eastern boundary of *P. oceanica*; the remaining three stations were all set to the east of this boundary with St-3 at 7.2 nm; St-4 at 46.5 nm; and St-5 at 121.1 nm.

The data loggers enabled to investigate a more precise temperature limit which may be affecting the growth of *P. oceanica*. The temperature recordings of St-1 were taken at 10 min intervals and were averaged over a day (00:00-24:00). The maximum daily average temperature measured at this reference station, with a representative healthy meadow, was set as the Maximum Tolerable Temperature Limit (MTTL). This limit provided the maximum temperature for *P. oceanica* growth in the Levant basin. For further analysis, numbers of warm pulses, which had higher temperatures than the MTTL, along with their duration, and frequency, were estimated for the rest of the stations.

Results

Turbidity and Salinity measurements:

The Secchi disk depths inside the *P. oceanica* covered zone ranged between 8 - 40 meters, which were wider than the depth ranges observed outside the zone. The Secchi depths measured east of the boundary were between 12 and 31 meters.

The depths of 10% surface irradiance, which was defined as the lower limit of *P. oceanica* growth (Duarte, 1991) were calculated using PAR/Irradiance profiles, at 69 stations. The results were mapped in Fig. 1. The areas near representing high light attenuation sites were found outside the *P. oceanica* boundary. But there is one area with low light attenuation site found outside the boundary where hypothetically light may be adequate for *P. oceanica* growth (e.g., north of the Iskenderun Bay).

The vertical salinity profiles in the warmest season of the years 1990 and 1991 inside and outside of the *P. oceanica* zone showed different ranges. The salinity profile measured outside, to the east of the *P. oceanica* boundary, during the warmest month of the year had a relatively narrow range, which was between 39.1‰ and 39.7 ‰. The salinity profile measured inside, to the west of the boundary, was between 38.7‰ and 39.9 ‰. The salinity range outside the boundary fell within the range inside the boundary.

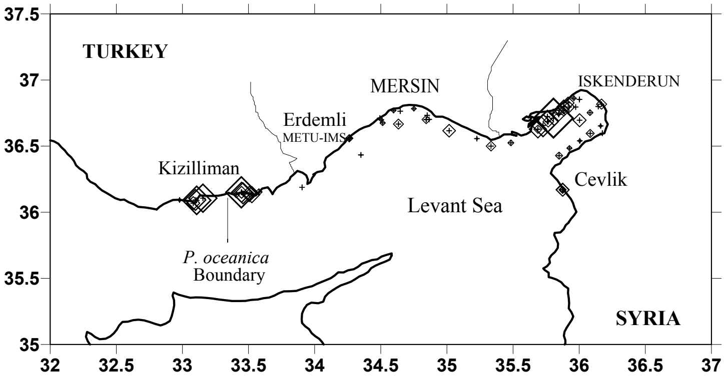

Fig. 1 - Map of Levant Sea where light penetration is adequate for growth of *P. oceanica* (squares indicate sutable areas)

Temperature measurements:

The vertical temperature profiles in the warmest season of the years 1990 and 1991 indicated distinctive ranges between the eastern and western sides of the border. The mean temperature of the eastern side was higher than the mean temperature of the western side in 1990 and 1991. Furthermore, the range of the eastern side for the water column up to 20 meter depth was narrower and closer to the maximum temperatures of the western range (27-29 ºC and 23-28 ºC, respectively). Considering these profiles the separation point of temperature ranges of western and eastern sides is found around 27.5ºC. Therefore, this temperature is presumed as the limit for the growth of the *P. oceanica* in the Levant Sea.

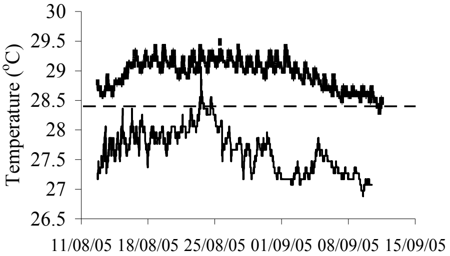

Fig. 2 - Continuous temperature recording data in 2005 (bold line: station in Cevlik, dashed line: MTTL and line: station in Kizilliman)

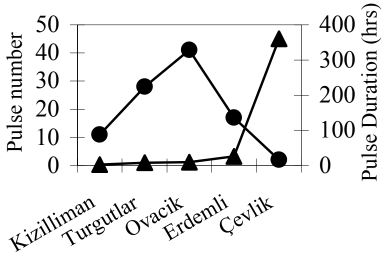

Fig. 3 - Pulse frequency and duration (● total number of pulses, ▲ mean pulse duration).

The continuous temperature measurements at fixed depths during the warmest season of 2005 represented an increasing trend from the west to the east (Fig. 2). From the continuous temperature recordings at the westernmost station, MTTL is calculated as 28.4 ºC. The total exposure time to warm pulses higher than MTTL showed same increasing trend from the west to the east.

Beside the mean pulse duration also the number of pulses higher than MTTL was found important for the growth and survival of the species on the western side of the border. Going eastwards the number of pulses increased in the first three stations paralleling the mean pulse duration, but it decreased sharply in the last two stations; while the mean pulse duration continued to increase (Fig. 3). This increase in the easternmost station reached 99 – 100 % of the entire recording period at each depth.

Discussion

The importance of temperature on the growth of *P. oceanica* in the western parts of the Mediterranean Sea has been highlighted by several studies (Zupo *et al*., 1997; Mayot *et al*., 2005). However, in the present investigation of the Levant Sea (Eastern Mediterranean), which encompasses the easternmost boundary of the species, temperature did not suggest such clear cut significance.

Nevertheless, the vertical temperature profiles inside and outside the boundary were distinctively separate and 27.5 °C seemed to be the highest temperature limit the meadows could survive. This analysis was done in the warmest month, August, and continuous data was necessary to further investigate the temperature limitations.

Therefore, continuous data loggers were deployed and the maximum daily average temperature measured at the reference station, representing a healthy meadow, was recorded as 28.4 °C. This temperature was set as the Maximum Tolerable Temperature Limit (MTTL) and it was assumed to be the maximum temperature for *P. oceanica* growth in the Levant basin (Fig. 2). For further analysis, numbers of warm pulses, which had higher temperatures than the MTTL, along with their duration, and frequency were estimated for the rest of the stations and the data suggested that the eastern side of the boundary had the highest exposure and the highest temperatures (Fig. 3).

Apart from temperature, the two other hydrological factors, turbidity (Fig. 1) and salinity profiles, revealed no differences between the two sides of the border. The matching profiles of these parameters on both sides may suggest that salinity and light availability were not the major factors hindering the growth of the species.

On the other hand, care should be taken when interpreting these results, because in this study each factor was evaluated individually for its separate effect, however their possible combined effects were disregarded. In literature, there are short term laboratory studies which investigate environmental factors and emphasize both antagonistic and synergistic impacts among factors, as well as projecting collective impacts on the growth of the species.

In conclusion, this preliminary study may provide the baseline information for future long-term studies from which models predicting the integrated effects of hydrological factors on seagrass distribution in the Levant Sea can be developed.

Acknowledgement

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A. Chiappetta, M. Greco, M.B. Bitonti

Dipartimento di Ecologia, Università della Calabria, Arcavacata di Rende, I-87030 Cosenza, Italy

CYTOPHYSIOLOGICAL FEATURES OF EMBRYOS IN *POSIDONIA OCEANICA* SEEDS

Abstract

Embryo cytophysiological features of Posidonia oceanica *mature seeds were investigated in relation to their utilization for in vitro propagation of this seagrass. Both direct and undirect nuclear metabolism were taken into account in distinct zones of quiescence-less embryos. In this contest, the first node is featured by a very high metabolic activity and its potential for regeneration is discussed in relation to programs of in vitro propagation of* Posidonia oceanica *germoplasm.*

Key-words**:** Posidonia oceanica**,** in vitro *regeneration, embryo metabolism, DNA methylation.*

Introduction

The *Posidonia oceanica* (L.) Delile meadow is widely recognized as a key and unique ecosystem in the Mediterranean coastal zone (Ferrat *et al*., 2003; Boudouresque *et al*., 2006). Moreover, due to its extreme sensitivity to disturbance factors, this seagrass is considered an effective bio-indicator species of environmental quality (Pergent, 1991; Boudouresque *et al.,* 2000; Ferrat *et al*., 2003). In the last decade a wide range of factors, mostly related to human activities (Peres, 1984; Pergent-Martini *et al.*, 1995), such as fish farming (Delgado *et al*., 1997; Ruiz *et al*., 2001) and coastal works (Ruiz *et al*., 1993; Guidetti and Fabiano, 2000), are causing a significant and widespread decline of *Posidonia* meadows. Given the ecological relevance of this acquatic phanerophyte in maintaining marine biodiversity, much effort has been made to protect and restore this habitat through the transplantation of both seedlings (Meinesz *et al*., 1990, 1993; Balestri *et al*., 1998, 2003) and vegetative material collected from existing beds (Meinesz *et al*., 1993; Piazzi *et al*., 1996). However, field data are not yet fully convincing. Thus, it becomes even more essential for management of meadows to develop novel strategies for *in vitro* propagation of *Posidonia oceanica* germoplasm. To this regard, very little is known about the reproductive fitness of this species either in relation to seed physiology or vitality (Cooper, 1981; Cay and Meinesz, 1984, 1989; Buia and Mazzella, 1991).

The work reported here, aimed to investigate the cyto-physiological features of *Posidonia oceanica* seeds. The work was mainly focused on the metabolic state and the potential developmental plasticity of embryos as powerful material for *in vitro* culture. With this aim, histological pattern formation, chromatin organization and total RNA abundance were investigated in mature embryos of seeds directly collected from plants. Since plant developmental plasticity could be achieved by mechanisms of reversible gene repression, the level of nuclear DNA methylation was also investigated by using an immumocytological approach. Namely, DNA methylation is very common in plant genomes and has been frequently associated to the repatterning of chromatin and the modulation of gene expression (Lewis and Bird, 1992; Finnegan *et al*., 1998; Fraga *et al*., 2002; Bitonti *et al*., 2002; Xiao *et al.*, 2006). In addition to broadening the knowledge on the developmental biology of *Posidonia oceanica*, our results provided useful information in relation to suitable programs for germoplasm preservation and propagation.

Material and methods

Twenty mature fruits of *Posidonia oceanica* were collected directly from plants growing in a meadow located at Diamante (Cosenza), along the Tyrrhenian coast of Calabria in the Southern Italy. Seeds were excised and fixed in 0.5%(v/v) gluteraldehyde and 3%(w/v) paraformaldehyde mixture in PBS (135 mM NaCl, 2.7mM KCl, 1.5mM KH_2PO_4,8mM K_2HPO_4, pH 7.2). Samples were dehydrated, embedded in Technovit 8100 resin and sectioned at 3μm with a tungsten knife using a Leica 2155 microtome. Some sections were used for cyto-histological analyses and other for 5-methylcytidine immunolabelling. For histological analysis sections were stained with toluidine blueO 0.1% (w/v) and Schiff reagent.

5-methylcytidine-immunocytolabelling

Preparation and specificity testing of the monoclonal antibody directed against 5-methylcytidine (5-mC) have been already described (Podestà *et al.*, 1993). Nuclear DNA was denaturated and sections were incubated with the primary antibody anti-5-mC (1:250 in 0.1M PBS, 1% BSA, 0.2%Tween 20). Afterwards, sections were exposed to goat anti-mouse gold conjugated antibody (1:250 in 0.1M PBS, 1% BSA, 0.2%Tween 20), subjected to silver-enhancement of gold signal and then stained with basic fuchsine (1%). Controls were: (1) omission of primary antibody; (2) replacement of the first monoclonal antibody with somatostatine antibody; (3) omission of DNA denaturation. The number of silver grains per nucleus and nuclear area were estimated by scoring all the serial sections using a Leica DMRB microscope and a Leica Q500/W image analyser equipped with a CCD camera. A labelling index (Li) was expressed as number of silver grains/ nuclear area and statistical differences were evaluated by ANOVA followed by Neuman Keuls post hoc.

Qualitative fluorescence

Acridine Orange (AO) staining was used for total RNA localization. AO is a metochromatic stain that interacts with both DNA, by intercalation and RNA, by electrostatic attraction, giving yellow and orange fluorescence, respectively. Five excised seeds were fixed as above described. Samples were dried, wax-embedded in Paraplast and sectioned at 8 μm. Rehydrated sections were -stained with AO solution for 15 min (stock AO 1% in the ratio 1/9 in PBS:$CaCl_2$ 100mg/l; KCl 200mg/l KH_2PO_4 200mg/l; $MgSO_4$ 59mg/l; NaCl 800mg/l; $NaHPO_4$ 1150mg/l) at pH 6.0. Samples were examined using a Leica DMRB epifluorescent microscope equipped with a 50W mercury lamp. A Leica excitation filter (BP 450-490nm) plus a barrier filter (LP 515nm) were used.

Results and discussion

A high level of embryo differentiation was observed in longitudinal sections of *Posidonia oceanica* seeds (Fig 1a). In particular, the Shoot Apical Meristem (SAM) exhibited a clear zonation, with the central zone (CZ) well distinguishable (Fig 1c) from the peripheral zone (PZ) and rib meristem (RM), as normally reported for adult higher plants (Medford, 1992). SAM showed an intense mitotic activity and interphase nuclei were featured as areticulate structures with prominent dense chromatin areas (chromocentres) (Fig 1d). Moreover, the embryo was characterized by the production of numerous leaf primordia (Fig 1a) that in cross section profiles displayed a fully differentiated vascular tissue (Fig 1e). At the opposite pole, a root apical meristem (RAM) was clearly organized (Fig. 1b). In all the seeds analyzed, an incipient adventitious root originating from the first node zone was also observed (Fig. 1a). Continuous procambial strands interconnected the shoot with both adventitious and primary roots (Fig 1a,b).

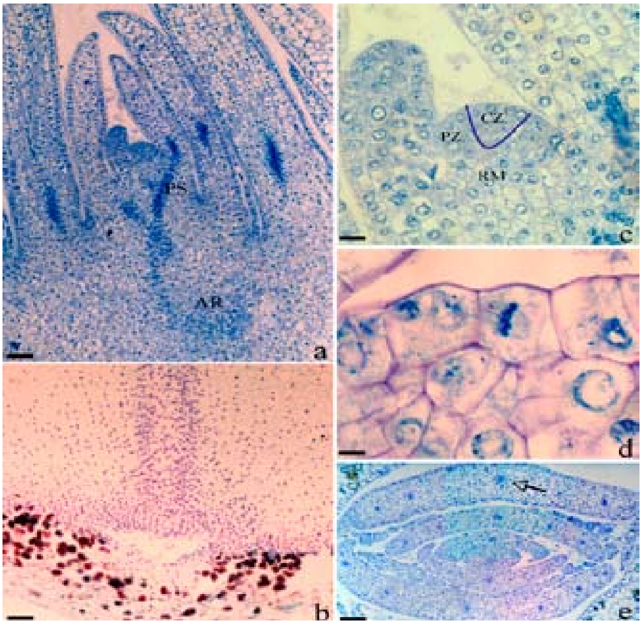


Fig. 1 - Longitudinal (a-d) and cross sections (e) of *Posidonia oceanica* seeds stained with toluidine blueO (a, c-e) and Schiff's reagent (b). CZ: central zone; PZ: peripheral zone; RM: Rib meristem. AR: adventitious root, PS: procambial strand, arrow on vascular tissues. Bars: 75µm (a); 25µm (b); 5 µm (c); 3.0 µm (d); 350 µm (a).

Acridine Orange staining provided an intense signal that was observed through the whole embryo (Fig. 2), thus indicating a high abundance of total RNA. This result is consistent with the absence of quiescence processes in *Posidonia oceanica* seeds, that indeed do not undergo dehydration during maturation. Notably, a more intense staining and consequently a higher metabolic activity characterized both the first node and adventitious root (Fig 2a,b) as compared to the SAM and above all, the RAM (Fig 2c).

Thereafter, the level of nuclear DNA methylation of embryonic tissues was evaluated as an indirect parameter of genome transcriptional activity (Finnegan *et al.*, 1998; Fraga *et al.*, 2002; Bitonti *et al.*, 2002; Xiao *et al.*, 2006). Central to this concept is the major role of methylation in changing DNA accessibility by chromatin remodelling. As a general rule, hypermethylation of DNA is associated to highly condensed chromatin and consequentely to low transcriptional gene

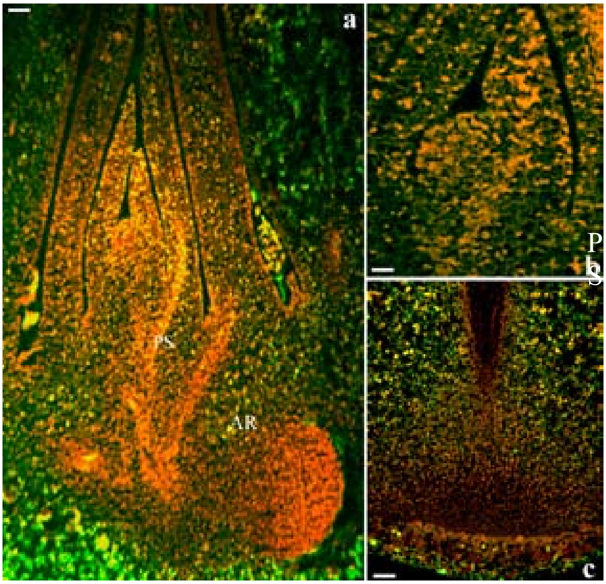
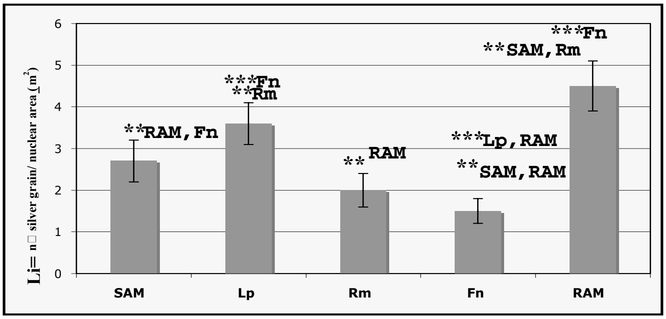


Fig. 2 - (a) Total RNA localization by Acridine Orange staining in longitudinal sections of *Posidonia oceanica* seeds. b, c) high magnification of SAM and RAM respectively. AR: adventitious root, PS: procambial strand. Bars: 140 µm (a); 7.5 µm (b); 60 µm (c);

activity (Lewis and Bird, 1992; Finnegan *et al.*, 1998; Fraga *et al.*, 2002; Bitonti *et al.*, 2002; Xiao *et al.*, 2006). In this context, methylated sites on interphase nuclei were detected by using an antibody against 5-methylcytosine. Labelling index (Li), expressed as number of silver grains/nuclear area (Fig. 3), was significantly different in the distinct embryonic zones, the lowest value being detected just in the first node.



Differences were obtained using ANOVA followed by Neuman Keuls post hoc; ***p< 0.001, **p< 0.01

Fig. 3 - DNA methylation levels espressed as Li of different embrionic zones. SAM: Shoot Apical Meristem; Lp: leaf primordia; Rm: rib meristem; Fn: first node; RAM: Root Apical Meristem

Differences in cytosine methylation among both different organs (Messeguer *et al.*, 1991; Zluvova *et al.*, 2001) and different developmental phases have been also observed in several other species (Finnegan *et al.*, 1998; Bitonti *et al.*, 2002;

Fraga *et al.*, 2002). Notably, a rapid reduction of DNA methylation was detected in seeds of unrelated species during germination in connection with an increasing metabolic activity and cell proliferation (Drozdenyuk *et al.*, 1976; Follmann *et al.*, 1990; Zluvova *et al.*, 2001). Hence, the low level of DNA methylation detected in the first node of *Posidonia oceanica* seeds further supports the very active state of this zone and its potential plasticity for regeneration. In this context it is worth noting that such feature has been previously verified in another relevant terrestrial monocot, such as *Triticum durum*. Indeed, *in vitro* culture of *Triticum* embryo first nodes were able to proliferate and regenerate new plantlets also when explants where excised from old seeds unable to undergo germination (Innocenti *et al.*, 1983). Good criteria for *in vitro* regeneration include high metabolic activity and high transcriptional activity together with proliferative activity linked to organ formation. Based on our results, embryonic tissue and in particular, the first node zone should be excellent starting material for *in vitro* regeneration in this seagrass.

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Y. Fernández Torquemada, J.L. Sánchez Lizaso

Department of Marine Sciences and Applied Biology, University of Alicante, P.O. Box 99; E-03080.
Alicante (Spain).

EFFECTS OF SALINITY ON GROWTH AND SURVIVAL OF *CYMODOCEA NODOSA* (UCRIA) ASCHERSON AND *ZOSTERA NOLTII* HORNEMANN

Abstract

The aim of the present study was to examine the effects of salinity on growth and survival of Cymodocea nodosa *(Ucria) Ascherson and* Zostera noltii *Hornemann. Leaf growth and survival responses to salinity were characterized by several short-term experiments under controlled conditions. For each experiment, ten marked shoots were placed in plastic aquariums of 5 l and were exposed to different salinity treatments (ranging from 2 to 72 PSU). Growth and survival of both species were significantly affected by salinity. Under these laboratory conditions it has been detected that* Zostera noltii *tolerates hyposalinity conditions better than* Cymodocea nodosa*, but both species were very sensitive to salinity increases (> 41 PSU), what suggests that these seagrasses could be adversely affected by brine discharges from seawater desalination plants.*

Key-words*: salinity effects, desalination impact,* Cymodocea nodosa*,* Zostera noltii.

Introduction

Salinity is considered a constant factor in open waters of the Mediterranean Sea, but the recent development of new seawater desalination facilities can produce significant increases of salinity that may affect benthic organisms. Previous studies have shown that *Posidonia oceanica* is quite sensitive to salinity increments (Buceta *et al.*, 2003; Fernández-Torquemada and Sánchez-Lizaso, 2005), but there is no information about the response of other Mediterranean seagrasses to this impact.

The aim of the present study was to examine the effects of salinity on growth and survival of *Cymodocea nodosa* (Ucria) Ascherson and *Zostera noltii* Hornemann.

Material and methods

Leaf growth and survival responses to salinity were characterized by several short-term (10 days) experiments under controlled conditions between February 2003 and August 2004. Both species were collected from a mixed shallow meadow (– 2 m) at Alicante (SE Spain), with an ambient salinity of 36.8-38.1 PSU. For each experiment, ten marked shoots were placed in plastic aquariums of 5 l with sediment and an additional overhead light, and were exposed to different salinity treatments (ranging from 2 to 72 PSU). Statistical analyses were performed with ANOVA and SNK post-hoc test.

Results

Growth and survival of both species were significantly affected by salinity. Maximum leaf growth occurred between 16 and 41 PSU for *C. nodosa* and between 2 and 41 PSU for *Z. noltii* (Fig. 1). *C. nodosa* plants sustained considerable mortality at salinities below 17 PSU and above 50 PSU, with 100 % mortality at 56 PSU, while *Z. noltii* showed maximum survival rates (> 90 %) at 2-43 PSU (Fig. 2).

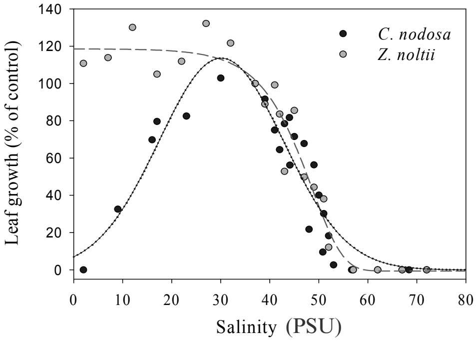

Fig. 1 - Leaf growth *versus* salinity as percentage of the growth of the control plants. Each point represents the mean for three aquaria at each salinity.

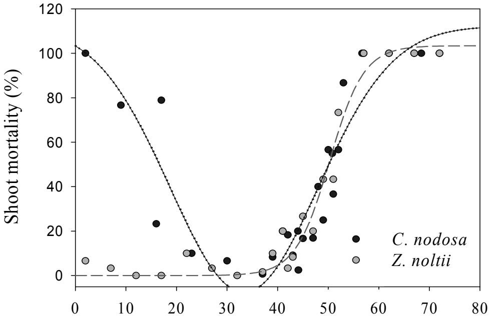

Fig. 2 - Shoot mortality of plants subjected to the different salinity treatments

Conclusions

Under these laboratory conditions it has been detected that *Zostera noltii* tolerates hyposalinity conditions better than *Cymodocea nodosa*, but both species were very sensitive to salinity increases (> 41 PSU).

Our results suggest that both seagrasses are more tolerant than *Posidonia oceanica* to salinity increase but also could be adversely affected by brine discharges from seawater desalination plants.

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C. Lafabrie, G. Pergent, C. Pergent-Martini

University of Corsica, Faculty of Sciences, Equipe Ecosystèmes Littoraux, BP 52, 20250 Corte, France

MEMORIZATION OF MERCURY BY THE SEAGRASS *POSIDONIA OCEANICA*

Abstract

The aim of this study is to appreciate the value of Posidonia oceanica *as a tracer of the past mercury contamination. Mercury concentrations were measured in living sheaths and in the corresponding dead sheaths. An overestimation is observed from the second lepidochronological year, it remains stable from the third lepidochronological year. Thus, it is possible from the mercury concentrations measured in the dead sheaths and thanks to corrective coefficients to estimate the previous mercury concentrations of the living sheaths. The interest of the use of* Posidonia oceanica *as a bioindicator of the past mercury contamination is thus reinforced.*

Key words: Posidonia oceanica*, mercury, memorization, dead sheaths, Mediterranean Sea*

Introduction

Mercury contamination has long been recognized as a serious marine environment concern (Clarkson, 1998). *Posidonia oceanica* (L.) Delile has been extensively used as a bioindicator of mercury (Maserti *et al.*, 1988; Pergent-Martini, 1998; Capiomont *et al.*, 2000) and some authors (Roméo *et al.*, 1995; Pergent-Martini, 1998) suggested that several trace metals could be memorized through its dead sheaths. The aim of this study is to compare mercury concentrations recorded in living sheaths and in the corresponding dead sheaths of *P. oceanica* shoots collected at different times to know if this dead part can be used as a good bioindicator for this metal.

Materials and methods

Shoots of *P. oceanica* were collected in Rosignano (Italy, impacted) and in Tonnara (France, non-impacted). Analyses of Hg were performed on: (i) the living sheaths for shoots collected monthly between Oct. 1995 and Oct. 1996; (ii) the dead sheaths of the lepidochronological year 1996 for shoots collected in Sept. 1997; (iii) the dead sheath showing the maximal thickness for the lepidochronological year 1994 for shoots collected in Oct. 1995, in Oct. 1996, in Sept. 1997 and in Nov. 2003.

Results

The mercury concentration in the living sheaths raises 54.7 ± 1.3 $ng.g^{-1}$ dry wt. for Tonnara and 289.3 ± 11.4 $ng.g^{-1}$ dry wt. for Rosignano. For both sites, it is similar to that recorded in the dead sheaths corresponding to the period (dead sheaths of the lepidochronological year 1996) of the shoots collected in September 1997 (47.0 ± 3.5 $ng.g^{-1}$ dry wt - Tonnara; 289.3 ± 11.4 $ng.g^{-1}$ dry wt - Rosignano; $p \geq 0.05$). Concerning Tonnara, the mercury concentration recorded in the dead sheath showing the maximum thickness for the lepidochronological year 1994 is similar for all the sampling dates considered (48.3 ± 3.9 – Oct. 1995; 46.7 ± 4.9 – Oct. 1996; 55.3 ± 1.9 – Sept. 1997; 49.5 ± 2.4 – Nov. 2003 $ng.g^{-1}$ dry wt; $p \geq 0.05$) whereas for Rosignano it increases with the sampling date but the increase is not significant from the shoots collected in Oct.1996 (208.4 ± 4.5; 230.4 ± 12.6; 297.3 ± 17.7; 324.6 ± 45.5 $ng.g^{-1}$ dry wt; $p \geq 0.05$). The biomass of the dead sheath showing the maximum thickness for the lepidochronological year 1994 decreases with the sampling date from 0.061 ± 0.002 to 0.028 ± 0.002 g dry wt. by shoot for Tonnara and from 0.046 ± 0.003 to 0.020 ± 0.005 g dry wt. by shoot for Rosignano.

Discussion and conclusion

The evolution of the mercury concentrations can be thus summarized as follow:

(i) The mercury concentration in the living sheaths of the year *n* remains stable for the year *n*+1 following the death of the sheaths in both sites: $[Hg]_n = [Hg]_{n+1}$.
(ii) The mercury concentration in the dead sheaths of the year *n*+1 remains stable for the years *n*+2 and *n*+3 in Tonnara: $[Hg]_{n+1} = [Hg]_{n+2} = [Hg]_{n+3}$ whereas it increases in Rosignano: $[Hg]_{n+1} < [Hg]_{n+2} < [Hg]_{n+3}$.
(iii) The mercury concentration in the dead sheaths of the year *n*+3 remains stable for the years going from *n*+4 until at least *n*+9 in both sites: $[Hg]_{n+3} = [Hg]_{n+4 \rightarrow n+9}$.

Therefore, mercury would be stored in the living sheaths throughout the life of the adult leaves and would stay stored in this tissue at the blade shedding. Besides, the value of the mercury concentration is overestimated for the dead sheaths from the second lepidochronological year, but it is important to point out that this overestimation is stabilized from the third lepidochronological year. Romero *et al.* (1992) described a decrease of the biomass of the dead sheaths with the lepidochronological years that is to say with the ageing of the shoots, and to model this decrease they proposed a simple negative exponential model. To resume, during the ageing of the shoots, on one hand there may be an apparent increase of the mercury concentrations in the dead sheaths and on another hand a loss of the biomass of the dead sheaths. Thus, the total quantity of mercury present in the dead sheaths may remain stable or may decrease but less than the biomass. Mercury would be so distributed in a not homogeneous way in the dead sheath tissue and would be preferentially linked to the part of the dead sheath being the less affected by the degradation due to the ageing of the shoots, that is to say the basal part. Therefore, using the data presented here, the past mercury concentrations can be determined applying the following relations:

(i) For the living sheaths of the year *n*: $[Hg]_n^{\text{"real"}} = [Hg]_n^{\text{measured}}$
(ii) For the dead sheaths of the lepidochronological year *n*-1: $[Hg]_{n-1}^{\text{"real"}} = [Hg]_{n-1}^{\text{measured}}$
(iii) For the dead sheaths of the lepidochronological year *n*-2: $[Hg]_{n-2}^{\text{"real"}} = \alpha\ [Hg]_{n-2}^{\text{measured}}$ ($\alpha = 95\%$)
(iv) For the dead sheaths of the lepidochronological years *n*-3 to *n*-9 : $[Hg]_{n-3\rightarrow n-9}^{\text{"real"}} = \beta\ [Hg]_{n-3\rightarrow n-9}^{\text{measured}}$ ($\beta = 74\%$).

Thus, this study shows that *Posidonia oceanica*, thanks to the use of the lepidochronology technique and corrective coefficients, allows to estimate more precisely the past mercury concentrations of the living sheaths and therefore to trace the evolution of the past mercury contamination of a site.

Acknowledgments

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M. Lenzi , R. Roffilli, M. De Pirro, P. Micarelli, E. Franchi, F. Borghini, S. Focardi

Lagoon Ecology and Aquaculture Laboratory (LEALab) OPLsrl, v. G. Leopardi 9,
58015 Orbetello (Grosseto, Italy).

THE MEADOWS OF *POSIDONIA OCEANICA* (L.) DELILE ALONG THE TUSCANY SOUTHERN COAST (MT. ARGENTARIO - CHIARONE RIVER, ITALY).

Abstract

A stretch of coast of 40 Km (from Porto S. Stefano-Mt. Argentario to the Chiarone River) was monitored in June and July 2004 for presence, range, biomass and biometry of meadows of Posidonia oceanica. *The survey was made by videocamera along 30 transects and quantitative samples were taken from 15 of the 30 transects, at the upper and lower limits (UL, LL). The data were analyses by ANOVA and MDS. Shoot density suggested widespread conditions of "normal density", while the Leaf Area Index at UL (11.9 ± 5.2* $m^2 \cdot m^{-2}$*) and LL (9.5 ± 4.1* $m^2 \cdot m^{-2}$*) and the Leaf Standing Crop at UL (1006,8 ± 389,8* $gdw \cdot m^{-2}$*) and LL (637.1 ± 528.0* $gdw \cdot m^{-2}$*) showed high values. The deepest LL was –28.5 m and the mean was –20.6±5.6 m. Between UL and LL, the percentage of bottom covered by meadow varied between 10 and 80, with a mean of 51.1 ± 20.5 %. Three of the sites sampled were relatively worse than the others. In two cases, it could be ascribed to a bottom consisting mostly of sandstone detritus; in the third site it could be due to erosion and bottom trawling. Meadow range along Mt. Argentario was limited by the steep slope of the coast, which drops sharply to depth below the lower limit of* P. oceanica.

Key-words*: Seagrass meadow, Monte Argentario,* Posidonia oceanica*, Tuscany coast, Tyrrhenian Sea.*

Introduction

Posidonia oceanica meadows are the most complex and widespread ecosystem of the Mediterranean coastal belt, playing a fundamental role in the ecology of neritic zone. *P. oceanica* beds are very sensitive to disturbance by human activity (e.g., coastal change, pollution, turbidity, anchoring, etc.) and reduction of these meadows has been observed in a number of Mediterranean region (Meinesz *et al*., 1991). To safeguard these meadows, it is necessary to define their range and density, follow their developments in time, and in the case od regression, discover the causes. Despite growing interest in this ecosystem, few stretches of the long Italian coastline have yet been well monitored. Regarding the coasts of southern Tuscany, Papi *et al*. (1992) studied the distribution of the phytobenthos in the islands of the Tuscan archipelago, and Lenzi (1987) and Lenzi *et al*. (1989; 1993) that of part of the Grosseto coast. The present study examines the meadows of the coast of Mt. Argentario, from the western tip at Porto Santo Stefano, where Lenzi *et al*. (1989) terminated theirs study, to the mouth of the Chiarone, a distance of 42 Km (Fig. 1). This coastal belt has a tourist flow of more than two million persons concentrated in just over three months of the year. This implies: 1) outflow of the wastewater from various water treatment plants; 2) thousands of pleasure boats that cast anchor indiscriminately; 3) major anthropogenic remodelling of the coastline. Some stretches of coast are subject to erosion and loss of sand. Major fishing fleets, that prevalently use drag nets, operate out of Porto Ercole and Porto S. Stefano.

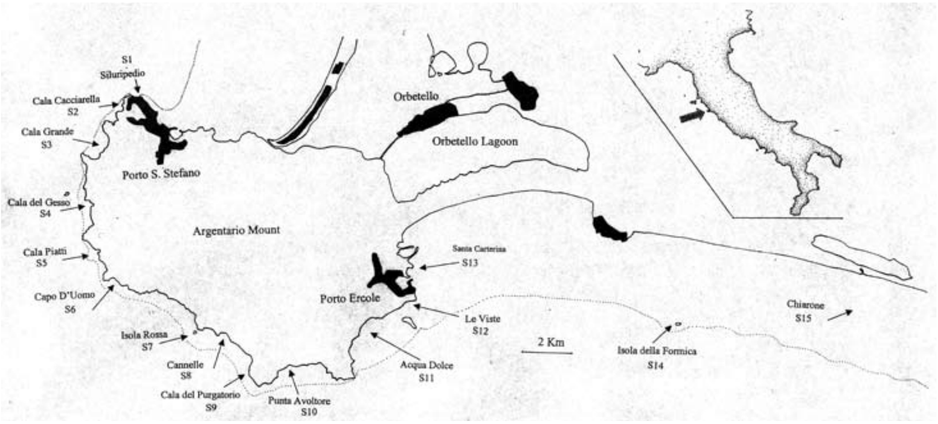

Fig. 1 - Stretch of coast from Porto Santo Stefano to the river Chiarone. Names of study sites and stations (S1-S15). Towns are shown in black.

Materials and methods

Fifteen sampling sites were established between Porto S. Stefano (42°26.743 N; 11° 06.799 E) to the Chiarone estuary (42°23.615 N; 11° 20.715 E). Two bionomic transects were identified in each site from the upper (UL) to the lower limit (LL) of any meadow present (Fig. 1). The transect was inspected by SCUBA divers along a positioned metric line (Bianchi *et al.*, 1991). One of the two transects of each site underwent quantitative sampling and both were recorded with a video-camera, as described by Somaschini *et al.* (1998). Sampling was performed at the two extremes UL, LL, of the 15 transects, making a total of 30 stations, randomly collecting seagrass in a 900 cm^2 (30x30cm) sampling unit. The following parameters were determined in samples as described by Giraud (1977a,b): number of shoots per square meter (shoot/m^2), number of leaves per shoot (l/shoot), lenght of adult leaves in cm (l-al); lenght of intermediate leaves in cm (l-il); lenght of young leaves in cm (l-yl), coefficient of breakage of tips of adult+intermediate leaves (A), percentage of leaf surface covered in epiphytes (E), leaf surface area in m^2 per m^2 (LAI) and dry weight of epigeal fraction per m^2 (Life Standing Crop, LSC) after drying in a oven at 80°C for 48 hours. From the videos, the following parameters were estimated (Grillas *et al.*, 2000): *P. oceanica* cover as percentage of substrate covered by meadow (CP), percentage of bottom covered by dead matte (d.m.), percentage of substrate with sandy intermattes (sand), percentage of rocky substrate (rock). Using video material and depth, we produced a profile of coastal morphology. According to Pergent-Martini *et al.* (1999), a density-depth scale (dds) was created to define meadow types. Similarity in shoot/m^2, l/shoot, LAI and LSC among UL stations and among LL stations (separately) was analysed by calculation of normalised Euclidean distance coefficients. Data was transformed by arch-tangent function before similarity matrix calculation. For graphical representation, a two dimensional MDS ordination was carried out with PRIMER-5 software. The differences between UL and LL stations were analysed for each parameter by one-way ANOVA, and Dunnett's test was applied for each parameter, using a control station identified by MDS for each limit group.

The paired t-test was applied to the LAI data set and the Chi-Sq test to the A data. SAS institute, JMP 5.1 statistical package was used.

Results and discussion

MDS analysis showed three groups of similarity for UL, including S1-S8, representing the best meadow conditions, and the group S6-S12-S13-S15, representing the worse. For LL, group S1-S6 represented the best conditions and S2-S15-S14-S8 the worse. However, distribution at LL stations was influenced by LAI values that varied widely at different depths. On the basis of this discriminant, S8 and S6 were used as references for UL and LL groups in Dunnett's post hoc test. S1 was discarded as misleading due to the shallowness of both limits. ANOVA one way results are reported in Table 1. Values of **l-al** were not significantly different between UL (47.4±17.9 cm) and LL (48.1±8.0 cm). Values of **l-il** were significantly higher at LL (53.5±8.2 cm) than at UL (43.8±18.2 cm). Values of **l-yl** were significantly higher at UL (0.6±0.2 cm) than at LL (0.4±0.2 cm). Values of **l/shoot** were not significantly different between UL (6.99±0.38) and LL (6.96±0.51). Coefficient **A** was significantly greater (Chi-Sq=468; P<0.0001) at UL (65±15) than at LL (34±18). Since values of **l-il** and **A** were lower and higher, respectively, at UL than at LL, shoots are presumably subject to greater stress in shallow water (hydrodynamics and grazing). This suggests that the greater length of **l-yl** at UL indicates faster growth to replace damaged leaves. Values of **E** were not significantly different between the two limits (Tab. 1) and were not high (UL: 22.5±6.5; LL: 19.0±9.4; Tab. 2). Epiphytes therefore do not seem to be the cause of the higher frequency of leaf renewal at UL. At LL, the post hoc test (d=2.7; $P<0.05$) showed groups S1-S2-S3 and S8-S14-S15 to be distant from the reference group with values of 36-38% and 2-8%, respectively. For the former group, these values could be due to relative closeness to the port and/or to shallow bottom; for the latter, to the relatively greater distance from anthropogenic impacts. At UL, E values were significantly higher at S4 and S6, probably due to low depth and high light, and at S12 and S13, due to closeness to Porto Ercole, than S8 (d=2.8; $P<0.05$) (Fig. 1).

Tab. 1 - ANOVA results for UL vs LL

Parameter	F	df	*P*	Parameter	F	df	*P*
l-al	1.8	4099	0.1819	$l \cdot shoot^{-1}$	2.1	1216	0.1447
l-il	39.1	1724	0.0001	$shoot \cdot m^{-2}$	14.5	83	0.0003
l-yl	38.3	2840	0.0001	LAI	1.42	81	0.2448
E	1.5	2893	0.2151	LSC	9.4	83	0.0029

LAI was only significantly different between UL (11.9±5.2 $m^2 \cdot m^{-2}$) and LL (9.5±4.1 $m^2 \cdot m^{-2}$) according to the t-test (t=1.64; P<0.05). The highest value (21.4 $m^2 \cdot m^{-2}$) was found in S8-UL, whereas the deepest station (-28.5 m) had a LAI of 7.9 $m^2 \cdot m^{-2}$ (Tab. 2). Though **LSC** was high (1006.8±389.8 $g_{dw} \cdot m^{-2}$ at UL; 637.1±528.0 $g_{dw} \cdot m^{-2}$ at LL), it was significantly different between the two limits (Tab. 1). Shoot densities were significantly different at UL (555.8±206.5 $shoot \cdot m^{-2}$) and LL (384.0±144.5 $shoot \cdot m^{-2}$) (Tab. 1). 32% of meadows had sub-normal high density (67% of which were at LL), 61% had normal density and only 7% had extremely low density (Tab. 2). Near the two ports (Fig. 1), meadows were much smaller and of variable density (Tab. 2), probably due to disturbance by boats and/or fine particles from the port, limiting meadows to rocky areas with stronger currents, especially in S1. **CP** varied from 10% in S13 to over 80% in S1 and S11 (mean 51.1±20.5 %). Substrate devoided of *Posidonia* was sandy bottom in 29.8% of cases, rocky in 15.9% and dead matte in 2.7%. The coastal stretch around Mt. Argentario did not have particularly extensive meadows. This is largely due to the small infralittoral zone off cliffs and narrow zone <30 m depth (Fig. 1), and to abundant sandstone detritus in many stretches. For the 26 Km from S1 to S13, estimating a mean transverse meadow length of about 81 m from the coastal profiles, it is possible to calculate an overall meadow area of about 210 ha. Adding the meadows of the 6 Km of coast in the northern part (Lenzi *et al.* 1989), we obtain about 270 ha of meadows. Although rela-

Tab. 2 - Means and standard deviations of the main utilised parameters. Density depth scale (dds): extremely low density (AD); normal density (ND); high subnormal density (HSD)

Abbr.	Limits	depth (m)	shoot/m^2	l/shoot	A (%)	E (%)	LAI (m^2/m^2)	LSC (g/m^2)	Dds
S1	UL	1.05	911±164	7.8±1.8	77.0	15±14	16.02	1602±959	DN
	LL	7.09	700±184	8.2±2.0	77.3	38±18	18.02	2038±339	DSS
S2	UL	8.00	681±105	7.1±1.0	74.7	14±13	14.00	1180±379	DSS
	LL	25.0	219±71	6.8±1.1	11.7	36±22	4.04	261±102	DN
S3	UL	8.00	507±101	6.9±1.2	63.2	16±13	13.04	1065±161	DN
	LL	17.00	330±36	7.0±1.2	40.9	38±19	6.04	471±152	DN
S4	UL	8.09	378±183	6.8±1.3	67.3	29±13	9.03	635±17	DN
	LL	26.7	359±210	6.3±0.9	17.0	13±13	8.00	710±602	DSS
S5	UL	11.00	444±40	7.1±1.1	66.8	12±11	14.00	1072±111	DN
	LL	25.0	304±23	7.0±1.0	46.9	22±15	9.06	611±90	DN
S6	UL	7.00	285±330	6.4±1.9	53.1	26±18	3.00	635±17	DA
	LL	13.9	544±109	6.8±1.5	53.7	20±15	15.04	1498±61	DSS
S7	UL	8.00	526±124	7.3±1.2	58.2	23±11	16.07	1336±399	DN
	LL	28.5	359±51	7.1±1.2	21.9	11±10	7.09	348±90	DSS
S8	UL	13.5	774±449	7.0±1.4	64.4	15±11	21.04	1480±809	DSS
	LL	27.0	248±23	6.5±0.9	27.5	8±8	6.02	297±6	DN
S9	UL	5.0	530±319	7.2±1.4	58.6	28±14	14.07	1526±339	DN
	LL	24.0	456±190	6.4±1.1	30.4	15±11	10.06	734±249	DSS
S10	UL	13.0	437±26	6.9±1.2	29.9	26±13	14.00	1064±149	DN
	LL	22.0	363±185	7.4±1.6	34.3	16±12	10.03	540±240	DN
S11	UL	7.0	659±55	6.3±1.2	72.4	18±10	12.04	998±256	DN
	LL	15.0	574±301	6.5±1.2	36.6	16±7	13.07	650±306	DSS
S12	UL	8.0	504±83	6.8±1.5	81.3	51±27	4.09	455±196	DN
S13	UL	5.0	367±67	7.3±1.1	85.1	39±25	4.05	469±4	DA
S14	UL	3.0	981±225	6.6±0.8	81.0	15±13	13.08	1081±184	DSS
	LL	21.0	259±23	7.3±0.8	25.4	2±10	7.01	420±27	DN
S15	UL	17.0	352±110	7.3±1.4	38.2	25±15	6.07	502±203	DN
	LL	18.5	278±38	7.2±1.4	14.7	3±4	6.02	327±99	DN

tively small, the significance of this important ecosystem should not be underestimated. The areas beyond Argentario, S14 and S15, the former with a bare, mostly rocky bottom (>50%), the latter with a sandy-muddy bottom (>40%), had relatively good density (Tab. 1). However, the meadow in S15, together with the S3 transect, had the largest amount of dead matte (6% e 13%, respectively) and eroding edges. The area adjacent S15 was previously subjected to trawling.

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M. Lorenti[1], A. Gera[1], J. Lassauque[1,2], F. Mattera[1], M.C. Buia[1]

[1]Laboratorio di Ecologia del Benthos, Stazione Zoologica 'A. Dohrn' di Napoli,
Punta S. Pietro, 80077 Ischia (Naples), Italy
[2]Laboratoire Environnement Marin Littoral, Université de Nice-Sophia Antipolis, F-06108, Nice, France

COMPARATIVE *IN SITU* ESTIMATES OF THE PHOTOSYNTHETIC ACTIVITY OF *POSIDONIA OCEANICA*: RLC AND MAXIMUM QUANTUM YIELD MEASUREMENTS

Abstract

Variation in fluorometry-derived parameters of the photosynthetic performance of Posidonia oceanica *was studied at two sites off the island of Ischia (Gulf of Naples, Italy) by performing RLCs (Rapid Light Curves) and measuring maximum quantum yield (F_v/F_m) on intact plants in situ by means of a Diving-PAM. Measurements on an annual scale revealed a decrease in photosynthetic efficiency in months when light availability was highest while ETR_{max} showed a peak in spring. At a spatial scale, the effect of depth on photosynthetic variables was evident although with less clear patterns. Overall, the use of PAM fluorometry in situ appears to yield a reasonable picture of the natural variability of photosynthetic activity in* P. oceanica.

***Key-words**:* Posidonia oceanica*, PAM fluorometry, photosynthesis.*

Introduction

The ability of PAM fluorometry as used *in situ* for detecting variations in photosynthetic activity has been approached for a number of seagrasses (e.g. Beer *et al*., 1998; Durako and Kunzelman, 2002; Lorenti *et al*., 2006). The instrument which has been generally employed in such studies is the Diving-PAM model produced by Walz (Effeltrich, Germany) in view of its portability, relative ease of use and software availability. The use of the Diving-PAM has also been the subject of standardization efforts (Beer *et al*., 2001; Ralph and Gademann, 2005) and is often involved in comparative photosynthetic measurements at various spatial and temporal scales.

The main aim of the present work was to describe the variability of photosynthetic activity in two beds of the Mediterranean endemic *Posidonia oceanica* as detected by Diving-PAM fluorometry *in situ* at one temporal (annual) and one spatial (depth) scale. An expeditive approach was adopted, mainly in view of the possible use of PAM fluorometry as a monitoring tool for *P. oceanica*. This involved the collection of Rapid Light Curves (RLCs), with irradiance durations of 10 seconds at each of eight light steps (Ralph and Gademann, 2005), and the measurement of optimal quantum yields (F_v/F_m) with dark-adaptation times of about 10 minutes.

Materials and methods

Measurements were carried out in *P. oceanica* stands at two sites off the island of Ischia (Gulf of Naples, Italy) using a Diving-PAM fluorometer operated by SCUBA divers. The annual variation in photosynthetic activity at the shallow (–3 m) site (San Pietro, henceforth indicated as SP) was assessed for five months (from March 2005 to February 2006). At the other site (Lacco Ameno, LA), characterized by the presence of a continuous meadow extending down to ca. 30 m depth, two stations were selected (5 and 22 m depth) at which measurements were performed for four months (from May 2005 to February 2006). The photosynthetic response was determined on the mid portion of the 2nd intermediate leaf, taken as representative of the whole *P. oceanica* shoot performance (Buia *et al.*, 1992). RLC parameters (ETR_{max}, maximum rate of electron transport; α, initial slope of the curve) were calculated by fitting an exponential function to experimental data. Statistical analyses were performed using Prism 4 (GraphPad Software, Inc.).

Results

With regard to the variation of photosynthetic variables as assessed on a seasonal basis at SP, the more obvious trends were shown by F_v/F_m and α, which assumed minimum values in September and maximum ones in February (Fig. 1, a and b). ETR_{max} showed a peak in May (Fig.1, c), while the other months did not differ significantly between each other. In spite of the high variability displayed within each seasonal sample, the statistical analysis showed a highly significant ($p < 0.001$; Kruskal-Wallis test) effect of the month on all the three considered variables.

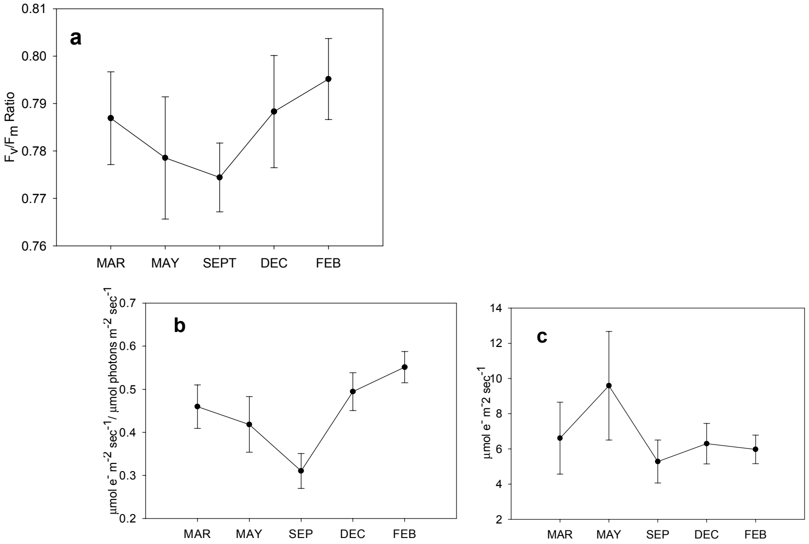

Fig. 1 - Annual variation in F_v/F_m (a), α (b) and ETR_{max} (c) in the *P. oceanica* population studied. Values are means of ca. 12 replicates ± S.D.

At LA, depth-related differences varied according to the sampling month (Tab. 1). While in September no significant differences occurred for any of the considered variables, in December highly significant differences were shown ($p < 0.001$; ANOVA). In the two other months, while F_v/F_m presented significantly lower values at 22 m, a difference in ETR_{max} was shown only in May, with higher values recorded at 5 m.

Tab. 1 - Results of ANOVA for the depth effect at LA conducted on each month. NS = non significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

	MAY	SEPT	DEC	FEB
F_v/F_m	*	NS	***	**
ETR_{max}	***	NS	***	NS
α	NS	NS	***	**

Discussion

PAM fluorometry *in situ* as applied to *P. oceanica* does detect variation at different scales and appears to yield to a good extent a reasonable picture of natural trends in photosynthetic activity. In most instances, the variability among replicates is high; reasons for this may be the intrinsic diversity of beds, some experimental pitfalls and the heterogeneity of the environment, particularly as far as irradiance is concerned (Zimmermann, 2006). The detection of within-population variability is however an advantage offered by *in situ* fluorometry and may originate from the individual acclimation status of different plants, as occurs in macroalgae (Hanelt *et al.*, 2003).

At shallow depth, the clearest trends on an annual scale are shown by α and F_v/F_m, variables which should describe the efficiency in light utilization in relation to either incident (α) or absorbed (F_v/F_m) radiation. Both show a decrease in months when the light availability is highest so that lower efficiency in light utilization and higher photoprotection are required. On the other hand, maximum rates of photosynthesis occur in May, which may reflect the peak observed in the growth of the second leaf in spring (Buia *et al.*, 1992).

While temporal gradients appear to have an interpretable pattern, depth-related differences are less clear in our data. Reports on the variation of photosynthetic activity of *P. oceanica* with depth are somewhat contrasting (Figueroa *et al.*, 2002; Olesen *et al.*, 2002) and species-specific determinants have been invoked for explaining the lack of a significant depth response. The generally lower values of F_v/F_m at 22 m as compared to 5 m ones are puzzling as a higher efficiency in light use and a lower need for photoprotection should be expected at the deep station. As F_v/F_m is also a stress indicator, this result may be related to a local situation translating in a general poorer status of the deeper stand due to either natural or anthropogenic disturbance and calls for a further comparison with truly pristine or low-impacted meadows.

As a final remark, a rather strict repeatability of conditions at which comparative measurements are made is probably required if the use of the Diving-PAM as a monitoring tool is envisaged. This is because of the many scales of variability reflecting a variety of acclimation times occurring in seagrasses.

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A.M. Mannino[1,4], A. Santulli[2], G. Sarà[3,4]

[1] Dipartimento di Scienze Botaniche, Università di Palermo,
Via Archirafi, 38 - 90123 Palermo, Italy.
[2] Dip. Scienze Biochimiche, Sez. Biochimica Marina-Policlinico, Palermo, Italy.
[3] Dip. Biologia Animale, Università di Palermo, Palermo, Italy.
[4] Polo Didattico di Trapani, Università di Palermo, Trapani, Italy.

THE SHALLOW SEAGRASS SYSTEM IN W-SICILY (ITALY): A LARGE RESERVOIR OF ORGANIC MATTER AVAILABLE TO UPPER CONSUMERS

Abstract

The effects of human activities related to salt production on the complexity of a shallow hyperhaline system (Sicily, Italy) were investigated, through monitoring of some physical, chemical and trophic factors involved in the origin of organic matter and its availability to apex consumers (fishes and migrating birds). The system was dominated by seagrass Ruppia cirrhosa *and large stands of macroalga* Chaetomorpha linum*; isopods and gasteropods were the most abundant taxa among benthic organisms, while* Aphanius fasciatus *and* Atherina boyeri *represented up to 90% of all catch among the small resident fishes.* R. cirrhosa *could function as a multidimensional framework able to increase the richness of algal epiphytes and in turn the richness of fish species. A positive relationship was in fact observed between levels of Ruppia coverage and fish abundance and between fish abundance and migrating bird occurrence. The* R. cirrhosa *represents a large reservoir of organic matter available for top consumers.*

Key-words: *Seagrass,* Ruppia cirrhosa*, organic matter availability, shallow waters, Mediterranean Sea.*

Introduction

The importance of most of the coastal habitats relies on the presence of seagrasses, which abundantly colonise almost all substrata. Seagrasses play a crucial role in the input of organic matter into the food web (Hemminga *et al.*, 1991; Thom and Albright, 1990) and in creating a physical multidimensional framework for algal epiphytes, which represent food sources (Borowitzka *et al.*, 2006), either directly or through detritus for an array of organisms (benthic invertebrates, insects and fishes). These, in turn, represent food items for upper consumers (e.g. fishes and migratory birds). Shallow systems of Western Sicily (Italy) represent an important ecological stopover site for Mediterranean migratory bird species, moving from Africa to Europe, reaching for breeding or wintering areas. These sites are composed by dozens of shallow ponds with fluctuant salinity around marine levels which are abundantly colonised by the seagrass *Ruppia cirrhosa* (Petagna) Grande. *R. cirrhosa* represents the main primary producer of organic matter in the area and from it depends the stability of the whole ecosystem (Mannino and Sarà, 2006). Any human induced disturbance, regarding the availability of space and resources for the species, may represent a serious threat to biodiversity in this kind of habitat.

The main aims of this paper were to verify: i) variability in submersed vegetation is related to the abundance of fish and ii) differences in abundance of fish and related abundance of avifauna.

Materials and methods

The study was carried out in the Natural Reserve of Trapani and Paceco (western Sicily) (37°52'N, 12°28'E). The area hosts the most important pond system of the western Mediterranean composed by about 20 basins covering a surface area of about 1,000 ha. Samples were collected seasonally from spring 2004 to winter 2005 in 9 of the 20 basins. The total plant coverage (seagrasses plus macroalgae, %) was estimated by visual census, while *R. cirrhosa* coverage (%) and macroalgal coverage (%) were estimated in triplicate using respectively 1 m^2 (1m x 1m) and 400 cm^2 (0.2 x 0.2 m) quadrates.

Depth, temperature, salinity were measured by means of a multiprobe sampler (SD6000; YellowSpring). Samples of waters in triplicate were collected using a 10-l Niskin bottle, filtered onto pre-washed, precombusted and pre-weighed Whatman GF/F filters. For each replicate, the total suspended material (TSM, mg l^{-1}) determination was carried out gravimetrically after desiccation (105 °C, 24 h) using a Sartorius M200 balance (accuracy ± 1 µg), while chlorophyll-a [CHLa, µg l^{-1}] was determined according to Lorenzen and Jeffrey (1980). Fish were seasonally collected in triplicate from each basin by using nets on a standardised area (20 m^2) and values reported to mean square, while avifauna abundance was obtained from data of a census conducted by the Natural Reserve. In this paper, for a summary, all data are reported as annual averages.

Results

Temperature values of waters, throughout the study area, ranged between 12 °C and 30 °C (on annual average 19.7 ± 1.0 °C), the salinity ranged between 20‰ and ~75‰ (summer peaks) with an annual average of 34.0 ± 5.7‰, total suspended material concentrations ranged between 30.9 mg l^{-1} and ~1000.0 mg l^{-1} (annual average 122.4 ± 84.2 mg l^{-1}), the organic fraction represented 60.2% and suspended chlorophyll-a ranged between 4.8 and 55.0 µg l^{-1} (annual average 21.5 ± 15.6 µg l^{-1}).

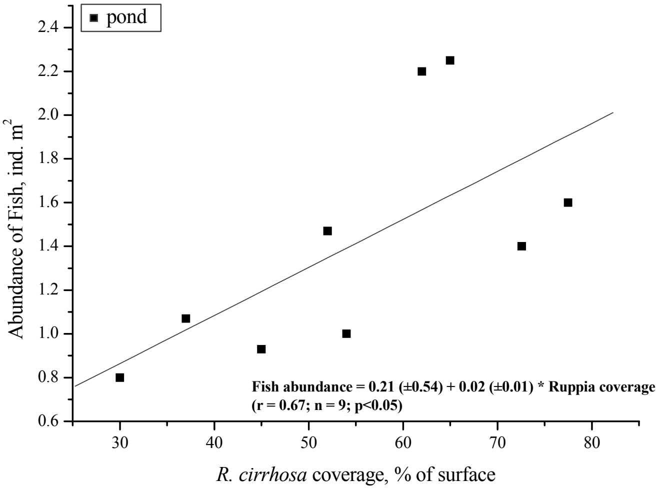

Fig. 1 - Correlation between *Ruppia cirrhosa* surface coverage and fish abundance in 9 ponds (r = correlation coefficient; n = sample size; p = probability level) .

Vegetated bottoms accounted on average for about 55.3 ± 14.8% of total surfaces, submersed vegetation was dominated by seagrass *R. cirrhosa* (50.7 ± 14.2 %) while stands of macroalgae dominated by *Chaetomorpha linum* (O.F. Müller) Kützing represented 44.5 ± 16.8% and number of associated algal taxa ranged between 7 and 15 (average 10.6 ± 2.8 taxa). The most represented fish was *Aphanius fasciatus* representing up to 90% of all catch among the small resident fishes. Other fishes as those belonging to Signatidae, Mugilidae and *Atherina boyeri* were only occasionally collected throughout the study period. Avifauna was mostly represented by 5 species: *Egretta garzetta* (the most abundant), *Sterna albifrons, Platalea leucorodia, Recurvirostra avosetta* and *Egretta alba*. A positive correlation between surface covered (percentage of coverage) by *R. cirrhosa,* as measured annually in 9 ponds, and the total fish abundance per mean square was observed (Fig. 1). Furthermore, also a correlation between fish abundance and bird presence was evident (Fig. 2).

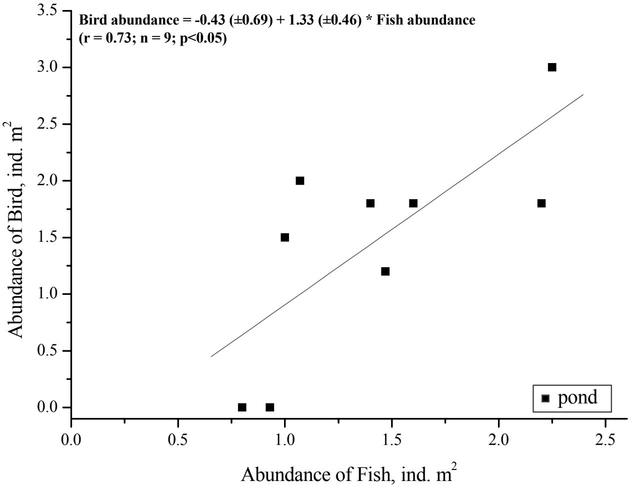

Fig. 2 - Correlation between fish abundance and bird presence in 9 ponds (r = correlation coefficient; n = sample size; p = probability level).

Conclusions

The Natural Reserve of Trapani and Paceco of Western Sicily is a large reservoir of organic matter available to top consumers. The measured concentrations of suspended matter and its organic fractions were the highest among those measured in the whole Mediterranean basin (Sarà, in press). The seagrass biomass values registered in this area, was amongst the highest reported for *Ruppia* meadows combined with the shallowness, appeared to be the major cause in making huge quantities of organic matter available to top consumers (Mannino and Sarà, 2006).

Intense production of *Ruppia* organic matter can fuel constantly, over time, the detritus cycle that in turn fuels the trophic web and provides a multidimensional framework able to increase the richness of algal epiphytes (Mannino and Sarà, 2006) and in turn the richness of fish species, which are preferred preys of migratory birds. Thus, the naturalistic value of the studied area is strongly correlated to presence and extent of *Ruppia*, which makes the area a large reservoir of organic matter available for top consumers, notwithstanding the area is subjected to constant anthropogenic disturbance.

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M. Migliaccio[1], A. Cavallini[2], L. Natali[2], G. Procaccini[1]

[1]Stazione Zoologica 'A. Dohrn', Laboratorio di Ecologia del Benthos, 80077 Ischia (Napoli) – Italy
[2]Università di Pisa, Facoltà di Agraria, Via Matteotti 1, 56124 Pisa (Italy)

NEW GENOMIC APPROACHES ON THE SEAGRASS *POSIDONIA OCEANICA* (L.) DELILE

Abstract

Posidonia oceanica *(L.) Delile is a seagrass adapted to a wide range of environmental conditions along broad latitudinal, longitudinal and bathymetric ranges. Its ecology and physiology, along with its capacity to adapt to different environmental conditions have been studied using a combination of manipulative field experiments with neutral molecular markers. The complex interactions among adaptive genetic diversity, selection, gene flow and ecological plasticity await the development of gene-linked markers. We report on the construction of a non-normalized EST (Expressed Sequence Tags) library for* P. oceanica *including preliminary annotation. 2591 single run sequences have been determined from cDNA clones taken at random in the library prepared. From these sequences 1363 were singlets, 196 were grouped in Tentative Consesus sequences obtained from clustering step. 32% of the sequences are no hits better than 0.0001.*

***Key-words**:* Posidonia oceanica*, cDNA, ESTs.*

Introduction

Seagrasses are monocotyledonous plants adapted to live completely submerged in the sea. They are key species in shallow coastal waters all over the world including polar areas (Green and Short, 2003). Their areal coverage is ca. 200 $\times 10^{6}$ ha. Seagrasses are declining worldwide and the decline is mainly associated with increasing anthropogenic pressure along the coastline, such as enhanced turbidity and sedimentation, eutrophication and industrial pollution (Short and Wyllie-Echeverria, 1996). Hence, there is a growing awareness that loss of seagrass meadows may have conspicuous impacts on the stability and morphogenesis of the coastal zone (Borum *et al.*, 2004). In general, reliable estimates of seagrass decline in Mediterranean Sea are not available but extensive regression for *Posidonia oceanica* has been reported (Procaccini *et al.*, 2003).

Existing population genetic data on *Posidonia oceanica* is based on neutral, microsatellite markers. Studies show that genetic diversity is highly structured at different hierarchical scales. Within the whole Mediterranean basin, major clusters correspond to recognized biogeographic sectors defined with the current regimes and post-glacial recolonization (Procaccini *et al.*, 2002, Pinardi and Masetti, 2000). At the single-meadow level, a recent analysis carried out at Lacco Ameno (Ischia, Gulf of Naples) confirmed the existence of a clear disjunction between plants growing above and below the summer thermocline, located at about 15 m depth (Procaccini and Mazzella, 1998; Procaccini *et al.*, 2001; Migliaccio *et al.*, 2005). There are clearly differences in both light and temperature experienced across this depth gradient as noted by Buia and Mazzella (1991), Buia *et al.* (1992) and Lorenti *et al.* (1995).

In order to investigate adaptation to different environmental conditions, both in different geographic localities and along the depth gradient in the same locality, new "selectively relevant" markers need to be developed. Here we present preliminary data from a study aimed to identify expressed sequences involved in adaptation processes. To accomplish this goal, a non-normalized EST (Expressed Sequence Tags) library, from total RNA extracted from *P. oceanica* shoots collected at different depths within the Lacco Ameno meadow (Ischia) has been constructed. Expressed gene regions will be annotated and given a specific function. Results will be compared with EST databases from phylogenetically related terrestrial species, in order to select genes responsible for the adaptation to marine conditions.

Material and methods

Total RNA extraction

Samples were collected by SCUBA diving from a meadow located in Lacco Ameno, Ischia (Gulf of Naples), from 5 and 25 meters depth. Samples were cleaned and shock frozen in liquid nitrogen on the boat soon after collection. Total RNA was isolated from young leaf tissue and meristematic portions of shoots using a hexadecyltrimetihyl ammonium bromide (CTAB) method (Chang *et al*., 1993). Lithium Chloride was used for precipitation of total RNA.

Construction of a standard cDNA library.

The cDNA was synthesized according to Vertis biotechnologie (Freising, Germany) standard protocol for full-length enriched cDNA synthesis from poly A+ RNA purified from the total RNA.

An oligo (dT)-linker primer was used for first strand synthesis. The resulting cDNA was amplified with 17 cycles of PCR. For cloning, cDNA was subjected to a limited exonuclease treatment to generate 5' overhangs at both ends of the cDNAs. After size fractionation on Agarose gel the cDNA fraction larger than 0.5 kb was directionally ligated into the *Eco*RI and *Bam*HI sites of the pBSII sk+ plasmid. The cDNA inserts can be released from the pBS II-vector by an *Eco*RI/ *Bam*HI digestion. Ligations were electrophorated into Phage T1 resistant TransforMax EC100 (epicentre) electro- competent cells. After transformation, glycerol was added to a final concentration of 15% (v/v) and cells were frozen at -70°C. After a freeze-thaw cycle, the titer of the library was determined to be about 1900cfu per µL bacterial suspension resulting in a total number of about 11,000,000 recombinant clones. To get a comprehensive impression on the distribution of the insert sizes within the library, about 1000 colonies of the library grown overnight on a Petri dish were suspended in water. With an aliquot of the bacterial suspension PCR analysis was performed with a primer pair that binds to the T3- and T7 promotor of the pBSII sk+ vector. Therefore, together with the inserts of 140bp vector sequences are co-amplified. PCR products obtained after 22 cycles were analyzed on a 1.3% agarose gel.

Sequencing and analysis of ESTs

Randomly selected clones from the library were isolated from plasmids (minipreps) and sequenced. Sequences were performed both at the Zoological Station (Naples) and at the Max Planck Institute of Molecular Genetics in Berlin (Germany). T7 primer was used to amplify the 2591 clones that were consequently sequenced.

Bioinformatics

Automatic annotation of the EST library was performed online with the SAMS (Sequence Analysis and Management System) software, at the Center for Biotechnology (CeBiTec) in Bielefeld, Germany. Using SAMS, tentative Consensus Sequences (TCs) and ESTs will be automatically annotated with BLAST searches against several database, such as KEGG and SwissProt, as well as InterPro and Pfam.

Results

The random sequencing approach yielded some interesting information on the structure and expression of gene families. The first results that we obtained from our cDNA library by using SAMS program are the following ones:

Two thousand five hundred and ninety-one single run sequences were determined from one end of the no-directional cDNA library. The average read length is about 670bp and the redundancy is about 47%.

One hundred and ninety six tentative consensus sequences (TCs) and one thousand three hundred and sixty three singlets were obtained. The average of ESTs/TCs was 6.27. 32% of the sequences are no hits better than 0.0001. The GC level was 47.14%.

We identified 149 SSRs (simple sequence repeats) among the ESTs, 57 of which were dinucleotides, 83 trinucleotides, 2 tetranucleotides, 1 pentanucleotides and 6 esanucleotides. Those can be useful markers for genetic diversity analysis and molecular mapping strategies.

All the tentative consensus sequences and singlets have been annotated. Metallothionein-like genes turned out to be the most abundant class in TCs, suggesting that they might perform essential functions of plant growth besides metal detoxification (Matsumura *et al.*, 1999).

Conclusions

Plant ESTs have been increasingly used in analyzing global gene expression and function, and as markers and probes in genome mapping (Ewing *et al.*, 1999). Random sequencing provides an overall view of genes expressed in different tissues. ESTs are usually short and relatively inaccurate (around 2% error), but have been shown to be an invaluable resource for the discovery of new genes involved in specific phenotype and crucial biological process (Donson *et al.*, 2002).

Until now, the genome of *Posidonia oceanica* has never been studied. Although this project represents only a first step, our goal was to give a contribution to discover genes that could be involved in adaptation to particular environmental conditions. We are currently completing the annotation of the 2591 ESTs sequenced and we will soon increase the number of sequences. After that, our results will be compared with EST databases from phylogenetically related terrestrial species, in order to select genes responsible for the adaptation to marine environment. We have recently begun subtractive expression libraries in order to identify genes which are up- (or down) regulated under different environmental conditions. This experiment will provide an important tool to understand adaptation processes in *Posidonia oceanica*.

Aknowledgements

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H. Molenaar, A. Meinesz, T. Thibaut

E.A. 3156 Gestion de la Biodiversité, Université de Nice-Sophia Antipolis,
Faculté des Sciences, 06108 Nice Cedex 2, France.

COMPETITION BETWEEN NATIVE *POSIDONIA OCEANICA* AND INVASIVE *CAULERPA TAXIFOLIA*

Abstract

The density, the structure and the rate of ramification of a Posidonia oceanica *meadow invaded by* Caulerpa taxifolia *at 20 m depth were studied from 1995 to 2004. Cover of the invasive* Caulerpa taxifolia *inside the seagrass bed remained low, from 5 % to 26 %. This low infestation did not significantly change the density, the structure and the ramification of the invaded bed. Densities remained normal and constant from 311 to 515 ± 32 shoot.m^{-2}.*

Orthotropic shoots were predominant and only ramified from 5 to 16 %; there were fewer plagiotropic shoots ramified from 34 to 65 %. This does not corroborate results from a similar study performed at 6 m depth where an important impact was observed in a highly invaded bed: the shoot density decreased strongly and the remaining orthotropic shoots transformed into abnormally ramified plagiotropic ones.

Key-words: *Mediterranean Sea,* Posidonia oceanica, Caulerpa taxifolia, *competition.*

Introduction

The endemic seagrass *Posidonia oceanica* (Linnaeus) Delile is the main coastal ecosystem of the Mediterranean Sea (Den Hartog, 1970). The species is undergoing constant human pressures in almost all the Mediterranean and this reduces the surface of this coastal key ecosystem (Peirano *et al.,* 2005). The competition with the introduced alga *Caulerpa taxifolia* Vahl C. Agardh (Meinesz and Hesse, 1991) is also one of the pressures. The seagrass have been found growing together with the introduced chlorophyte *Caulerpa taxifolia* for two decades. The alga is actually found on 11 countries in the Mediterranean Sea and at the end of 2005, in France, 8687 hectares of sea floor are concerned by *C. taxifolia* invasion with 86 zones of competition (Javel and Meinesz, 2006, www.caulerpa.org). Sparse *P. oceanica* beds are known to be easily invaded by *C. taxifolia* (Villèle and Verlaque, 1995) due to a positive effect of the seagrass on the alga (Ceccherelli and Cinelli, 1999). Interactions between the two macrophytes induce an increase in the seagrass phenol production (Villèle and Verlaque, 1995), a decrease in leaf length and longevity and an increase in the number of leaved produced each year (Dumay *et al.,* 2002). All these observations were done punctually or over short-term experiments which did not allow knowing if *C. taxifolia* can modify the structure of an invaded *P. oceanica* bed. To this aim, we compared the evolution of the structure of invaded and non invaded beds of *P. oceanica* over a 9 years period at 20 m depth.

Material and methods

The invaded seagrass bed is located at the end of Cap Martin (Alpes Maritimes, France, 7°30' E; 43°45' N) and the non invaded bed is located at the Cap Ferrat (Alpes Maritimes, France, 7°07'E; 43°41'N). Both sites are at 20 m depth, they are frequently subjected to swell and, the *P. oceanica* shoot densities are qualified as Normal according to Pergent-Martini (1994)'s classification. *C. taxifolia* settled in

1995 in the invaded zone, and was never observed in the reference site in Cap Ferrat.

In each site, 20 permanent quadrats (25 x 25 cm) were set inside *P. oceanica* meadows. Within each quadrat the surface covered by *C. taxifolia*, the shoot density, the number of orthotropic and plagiotropic shoots and the number of ramifications of *P. oceanica* were measured yearly in July-August from 1995 to 2004, except in 1996 and 1997.

Results

The percentage of colonisation by *C. taxifolia* in the permanent quadrats remained low from 1995 to 2004 and ranged from 4.5 to 26.4 %. The minimum infestation was observed in 1999 and 2004 (Fig. 1).

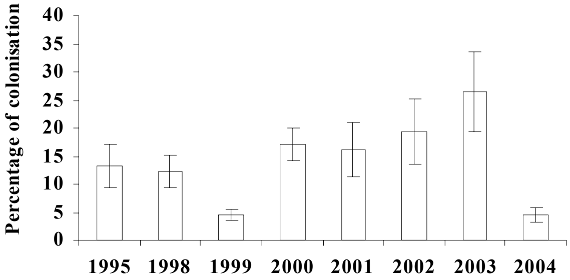

Fig. 1 - Colonisation of the permanent quadrats by *C. taxifolia* (Mean % ± s.e., n = 20) at 20 m depth in Cap Martin (invaded site).

Densities remained significantly higher in Cap Martin than in Cap Ferrat throughout the study period (Fig. 2).

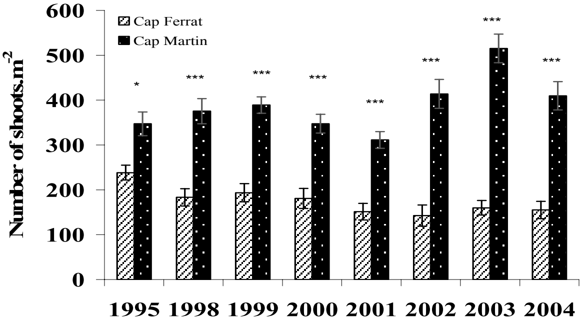

Fig. 2 - Evolution of *P. oceanica* density (Mean number of leaf bundle. m^{-2} ± s.e., n = 20) at 20 m depth in Cap Martin (invaded site) and in Cap Ferrat (control site). Significant difference, t or U-tests, *=0.05; **=0.01, ***=0.001.

Shoot densities in Cap Ferrat remained constant throughout the study (from 142 to 238 shoot· m^{-2}). In Cap Martin, from 1995 to 2001, densities remained constant (from 311 to 388 shoots. m^{-2}), and then increased to a maximum in 2003 (515 shoot· m^{-2}).

The structure (number of plagiotropic and orthotropic shoots) of the invaded and non invaded beds did not change through time (Fig. 3 a, b) with a higher proportion of orthotropic shoots. Over the 9 years, the percentage of ramified orthotropic shoots was low in the invaded and in the non invaded site.

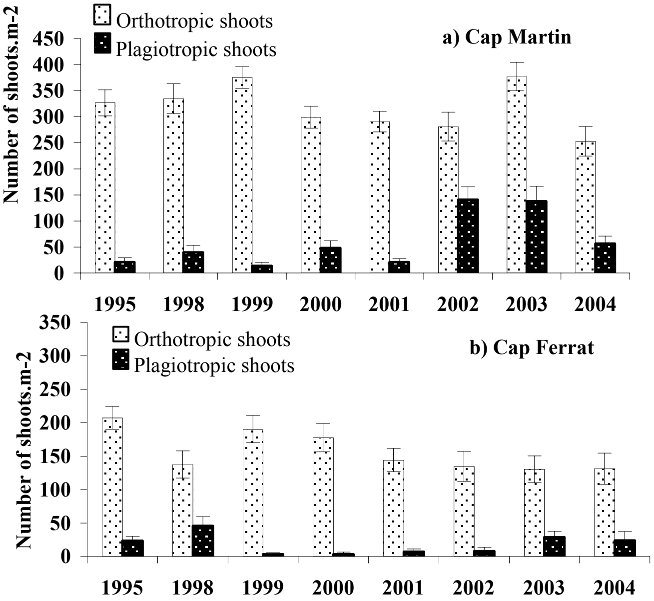

Fig. 3 - Evolution of *P. oceanica* structure (Mean number of orthotropic or plagiotropic shoots.m^{-2} ± s.e., n = 20, at 20 m depth in a) Cap Martin (invaded site), b) Cap Ferrat (control site)

The percentage of ramified plagiotropic shoots was high in the two sites (Table 1). These results are in conformity with a normal meadow structure (Molenaar *et al.*, 2000).

Discussion and conclusions

At 20 m, the presence of low percentages of *C. taxifolia* did not seem to affect the density, the structure and the ramification of the invaded Posidonia quadrats. The shoot densities of *P. oceanica* in the invaded site remained within the range of bed with a "Normal" density (from 173 to 397 shoot· m^{-2}) according to Pergent-Martini (1994).

These results are contrasting respect to those observed in a similar experiment at 6 m depth, where in the invaded quadrats (up to 93 % of infestation), the shoot densities strongly decreased, and all the orthotropic rhizomes transformed into abnormal ramified plagiotropic shoots (Molenaar *et al.,* 2005). In shallow water the higher biomass of *C. taxifolia* increased sedimentation and anoxia (Finzer and Poizat, 1996). This could change the structure of the invaded bed which is not the case at 20 m depth.

Tab. 1 - Orthotropic and plagiotropic ramification (Mean % ± s.e., n = 20, at 20m depth at a) Cap Martin (invaded site), b) Cap Ferrat (control site)

a) Invaded Cap Martin	1995	1998	1999	2000	2001	2002	2003	2004
Orhtotropîc ramification	**14.5** ± 3.5	**7.9** ± 1.9	**11.8** ± 2.4	**5.6** ± 2.7	**5.01** ± 1.2	**16.16** ± 2.5	**12.9** ± 2.7	**7.16** ± 2.0
Plagiotropic ramification	**34.3** ± 15.5	**60.5** ± 6.8	**61.7** ± 7.3	**42.08** ± 8.9	**64.3** ± 3.5	**65.4** ± 1.4	**57.1** ± 3.7	**53.6** ± 5.4

b) Non invaded Cap Ferrat	1995	1998	1999	2000	2001	2002	2003	2004
Orhtotropîc ramification	**14.06** ± 5.13	**17.79** ± 3.95	**2.72** ± 1.67	**0**	**1.46** ± 1.02	**1.04** ± 1.04	**15.37** ± 5.37	**12.03** ± 2.68
Plagiotropic ramification	**45.83** ± 9.72	**39.3** ± 9.3	**83.3** ± 16.6	**75**	**35.42** ± 20.52	**50**	**56.06** ± 6.26	**51.9** ± 11.3

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H.M. Mostafa

Department of Oceanography, Faculty of Science, University of Alexandria, Moharam Bey-21511, Alexandria - Egypt

PRELIMINARY ECOLOGICAL SURVEY OF SEAGRASS BEDS AT MARSA MATROUH, WEST OF ALEXANDRIA-EGYPT

Abstract

The seagrass meadows of Posidonia oceanica, Cymodocea nodosa *and* Halophila stipulacea *are recorded along two main transects in the vicinity of Marsa Matrouh (270 km west from Alexandria). The* Posidonia *beds are located in open deeper wat ers while* Cymodocea *and* Halophila *are much more confined to the calm warm waters of shallow enclosed areas. Marine algal associations were recorded in the sublittoral region.* Cystoseira*,* Laurencia*,* Padina *and* Caulerpa prolifera *are the most dominant species present. The results showed that* Halophila stipulacea *is mainly located at depths ranging between about 2 and 7 m in calm enclosed areas. The present work indicated the presence of successive communities at shallow depths in addition to the unspreading of* H. stipulacea *in other neighbored enclosed areas.*

Key-words*:* Posidonia oceanica*,* Cymodocea nodosa*,* Halophila stipulacea*, Marsa Matrouh, NW Egypt.*

Introduction

In the last five decades, there has been precipitous decline in the areal extent of seagrass beds in many parts of the world (Short, 1987). These declines have been linked to reduction in water quality and increased turbidity that decreases the amount of light available to benthic plants (Mostafa *et al.,* sumitted). The Mediterranean Sea and its coastal ecology face common environmental challenges as pollution problems worsen and the pressure on resources increases. Pollution of the sea, particularly transnational problems such as oil pollution, persistent chemicals and eutrophication are of international concern (Mostafa, 1996; 1998).

The coastline of Egypt, along the Mediterranean Sea is undergoing rapid development, where every section of the coast is faced with rapid, large scale environmental change. The coastal challenges include road construction, drainage from wastewater and irrigation, lagoon management and land reclamation. Major settlements are being constructed along coastal areas with little land planning

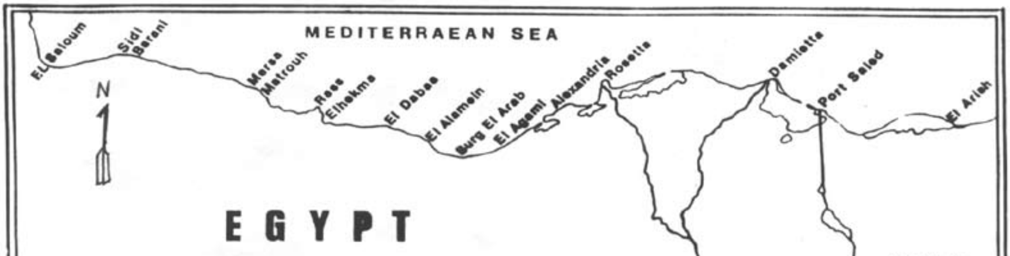

Fig. 1 - Different location along the NW Egyptian Mediterranean coastal waters

and little infrastructure (Mostafa, 2000). There is an increasing intensive pressure, where coastal lands are being rapidly urbanized and the increasing use of water, fertilizers, and pesticides in new agricultural land poses danger to the area. Both natural and urbanized coastline are undergoing a process of rapid degradation as the concentration of population and economic activities in the coastal area increases (Mostafa, 2004).

The NW Mediterranean coast of Egypt (Fig. 1) is an ecologically vulnerable coastal area that extend from west of Alexandria to the Libyan boarders (about 500 km in length) (Mostafa, 2003). The area is characterized by *Posidonia oceanica* forming large and dense meadows that cover the bottom down to about 27 m depth (Mostafa, 2000).

These meadows are being under the stress of many pollutants. Little work has been done in the area and only at certain localities (Mostafa, 1997, 2000; Thelin *et al.*, 1985). The present work is a preliminary attempt to describe the different communities in the vicinity of Marsa Matrouh (280 km west of Alexandria).

Materials and methods

Two main transects (Boudouresque *et al.*, 1980) perpendicular to the coast using skin diving during summer 2004 were established as representing the bay area near the harbor (Fig. 3). Different seagrass beds of *Posidonia oceanica, Cymodocea nodosa* and *Halophila stipulacea* were recorded olong a depth gradient. The length of each transect was about 50 meters from the shoreline, with a maximum depth from 7 to 11 meters.

In addition, different algal communities associated with the seagrass beds were collected for identification. The depth was recorded using a depth gauge and the distance from shore and the bottom type was recorded on a plastic slate during the survey. Another free skin diving was made along several points on the bay

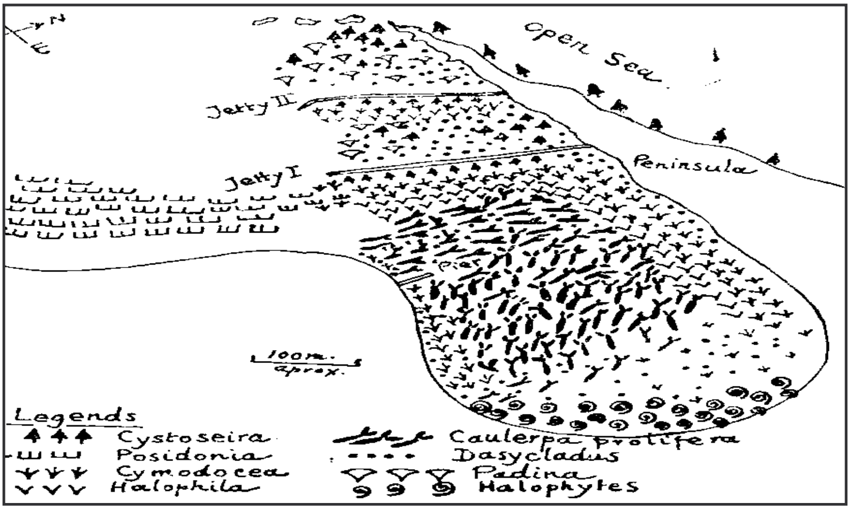

Fig. 2 - The distribution of different plant communities in Marsa Matrouh Harbor (from Aleem, 1960)

so as to monitor the rest of the bay and to check for the distribution of other seagrass meadows present.

Results and discussion

The harbor bay of Marsa Matrouh was fundamentally changed as the main bay was deepened (Fig. 3). Major plant communities had disappeared from the bay as a direct result of dredging the main channel (Fig.s 2 and 3). This process has affected the distribution of the seagrass beds and algal association within the bay area. This change in distribution could be related to the removal of one of the two jetties of the harbor (Fig.s 2 and 3) that caused the area next to the first jetty to be calm and accumulate more sediment in addition to other environmental factors (Perez and Romero, 1992).

The removal of one of the two jetties in turn, increased the density of *Cymodocea nodosa* meadows and decreased the density of *Halophila stipulacea* that might have been affected by the turbidity of the water column. *Caulerpa prolifera* was removed form the bottom area of the bay but still exists at about 10-12 m depth. According to Aleem (1960), the bay hosted three different seagrass species, *Posidonia oceanica, Cymodocea nodosa* and *Halophila stipulacea*. The present study documented the occurrence of the three seagrass species in the area and their distribution along depth gradient.

On the sloping sides of the two transects of the study site (Fig.s 4 and 5), a more or less regular depth zonation consisting of successive belts of the following communities, has been recognized:

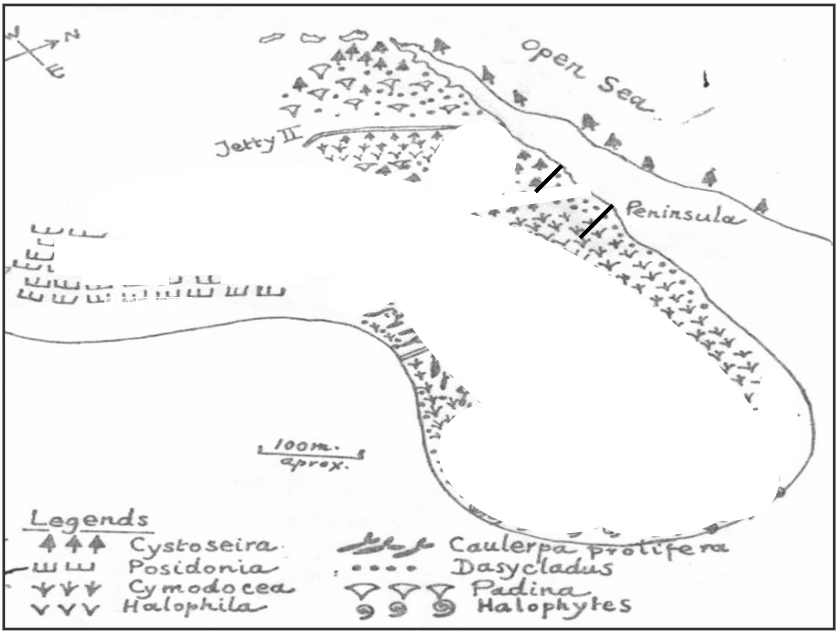

Fig. 3 - The major changes that took place in Marsa Matrouh Harbor. The black lines are the two transects of the study site. (Modified after Aleem, 1960).

The *Cystoseira* - *Laurencia* community, followed by the *Cymodocea nodosa* community and then the *Halophila stipulacea* community followed by the deep dense layer of *Caulerpa prolifera* community intermingled with *P. oceanica* dead remains.

Thus *Halophila stipulacea* seems to occupy an intermediate position between the *Cymodocea nodosa* and the *Caulerpa prolifera* (Fig.s 4 and 5). It is concluded that *Posidonia oceanica* and *Cymodocea nodosa* are the most common species in the area. The less dense *Halophila stipulacea* (most probably due to water turbidity) occupies an intermediate position between the dense *Cymodocea nodosa* and

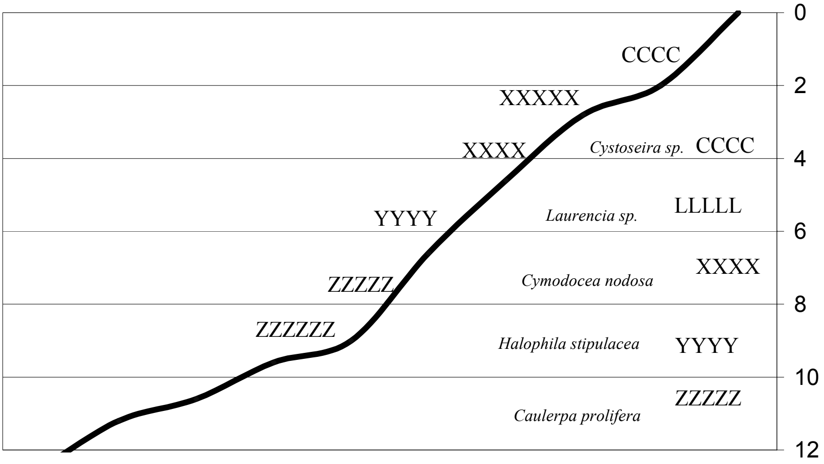

Fig. 4 - Distribution of algal and sea grass-communities along a depth gradient (Transect one)

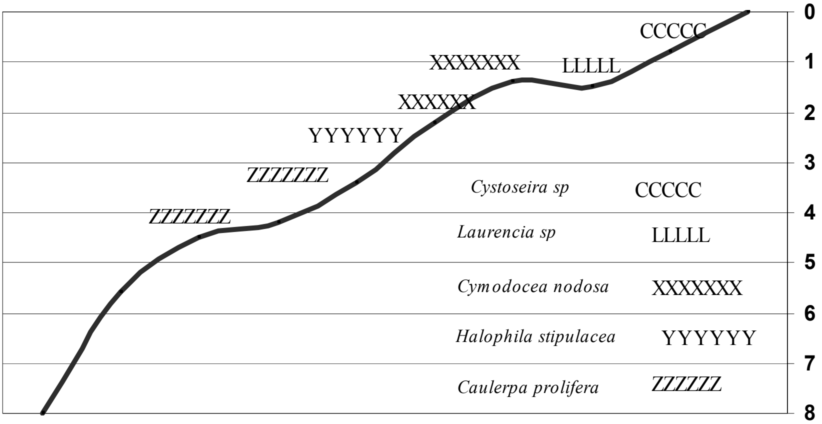

Fig. 5 - Distribution of algal and sea grass-communities along a depth gradient (Transect two)

Caulerpa prolifera.

It is most likely that this succession of communities has survived the deepening of the bay of Marsa Matrouh harbor or might be established again after the process of deepening of the bay (Pirc, 1984).

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I. Olivé, M.P. García-Sánchez, J.J. Vergara, J.L. Pérez-Lloréns

Departamento de Biología. Área de Ecología. Facultad de Ciencias del Mar y Ambientales.
Universidad de Cádiz. Polígono Río San Pedro s/n. 11510. Puerto Real. Cádiz. (Spain).

ANNUAL PHOTOSYNTHETIC CHARACTERIZATION OF THE SEAGRASS *CYMODOCEA NODOSA* ALONG DEPTH AND WITHIN LEAF GRADIENTS

Abstract

Annual monitoring of the physiological state of the seagrass Cymodocea nodosa *(Ucria) Ascherson. (Fam. Cymodoceaceae) growing along a depth gradient (from intertidal to subtidal range) in Cadiz Bay Natural Park (SW Spain) was carried out from November 2004 until August 2005. Photosynthetic measurements at different positions within a leaf of plants collected at several depths were carried out through measurements of oxygen evolution in the laboratory. The photosynthetic parameters obtained were higher in spring and summer than in winter. Intertidal plants had a higher variability in α (photosynthetic efficiency), Pmax (maximum photosynthetic rate) and R (dark respiration) than subtidal plants throughout the year. Nevertheless, seasonality in pigment content was higher in the subtidal meadow than in the intertidal one. Considering the three sources of variability studied, the physiological status of the photosynthetic machinery of* C. nodosa *is affected by seasonality and depth, while leaf position did not represent an important source of variability.*

Key-words: Cymodocea nodosa*, seagrass, photoacclimation.*

Introduction

Photosynthesis has been studied in a wide range of seagrass species. Most of the variability in photosynthetic performance has been attributed to seasonality, depth (Dennison and Alberte, 1986; Dennison, 1987) and leaf position (Mazzella and Alberte, 1986). A common seasonal pattern is maximum photosynthetic rates in summer and minimum in winter (Plus *et al.*, 2005). Maximum photosynthesis tends to decrease with depth according to a light attenuation pattern (Dennison and Alberte, 1986); whereas it increases from basal (younger) towards apical (older) parts of the leaf (Mazzella and Alberte, 1986).

The effect of tissue age on photosynthetic rates has been considered important in long-lived species (i.e. *Posidonia oceanica* (L.) Delile) (Alcoverro *et al.*, 1998) while short-lived seagrasses tend to have higher seasonal and depth related variability than long-lived ones (Enríquez *et al.*, 2004; Olesen *et al.*, 2002). Besides tissue age, canopy structure and leaf density can seriously attenuate light availability within the seagrass meadow, resulting in a gradient of photosynthetic activity along the leaf length (Enríquez *et al.*, 2002).

Another important variable to assess the physiological status of the photosynthetic apparatus is the pigment content. Seasonal, depth and within leaf differences in pigment content have been previously reported for seagrasses (Dalla Via *et al.*, 1998; Enríquez *et al.*, 2002; 2004). Since pigments are principally responsible for determining the absorptance of the leaf (Zimmerman, 2006) such differences partly explain the mechanism of seagrass photoacclimation.

Previous studies have been focussed only on one or two environmental factors affecting seagrass photosynthesis; this work goes a step further evaluating the role of three factors (i.e. season, depth and tissue age) affecting the photosynthetic performance of *Cymodocea nodosa*. From a laboratory approach, the data obtained here provide information on the potential for photosynthesis that this species possess to benefit from *in situ* environmental conditions.

Materials and methods

From November 2004 until August 2005, intact ramets of *C. nodosa* were collected seasonally at Cadiz Bay Natural Park (SW Spain). Three stations were selected along a depth gradient: intertidal (I), at 0 m relative depth emerged in every low tide, near subtidal (NS), at 0.4 m relative depth in emersion only in spring tides, and far subtidal (FS), at 0.9 m relative depth always submerged. Each week, 10-15 intact ramets were randomly collected from one station. Plants were transported to the laboratory and kept in darkness at 20 ºC. Each day, three leaf sections (apical, middle and basal) of 2.0 cm long each one were cut from the second or third oldest leaf (mature but not photodamaged) on a single shoot. The photosynthesis *vs*. irradiance (PE) response was studied in every leaf section. Another leaf segment (1.5 cm long) was cut contiguous to the previous one and kept at -80 ºC for pigment measurements. A total of 4 shoots were measured from each station.

The PE curves of *C. nodosa* were determined following Enríquez *et al.* (2002) at 20 ºC using 14 increasing incident irradiances, ranging from 0 (dark respiration rate) to 700 µmol quanta m^{-2} s^{-1}, plus a final dark incubation period for estimate respiration in light acclimatized tissues. Irradiance was provided by a LED (LS3/LH36U, Hansatech Ltd, UK). At each irradiance, oxygen concentration was measured during 7 min (except for respiration measurements, 15 min), and the rates were computed when the slope had stabilized. Maximum photosynthetic rates (P_{max}), calculated from net photosynthesis, were obtained from the averaged maximum values above saturating irradiance (last 4 irradiances). The photosynthetic efficiency (α) was estimated from the initial slope of the light response curve by linear least-squares regression analysis. Dark respiration was estimated from oxygen consumption in the initial darkness period.

Pigments were extracted in 80% acetone at low temperature (Dennison, 1990) and Chlorophyll *a+b* was calculated using the equations of Lichtenthaler and Wellburn (1983).

Results

Cymodocea nodosa showed a clear seasonal trend both in α (Fig. 1A) and P_{max} (Fig. 1B) with lower values in autumn-winter and higher in spring-summer. This pattern also changed with depth. For intertidal plants (St. I), α and P_{max} peaked in spring and dropped in summer (Fig. 1A, B) while in the two subtidal stations (St. NS and St. FS) the maximum values were extended until summer. A smoothed trend in P_{max} was also observed with depth (from St. I towards St. FS). No significant differences were detected among leaf portions. Initial dark respiration rates (Fig. 1C) also showed significant differences throughout the year and were also affected by depth, but no significant differences were detected among leaf portions. Respiration was significantly higher in spring and summer than in winter. A marked increase in respiration was detected during spring in the intertidal plants, in contrast to subtidal ones.

In intertidal plants, the seasonal pattern was similar for P_{max}, α and respiration rate (St. I). However, for subtidal plants (St. NS and St. FS), photosynthetic parameters and respiration did not match, P_{max} and α increased from winter towards summer while respiration was quite constant throughout the year, and lower than in the intertidal station.

There were seasonal differences in Chlorophyll *a+b* content between intertidal and subtidal plants (Fig. 1D). There was also an effect of leaf portion, as the basal parts showed a lower chlorophyll content than middle and apical parts, especially in summer.

Regarding seasonality and depth, the pigment content showed an opposite pattern respect to the photosynthetic parameters; intertidal plants showed a quite constant pigment concentration along the year while subtidal plants presented higher variability along the year.

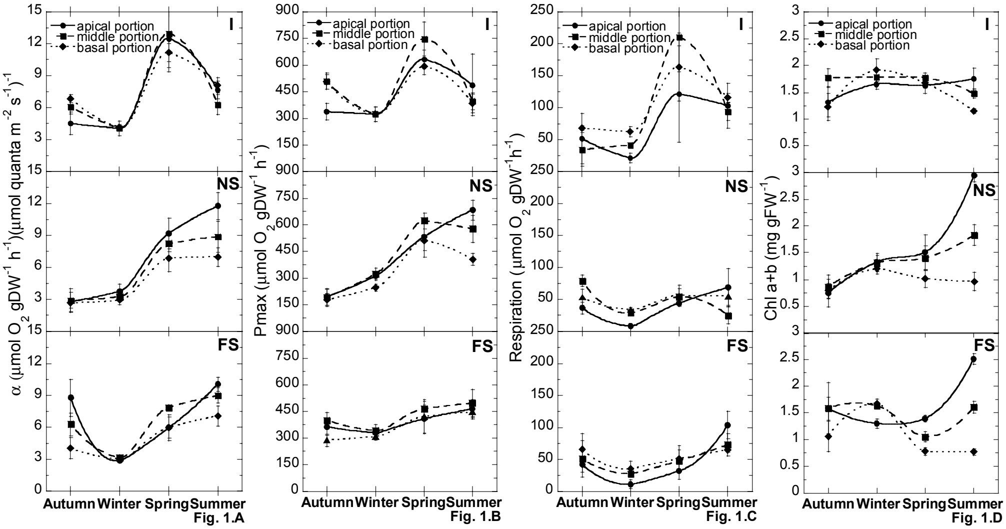

Fig. 1.A Fig. 1.B Fig. 1.C Fig. 1.D

Fig. 1 - Seasonal variations in photosynthetic parameters (α (A), P_{max} (B), dark respiration (C) and Chlorophyll *a+b* content (D) along a depth gradient (intertidal (I), near subtidal (NS) and far subtidal (FS) and along leaf length (apical (●), middle (■) and basal (♦)) in *C. nodosa*. Data are expressed as mean±SE.

Discussion

Seagrass photosynthetic performances have been previously related to seasonal cycles (Enríquez *et al.*, 2004), depth location (Olesen *et al.*, 2002) and tissue age (Mazzella and Alberte, 1986). This study has assessed the relative simultaneous contribution of these factors on the photosynthetic machinery of *C. nodosa* from a physiological point of view. Despite the homogeneous laboratory conditions, and discarding the short-term effects of the environmental variables (i.e. light and temperature), the described differences suggest that seasonality is a major factor affecting the photosynthetic parameters (i.e. α, P_{max} and dark respiration) of *C.*

nodosa. These results agree with previous references on temperate seagrasses (Dennison, 1987), showing minimum values of α and P_{max} in winter and maximum in spring-summer, when light availability and growth rate are maxima (Peralta *et al.,* 2005).

Depth is another important parameter affecting seagrass photosynthesis (Dennison and Alberte, 1986). In this work *C. nodosa* showed different strategies to seasonality depending on depth. The typical summer drop observed at the intertidal site could be due to the strong environmental stress of the emersion periods (i.e. high light, temperature and desiccation), probably causing damage in the photosynthetic apparatus (Armond *et al*., 1980). Without the emersion stress, plants at near and far subtidal sites showed a smoothed response, extending the spring maximum until summer, which would explain the large amount of pigments observed in the apical parts of these plants in the summer. The increase of P_{max} and α could be due to the increase in density of reaction centres associated to the peak of Chlorophyll *a+b* (Prézelin, 1981).

In contrast to previous seagrass studies (Mazzella and Alberte, 1986), tissue age (i.e. leaf portion) does not seem to affect the photosynthetic potential in *C. nodosa*. The leaf portion effect has been demonstrated in long-lived species with a low leaf turnover rate and large differences in tissue age along the leaf (Alcoverro *et al.*, 1998), whereas the leaf turnover rate of *C. nodosa* is relatively high. Our results showed some differences in photosynthesis and pigment content among leaf portions in the spring-summer period, when leaf length and growth rate are maxima. Therefore, leaf position can be an important factor in the photosynthetic response in *C. nodosa* when growing at high rates.

In seagrass meadows, depth acclimation is determined by the light attenuation through the water column and the self-shading of the canopy (Dalla Via *et al*., 1998). For temperate seagrasses, light availability is regulated by seasonality and by the own meadow structure (i.e. size and density of shoots). Reductions in shoot density along depth gradients can substantially enhance light availability for seagrass shoots (Mazzella and Alberte, 1986). In spring-summer period irradiance is higher and the shoot density and the length of the leaves increase as a consequence of the growth period (Dennison, 1987) increasing the potential range of light attenuation through the canopy. Therefore, the higher differences in α, P_{max}, R and total pigment content detected along the leaf in spring-summer can be a consequence of the higher differences in light levels through the canopy caused both by higher environmental irradiances and strong attenuation of light through the canopy

In conclusion, these results suggest that the photosynthetic apparatus of *C. nodosa* is long-term regulated by seasonality and depth. Nevertheless, the performance of this photosynthetic machinery will be finally determined by the local environmental factors, as light availability and temperature. Further *in situ* measurements will be necessary for the whole understanding of the meadow performance

Acknowledgements

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M. Polifrone, M. Rosell-Fieschi, M. Abelardo-González

Departamento de Biología, Universidad de Las Palmas de Gran Canaria, Campus Universitario de Tafira,
35017 Las Palmas, Spain.

A COMPARATIVE STUDY OF THE STRUCTURE OF *CYMODOCEA NODOSA* (UCRIA) ASCHERSON MEADOWS IN CANARY ISLANDS (SPAIN, ATLANTIC OCEAN)

Abstract

Seven seagrass meadows of Cymodocea nodosa *(Ucria) Ascherson (Magnoliophyta) from the Las Palmas de Gran Canaria Province, which includes the islands of Gran Canaria, Fuerteventura and Lanzarote, were compared. We analysed total biomass, plagiotropic/orthotropic biomass ratio, epiphytes biomass, mean shoot and blade density, shoot and blade length, photosynthetic area/m^2 and association with algae.*

Key-words: Cymodocea nodosa*, biomass, phenology, Canary Islands.*

Introduction

Conservation biology requires an effective management that involves a deep scientific knowledge based on the exhaustive characterization of natural populations. The importance of seagrasses in the constitution of complex marine ecosystems has been known since decades (Wood *et al*., 1969, Larkum *et al.,* 1989) and strategies for their conservations have been applied worldwide.

Cymodocea nodosa (Ucria) Ascherson is the most abundant seagrass in Canary Islands, where it mainly distributes in the eastern islands, reaching up to 40 m depth (Reyes, 1993). In the present study, we have analyzed the structural characteristics of seven meadows in the Gran Canaria Province, which comprises Fuerteventura, Gran Canaria and Lanzarote islands, in order to detect differences among meadows with different sizes and/or subjected to anthropogenic impact.

Materials and methods

Plants have been sampled at Gando (27º56’04.92” N, 15º22’34.44” W, surface about 399 ha), Playa del Cabrón (27º52’14.27” N, 15º23’03.14” W, surface about 1.2 ha), Risco Verde (27º51’42.60” N, 15º23’23.70” W, surface about 1.5 ha), Tiritaña (27º48’26.07” N, 15º44’44.13” W, surface about 0.56 ha) and Tufia (27º57’43.53” N, 15º22’45.24” W, surface about 0.13 ha) in Gran Canaria, at Gran Tarajal (28º12’38.80” N, 14º01’33.00” W, surface about 139.04 ha) in Fuerteventura and at Playa Blanca (28º52’00.00” N, 13º50’00.00” W, surface about 8.36 ha) in Lanzarote. Sampling has been done during spring 2004 and only at Tiritaña in 2005, in association with the period of major development of the plant recorded for Canary Islands (Reyes, 1993; Polifrone, 2005). The following parameters have been considered in the different meadows: biomass of plagiotropic and orthotropic part of the plant, plagiotropic/orthotropic biomass ratio, epiphytic biomass, shoot density, blade length and photosynthetic area/m^2. A Principal Component Analysis (PCA) based on the variance among populations for the different parameters and a Cluster Analysis based on mean values recorded for each populations have been performed on the data by means of the software SPSS v.12.0S for Windows (SPSS Inc. 1998-2003).

Results

The highest value of shoot density has been observed at Risco Verde (800 shoots/m^2), while Gando showed the lowest values (Tab. 1). Despite the low shoot density, Tiritaña has been characterized by plants with long leaves, which combined with a high number of blades/shoot resulted in a high photosynthetic area/m^2. Plants from Tufia showed a higher density than Tiritaña but a lower photosynthetic area/m^2, probably due to their reduced blade length. Playa Blanca showed a high shoot density and the lowest blade length, while Gran Tarajal showed high values for all parameters (Tab. 1).

Tab. 1 - Parameters observed for the Canary Islands meadows.

	Mean shoot density/ m^2	**Mean blade number/ shoot**	**Mean blade length (cm)**	**Photosynthetic area (cm^2/m^2)**
Gando	166.7 ± 15.9	2.3 ± 0.9	9.07 ± 0.6	360.8
Playa del Cabrón	337.0 ± 25.7	2.3 ± 0.7	21.6 ± 3.3	4074.7
Risco Verde	800 ± 75.2	2.1 ± 0.5	33.0 ± 2.1	8484.3
Tiritaña	238.9 ± 12.6	2.8 ± 0.7	37.5 ± 9.1	2907.9
Tufia	433.3 ± 41.3	2.7 ± 0.8	20 ± 3.0	2374.2
Gran Tarajal	611.1 ± 54.0	2.5 ± 0.6	46.5 ± 11.0	13693.3
Playa Blanca	751.9 ± 22.8	2.5 ± 0.6	19.9 ± 0.5	5732.2

The PCA (Fig. 1), which explain the 99.9% of variance in its two first components, clearly showed a similar pattern at Tiritaña, Risco Verde and Playa del Cabrón meadows, as well as evidenced the different pattern of Gando, separating it from all the meadows analyzed in this study. This pattern is mostly influenced by the mean shoot density and the blade length which in this meadow are particularly low. In the Cluster Analysis (Fig. 2), where the mean values are considered, the smallest meadows cluster in a group, while, another, group is characterized by Risco Verde and Gran Tarajal. Despite it was the meadow with the highest extension, Gando had similar values of the smallest meadows in the Cluster Analysis.

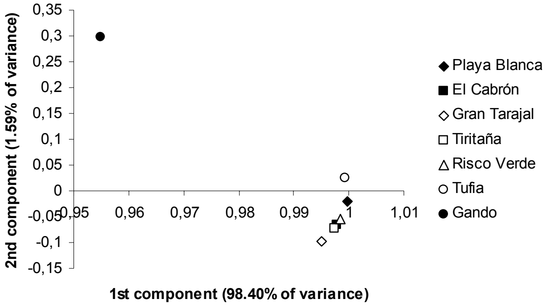

Fig. 1 - PCA of *C. nodosa* meadows of Canary Islands.

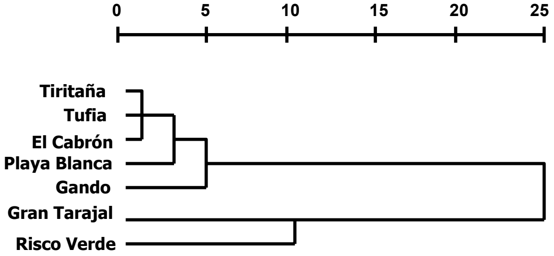

Fig. 2 - Cluster Analysis of *C. nodosa* meadows of Canary Islands.

At Gando, *C. nodosa* showed a high plagiotropic biomass (85.88 g/m^2), which was confirmed by the plagiotropic/orthotropic biomass ratio (16.8) and an high proliferation of the green algae *Caulerpa prolifera* was also observed (73.56 g/m^2). At Playa Blanca and at Playa del Cabrón the plagiotropic/orthotropic biomass ratio was also high (1.49 e 1.48 respectively) than the one observed in other meadows (0.44 - 1.28), but it was mainly due to the high development of shoots and blades (Tab. 1).

Conclusions

The data obtained reflected the general condition of the studied meadows, defining similar values for meadows with similar extension or which are affected by a different degree of deterioration, such as Gando where the high extension was not related to a high shoot density and where the presence of *C. prolifera* was important. Gran Tarajal (Fuerteventura) and Risco Verde (Gran Canaria) meadows, which have the higher extension, showed maturity conditions comparable between them. The parameters considered in the period of major development of the plant, hence, resulted useful in the analysis and comparison of the health conditions of these important coastal ecosystems.

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Y-R. Sghaier, R. Zakhama-Sraieb, F. Charfi-Cheikhrouha

Unité de Recherche de Biologie Animale et Systématique Evolutive. Département de Biologie.
Faculté des Sciences de Tunis. Campus Universitaire. 2092 Manar II. Tunisia.

STATUS OF *POSIDONIA OCEANICA* MEADOWS ALONG THE EASTERN COAST OF TUNISIA

Abstract

Posidonia oceanica *meadows at four sites along the eastern Tunisian coast (Hergla, El Kantaoui, Monastir, Mahdia) were sampled at three depths (-2, -5 and -10 m) between June and October 2004. They were compared by means of shoot density and biomass measurements as well as morphological and lepidochronological observations. The status of the seagrass was examined in view of local stresses, coastal constructions, fish farming and other anthropogenic impacts. Shoot density and lepidochronology showed a tendency towards degeneration of the meadows exposed to high levels of anthropogenic impacts (El Kantaoui, Monastir and Hergla). The Mahdia meadow displayed the highest values of shoot density and rhizome growth whereas El Kantaoui appeared to be the most stressed site exhibiting the lowest rhizome growth and shoot density values. Leaf and epiphyte biomasses of* P. oceanica *were related to the status of the meadow and anthropogenic disturbances. The El Kantaoui meadow exhibited an epiphyte biomass higher than leaf biomass and showed a trend towards degradation. A cluster analysis of similarity, taking into account all these parameters, allowed identification of three main groups of sites according to the degree of anthropogenic disturbance.*

Key-words: Posidonia oceanica*, anthropogenic disturbance, shoot density, lepidochronology, Tunisia.*

Introduction

Meadows of *Posidonia oceanica*, a marine phanerogam endemic to the Mediterranean Sea, are used as a bio-indicator for coastal water quality and as a quality element under the European Water Framework Directive (Romero *et al.*, 2005). Although widespread along the Tunisian coast, these meadows are particularly sensitive to pollution and to the impact of human activity.

P. oceanica beds in Tunisia have been the object of relatively few and scattered studies (Ben Mustapha and Hattour, 1992; Djellouli-Al Asmi, 2004; Zakhama and Charfi, 2005), compared to the studies in the Gabes Gulf (Ramos-Espla *et al.*, 2000; Ben Mustapha *et al.*, 2002a; 2002b). Le Danois (1925) was the first author to record the presence of *P. oceanica* on the eastern coast of Tunisia. A more complete description of this seagrass is in Ben Mustapha and Hattour study (1992) describing the *P. oceanica* beds in this area as in good health status.

The aims of our study were: a) to update the distribution of *P. oceanica* along the eastern coast of Tunisia; b) to assess the vitality of the meadows using phenological and lepidochronological approaches and c) to classify the meadows investigated according to their “ecological status.”

Materials and methods

Posidonia oceanica orthotropic rhizomes were sampled by SCUBA diving in October 2004 in four meadows along the eastern coast of Tunisia: Hergla, El

Kantaoui, Monastir and Madhia (Fig. 1). Hergla (36°02'165"N, 10°30'595"E) is a small coastal village, with a traditional fishing port, a fish farm and an offshore tuna farm. El Kantaoui (35°53'606"N, 10°30'052"E) is among the most attractive tourist cities in Tunisia with many popular developments and a marina. A drainage channel, located at Hammam Sousse, occurs 3 km south of the El Kantaoui sampling site. Monastir (35°47'266"N, 10°49'963"E) is also a coastal tourist city with a marina and a fishing port. Finally, Mahdia (35°30'500"N, 11°04'979"E) is a coastal tourist city with an important fishing port and archaeological vestiges. sampling was performed near the archeological site.

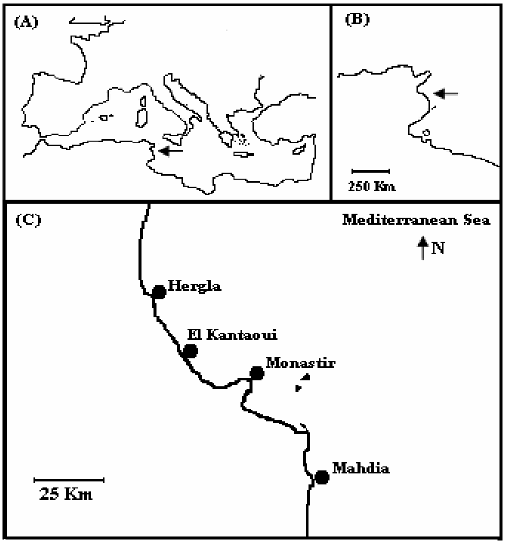

Fig. 1 - Map showing Tunisia location (A), the study area (B), and (C) the four *P. oceanica* meadows sampled.

Three stations were selected at each site at depths of -2, -5 and -10 m in order to assess the seagrass quality; shoot density was estimated *in situ* at each station by counting the number of shoots present in a 40x40 cm quadrat with ten replicates. Twenty rhizomes were collected at each station.

In the laboratory, *P. oceanica* morphometric parameters were estimated by 1) separating the leaves from the rhizomes 2) classifying them according to Giraud's (1979) classification as 'adult', 'intermediate' or 'juvenile' and 3) counting the number of leaves belonging to each class. Leaf widths and lengths (±1 mm) were recorded for the different leaf categories. The Leaf Area Index (LAI) and the Coefficient A (a percent of broken leaves) were estimated for each station. The epiphytic biomass was estimated by scraping epiphytes from the leaves using a blunt blade and weighing after drying in an oven at 70 °C for 48 h. The scraped leaves were also dried at 70 °C for 48 h and weighed (± 0.1 mg) to estimate mean shoot dry weight at each station. The annual rhizome elongation (cm year^{-1}) and leaf formation rate (number of leaves year^{-1}) were determined following the standardized procedure of the lepidochronological analysis (Pergent *et al.*, 1995). For each rhizome, dead sheaths were detached starting from the older to the more recent ones (near the living tissue) and the thickness of each sheath was measured

Tab. 1 - Shoot density, number of leaves per shoot, number of adult leaves per shoot, number of intermediate leaves per shoot, number of juvenile leaves per shoot, mean adult leaf length, mean adult leaf width, mean leaf length (intermediate), leaf area index (m^{2} m-^{2}), coefficient A, shoot biomass, epiphyte weight, mean number of leaves per year, and mean rhizome growth, in four Tunisian *Posidonia oceanica* meadows.

	-2m				-5m				-10m			
	El Kantaoui	**Monastir**	**Hergla**	**Mahdia**	**El Kantaoui**	**Monastir**	**Hergla**	**Mahdia**	**El Kantaoui**	**Monastir**	**Hergla**	**Mahdia**
Density	388 ± 41.6	456 ± 32.8	631 ± 16.3	984 ± 160	362 ± 14.3	402 ± 21.6	496 ± 29	487 ± 40.3	144 ± 20.5	221 ± 16.1	267 ± 15.1	441 ± 61.2
Number of leaves per shoot	3.8 ± 0.3	5.5 ± 0.5	4.9 ± 0.5	5.0 ± 0.6	4.1 ± 0.4	4.9 ± 0.3	5.1 ± 0.6	5.7 ± 0.4	2.8 ± 0.4	5.6 ± 0.6	4.8 ± 0.5	5.7 ± 0.4
Number of adult leaves per shoot	2.8 ± 0.3	2.4 ± 0.5	2.8 ± 0.3	3.2 ± 0.3	2.7 ± 0.3	2.4 ± 0.27	3 ± 0.4	3.5 ± 0.3	2.4 ± 0.3	2.9 ± 0.2	3 ± 0.4	3.5 ± 0.3
Number of Intermediate leaves per shoot	1 ± 0.2	3.1 ± 0.2	2.2 ± 0.35	2.7 ± 0.52	1.5 ± 0.3	2.6 ± 0.2	2.1 ± 0.4	2.2 ± 0.2	0.4 ± 0.3	2.7 ± 0.4	1.8 ± 0.3	1.7 ± 0.3
Number of juvenile leaves per shoot	2.7 ± 0.2	0.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.4	2.2 ± 0.2	0.9 ± 0.2	1 ± 0.2	0.7 ± 0.4	2.5 ± 0.3	0.9 ± 0.3	1.5 ± 0.4	0.7 ± 0.4
Mean adult leaf length (mm)	242 ± 28.9	253.1 ± 17.5	346.6 ± 25.7	284 ± 22	158.9 ± 13.2	241.7 ± 12	379.2 ± 36.5	447.8 ± 37.3	200.3 ± 29.2	245.5 ± 22.5	301.5 ± 42.8	637.5 ± 61.5
Mean adult leaf wide (mm)	11 ± 0.2	10.5 ± 0.34	9.5 ± 0.1	7 ± 0.2	9.5 ± 0.1	9.5 ± 0.2	9.4 ± 0.1	9 ± 0.1	9.3 ± 0.3	10.35 ± 0.3	9.7 ± 0.2	9.7 ± 0.3
Mean intermediate leaf length (mm)	69.8 ± 4.7	146.6 ± 22.5	157.9 ± 20.8	281 ± 37.5	93.3 ± 9.4	125.5 ± 13.62	182.7 ± 25.1	337± 92.9	69.3 ± 7.7	130 ± 15.9	92.3 ± 10.5	451 ± 37.6
Leaf area index (m^{2} m-^{2})	3.8 ± 0.4	4 ± 0.1	7.8 ± 0.2	10.5 ± 1.4	2.1 ± 0.1	2.2 ± 0.1	7.2 ± 0.4	8.2 ± 0.5	0.7 ± 0.1	1.9 ± 0. 1	2.6 ± 0.1	12.8 ± 1.3
Coefficient A	60.8%	28.0%	40.7%	70.0%	51.5%	34.8%	42.3%	46.0%	60.00%	25.64%	48.00%	41.00%
Shoot biomass (g shoot^{-1})	0.4 ± 0.03	0.3 ± 0.1	0.6 ± 0.03	0.6 ± 0.1	0.25 ± 0.03	0.3 ± 0.03	0.8 ± 0.04	0.78 ± 0.05	0.25 ± 0.02	0.35 ± 0.05	0.5 ± 0.03	1.5 ± 0.2
Epiphyte weight (mg cm-^{2})	5.66 ± 0.8	1.7 ± 0.1	2.5 ± 0.2	0.25 ± 0.1	2.7 ± 0.3	0.3 ± 0.03	1.4 ± 0.5	0.25 ± 0.01	3.3 ± 0.5	0.8 ± 0.1	1.6 ± 0.1	0.45 ± 0.06
Mean number of leaves per year	6.8 ± 0.4	7.7 ± 0.3	7.3 ± 0.5	7.7 ± 0.3	7.7 ± 0.3	7.1 ± 0.2	7.5 ± 0.4	7.5 ± 0.4	6.9 ± 0.5	7 ± 0.4	7.8 ± 0.3	7.4 ± 0.8
Mean rhizome growth (mm year^{-1})	6.1 ± 0.6	7.2 ± 0.6	6.5 ± 0.5	6.1 ± 0.5	4.2 ± 0.7	5.4 ± 0.5	9.4 ± 1.4	7.7 ± 2.16	3.8 ± 0.5	4.5 ± 0.4	7.6 ± 0.3	8.1 ± 1.3

microscopically. The number of leaves produced annually was derived from the mean number of leaf sheaths produced between two pairs of sheaths with minimum thickness in each shoot (lepidochronological year). The annual vertical rhizome elongation rate for each shoot was calculated as the length of the rhizome segment between the two sheaths of minimal thickness.

One-way analysis of variance (ANOVA) was used to test significant differences (0.05 level of significance) among the four meadows parameters between stations at the same depth. For the number of leaves, the Kruskal-Wallis test was used (0.05 level of significance).

Results

Morphological features of *P. oceanica* are reported in Table 1. At the same depths, shoot density in the four meadows was significantly different (ANOVA, $p < 0.05$). According to the classification of Pergent *et al.* (2005), the El Kantaoui meadow, exhibiting the lowest values, between 144 shoots m^{-2} at -10 m and 388 shoots at -2 m, is “bad.” The Monastir and Hergla meadows also had low shoot densities (221 shoots m^{-2} at -10 m to 456 shoots m^{-2} at -2 m at Monastir and 267 shoots m^{-2} at -10 m to 631 shoots m^{-2} at -2 m at Hergla), and received a classification of “poor.” Finally, Mahdia with the highest values (441 shoots m^{-2} at -10 m to 984 shoots m^{-2} at -2 m) was classified as “normal” and “good”.

Many morphological variables had mean values significantly different between the El Kantaoui meadow and the other sites. Indeed, values of mean total number of leaves per shoot were significantly different between stations at the same depth for the four meadows (ANOVA, $p < 0.05$). The El Kantaoui meadow exhibited a low number of leaves per shoot at the three depths (Kruskal-Wallis, $p <0.05$) and there were less than three photosynthetically active leaves per shoot at -10 m (2.8 ± 0.4 leaves per shoot). The number of intermediate leaves was significantly lower at the El Kantaoui meadow (Kruskal-Wallis, $p <0.05$), as opposed to the juvenile leaves which were the most abundant (Table 1). Furthermore, the El Kantaoui meadow displayed the shortest adult leaf lengths while Mahdia had the longest ones (ANOVA, $p < 0.05$). El Kantaoui and Monastir had the widest leaves at -2 m (ANOVA, $p < 0.05$). Excepting Mahdia (-2 m), the El Kantaoui meadow exhibited the highest Coefficient A. The LAI as very low (from 0.7 to 3.8 m^2m^{-2}) at El Kantaoui compared with the other stations (ANOVA, $p < 0.05$). Unlike other parameters, the highest epiphytic cover was recorded at El Kantaoui whereas the lowest was observed at Mahdia (ANOVA, $p < 0.05$).

The annual leaf production was significantly different only at -2 m (ANOVA, $p < 0.05$) where EL Kantaoui had significantly low values. Rhizomes collected in the four meadows showed an overall mean growth rate of 9.4 mm $year^{-1}$. The minimum growth rate (3.8 mm $year^{-1}$) was observed at El Kantaoui at -10 m. According to Pergent-Martini *et al.* (1999), both El Kantaoui and Monastir densities at -5 and -10 m can be considered as “sub-normal.”

Discussion and conclusions

Cluster analysis of similarity between all the parameters investigated showed three main groups of meadows (Fig. 2). The first includes exclusively the three stations of Madhia, characterized by high values of density, leaf length, LAI and shoot biomass (Table 1). The Madhia meadow can be considered therefore as the site with the best health status. The second group includes the deepest stations, with very low values of all the parameters, a group with meadows classified as bad. The third cluster includes the shallow stations except Madhia and can be divided into two subgroups:

- Hergla, characterized by intermediate values of shoot density, adult leaf length, LAI, shoot biomass and high values of rhizome growth, classified as moderate.
- The second subgroup is represented by Monastir and El Kantaoui characterized by low values of all parameters, and thus classified as poor meadows.

Our study underscores differences between meadows of *P. oceanica* along the eastern coast of Tunisia. Only Madhia meadow remains in good health; the three other meadows exhibit a tendency toward regression due to increasing anthropogenic pressure. The El Kantaoui meadow is the most degraded of the four sites, with mean values of several morphological variables significantly different from those of the other localities. Examination of the main phenological parameters reveals that El Kantaoui exhibits very low vitality. Indeed, seagrass bed density values are markedly lower than those usually recorded at the three sampling depths in the other sites.

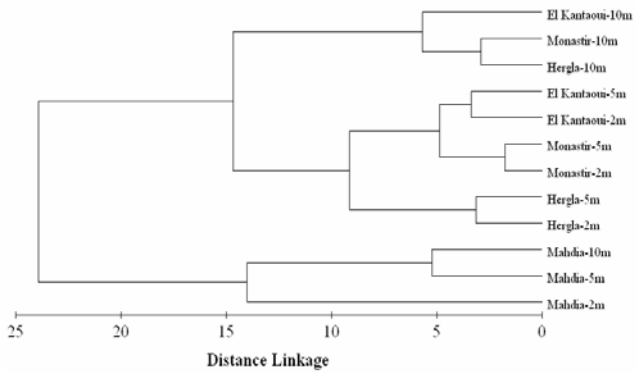

Fig. 2 - Cluster analysis of the four meadows investigated based on the similarity of all the parameters, measured through squared Euclidean distances.

The reduced mean number of intermediate leaves, associated with the high juvenile mean number of leaves at El Kantaoui has been observed in other meadows exposed to urban and industrial wastes (Balestri *et al.*, 2004). These authors argued that high intermediate shoot numbers could be the result of overproduction related to physiological responses under stressful conditions. Another hypothesis that could explain this result is a delay in *P. oceanica* leaf development and growth from the juvenile to the intermediate stage.

Leaf width was more important at El Kantaoui and Monastir at -2 m depth, and is probably a response to strong hydrodynamics (Semroud, 1993) or to a combined effect of the wealth in high nutrients and turbidity (Pergent-Martini, 1994). As for epiphyte biomass, the very high value at El Kantaoui is probably due to nutrient enrichment of the environment from urban waste (Dimech *et al.*, 2002, Cancemi *et al.*, 2003). The extensive development of epiphytic organisms on the leaves of seagrasses represents a source of disturbance to the plants, limiting the quantity of light available (Sand-Jensen *et al.*, 1997). Abundant epiphytic cover may also help explain the greater Coefficient A in El Kantaoui (Alcoverro *et al.*, 1997, Ruiz and Romero, 2003). As a whole, the distribution of *P. oceanica* along the eastern coast of Tunisia, showing a generalized state of regression, shows changes since the Ben Mustapha and Hattour study (1992). The vitality parameters of the meadows (phenological and lepidochronological approaches) show differences between localities probably due to anthropogenic disturbance. Finally, a classification of the four meadows, from poor to high quality, was established according to their “ecological status” based on several vitality parameters.

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I.A. Serra[1], G. Procaccini[2], M.C. Intrieri[3], M. Migliaccio[2], S. Mazzuca[1],
A.M. Innocenti[1]

[1]*Dipartimento di Ecologia, Università della Calabria, 87036 Arcavacata di Rende (CS), Italy*
[2]*Stazione Zoologica 'A. Dohrn' Laboratorio di Ecologia del Benthos, 80077 Ischia(Na), Italy*
[3]*Dipartimento di Biologia Animale e Genetica, Università di Firenze, 50125 Firenze, Italy*

COMPARATIVE ANALYSIS OF GENETIC DIVERSITY IN *POSIDONIA OCEANICA* (L.) DELILE USING ISSR AND SSR MARKERS

Abstract

Posidonia oceanica *(Posidoniaceae) is an endemic species in the Mediterranean Sea. In our analysis we compared the efficiency of two different classes of molecular markers (ISSR and SSR) in population genetic analysis of this seagrass along the Calabrian coasts (Italy, South Tyrrhenian Sea and Ionian Sea). Dinucleotide microsatellites were the most polymorphic set of markers, with respect to tri- and heptanucleotide microsatellites and to ISSR markers. Nevertheless, a discriminatory power analysis showed that some trinucleotide microsatellite and ISSR primers were more powerful than dinucleotides. Our analysis also indicated that clonal diversity values saturate with few selected markers. Both marker categories suggested the role of the Calabria peninsula as important biogeographic barrier between the Western and the Eastern side of the Mediterranean basin.*

Keywords*:* Posidonia oceanica*, ISSR, SSR, genetic diversity, biogeographic barrier.*

Introduction

Posidonia oceanica (L.) Delile (Posidoniaceae) is a Mediterranean endemic seagrass which, due to its ecological importance, has been the object of several genetic studies (e.g Arnaud-Haond *et al,*. 2005, Migliaccio *et al*., 2005) using different types of markers. Unsurprisingly, different levels of genetic variability have been found with different molecular markers (Arnaud-Haond *et al.,* 2005). Recent data obtained with tri- and eptanucleotide microsatellites (or SSR - Simple Sequence Repeats) and with highly polymorphic dinucleotide microsatellites has altered our general perception of *P. oceanica* as a species with low genetic variability; some areas show very high levels of genetic diversity (Arnaud-Haond *et al*., 2005, Migliaccio *et al*., 2005).

The aim of this paper was to compare the efficiency of two different classes of molecular markers (ISSR and SSR) in population genetic analysis of *P. oceanica* along the Calabrian coasts (Italy, South Tyrrhenian Sea and Ionian Sea). SSRs (simple sequence repeats) or microsatellites (Jarne and Lagoda, 1996) are highly reproducible codominant markers, in which a specific pair of PCR primers flanking a short repeated sequence produces polymorphic allelic patterns, depending on the number of repeated units. In contrast ISSR (inter-simple sequence repeats) markers (Zietkiewitcz *et al.,* 1994), rely on the use of arbitrary primers represented by short repeated sequences. Allelic polymorphisms occur whenever the repeated sequence is missing or insertions/deletions modify the distance between repeats. ISSRs have the advantage to analyze multiple loci in a single reaction and to be more accessible to ecology laboratories, not requiring sophisticated equipment and/or high-level expertise.

Material and methods

Plant material and DNA extraction: individual shoots of *Posidonia oceanica* (L.) Delile were sampled in five populations along the coast of Calabria (Italy): Praia a Mare, San Nicola Arcella and Belmonte Calabro in the Tyrrhenian Sea; Crotone and Cariati in the Ionian Sea. About 25 individual shoots for each meadow were collected randomly at a reciprocal distance higher than 8 meters.

DNA was purified according to the method devised by Doyle and Doyle (1987).

ISSR and SSR analysis: Eight, 3’-anchored primers (MWG-Biothec) were used for ISSR amplifications in *P. oceanica* (ISSR2: 5’-$(CT)_8$RG-3’; ISSR3: 5’-$(GT)_6$YR-3’; ISSR5: 5’- $(CA)_6$RY-3’; ISSR6: 5’- $(CA)_7$YC-3’; ISSR7: 5’- $(AG)_7$YC-3’; ISSR8: 5’- $(GA)_7$RG-3’; ISSR10: 5’- $(CTC)_4$RC-3’; ISSR11: 5’- $(GAG)_4$RC-3’). Amplification reactions were carried out in volumes of 25 µl containing 37.5 ng of template DNA, 1 unit of Taq-polimerase (Roche), 0.2 mM dNTPs (Roche), 1.5 pmol/µl –1 of primer (MWG-Biothec), 10mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50mM KCl. PCR reactions were performed under the following conditions: 3 min at 94°C for initial denaturation, 35 cycles of 60 sec at 94°C (denaturation), 60 sec at 44°C (annealing), and 90 sec at 72 °C (extension), followed by 5 min at 72°C for final extension. PCR products were separated on 2.5% Agarose 0.5 X TBE gel, ethidium-bromide stained and visualized on a UV light box. ISSR bands were scored as absent (0) and present (1) assuming that each band position corresponds to one “locus” with two “alleles”. SSR analysis of thirteen polymorphic microsatellite regions, twelve nuclear and one chloroplastic, were carried out as in Migliaccio *et al.* (2005). We refer to the six loci previously isolated by Procaccini and Waycott (1998), with a dominance of trinucleotide repeats, as T-SSR. The seven loci isolated by Alberto *et al.* (2003), with a dominance of dinucleotide repeats, are referred to as D-SSR.

Data analysis: the following parameters were estimated: (i) Number of polymorphic Molecular Variants (MV are represented by bands for ISSR and alleles for SSR markers); (ii) Number of banding patterns for each SSR locus/ISSR primer; (iii) The probability that two randomly chosen individuals have identical banding pattern (Confusion probability ,C*j*) calculated as: $Cj= \sum^{I}_{i=1} p_i [(N p_i-1)/(N-1)]$ (were *pi* is the frequency of the *i*-th pattern, *N* the sample size and *I* the total number of patterns generated by the jth assay unit); (iv) Discriminanting power (D*j*) of the *j*th assay unit : $Dj = 1 – Cj;$ (v) Limit of Dj as N tends towards infinity: $DL = \lim (Dj) = 1-\sum^{I}_{i=1} p_i^2$ (Tessier *et al.* 1999).

Clonal diversity was calculated in each population (for each marker) as the proportion of distinguishable genets (G/N), where G is the number of genets detected and N is the number of samples (Pleasant and Wendel 1989). In order to find the best possible marker combination to utilize for assessing intra-population variability we plotted G/N values obtained adding sequentially one marker at time starting from the one having the highest D value. G/N *Vs* number-of-markers curves were obtained for each single population and for the overall value. Nei’s (1978) genetic distance, was calculated using POPGENE genetic software (Yeh *et al*. 1997). Dendrogram based on Nei’s (1978) genetic distance were also constructed using POPGENE.

Results

The total number of molecular variants (MVs) varied from 75 for ISSR to 21 for T-SSR (Table 1). All MVs are polymorphic both in D-SSR and in T-SSR, while only the 20.4% were polymorphic among the ISSR bands (if we consider only the polymorphic ISSR primers). Discriminating power is lower for polymorphic ISSR (mean D = 0.466) than T-SSR and D-SSR, respectively (mean D = 0.484 and 0.625, Table 1). Confusion probability values show an opposite trend, with higher values in ISSR and lower values in SSR (Table 1). Nevertheless, discriminating power analysis showed that some trinucleotide microsatellite and ISSR primers were more powerful than dinucleotides.

Considering the values of G/N over the whole set of samples, D-SSR markers detect higher clonal diversity and T-SSR lower (G/N = 0.50 and G/N =0.40 respectively, Table 2). The value increases to 0.64 considering the SSR markers altogether and to 0.75 utilizing all markers. Tyrrhenian populations were always more diverse than Ionian ones also with microsatellite markers (Table 2).

Tab. 1 - Molecular Variants (MVs) are represented by bands in ISSR and alleles in SSR. C = Confusion probability; D = Discriminating power; DL = Limit of discriminating power; D class = order in the score of D.

Marker	MVs	Polymorphic MVs	patterns	C	D	DL	D class
ISSR2	7	2	3	0.423	0.577	0.575	9
ISSR3	8	2	3	0.549	0.451	0.447	12
ISSR5	16	2	4	0.507	0.493	0.489	11
ISSR6	9	0	1	-	-	-	-
ISSR7	12	0	1	-	-	-	-
ISSR8	8	1	2	0.807	0.193	0.192	16
ISSR10	10	3	6	0.384	0.616	0.616	6
ISSR11	5	0	1	-	-	-	-
			Mean values	0.534	0.466	0.464	
T-SSR							
Poc-trn	2	2	2	0.983	0.017	0.017	18
Poc-42	2	2	3	0.438	0.562	0.558	10
Poc-5	3	3	4	0.566	0.434	0.441	13
Poc-45	6	6	10	0.311	0.689	0.683	5
Poc-35	5	5	11	0.145	0.855	0.848	3
Poc-26	3	3	3	0.656	0.344	0.341	15
			Mean values	0.516	0.484	0.481	
D-SSR							
Po5-39	3	3	4	0.635	0.365	0.362	14
Po5-10	4	4	7	0.389	0.611	0.674	8
Po5	4	4	7	0.373	0.627	0.697	7
Po-15	8	8	18	0.093	0.907	0.903	2
Po5-40	15	15	24	0.076	0.924	0.918	1
Po5-49	7	7	12	0.167	0.831	0.853	4
Po4-3	3	3	3	0.889	0.111	0.110	17
			Mean values	0.375	0.625	0.645	

Tab. 2 - Clonal diversity values (genotypes/samples) for population groups. T-SSR and D-SSR, see Table1; Tot-SSR= total number of microsatellites markers. All markers = ISSR+SSR markers

Populations/Geographic areas	ISSR	D-SSR	T-SSR	Tot-SSR	All markers
Tyrrhenian Sea	0.70	0.55	0.45	0.70	0.94
Ionian Sea	0.06	0.42	0.34	0.54	0.52
overall	0.44	0.50	0.40	0.64	0.75

By plotting G/N values obtained with marker combinations that include markers with the first ten D values (eight SSR and two ISSR; Table 1, curves not shown) it results that a minimum of 4-5 loci are necessary to reach G/N saturation.

Adding ISSR10 and ISSR2 to the first 8 SSR loci it resulted in a slight increment of G/N values only in the more polymorphic populations.

Values of Nei genetic distance were utilized to build the dendrograms shown in Fig. 1. In all cases dendrograms showed identical topologies with the two main clusters grouping Tyrrhenian and Ionian populations as shown.

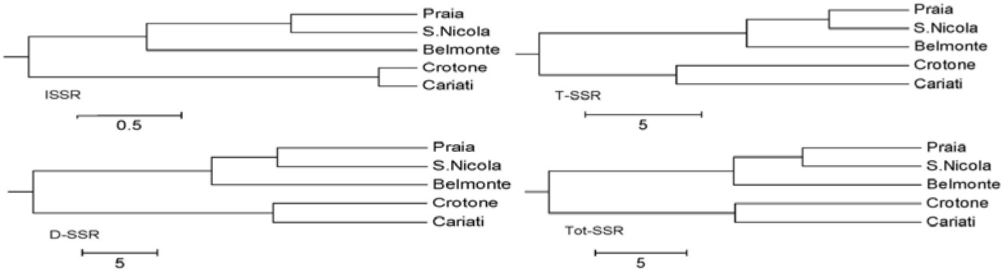

Fig. 1 - Representation of UPGMA dendrograms obtained for Nei's (1978) distance values.

Conclusion

ISSR and SSR markers showed variable levels of polymorphism in the *P. oceanica* populations, with Tyrrhenian populations always more polymorphic than Ionian ones. Our analysis also demonstrated that a reduced set of markers could be sufficient to analyze genetic polymorphism in this seagrass. SSR markers were able to detect higher levels of clonal diversity, although the use of ISSR and SSR markers together increases clonal diversity values in the most polymorphic populations. Finally, results from both marker categories suggest that the Calabrian peninsula is an important biogeographic barrier between the Western and the Eastern side of the Mediterranean basin. According to the higher polymorphism and greater statistical power, the use of SSR markers for further genetic diversity studies in *Posidonia* is recommended. Nevertheless, ISSR markers have given comparable results with the advantage of a technique that is simple, cheap and more accessible to smaller field-oriented ecology laboratories.

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F. Vallerini, E. Balestri

Dipartimento di Biologia, Unità di Biologia Marina ed Ecologia, via Volta 6, 56126, Pisa

SPATIAL VARIATIONS IN VEGETATIVE AND REPRODUCTIVE SHOOT DENSITY IN TWO CO-OCCURRING MEDITERRANEAN SEAGRASSES *CYMODOCEA NODOSA* AND *ZOSTERA NOLTII*

Abstract

Variations in vegetative and reproductive shoot density of Cymodocea nodosa *(Ucria) Ascherson (Cymodoceaceae) and* Zostera noltii *Hornemann (Zosteraceae) were examined in a mixed meadow at different spatial scales, across depths and over the reproductive season. Results of analyses of variance showed significant differences in the abundance of flowering shoots across depths for both the species. The spatial pattern of vegetative shoot density did not reflect those of flowering shoots. The results suggest that the species exhibit different allocation strategies, and that flowering shoot abundance is not related to vegetative shoot density.*

Key-words*:* Cymodocea*,* Zostera*, reproductive and vegetative shoots, spatial scales.*

Introduction

Cymodocea nodosa (Ucria) Ascherson and *Zostera noltii* Hornemann are relatively small, fast-growing colonizing species which commonly co-occur in mixed meadows in the Mediterranean Sea. Both the species can reproduce sexually through seeds and vegetatively through clonal propagation, but differ in morphological and phenological features, and reproductive strategies (Buia and Mazzella, 1991). *Cymodocea nodosa* is a dioecious species with sessile male and female flowers (Caye and Meinesz, 1985) which shows repeated seedling recruitment strategy. *Zostera noltii* is a monoecious, hermafroditic species, which produces flattened inflorescences with spathe-spadix arrangements (Ackerman, 2006) and exhibits initial seedling recruitment strategy. Increasing evidence indicates that in seagrasses sexual reproduction is highly variable both in space and time (Balestri, 2004). However, little is known about spatial and temporal variations in the distribution and abundance of reproductive shoots of both the species.

The aims of present study were to (1) examine variations in the abundance of reproductive shoots of co-occurring *C. nodosa* and *Z. noltii* at different spatial scales, ranging from centimetres to hundred of meters; (2) detect whether reproductive shoot abundance differs among depths (20, 50, 100 cm) and over the reproductive season; and (3) examine whether spatial patterns of reproductive shoot density are related with those of vegetative shoots.

Materials and methods

This study was carried in a mixed meadow of *C. nodosa* and *Z. noltii* during the spring 2003, at Antignano (Livorno, Italy, 43° 30’N, 10° 20’E). Shoots were collected at three depths (20, 50 and 100 cm) in May and June. On each date and depth, 4 sites separated by 100 meters were randomly chosen within the meadow. In each site, two areas, 10-15 mt apart, were randomly selected. In each area, four cores (25 x 25 cm) were randomly collected and all seagrass material, including roots and rhizomes, was fixed in 4% formalin in seawater. In the laboratory each core was processed to determine the density (number of shoots per square meter) of vegetative and reproductive shoots of *C. nodosa* (i.e. female flower) and *Z. noltii* (i.e. inflorences). A hierarchical, mixed-model ANOVA was used to detect significant differences in mean densities of reproductive and vegetative shoots among months, depths, sites and areas. The homogeneity of variance was examined using Cochran's *C*-test before running the analysis. Data were transformed when necessary to meet the assumptions for ANOVA. The Student-Newman-Keuls' (SNK) test was used for *post hoc* multiple comparisons. Estimates of variations in vegetative shoots at each of the scales examined were calculated as variance components used untransformed data. Negative components of variation were set to zero (Underwood, 1997).

Results

Differences in the spatial patterns of distribution of flowering and vegetative shoots between the species were detected.

Results of ANOVA showed significant differences on the reproductive shoot density among depths for both the species. Flowering shoots of *C. nodosa* were more abundant at 50 cm compared to the other depths (20 and 100 cm), while the density of inflorescences of *Z. noltii* was higher at the deepest sampling station compared to the intermediate one (Fig. 1 and 2; Table 1). No differences in the density of reproductive shoots among the spatial scales investigated were observed.

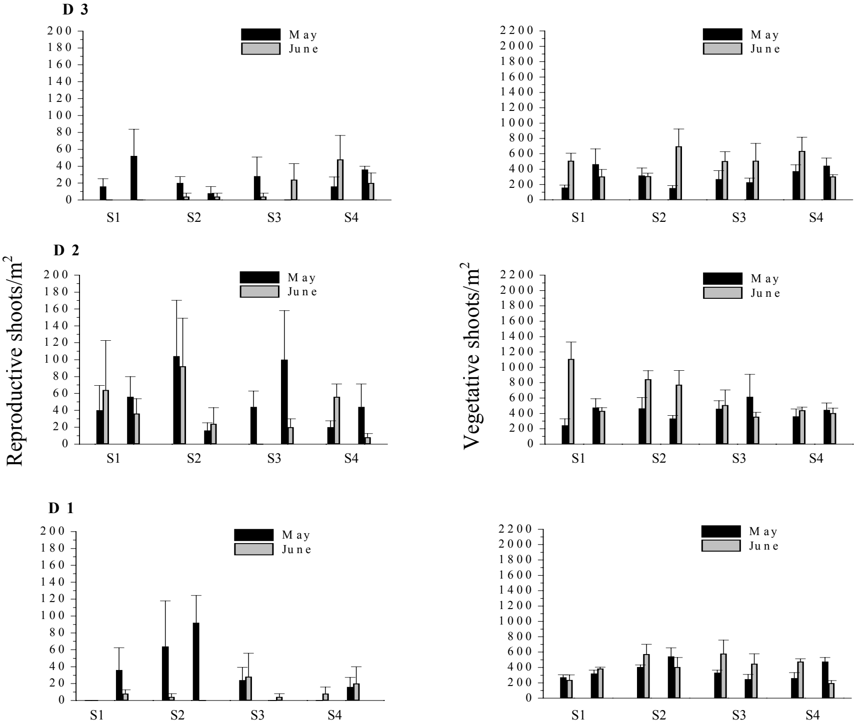

Fig. 1 - Mean (± SE) density of reproductive and vegetative shoots of *C. nodosa* in the three sampling stations. n= 4. D1= 20 cm; D2= 50 cm and D3= 100 cm.

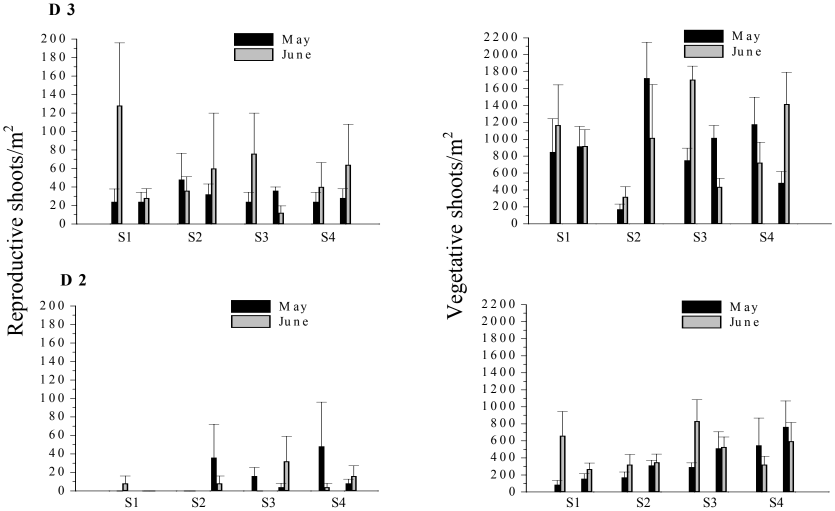

Fig. 2 - Mean (± SE) density of reproductive and vegetative shoots of *Z. noltii* in the two sampling station. n= 4. D2= 50 cm and D3= 100 cm

Results of ANOVA on vegetative shoot density of *C. nodosa* showed a significant interaction month x area and depth x site effects (Fig. 1; Table 1). There was also a significant interaction month x area effect for the density of *Z. noltii* (Fig. 2; Table 1). While the density of *C. nodosa* was highest at the intermediate depth, vegetative shoots of *Z. noltii* were more abundant at 100 cm compared to the other depth. In both species vegetative shoot density reached maximum values in June (Table 1). As *Z. noltii* was not present at the shallowest depth in both dates of sampling, this depth was excluded from the analyses. Components of variation calculated on each of the spatial scale investigated indicated that centimetres (i.e. among cores) were the most important in explaining total variances for shoot density (Table 1).

Discussion and conclusions

Results of this study showed that patterns of distribution and abundance of vegetative shoots did not reflect those of flowering shoots in both species, except for depth. This finding suggests that environmental factors (i.e., light and hydrodynamics) could be involved in the flowering control. They also suggested that patchiness in the distribution of vegetative shoots existed on a small scale (i.e. centimetres), indicating that environmental heterogeneity (i.e. nutrients, light and sediment texture) may affect spatial distribution of both species on a very local scale.

A previous study on a mixed meadow of *C. nodosa* and *Z. noltii* has showed that, despite their similarity in the pattern of leaf biomass, these species differed in acquisition, storage and utilization of tissue N (Kramer and Mazzella, 1999). Variations in the density of vegetative shoots across depths could explain the contrasting pattern observed in the abundance of reproductive shoots of the two species. They also suggests an alleviation of competitive interactions which may explain the coexistence of these two seagrasses in the study meadow.

Tab. 1 - Results of ANOVA on vegetative and reproductive shoot density of *C. nodosa* and *Z. noltii.* Bold print indicates significance

		C. nodosa vegetative shoot density			*C. nodosa* reproductive shoot density				*Z. noltii* vegetative shoot density			*Z. noltii* reproductive shoot density	
Source	df	F	p	% Var	F	p	Source	df	F	p	% Var	F	p
Month= M	1	10.04	>0.05	9.3	6.09	>0.09	Month= M	1	1.03	>0.38	0	0.01	>0.91
Depth= D	2	4.07	>0.07	3.5	24.19	**<0.01**	Depth= D	1	29.03	**<0.05**	4.7	55.8	**<0.01**
Site= S	3	1.39	>0.36	0.4	0.84	>0.53	Site= S	3	1.84	>0.27	0	2.76	>0.17
Area (S)= A (S)	4	0.43	>0.78	0	1.31	>0.27	Area (S)= A (S)	4	0.69	>0.59	0	0.23	>0.91
M x D	2	0.55	>0.60	0	0.21	>0.81	M x D	1	1.07	>0.37	0	0.06	>0.81
M x S	3	0.36	>0.78	0	0.36	>0.78	M x S	3	0.39	>0.76	0	0.95	>0.49
M x A (S)	4	5.97	**<0.001**	21.9	2.28	>0.06	M x A (S)	4	2.90	**<0.05**	16.7	0.63	>0.64
D x S	6	6.10	**<0.05**	1.2	0.65	>0.69	D x S	3	2.57	>0.19	9.4	1.03	>0.46
D x A (S)	8	0.21	>0.98	0	0.75	>0.64	D x A (S)	4	0.55	>0.69	0	0.58	>0.68
M x D x S	6	1.16	>0.40	5.2	1.77	>0.22	M x D x S	3	1.25	>0.40	0.6	0.06	>0.97
M x D x A (S)	8	1.39	>0.20	7.2	1.12	>0.35	M x D x A (S)	4	1.05	>0.38	3.6	1.36	>0.25
Residual	144			51.1			Residual	96			64.9		
Cochran's *C* test		*C* = 0.1104			*C* = 0.0618		Cochran's *C* test		*C* = 0.1445			*C* = 0.0711	
Transformation		Ln (x+1)			Ln (x+1)		Transformation		Sqrt (x+1)			Ln (x+1)	
SNK Test		In May S1: A1<A2 In June S1: A2<A1 In S1A1, S2A2, S4A1: May < June In S1, S2: D3<D1= D2 In D3: S1=S2=S3<S4			D1=D3<D2		SNK Test		D2<D3 In June S3: A2<A1 In S1A1,S3A1: May < June			D2<D3	

Description of the relative scale and pattern of variability in the relative abundance of reproductive and vegetative shoots is of considerable importance for future sampling design and provides useful information on reproductive ecology of these species.

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A. Vela, V. Leoni, G. Pergent, C. Pergent-Martini

EqEL - Faculty of Sciences, University of Corsica, BP 52, 20250 Corte, France

RELEVANCE OF LEAF MATTER LOSS IN THE FUNCTIONING OF *POSIDONIA OCEANICA* SYSTEM.

Abstract

Annual primary production of Posidonia oceanica *shallow meadows was investigated in four sites: two situated along island coastlines (Corsica and Sardinia) and two along continental coastlines (France and Tunisia). The number of leaves produced per year ranged from 7.0 (Corsica) to 8.1 (Sardinia). The leaf production (blade and sheath) ranged from 919.3 to 2055.7 mg DW·yr^{-1}.shoots^{-1} and rhizome production from 27.7 to 150.7 mg DW·yr^{-1}·shoots^{-1}. Both island stations are characterized by a high rhizome growth and production and a low leaf production.*

Key-words*:* Posidonia oceanica*, Primary production, Mediterranean, Island, Shallows meadows.*

Introduction

Marine Magnoliophyta are structuring species which are among the most useful (Costanza *et al.*, 1997) and productive components of the biosphere (Pergent *et al.*, 1994; Thayer *et al.*, 1997). Marine Magnoliophyta form a quasi-continuous belt all along the coasts of the Mediterranean (Procaccini *et al.*, 2003), and among them *Posidonia oceanica* (L.) Delile is the most widespread species (Short *et al.*, 2001). Islands have always had a particular significance in ecology and in evolutionary studies (Denslow, 2001). The islands cover approximately 4% of the Mediterranean, and are unequally distributed in each of the two basins. These islands benefit from particular environmental conditions, as much from a natural point of view (*i.e.* biological, physicochemical) as from an artificial point of view (*i.e.* demographic pressure, pollution, urbanization). In order to further understand the mechanisms involved in the development of *Posidonia oceanica* meadows, the aim of this work was to consider precisely the primary production and the functioning of four shallow meadows in relation with their environments and location.

Materials and methods

Four meadows, located in the Western Mediterranean basin, are studied. Two sites are located on the continental coasts (Méjean, South France and Sidi Raïs, North Tunisia), the others two are located on insular coasts (Alga, North-West Corsica and Coda Cavallo, North-East Sardinia). The meadow density (number of shoots per m^2) is measured for each station while 20 ± 5 orthotropic shoots of *Posidonia oceanica* are collected each month, from March 2002 to April 2003. A phenological analysis (Giraud, 1979), and a lepidochronological study (Pergent, 1990) are performed. The primary production is estimated according to the crossed lepidochronological method (Vela, 2006), derived from the lepidochronological method (Pergent and Pergent-Martini, 1990). The blade primary production is estimated considering: the average number of annually produced leaves, the average length of the rank 3 adult blades, the average width of the rank 1 adult blades, the average density of the rank 1 adult blades. The use of the rank 3 adult leaves enables us to estimate the average leaf matter loss on the living leaves (due to the action of macro-herbivores and/or hydrodynamics), during the year (Vela, 2006). The nitrogen and carbon content (% of dry weight) of adult blades and sheaths and intermediate leaves are determined every 2 months using an analyzer CHN Carlo-Erba®. The statistical processing is carried out using Statgraphics ® software.

Results

The density of the meadows varies significantly according to the site ($p<0.001$, ANOVA; Table 1).

Tab. 1 - Density of the meadows in the various studied sites (mean ± s.d.)

	Méjean	Alga	Coda Cavallo	Sidi Raïs
Density (no. shoots·m^{-2})	749.6 ± 42.7	621.9 ± 54.6	774.6 ± 48.2	547.3 ± 76.3

Leaf formation and primary production (per shoot and per m^2) vary according to sites (Table 2). The higher primary production are found in Sidi Raïs (per shoot) and in Méjean (per m^2), while the lowest is always recorded in Coda Cavallo. The nitrogen involved in the produced leaf tissues also varies according to sites with a maximum N observed in Sidi Raïs (48.30 mg shoots^{-1} ·yr^{-1}), and a minimum observed in Coda Cavallo (17.26 mg shoots^{-1}·yr^{-1}).

Tab. 2 - Tissue primary production and leaf matter loss for each site for the lepidochronological year 2002-2003.

Sites	**Tissues**	**No. of produced leaves**	**Primary production**		**Leaf matter loss**		
			mg DW.shoots^{-1}.yr^{-1}	g DW.m^{-2}.yr^{-1}	%	mg DW.shoots^{-1}.yr^{-1}	g DW.m^{-2}.yr^{-1}
Méjean	**Blade**	7.06	1636.8	1227.0	69	1132.1	848.6
	Sheath	7.06	238.4	178.7			
	Rhizome	-	27.6	20.7			
	Total		**1902.9**	**1426.4**			
Alga	**Blade**	6.96	1068.1	664.2	40	425.3	264.5
	Sheath	6.96	297.6	185.1			
	Rhizome	-	150.7	93.7			
	Total		**1516.3**	**943.0**			
Coda Cavallo	**Blade**	8.05	730.0	565.4	34	250.9	194.4
	Sheath	8.05	189.3	146.7			
	Rhizome	-	140.1	108.5			
	Total		**1059.4**	**820.6**			
Sidi Raïs	**Blade**	7.62	1682.6	920.9	39	650.5	356.0
	Sheath	7.62	373.1	204.2			
	Rhizome	-	62.0	33.9			
	Total		**2117.7**	**1159.0**			

The nitrogen content of the adult and intermediate leaves presents significant differences according to season in each site ($p<0.05$, Kruskall-Wallis). A maximum content is observed, for the adult and intermediate leaves, in February in

Méjean (2.8 % and 3.0 %, respectively). The minimum N content of adult leaves is observed in June and in August in Coda Cavallo (1.7 %), and the minimum N content of intermediate leaves is observed in August in the same site (2.0 %). The N content decreases with the age of tissues in all sites. The N content of adult and intermediate leaves present significant differences according to sites ($p<0.001$, Kruskall-Wallis; Table 3).

Tab. 3 - Post-hoc test (Student-Newman-Keuls) for N content of intermediate leaves (gray) and adult leaves (white).

	Méjean (M)	**Alga (A)**	**Coda Cavallo (CC)**	**Sidi Raïs (SR)**
Méjean (M)		-	M>CC	-
Alga (A)	-		-	-
Coda Cavallo (CC)	CC<M	CC<A		-
Sidi Raïs (SR)	-	-	SR>CC	

Discussion

The meadow densities are comparable with the values of other sites of the Mediterranean at equivalent depths (Alcoverro *et al.*, 1995; Pergent *et al.*, 2005). According to the classification of Pergent *et al.* (2005), which takes into account the requirements of the Water Framework Directive (N° 2000/60/EC, of the European Parliament and Council), two sites (Méjean, Coda Cavallo) have a "Normal" density, whereas the other two (Alga, Sidi Rais) have a "Poor" density.

The leaf N content is suitable to evaluate the availability of N for the plant (Invers *et al.*, 2004; Leoni, 2005; Touchette *et al.*, 2003). Coda Cavallo has the lowest value for N content in leaf tissues, the annual average values recorded in leaf tissues are close to the threshold value, which is likely to involve a strong N limitation (*i.e.* 1.80 %; Duarte, 1990). The decrease of leaf N content (blades and sheaths), according to the age of the leaves, could be linked, at least in part, to the re-translocation of nutrients from the oldest leaves before shedding (Lepoint *et al.*, 2002). The N re-mobilisation belongs to the mechanisms allowing *Posidonia oceanica* to support an important primary production under oligotrophic conditions (Gobert *et al.*, 2002; Lepoint *et al.*, 2002).

The values recorded for the formation of new leaves are in agreement with those in the literature (Leoni, 2005; Pergent and Pergent-Martini, 1990; Rico-Raimondino, 1995).

The leaf primary production measured for each site is comparable to those measured in other sites of the Mediterranean at similar depths (Cebrian *et al.*, 1997; Guidetti *et al.*, 2000; Pergent-Martini *et al.*, 1994) but the values recorded along continental coasts (Sidi Raïs: 781.2 mg C·shoots^{-1}·yr^{-1}; Méjean: 689.3 mg C·shoots^{-1}·yr^{-1}) are higher that those observed along insular coasts (Alga: 534.1 mg C·shoots^{-1}·yr^{-1}; Coda Cavallo: 358.2 mg C·shoots^{-1}·yr^{-1}). The share of leaf production represented by the sheaths varies from 13 to 23 % according to sites. The same trend is observed for the leaf production per m^2, with lower values along insular coasts (Alga: 295.0 g C·m^{-2}·yr^{-1}; Coda Cavallo: 172.6 g C·m^{-2}·yr^{-1}).

The site of Méjean, in which the leaf production is the highest (per m^{-2}), presents the strongest leaf matter loss (69 %; 311.7 gC·m^{-2}·yr^{-1}), that is linked to the strong pressure of herbivores recorded in this site (Vela, 2006). The other sites present a weaker annual leaf matter loss (101.4 gC·m^{-2}·yr^{-1} for Alga; 76.4 gC·m^{-2}·yr^{-1}

for Coda Cavallo; 136.7 $gC \cdot m^{-2} \cdot yr^{-1}$ for Sidi Raïs). Minimal leaf matter loss recorded at Coda Cavallo corresponds to the weakest leaf production. A correlation between leaf matter loss and N content of adult and intermediate leaves is highlighted ($r = 0.64$ and $r = 0.71$, respectively; $p<0.01$). This observation is likely to confirm the assumption that grazing pressure increases in tissues with higher food value (Ruiz, 2000). Taking into account the grazing potential impact on growth (Cebrian *et al.*, 1998) on our Méjean results, the herbivores pressure and/or hydrodynamics action visualized by the leaf matter loss, can result in an increase in leaf primary production. A significant part of energy, allocated to the compensation of leaf matter loss, is likely to give the meadow brittleness characteristics, especially when facing other environmental disturbances.

The share of total primary production devoted to the rhizome production is variable but consistent with previous data (Pergent-Martini *et al.*, 1994, Cebrian *et al.*, 1997; Guidetti *et al.*, 2000). This proportion varies, for the studied year, between 1 %, in Méjean, and 13 %, in Coda Cavallo. For the two insular sites (Alga and Coda Cavallo), the rhizome production is particularly high. In general terms, the production of rhizomes is significantly correlated to the growth speed (Pergent and Pergent-Martini, 1990; Rico-Raimondino, 1995), and thus seems related to the sedimentary dynamics of each site. Indeed, in the site of Alga, the withdrawal of the beach litter deposits of *Posidonia oceanica* and of the back beach vegetation could be the cause of a sediment input to the marine environment, thus increasing sedimentation rate on the meadow.

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SECTION 2

ECOLOGICAL SERVICES OF SEAGRASSES

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C.F. Boudouresque[1], N. Mayot[1], G. Pergent[2]

[1]Centre d'Océanologie de Marseille, Campus de Luminy, 13288 Marseille cedex 9, France
[2]Ecosystèmes littoraux, Université de Corse Pasquale Paoli, BP 52, 20250 Corte, France

THE OUTSTANDING TRAITS OF THE FUNCTIONING OF THE *POSIDONIA OCEANICA* SEAGRASS ECOSYSTEM

Abstract

The biomass, primary production, nutrient and organic carbon inputs and outputs and food webs of the Posidonia oceanica *ecosystem are compared with those in other marine and terrestrial ecosystems. It exhibits some striking similarities with terrestrial ecosystems (e.g. forest ecosystems), such as the amount of biomass of the primary producers, the presence of litter and the prominent role of detritus based food webs. Other traits are not uncommon in the marine realm, e.g. the sequestration of organic carbon (sink). In addition, it exhibits two further uncommon features: (i) the juxtaposition of two sets of primary producers, the seagrass, which features material that may require months or years to become degraded and enter the detritus food webs, and the epiphytes, with material which is degraded more easily and which enters the herbivorous food webs; (ii) the occurrence of a relatively high rate of primary production despite low nutrient availability (a LNHC system).*

Key-words: Posidonia oceanica*, Ecosystem functioning.*

Introduction

The seagrass *Posidonia oceanica* is endemic to the Mediterranean Sea, where it constitutes extensive meadows from the sea level down to 25-40 m depth. Overall, the *P. oceanica* meadow carpets 1-2% of the Mediterranean seabed, which represents a surface area of the order of 37 000 km² (Boudouresque *et al.*, 2006).

The functioning of the *Posidonia oceanica* ecosystem

The average biomass of *Posidonia oceanica* is 501 gDW/m² aboveground and 1 611 gDW/m² belowground (Duarte and Chiscano, 1999). Higher values have been measured: up to 1 640 and 5 500gDW/m² for leaves and rhizomes, respectively (Boudouresque *et al.*, 2006). No other marine MPO (Multicellular Photosynthetic Organism) key species attains such a high overall biomass. In addition to the seagrass biomass, *P. oceanica* leaves and rhizomes harbour autotrophic epiphytes, belonging to Chlorobionta, Rhodobionta and Stramenopiles; their average biomass ranges between 160 and 420 gDW/m².

The primary production of *P. oceanica* ranges between 400 and 2 500 gDW/m²/y^{-1} and decreases with depth. Autotrophic epiphytes also contribute to the primary production: up to 500-900 gDW/m²/y^{-1} (Cebrián and Duarte, 2001; Romero, 2004; Boudouresque *et al.*, 2006). It is the sum of these two compartments, the seagrass and its epiphytes, with values ranging from 2 000 to 3 000 gDW/m²/y^{-1}, which can approach the highest values of net primary production observed in the terrestrial realm. However, it is worth that such very high values only concern shallow water meadows.

In the context of the very low nutrient (N and P) concentrations which characterize most of the Mediterranean, the very high biomass and the relatively high primary production rate displayed by *P. oceanica* meadows are unexpected. Gobert *et al.* (2002) classify this ecosystem within the category of LNHC ecosystems (Low Nutrient High Chlorophyll). The reasons for this paradox may lie in the functioning of the nutrient machine within the system (Fig. 1): (i) the extraction by roots of nutrients buried within the sediment; (ii) the uptake of nutrients both by roots and by leaves; (iii) the luxury consumption of inorganic nitrogen, taken up in excess with respect to the plant requirements and stored within the rhizomes, for further utilisation (Romero, 2004; Mateo *et al.*, 1997); (iv) the trapping by the canopy of POM (Particulate Organic Matter) from the water column, and its subsequent mineralization (Duarte *et al.*, 1999); (v) the trapping within the canopy water of the nutrients released by the mineralization of POM and *P. oceanica* detritus; the canopy water therefore acts as a nutrient reservoir (Gobert *et al.*, 2002); (vi) the internal recycling of nutrients from senescent leaves towards young leaves; (vii) the possible presence of N_2 fixing bacteria on leaves and/or within the rhizosphere (Béthoux and Copin-Montégut, 1986).

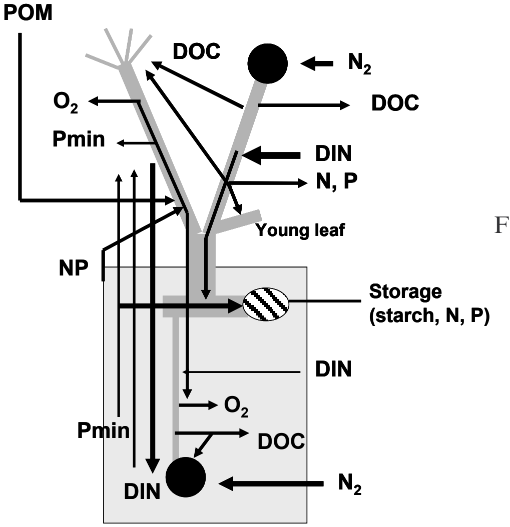

Fig. 1 - Nutrient and carbon fluxes in a *Posidonia oceanica* ecosystem. When not available for *P. oceanica*, some fluxes are extrapolated from other seagrasses. Black circles: N_2 fixing bacteria. Light grey: the belowground compartment. Dark grey: *P. oceanica* (roots, rhizomes, leaves) and leaf epiphytes (both autotrophic and heterotrophic). DIN: dissolved inorganic nitrogen. DOC: dissolved organic carbon. N: nitrogen. P: phosphorus. POM: Particulate organic matter from the water column.

Dissolved Organic Carbon (DOC) can be leached towards the water column and the rhizosphere (Fig. 1), which stimulates the bacterial development (García-Martínez *et al.*, 2005). An interconnected system of gas spaces (aerarium) crosses the whole plant and makes possible the recycling of CO_2 (from photorespiration) and the leaking by the roots of O_2 (from photosynthesis). Carbohydrates derived from the photosynthesis are for a large extent rapidly exported to the rhizomes, then redistributed to the surrounding leaf shoots (Libes and Boudouresque, 1987). This constitutes an insurance mechanism: even if the production rate of a shoot is low, it will receive an amount of carbohydrates corresponding to the needs of the growing leaves. In summer, large amounts of carbohydrates are stored in the rhizomes (Alcoverro *et al.,* 2001).

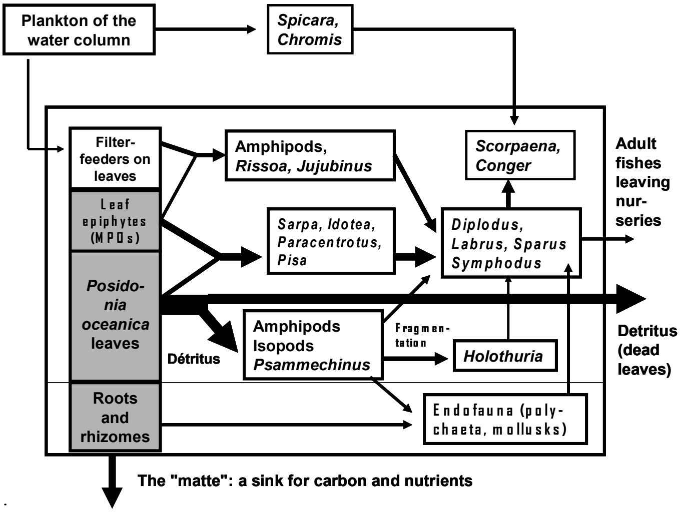

Fig. 2 - A simplified conceptual model of the functional compartments and food webs in a *P. oceanica* ecosystem (enclosed within the rectangle), with the main inputs and outputs. The width of the arrows is proportional

The *P. oceanica* ecosystem comprises two main organic carbon inputs (Fig. 2): (i) heterotrophic plankton and POM proceeding from the pelagic ecosystem; (ii) teleosts which feed on zooplankton of the pelagic ecosystem in the daytime and shelter at night below the leaf canopy. The ecosystem comprises three main organic carbon outputs: (i) a large part of the leaf production is exported, as dead leaves (outwelling); (ii) adult fishes leave the seagrass nurseries; (iii) organic carbon sequestrated within the matte (sink) for millennia. Within the ecosystem, it is generally considered that a small part (less than 10%) of the leaves are directly consumed by herbivores (Cebrian and Duarte, 2001). However, the paradigm of the low consumption of the leaves by herbivores could be an artefact linked to overfishing (Pergent *et al.,* 1993). The leaves which are not consumed are either exported or accumulate in the litter. The mineralization of the litter is a long-term process, which requires months to years. The detritus based food web is the main mechanism of energy transfer from the leaves towards the higher trophic levels. Contrarily to the *P. oceanica* leaves, autotrophic leaf epiphytes are largely consumed by herbivores and their mineralization is a short-term process (Boudouresque *et al.*, 2006).

For the purposes of our comparison of the functioning of the *P. oceanica* ecosystem with other marine and terrestrial ecosystems (Table 1), (i) we have not taken into consideration lakes and rivers; (ii) we compare this ecosystem with the majority of the ecosystems of a realm, but we are aware that some ecosystems do not fit the general case; (iii) we have set aside the other seagrass ecosystems, which obviously share most of the characteristics of the *P. oceanica* ecosystem.

The *P. oceanica* ecosystem shares several functional characteristics either with many terrestrial (e.g. forests), or marine ecosystems. In addition, it exhibits two more uncommon features: (i) the juxtaposition of two sets of primary producers, the seagrass (Magnoliophyta, Plantae), which features material that requires months or years to be degraded and enter the detritus food webs, and the epiphytes belonging to Chlorobionta, Rhodobionta (Plantae) and Stramenopiles, whose material is more easily degraded and quickly enters the herbivorous food webs. Most terrestrial ecosystems rely on the first type of primary producers while most benthic photophilous marine ecosystems are based upon the second; (ii) the occurrence of a relatively high rate of primary production despite low nutrient availability (a LNHC system). This characteristic is shared by coral reefs, some pelagic ecosystems and by grasslands based upon the symbiosis between N_2 fixing procaryotes and Fabaceae.

None of the characteristics of the functioning of the *P. oceanica* ecosystem are strictly speaking unique. In fact, what makes it original is the grouping of traits which characterise either terrestrial or marine ecosystems. As far as the marine realm is concerned, *P. oceanica* beds could be viewed as a terrestrial ecosystem in the wrong place. This is not a surprise as seagrasses are terrestrial plants whose ancestors colonized the sublittoral zone.

Tab. 1 - Some features of the *P. oceanica* ecosystem which are shared – or not – with marine (ME) and terrestrial ecosystems (TE).

Features of the *P. oceanica* ecosystem	Common in ME	Common in TE
Huge primary producers (P1) biomass	No	Yes
High belowground P1 biomass	No	Yes
Relatively high primary production	Yes/No	Yes/no
Juxtaposition of P1s: hard vs easy to degrade	No	No
A LNHC system	No	No
Recycling of the nutrients within the ecosystem	No	Yes
Presence of a litter of dead leaves	No	Yes
Prominent role of detritus based food webs	No	Yes
Outwelling of a part of the primary production	Yes/No	No
A sink for carbon	Yes/No	No
Low biomass of the secondary producers (P2)	Yes/No	Yes
Low P2/P1 ratio	Yes/No	Yes

Whatever its originality, the *P. oceanica* ecosystem clearly represents a success story, as it occupies a large part of the Mediterranean sublittoral zone. Two features may account for this success: (i) the traits which characterize the Magnoliophyta, that are the basis for the "nutrient machine"; (ii) the maximization of the biomass, with respect to the energy flow, which constitutes a strategy which enhances the ecosystem's resilience.

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S. Acunto, D. Balata, G. Pardi, I. Papi, L. Piazzi, M. Burgassi, F. Cinelli

Dipartimento di Biologia, Unità di Biologia Marina ed Ecologia
Università di Pisa – via A. Volta, 6 – 56126 Pisa, Italy

SPATIAL VARIABILITY IN THE EPIPHYTIC ASSEMBLAGES OF *POSIDONIA OCEANICA* (L.) DELILE BEDS AROUND MALTESE ISLANDS

Abstract

The patterns of spatial variability in the epiphytic assemblages of leaves and rhizomes of Posidonia oceanica *along the coasts of Malta, Comino and Gozo islands were investigated in June 2002. This was accomplished through a hierachical sampling design that enable comparisons at scales ranging from metres (areas) to kilometres (meadows). Data of cover were analysed by multivariate and univariate techniques.*

MDS ordination did not show a clear separation among islands or coast exposure. Univariate analysis carried out on the most abundant epiphytic groups of leaves and rhizomes, detected significant differences among meadows and areas for all the groups considered. The epiphytic assemblages of P. oceanica *studied around the Maltese Islands showed a similar structure. However, the epiphytic assemblages of four meadows appeared different from the others. These differences did not appear linked to the impact of human activities and they could be determined by different environmental conditions to be investigated.*

Key-words: *Maltese Islands,* Posidonia oceanica*, epiphytes, spatial variability.*

Introduction

One of the most important seagrasses in the Mediterranean Sea is *Posidonia oceanica* (L.) Delile, an endemic species that can form wide meadows. Assemblages of epiphytes of *P. oceanica* have been widely studied both on leaves (Van der Ben, 1971; Cinelli *et al*., 1984; Casola *et al.,* 1987; Mazzella *et al*., 1989) and rhizomes (Boudouresque, 1974; Piazzi *et al*., 2002). Spatial patterns have been investigated mostly in relation to depth (Mazzella *et al*., 1989), whereas little is known about horizontal scales of variability in spatial distribution of epiphytes.

This study is a contribution to the understanding of spatial variability of *Posidonia oceanica* epiphytes. In particular, patterns of distribution of several categories of animals and algae were studied along the coasts of Maltese Archipelago. The knowledge of spatial variability is essential to design and to optimise any environmental sampling programme that can discriminate between natural and anthropogenic disturbances (Benedetti-Cecchi, 2001; Piazzi *et al*., 2004; Fraschetti *et al*., 2005).

Materials and methods

The study was carried on in June 2002 along the coast of Malta, Comino and Gozo Islands. This was accomplished through a hierachical sampling design that enables comparisons at scales ranging from metres (areas) to kilometres (meadows). Samples of *P. oceanica* were collected in 15 meadows randomly chosen along the coasts of the islands (Fig. 1).

Within each meadow 3 sites hundreds of meters apart were randomly chosen at the depth of 10 meters, within each site 3 areas were sampled and 5 orthotropic shoots were randomly collected in each area.

Data of cover of epiphytic macroalgal morphological groups (red crustose algae, brown crustose algae, filamentous algae, corticated-terete algae, foliose algae, articulated algae) and animal phyla of the two external leaves of each shoots (Piazzi *et al.*, 2004) were analysed by multivariate (nMDS) techniques.

Univariate analyses of variance (3 way-ANOVA) were carried out for the most abundant groups or phyla on leaves (red crustose algae, brown crustose algae, corticated-terete algae and filamentous algae) and rhizomes (filamentous algae, foliose algae, bryozoans and foraminifers). Data were Ln(X+1) transformed when necessary.

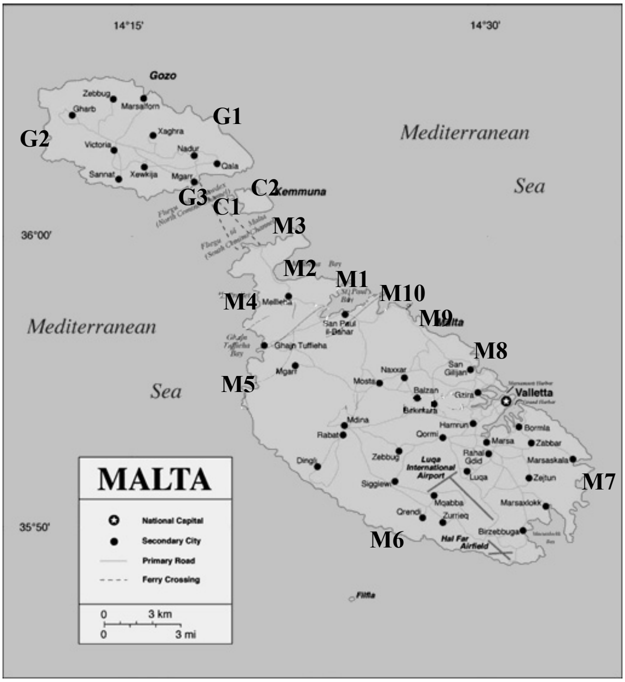

Fig. 1 - Sampling locations: M1: St. Paul Island; M2: Mellihea Bay; M3: Ahrax; M4: Anchor Bay; M5: Tomm Ir Rih; M6: Gharlapsi; M7: Munx Ar Reef; M8: St George Bay; M9: Qalet Marhu; M10: Qwara Point; C1: Cominotto; C2: Maria Caves; G1: Ir Ramla Bay; G2: Dwejra; G3: Sat L'Ahmar.

Results

Leaf epiphyte

Leaf epiphyte assemblage of *Posidonia oceanica* meadows of Malta Islands was dominated by encrusting algae in all the studied locations; filamentous and corticated-terete algae and foraminifers were widely distributed with low cover. The other groups considered were occasionally present.

The nMDS showed that the majority of the meadows studied were grouped together and only four locations resulted separated from the others: C2, G2, M1 and M6 (Fig. 2).

3 way-ANOVA detected significant differences among locations and areas for all the groups considered (Tab. 1).

Tab. 1 - Results of 3 way ANOVA examining percent cover of epiphyte assemblage. Significant values are in bold.

Leaf epiphytes

		RED CRUSTOSE ALGAE		BROWN CRUSTOSE ALGAE		FILAMENTOUS ALGAE		CORTICATED TERETE ALGAE	
Source	**df**	**MS**	***F***	**MS**	***F***	**MS**	***F***	**MS**	***F***
Locations	14	21819.280	**13.61**	36.266	**8.93**	40.861	**11.84**	13.072	**7.75**
Sites (L)	30	1603.650	**1.64**	4.059	1.04	3.451	1.28	1.686	0.80
Areas (LxS)	90	978.644	**2.45**	3.895	**2.62**	2.695	**2.10**	2.095	**3.04**
Residual	1215	400.056		1.487		1.283		0.810	
Test *C* Cochrans		0.032 p<0.001		0.017 n.s.		0.021 n.s.		0.027 n.s.	
Trasformation		-		Ln (x+1)		Ln (x+1)		Ln (x+1)	

Rhizome epiphytes

		FOLIOSE ALGAE		FILAMENTOUS ALGAE		BRYOZOANS		FORAMINIFERS	
Source	**df**	**MS**	***F***	**MS**	***F***	**MS**	***F***	**MS**	***F***
Locations	14	12.732	**2.97**	17.218	**6.27**	10.057	**7.09**	5.158	**3.45**
Sites (L)	30	4.286	1.60	2.746	0.78	1419	1.38	1.494	0.98
Areas (LxS)	90	2.683	**2.58**	3.501	**3.44**	1.031	**1.32**	1.527	**1.75**
Residual	540	1.040		1.017		0.783		0.873	
Test *C* Cochrans		0.030 n.s.		0.027 n.s.		0.031 n.s.		0.023 n.s.	
Trasformation		Ln (x+1)		Ln (x+1)		Ln (x+1)		Ln (x+1)	

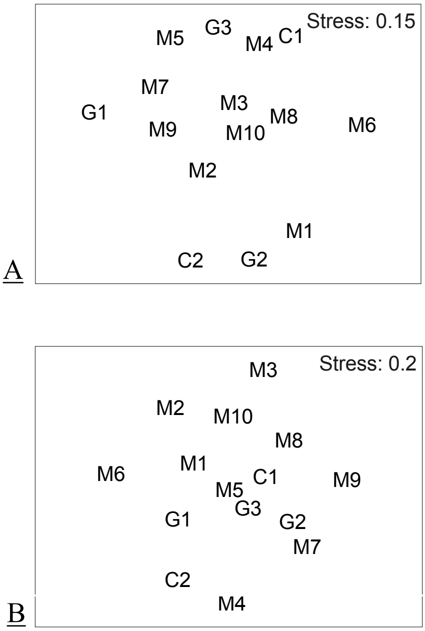

Fig. 2 - Multidimensional scaling (MDS) ordination based on Bray-Curtis similarity, applied to meadows-morphological groups matrix, related with the epiphytic assemblages of leaves (A) and rhizomes (B) of *Posidonia oceanica*.

Rhizome epiphyte

Rhizome epiphyte assemblage resulted mostly constituted by filamentous and foliose algae; encrusting bryozoans and the foraminifer *Miniacina miniacea* were also well represented.

The nMDS showed that the meadows studied were closed each other. However, some locations appeared softly separated: M3, M4, M6, M9, C2 and G1 (Fig. 2).

3 way-ANOVA detected significant differences among locations and areas for all the groups considered (Tab. 1).

Discussion

Epiphyte assemblages of *Posidonia oceanica* of Malta Archipelago showed significant variability among locations (km apart) and among areas (m apart). Variability at small scale is considered a common trend for *P. oceanica* epiphytes, while differences among locations are determined by some meadows showing different characteristics compared to the others.

Leaf epiphyte assemblage was characterized by encrusting algae, with the exception of the meadow of Dwejra (G2), where cover was very low for all the groups considered. Gharlapsi (M6) assemblage was characterized by a high cover of red crustose algae and a very low abundance of all the other groups. St. Paul's Island (M1) and St. Maria caves (C2) were separated from the others mostly due to the low cover of the bryozoan *Electra posidoniae*.

Rhizome epiphytes showed higher diversity, even if cover was low in some meadows (G1, M6, M5).

In general, epiphyte assemblages of Malta Archipelago meadows showed a similar structure, with only few locations separated from the others. This separation does not appear linked to the impact of human activities and it is not clearly explainable with the data obtained, thus further specific investigations should be useful.

Possible different environmental conditions and geographical distance could be taken into account. As it is evident in Dwejra (G2), that is a little meadow isolated from the others by wide zones characterised by rocky cliffs, and in Gharlapsi (M6), that is the southernest studied meadow. Distance and segregation may influence recruitment and settlement of both algae and sessile animals. On the contrary, the low cover of bryozoans and *Miniacina miniacea* in rhizome assemblage at Anchor Bay (M4) may be linked to eutrophic waters related with the sewage present in this location.

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E. Apostolaki [1,2], T. Tsagaraki[1], M. Tsapakis[1] I. Karakassis[2]

[1]Institute of Oceanography, Hellenic Centre for Marine Research, PO Box 2214, GR-71003,
Heraklion, Crete, Greece
[2]Marine Ecology Laboratory, Biology Department, University of Crete,
PO Box 2208, GR-71409, Heraklion, Crete, Greece

FISH FARMING EFFECTS ON MACROFAUNAL COMMUNITIES ASSOCIATED WITH MEDITERRANEAN SEAGRASS MEADOWS

Abstract

Fish farming impact on macrofauna associated with Posidonia oceanica *meadows was studied in three coastal areas (Spain, Italy and Greece) in the Mediterranean. Data analysis showed that stations were characterized by coarse sediment with low percentage of silt/clay and oxic conditions. Distance seemed to be a feeble distinguishing factor between stations at each site, implying low intensity of disturbance to macrobenthic communities due to fish farming.*

Key-words: *macrofauna,* Posidonia oceanica*, Mediterranean, fish farming.*

Introduction

Posidonia oceanica (L.) Delile, an endemic seagrass species of great importance for the Mediterranean, suffers from intensive aquaculture activity (Duarte, 2002). Relevant studies in the past have mainly focused on alterations in seagrass demography (e.g. Marba *et al.,* 2006) while studies on the impact on associated macrofauna have been partly assessed (Dimech *et al.,* 2002). The aim of the present study was to investigate the impact of fish farming on macrofaunal communities associated with *P. oceanica* meadows along the Mediterranean.

Materials and methods

Macrofaunal sampling was carried out at three exposed coastal bays during the warm season in the Eastern Mediterranean (Sounio, Greece in June 2003) and the Western Mediterranean (Sicily, Italy in September 2002 and Alicante, Spain in September 2003). Three vegetated stations were selected at increasing distance from the fish cages, under the cages, intermediate distance (not exceeding 40 m) and control station, at each site. Replicate macrofaunal samples were collected by means of SCUBA diver operated suction devise, using a metal frame of $0.2m^2$ surface area. All macrofaunal samples were sieved *in situ* using a 0.5 mm mesh size sieve, stained with rose Bengal, to facilitate sorting, and preserved with 10% formalin. Macrofauna samples were then sorted and animals were weighted, counted and identified to species level, when possible.

Results

Redox potential ranged between 69 and 353 mV. Stations were characterized by coarse sediment (mean diameter= 0.3-0.9 mm), with low percentage of silt/clay (5-20%). A total of 20,037 individuals belonging to 395 species were collected during the present study. Their mean abundance ranged from 1,656 to 3,979 individuals m^{-2}. Distance seemed to be a feeble diversification factor between stations at each site. A tenuous clustering was encountered in Sounion and Alicante, whereas no such pattern was observed in Sicily (Fig. 1).

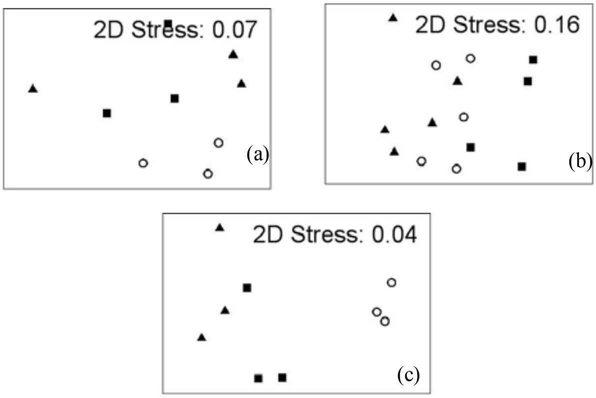

Fig. 1 - MDS plots for the areas studied (a) Alicante, (b) Sicily and (c) Sounion (▲: impacted, ■: intermediate, ○: control station).

Discussion

Low dissimilarity between stations in all sites, which was attributed to quantitative rather than to qualitative changes, could suggest low intensity of disturbance (Pearson and Rosenberg, 1978). Gray *et al.* (2002) suggested that hypoxia is the major factor altering benthic fauna rather than organic enrichment per se, the former being mainly correlated with the hydrographic regime of the study area. It seems that coarse sediments in combination with intense currents which prevail at the sites studied allow for oxic conditions, as also indicated by the positive Eh values. In such sites, the microbial processes related to decomposition of deposited material do not result in severe chemical stress for the macrofauna. Provided additional food supplied by fish farm effluents, environmental conditions encountered at the sites allow the existence of diverse communities of high abundance and biomass, despite high mortality and low recruitment rate of *P. oceanica* shoots measured under the cages at the studied sites (Diaz-Almela *et al.*, submitted).

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M. Ben Brahim, A. Hamza, A. Bouain

Faculté des Sciences de Sfax, Institut National des Sciences et Technologies de la Mer,
Sfax, Tunsia

DISTRIBUTION AND TIME VARIABLITY OF MICROEPIPHYTES AND PHYTOPLANKTON IN *POSIDONIA OCEANICA* MEADOWS OF THE KERKENNAH ISLANDS (TUNISIA)

Abstract

Studies of micro-epiphytic organisms on the leaves of Posidonia oceanica *and phyptoplankton in water column near meadows during different periods in Kerkennah island (Southeast Tunisia) showed that diatoms are the dominant group. They are composed by 22 families and about 50 species. For Dinoflagellates we identified 11 families and 20 species among which three are toxic taxa. Epiphytes showed often high biomass but diversity was relatively low. Cysts are also abundant in our samples, and occasional occurrence of temporary cysts may indicate some degree of stress in this area.*

***Key-words*:** Posidonia oceanica*, epiphytic microflora, phytoplankton, distribution.*

Introduction

Studies on *Posidonia oceanica* meadows in Tunisia are mainly focused to distribution and dynamics of these systems (Ben Mustapha and Hattour, 1992). A few studies have determined and quantified the epiphytic microalgal component. Survey of the epiphytic microalgae on *Posidonia oceanica* leaves have an important implication along our region because these populations can influence phytoplankton status in areas where shellfish are harvested (Hamza, 2003).

The aim of this study is to provide additional data on composition and distribution of epiphytic microflora and phytoplankton in *P. oceanica* meadows off the Kerkennah islands (Southeast Tunisia)

Materials and methods

Samples were collected in different periods in *P. oceanica* meadows situated in 3 areas around Kerkennah islands at depths from 0.5 to 1.90 m (Fig. 1, Tab.1).

The meadow of *P. oceanica* in Hassar is “tiger-shaped”, constituted with cords of seagrass from 1 to 3 m long and spaced about 1-1.5 m.

In Ras El Besch the meadow is regular and continuous, and in Gremdi it is shaped with micro-atolls with a diameter between 2 and 5 m.

At each station and in each period, 30 plants were collected to calculate the leaf length of *P. oceanica* and L.A.I. Samples of sea water were collected by bottle from each station and in different period. The volume of each sample was 1 liter. To study of epiphytic phytoplankton we take 10 ml of the washing water of 100 to 300g of plant (Turki, 2005). To identify phytoplankton, water column and washing water of *P. oceanica* leaves collected simultaneously in each station were analyzed by sedimentation method (Sournia, 1978).

The index of species diversity (H’, Shannon-Weaver) was calculated for each sample in each area and sampling period.

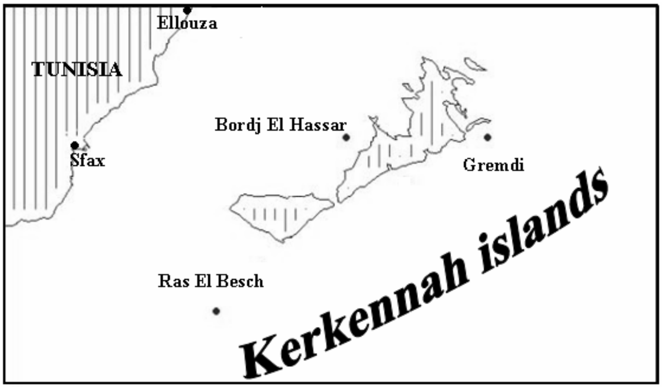

Fig. 1 - Location of the sampling stations on *Posidonia oceanica* meadows in Kerkennah island (Gulf of Gabes, Tunisia).

Tab. 1 - Characteristics of the studied *P. oceanica* meadows in Kerkennah islands (Classification according to Giraud, 1977)

Station	**coordinates**	**Depth**	**Shape of meadow**	**Season of sampling**	**Density**	**Type of meadow**
Hassar	34° 44'29" N 11°08' 59" E	1.10 to 1.72 m	Tiger-shaped	Winter Spring	352 976	Type III Type I
Ras El Besch	34° 32' 30" N 10° 57' 79" E	0.8 to 1.20 m	Continuous	Spring Summer	832 1792	Type I Type I
Gremdi	34° 44' 76" N 11° 20' 06" E	0.5 to 0.7 m	Micro-atolls and tuffs	Summer	704 1184	Type I Type I

Results

Diatoms are the dominant group of microalgae in all sampling periods, both in water column and epiphytic on leaves. The proportions of dinoflagellates are more important in the water column than in the leaves, and usually we found the same species regardless of the habitat. This can be due to the fact that microalgae are driven by the intensive hydrodynamics. In winter and spring the numbers to species increase in the medium and also in stations which are exposed to high current, diversity in the water column are higher than in leaves.

The trend dinoflagellates/diatoms in each station reveal the abundance of the diatoms in whole samples.

This increases in spring in water column, mainly in Ras El Besch, is due to dinoflagellates. For the epiphytic component, the high values in winter, at Hassar, are again due to dinoflagellates

Diatoms are represented in the water column by various families (Naviculaceae Fragilariaceae, Rhyzoseniaceae, Cymbellacea, Nitzschiaceae), while for epiphytic component only Naviculaceae occurred. Dinoflagellates in water column are dominated by cysts. In the leaves cysts were detected only in winter, mainly represented by Prorocentraceae and Gymnodiniaceae. The toxic specie *Prorocentrum lima* occurred in the leaves in winter and in the water column in summer. The highest number of epiphytic microalgae is registered in Ras El Bech in summer, where in this period this area is sheltered form currents and is protected by traditional fish catching gears. In this station diversity and number of dinoflagellate species were high. Together with cysts and *P. lima*, other families occurred (Peridiniaceae, Gymnodiniaceae, Polykrikaceae).

Index of species diversity (H') was relatively high in spring for all stations, except in Ras El Besch. In this season the leaves of *P. oceanica* are longer and more numerous with a consequent high Leaf Area Index, this provides higher space for epiphyte colonization (Drew, 1971; Drew and Jupp, 1976; Giraud, 1977; 1979) (Tab. 2).

Tab. 2 - Seasonal variation of leaf length and L.A.I. of *P. oceanica* meadows in different station in Kerkennah.

Stations	Period	Leaf length (cm)	L.A.I. (m²/m²)
Bordj El Hassar	Winter	22.169 ± 09.39	6.52
	Spring	19.58 ± 08.50	14.84
	Summer	11.33 ± 05.82	19.34
Ras El Besch	Spring	24.20 ±11.80	18.39
	Summer	19.84 ±11.62	29.51
Gremdi	Spring	35.58 ±12.61	20.20
	Summer	25.39 ± 13.95	26.26

The L.A.I. seems to increase from West to the East and form the North to South of the islands.

Conclusions

The main features of the epiphytic micro-algae populations of the *Posidonia oceanica* leaves in Kerkennah meadows are:

- Phytoplankton composition in water column depends of epiphytic population and also of degree of hydrodynamics in each station.
- Diatoms are the major micro-epiphytes in the whole period, while dinoflagellates increase in abundance in spring.
- Diversity and abundance show the highest values in spring.
- Leaves host many species, but limit somehow the growth; encistement of dinoflagellate in fact is high.

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M. Ben Brahim, A. Hamza, A. Bouain

Faculté des Sciences de Sfax
Institut National des Sciences et Technologies de la Mer- Centre de Sfax -BP. 1035-3018 Sfax- Tunisia

SPATIO-TEMPORAL DISTRIBUTION OF MACRO-EPIPHYTIC ORGANISMS IN *POSIDONIA OCEANICA* LEAVES IN THE GULF OF GABES (TUNISIA)

Abstract

The macro epiphytic populations of leaves of Posidonia oceanica *meadows in the Kerkennah islands (Gulf of Gabes, Tunisia) are composed essentially by Bryozoa, Hydrozoa and Annelida. A variable distribution can be observed according to station, sampling period and position in the leaves. Hydrozoans were more abundant in the cold season. Bryozoans occur mainly in summer, generally are localized in the external side of the leaves, and occupied the medium or high leaf portions. Spatial difference in epiphyte biomass were observed between north and south locations of the studied area.* P. oceanica *meadows of Kerkennah islands present high epiphyte diversity but low biomasses.*

Keys words: Posidonia oceanica*, epiphytes***,** *Bryozoa, Hydrozoa, Annelida, Tunisia.*

Introduction

The epiphyte organisms living on the *Posidonia oceanica* leaves are represented by a rich assemblage of both plant and animal species. Their distribution change at various scale both in space and time (Anonyme, 2003; Ben Mustapha *et al.*, 1999). In this survey, carried out in the Kerkennah islands (Gulf of Gabes, Tunisia), we studied the distribution of these organisms in different meadows, for leaf of different age and in different leaf position. This study provides the first data on this important component of the *Posidonia* system for this geographic area.

Study area, material and methods

The study was conducted at the Kerkennah islands (north Gulf of Gabes) (Fig. 1).

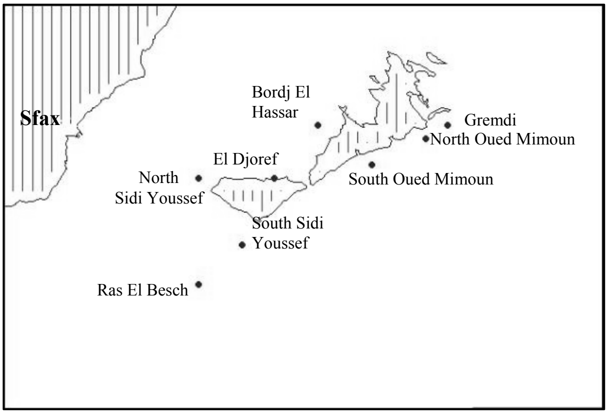

Fig. 1 - Map of the Kerkennah islands (Gulf of Gabes, Tunisia) with location of the *Posidonia oceanica* meadows studied.

Samples were taken in three periods (winter, spring and summer from December 2002 to July 2003) in different stations all located in the shallow stands of *Posidonia* meadows (depth between 0.5 - 1.75 m) (Tab. 1).

Tab. 1 - Characteristics of the studied *P. oceanica* meadows in Kerkennah islands (North Gulf of Gabes) (Classification according to Giraud, 1977)

Stations	Coordinates	Depth (m)	Shape of Meadow	Sampling season	Type of Meadow	Density n. shoots/m^2
Hassar	34°44’29’’N 11°08’59’’E	1.10 – 1.7	Tiger-shaped	Winter Spring	Type I	976
El Djoref	34°40’62’’N 11°04’15’’E	1.5	Plain-shaped	Winter Spring	Type I	1114
North Sidi Youssef	34°40’53’’N 10°57’30’’E	1 - 1.7	Plain-shaped	Spring Summer	Type I	1560
Ras El Besch	34°32’30’’N 10°57’79’’E	0.80 - 1.20	Plain-shaped	Spring Summer	Type I	1311
South Sidi Youssef	34°35’30’’N 10°01’18’’E	1 - 1.40	Plain-shaped	Spring Summer	Type I	1040
South Oued Mimoun	34°40’68’’N 11°20’72’’E	1.30 - 1.70	Tiger-shaped	Spring Summer	Type I	1448
North Oued Mimoun	34°40’78’’N 11°18’72’’E	1.20 - 1.90	Tiger-shaped	Spring Summer	Type I	1472
Gremdi	34°44’76’’N 11°20’06’’E	0.5 - 0.7	Atoll	Spring Summer	Type I	1444
Gannouch	34°10’30’’N 10°02’41’’E	1.50	Plain-shaped	Spring Summer	Type III	311

In each station 30 *Posidonia* shoots were sampled and analyzed. The numbering of leaves was done taking care to detach every leaf respect to the order of its insertion in the shoot, and therefore its age. For each epiphytic species, position and abundance were annotated in each leaf’s type, distinguishing also the basal, the intermediate, and the apical portion, as well as the internal and external leaf sides.

As a whole, 120 adult and intermediate leaves were analyzed.

Results and discussion

The macroscopic epiphytic species identified on the leaves of *Posidonia oceanica* in meadows located in the studied area are characterized by Bryozoa, Hydrozoa, Ascidians, Annelida and macroalgae. The Bryozoa are represented manly by *Lichenopora radiata*, *Electra posidoniae*, *Alcylodium* sp. and *Micropora complanata*. The Hydrozoa are represented by *Dynamena cavolinii, Obelia genicuculata* and *Monotheca* sp. The Ascidians with 2 different species, and the Annelida with only the polychaete Spirorbidae, *Spirorbis* sp.

Bryozoa: *Electra posidoniae* is the most abundant species; it occurs in all stations except the North Sidi Youssef. In winter, this species has been recorded with a high frequency in the apical and intermediate portions of the adult leaves. In spring it occupies all the leaf surface, while in summer it tends to settle on the median part of the leaf (Fig. 2).

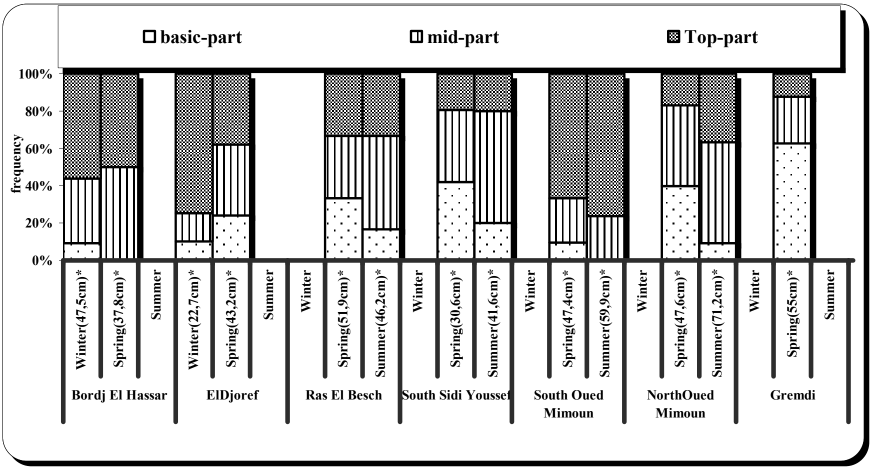

Fig. 2 - Temporal distribution of *Electra posidonia* on the leaves of *Posidonia oceanica* on different stations (*: Average length of *Posidonia* leaf)

The bryozoan *Lichenopora radiata* is the most frequent and abundant epiphytic species (Fig. 3).

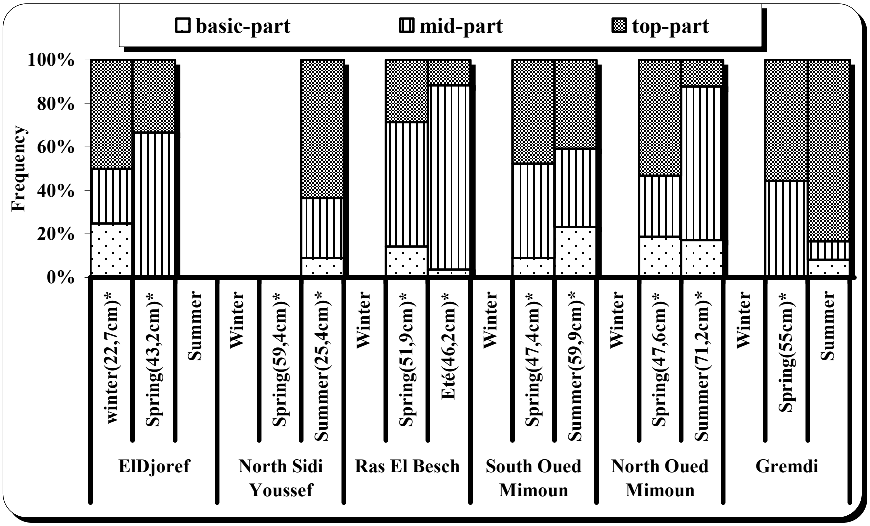

Fig. 3 - Temporal distribution of *Lichenopora radiata* on the leaves of *Posidonia oceanica* on different stations (* : Average length of *Posidonia* leaf)

Regardless of season and leaf category, this species covered the whole leaf surface.

Micropora complanata is covering all the leaf portions in all seasons, although it is more abundant in the apical parts.

Finally, *Alcyonidium* sp. occurred in all seasons and stations mainly located in the median and superior portion of the adult and intermediate leaves on both sides.

Survey established by Kocak *et al.* (2002), for epiphytic bryozoan of the seagrass meadow of *P. oceanica* in Cyprus shows these organisms more numerous on the rhizomes than on leaves.

Hydrozoa: The two most abundant species, *Dynamena cavolinii* and *Monitheca* sp. occupy all the leaf surface mainly in spring and summer.

Annelida: *Spirorbis* sp. is a polychaete living in a calcified tube, and prefers the median part of the adult leaves with a frequency up to 66.6% in summer (Fig. 4).

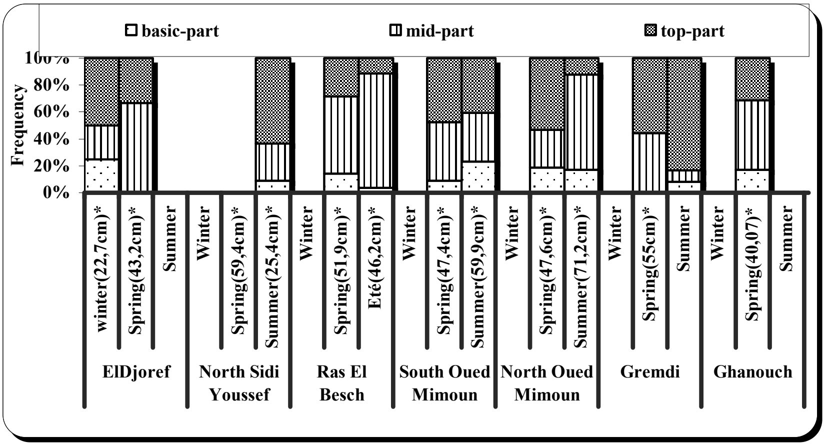

Fig. 4 - Temporal distribution of *Spirorbis* sp. on the leaves of *Posidonia oceanica* on different stations (* : Average length of *Posidonia* leaf)

Conclusions

In this study we detected no differences in the distribution of the macroepiphytic species of the leaves and the same groups are dominant in all seasons, in all stations and in the whole leaf portions examined.

The most external leaf, the oldest one, and the apical portion in particular, is colonized by all species. The epiphytic load weakens these leaves and favor their break. In Gremdi, the leaves reach the surface level, and they are more exposed to the thermal and hydrological variations of the water. In this station the epiphytes concentrated in the intermediate and basal leaf portions.

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M. Dimech, J.A. Borg, P.J. Schembri

Department of Biology, University of Malta, Msida MSD06, Malta

MOTILE MACROINVERTEBRATE ASSEMBLAGES ASSOCIATED WITH SUBMERGED *POSIDONIA OCEANICA* LITTER ACCUMULATIONS

Abstract

In the Mediterranean, seagrass leaf litter derived from Posidonia oceanica *beds constitutes an important source of detritus. Large areas with* P. oceanica *leaf litter are ubiquitous in the infralittoral, but data on the species assemblages characterising this habitat are lacking. Samples of leaf litter were collected in December 2001 from 8 stations in each of two bays on the northern coast of Malta, using a suction sampler. A total of 4794 motile macroinvertebrates comprising 43 species were recorded. Overall, diversity and evenness values were low, however, abundance values were very high. Detritivorous crustaceans were the most abundant taxon (>97 %), with amphipods having the highest abundance (81.8 %). Our results indicate that* P. oceanica *leaf litter is a distinct habitat that supports characteristic motile macroinvertebrate assemblages dominated by gammarid amphipods. Mediterranean marine* Gammarus *spp. are typically found in brackish habitats, and only infrequently occur in fully marine waters. However, no sources of brackish water were present in the vicinity of our study sites, which seems to indicate that the occurrence of* Gammarus *spp. in* P. oceanica *leaf litter accumulations may be widespread. We suggest that the* P. oceanica *leaf litter habitat supports unique macroinvertebrate assemblages composed mostly of motile detritivores and which may constitute an important link in transferring production from* P. oceanica *leaves to higher trophic levels.*

Key-words: *seagrass,* Posidonia oceanica*, leaf litter, crustaceans,* Gammarus *spp., Maltese islands.*

Introduction

Most of the primary production in seagrass ecosystems is not consumed directly by herbivores (Pergent *et al.*, 1997; Cebrián and Duarte, 1998) but is decomposed within the meadow to form litter and detritus, a proportion of which may be exported to other ecosystems (40–80%, Cebrián and Duarte, 2001; 10–20%, Mateo and Romero, 1997). Seagrass litter exported to adjacent biotopes adds complexity to the habitat, particularly when it settles on sandy bottoms. The exported drifting macrophyte litter provides an unlimited supply of food and refuge from predators to benthic invertebrates (Vetter, 1995; Norkko *et al.*, 2000), while the latter serve as a valuable food resource for higher trophic levels (Vetter, 1995). Drifting macrophyte litter can also affect the distribution (or redistribution) of fauna, since some species utilise it as a transport medium through rafting (Norkko and Bonsdorff, 1996). The dominant macrofauna in drifting macrophyte litter consists of motile macroinvertebrates (Vetter, 1995; Norkko *et al.*, 2000), mainly detritivorous and omnivorous species (Gore *et al.*, 1981). Sublittoral accumulations of drifting macrophytes may have high abundances of motile macroinvertebrates, with the highest values recorded for accumulations of kelp and surfgrass detritus off the West Coast of the USA (Vetter, 1995), and from intertidal (Norkko *et al.*, 2000) and pelagic (Ingólfsson, 1998) drift algal mats.

In the Mediterranean, few studies have addressed the motile macroinvertebrate assemblages associated with drifting macrophyte litter, despite the presence of vast quantities of such material in shallow coastal waters that originates mainly from *Posidonia oceanica* beds. As in other parts of the Mediterranean, drifting macrophyte litter accumulations occur around the Maltese Islands. Litter derived from Maltese *P. oceanica* meadows is present at different depths and on different bottom types, and represents a significant repository of biomass. The present study aimed to characterise the motile macrofaunal assemblages associated with drifting seagrass litter present in shallow waters (3-6 m) on sandy bottoms.

Material and methods

The study was conducted in two bays: Qortin Bay (14°21´N, 36° 00´E) and Armier Bay (14°21.5´N, 36° 00´E), located on the northern coast of mainland Malta (Fig. 1). Both bays have a sandy bottom in shallow waters (2–6 m) and a similar exposure to northerly and northwesterly winds. Preliminary surveys indicated that *P. oceanica* litter starts to accumulate on the sandy bottoms from late summer and persists until late spring, with very small isolated patches (~1 m^2) remaining during the summer period (Dimech, 2003). The accumulations have a thickness of 5-17 cm and consist mainly of *P. oceanica* leaves (>94% dry weight), roots and rhizomes (2% w/w) of the same seagrass, and various algae (4% w/w). In winter these accumulations may cover up to 80% of the otherwise bare sandy bottom (Dimech, 2003).

Eight replicate samples (0.05 m^2) of leaf litter, together with the associated macrophyte debris and macrofauna, were collected in December 2001 from each bay using a suction sampler. The samples were first washed on a 0.5 mm mesh sieve to remove fine sand, and then transported to the laboratory where they were fixed and stored in 10% formal saline. The samples were washed with tap water and sorted to extract the motile macroinvertebrates, which were identified and counted.

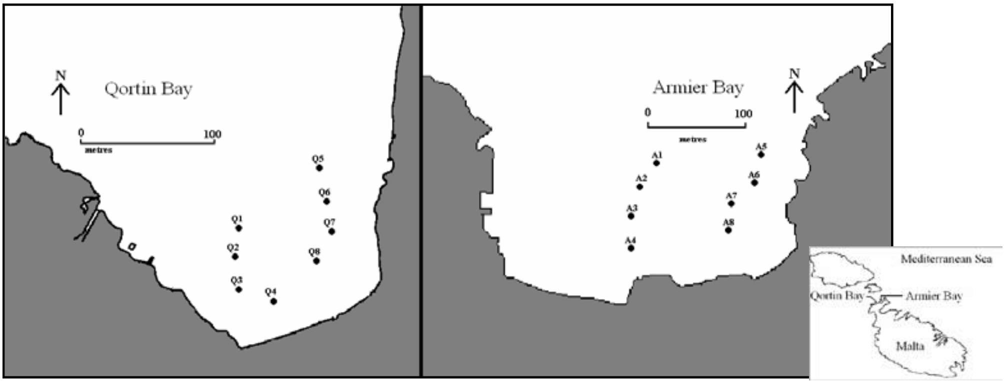

Fig. 1 - Location of Qortin Bay and Armier Bay, showing the distribution of eight replicate stations in each bay. Note the slight difference in scale.

Results

A total of 4794 individuals comprising 43 species were identified. In terms of abundance, Crustacea were most numerous (>96 % of all individuals), of which amphipods accounted for 81.8% of the total abundance. Eight species accounted for 96.9 % of the total abundance (Tab. 1). Three of the four Mediterranean marine *Gammarus* species (*G. aequicauda*, *G. subtypicus* and *G. crinicornis*) were present, however, due to the large number of juveniles and the difficulty in identifying them, individuals of the three species were grouped as '*Gammarus* spp.'

Tab. 1 - Mean total abundance of species recorded from the *P. oceanica* litter samples. (a) Amphipoda, (i) Isopoda, (p) Polychaeta.

Species	Mean Total Abundance (ind/m^2) n = 16	Standard deviation	Relative Abundance (%)
Gammarus spp. (a)	3553	3593	59.28
Atylus swammerdami (a)	836	1031	13.95
Idotea baltica (i)	520	656	8.68
Ostracoda sp.	381	576	6.36
Atylus guttatus (a)	240	413	4.01
Scolelepis sp. (p)	134	258	2.23
Idotea hectica (i)	75	98	1.25
Melita hergensis (a)	66	193	1.11
Others	188	136	3.13

Discussion

In terms of abundance, detritivorous crustaceans, mostly amphipods (81.8%), were dominant in the *P. oceanica* leaf litter from the study area, as noted in other studies on shallow water drifting macrophyte detritus habitats (Vetter, 1995; Norkko *et al.*, 2000). However, outside the Mediterranean, other crustacean groups may be dominant (e.g. Leptostraca; Vetter, 1995; Ostracoda; Norkko *et al.*, 2000). Although the mean abundance recorded in this study (5.9×10^3 ind./m^2) is much lower than that found by Vetter (1995) for kelp and surfgrass detritus (1.1×10^6 ind./m^2), abundance values from the present study are higher than those for local *P. oceanica* meadows (2×10^3 ind./m^2; Borg *et al.*, 2006). On the other hand, mean diversity and evenness values ($H' = 1.29$, $J' = 0.54$) were much lower than for local *P. oceanica* meadows ($H' = 3.45$, $J' = 0.91$; Borg *et al.*, 2006), due to dominance of amphipods (mainly *Gammarus* spp.) and isopods (mainly *Idotea baltica*) in the leaf litter. The relatively low diversity in the drifting *P. oceanica* leaf litter may be attributed to disturbance and transport of the litter by currents in the bays, as most of the litter is eventually washed ashore or transported to deeper waters. Vetter (1995) also found a low diversity in disturbed leaf litter patches and attributed these to disturbance by currents. The large proportion of relatively indigestible fibre present in *P. oceanica* may contribute since only a small number of species can consume the litter. In Vetter's (1995) study, the leaf litter was mainly composed of kelp, which is more digestible than *P. oceanica* litter. Despite their low diversity, *P. oceanica* leaf litter accumulations enhance the diversity of motile macrofauna on otherwise bare sandy bottoms that typically have an impoverished epifauna, and contribute to increase diversity in shallow coastal waters.

The leaf litter also supports unique benthic assemblages, predominant members of which are motile detritivores characteristic of this habitat but rarely found elsewhere. A number of these species are considered rare (e.g. *Idotea hectica*, Charfi-Cheikhrouha, 2000), or absent (e.g., *Gammarus aequicauda*, *Gammarus crinicornis*, *Gammarus subtypicus*; Scipione, 1998) from *P. oceanica* beds. *Gammarus* spp., the most abundant in this study, usually occur in brackish water habitats or in waters subjected to fluctuating salinity (Mancinelli and Rossi, 2002), and only rarely in fully marine environments. The dominance of this taxon in fully marine seagrass litter habitats appears to have been overlooked. Other studies in the Mediterranean (Mancinelli and Rossi, 2002) found relatively high abundances of *Gammarus* spp. (e.g. *Gammarus aequicauda*) in sediments enriched with leaf litter, which were attributed to passive dispersal of the gammarids from nearby brackish lagoons. Accumulations of *P. oceanica* leaf litter are present mostly in winter, and by summer much of the litter is either washed onshore or has decomposed, therefore, the habitat is somewhat transient. The most abundant species in the litter are not common in other habitats, and since there is evidence that colonisation of decaying drifting algae and seagrass by invertebrates is rapid (Norkko and Bonsdorff, 1996), there must be a source for the litter macrofauna. A likely candidate are the isolated pockets of leaf litter that persist during the summer period in depressions on the seabed, implying that *Gammarus* spp. and the rest of the litter macrofauna live permanently in fully marine conditions, either in large litter accumulations or in refugia. The present results demonstrate the importance of the *P. oceanica* litter accumulations – a decaying necromass that supports unique and diverse assemblages of motile macrofauna.

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A.M. Folkard

Catchment and Aquatic Processes Group, Lancaster Environment Centre, Lancaster University,
Lancaster LA1 4YB United Kingdom

MODELLING HYDRODYNAMICS AND SUSPENDED PARTICLE DISPERSION IN FRAGMENTED SEAGRASS MEADOWS

Abstract

Fragmentation of seagrass meadows is a well-known, widespread phenomenon which is caused by a wide range of physical and physiological stress mechanisms and which has important effects at both the landscape and patch scales. This paper reports and reviews our work on improving understanding of the patch-scale hydrodynamics of fragmented seagrass environments, which has focussed on Posidonia oceanica *as a test-case species. The detailed structure of the hydrodynamic field at canopy edges and within gaps in otherwise homogeneous meadows has potentially serious implications for the ecosystems supported by seagrasses. We have approached this work through the use of idealised (quasi-2D) laboratory flume simulations of seagrass meadows, which yielded maps of hydrodynamic parameters describing mean flow and turbulence fields for a range of flow conditions and seagrass configurations. From these data, we have constructed numerical models used to investigate the fate of organic and inorganic particulate matter, and thus infer ecosystem implications. Key parameters quantifying the relationship between the structure of the mean flow and turbulent fields and the patch separation are identified.*

Key-words: *hydrodynamics, seagrass, fragmentation, particulate matter, modelling, flume simulations.*

Introduction

Seagrass meadows are often fragmented. This affects meadows at many different scales. At the landscape scale, it can compromise meadow sustainability and expansion. At smaller scales, it provides a matrix of patches, edges and corridors that alter species diversity, abundance and distribution. As well as direct ecological effects, fragmentation also has hydrodynamic effects which in turn may affect ecology. At landscape scale, fragmentation alters the way in which meadows affect waves and currents. At smaller scales, it causes accelerated flows around or over patches and enhanced turbulence. These alter the transport of organic material, enact physical stresses on the plants and play a role in habitat definition.

The importance of hydrodynamics to seagrass ecology is well-established through a diverse range of studies (e.g., Fonseca *et al.*, 1982; Gambi *et al.*, 1990; Orth *et al.*, 1994; Granata *et al.*, 2001; Peterson *et al.*, 2004). The importance of meadow fragmentation to ecology is also well-established (e.g., Irlandi, 1997; Barbera-Cebrian *et al.*, 2002; Hovel, 2003; Tanner, 2003). However, there is less understanding of the effects of meadow fragmentation on hydrodynamics, and the follow-on ecological implications. Key questions in this area can be framed. Does the effect of a fragmented meadow on waves and currents depend only on the percentage coverage of the meadow, or does the spatial distribution of that coverage play a role? How does this affect seagrass meadows' ecological functions and their role in coastal protection? What information do we need to be able to predict these effects?

In addressing this topic, we identify the presence of edges – of either large expanses or small patches of seagrass – as the primary difference between continuous and fragmented meadows. These edges greatly complicate the hydrodynamics by creating non-uniform and unsteady flow structures: turbulent wakes, free shear layers and eddies. The situation is further complicated since these edges will be oriented at all angles to the incident flow and/or wave fields and have different spatial densities (edge length per unit area) within and between meadows. The work described has made some first steps towards addressing this issue using idealised configurations of flow and meadow edges in flume experiments. Further details are given in Folkard (2005), Folkard *et al.* (2006), and Maltese *et al.* (2006).

Materials and methods

The experiments considered quasi-2D flow (i.e. assuming no variation in flow conditions in the cross-flume direction) over 1D patterns (i.e. patterns which were uniform across the flume) of simulated seagrass. The simulated seagrass was constructed from polyethylene sheeting and tested to ensure that all its relevant mechanical properties were as similar as possible to natural seagrass (see Folkard, 2005). For this, comparisons were made with properties of *Posidonia oceanica*, although the experiments can be considered as generic representations of all ligulate seagrass species. Seagrass meadow patterns shown in Fig. 1 were used, encompassing “patchy” and “gappy” arrangements with different spacings between the pattern elements.

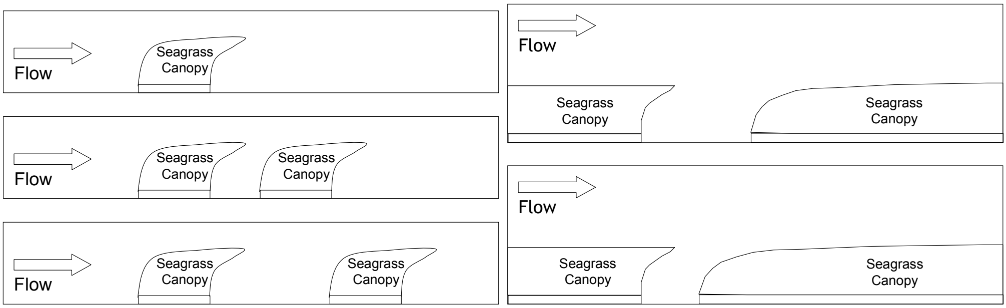

Fig. 1 - Simulated seagrass pattern configurations used in the experiments described

Flow depth was kept constant throughout all runs at 35 cm (c.f. the maximum leaf length, 50 cm). Flow speed was varied from a few cm.s^{-1}, representing typical tidal flows, to a maximum of 24 cm.s^{-1}, representing storm-driven flows. Measurements were made using an Acoustic Doppler Velocimter (ADV) at a grid of nodes separated every 10 or 20 cm horizontally and every 2 cm vertically within the central longitudinal plane of the flume. These were interpolated to provide full-field flow maps and contour maps of turbulent parameters. The maps were analysed to identify key flow processes and structures in the vicinity of the seagrass canopy edges.

Results

The mean flow vector maps identify a number of key features (Fig. 2). As expected, the flow had a rapid overflowing layer and a recirculation cell formed by the shedding of the shear layer from the top of the canopy. However, because of the presence of the overhanging canopy, this cell formed some way downstream of the canopy edge, and a stagnant region formed at the upstream end of the gap. Downstream of the recirculation cell, the flow profile gradually recovered a classical boundary layer profile. Clearly the elements of the flow structure (stagnant region, recirculation cell, transition region, recovered boundary layer profile) determine the stresses applied to the substrate and thus the rate of re-suspension in the gap. Thus the ratios of their horizontal length scales to the length of the gap are key parameters in determining the overall re-suspension budget within the gap. Our experiment indicate that a threshold occurs when the length of the recirculation cell is approximately 50% of the full length of the gap: if it is larger than this, there is no room for the boundary layer profile (which is associated with strong bed shearing forces and thus relatively high re-suspension rates) to re-form, whereas if it is smaller, the profile does re-form.

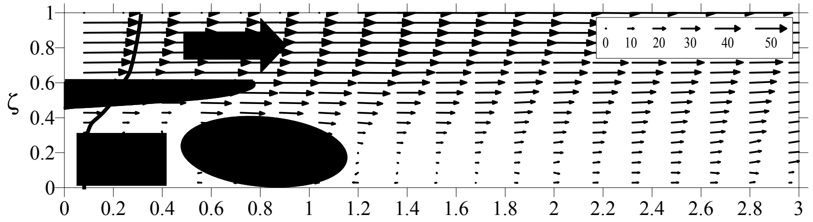

Fig. 2 - Flow field features downstream of canopy edge: rapid overflow (arrow), recirculation cell (oval), stagnant region (rectangle). Black feature to the left is the overhanging canopy.

The turbulence field was dominated by the wake downstream of the canopy edge (Fig. 3).

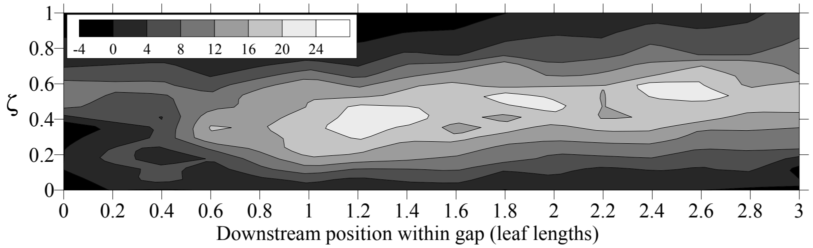

Fig. 3 - The Reynolds stress ($-\overline{u'w'}$) field downstream of the canopy edge, illustrating the turbulent wake (values shown are in cm^{2}s^{-2})

Analysis of the turbulence in the wake shows that there is a region immediately downstream of the canopy edge where turbulence production due to mean flow shearing is stronger than turbulent dissipation, and as a result turbulence intensity increases. However, in the absence of the canopy the shear layer weakens and turbulent production decreases. As a result, beyond a certain point, where turbulence intensity peaks, there is a region where dissipation is stronger than production and the turbulence decays. Thus the intensity of turbulence in a seagrass patch downstream of a canopy edge is determined by the distance from the turbulence intensity maximum in the wake to the start of the downstream patch. Our experiments show that the turbulence maximum moves further downstream as the mean flow speed decreases.

Conclusions

The results presented represent only a first step towards full understanding of the impacts of fragmentation of seagrass meadows on the hydrodynamics within them. The flow complexities caused by canopy edges are of key importance here. This work has identified the generic flow structure that occurs at these edges, and two parameters which we hypothesise play a role in determining its ecological and sedimentary impacts. These are the ratio of the recirculation cell size to the gap length, and the ratio of the gap length to the distance from the canopy edge to the wake turbulence maximum. Respectively, these play roles in characterising substrate re-suspension and the turbulence intensity in the downstream canopy. Although the lateral dimension is not considered in these experiments, in shallow water the acceleration of the flow over the seagrass is likely to be much greater than the acceleration around the side of the patch, so we may expect the flow structures caused by the former process to dominate. Thus we can reasonably expect the results obtained to have relevance in natural settings.

Future work in this area will consider variations in shoot density, leaf length and flow depth; 2D distributions of seagrass, wave action and direct measurements of particulate processes. It will transfer these flume-derived results to natural settings by measuring *in situ* hydrodynamics in tandem with ecological and sedimentary distributions. Finally, it will scale-up our small scale findings to elucidate their concerted landscape-scale implications.

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M.C. Gambi, B. Trani, M. Cigliano, V. Zupo

Laboratorio di Ecologia del Benthos – Stazione Zoologica "Anton Dohrn", Napoli (Italy)

THE "KRIGING" APPROACH TO STUDY THE RELATIONSHIPS BETWEEN *POSIDONIA OCEANICA* MEADOW STRUCTURE AND DISTRIBUTION OF ASSOCIATED FAUNA: AN EXAMPLE WITH BORER POLYCHAETES

Abstract

A small spatial scale relationship between shoot density and distribution of borer polychaetes was studied in a Posidonia oceanica *meadow off Lacco Ameno (Tyrrhenian Sea Italy). Twenty-one sampling points, at depths between 2.5 and 29 m, were analysed by means of the Kriging technique. The analysis revealed nest-like structures with shoot density increasing towards the centre. Borer frequency index (IB) showed similar nest-like structures with borer frequency increasing towards the centre, and was well matched with* P. oceanica *density 'nests'. IB values had a very similar range regardless of depth and absolute density values. The possible causes of the similar distribution patterns are discussed.*

Key-words*:* Posidonia oceanica*, polychaetes, borers, spatial structure, Kriging technique, Tyrrhenian Sea.*

Introduction

The spatial structure of *Posidonia oceanica* beds, and the scale at which it is studied, is a crucial element to evaluate both expansion/regression of the meadows and the structure of associated communities. Recent findings demonstrate that *P. oceanica* beds are characterized by high patchiness at medium and small spatial scales (Balestri *et al.,* 2003; Gobert *et al*., 2003; Borg *et al*., 2005; Kendrik *et al*. 2005; Zupo *et al*., 2006a); therefore, generalization of trends for a given biological variable can be biased if studied at a different spatial scale.

Variation in bed structure (e.g., patchiness), in shoot density and plant morphology can alter the structure and function of associated biotic communities. Although extensive literature is available on the distribution of fauna in seagrass beds in the Mediterranean, at different spatial and temporal scales (see Buia *et al.*, 2000, for a review), relatively few studies have focused on the spatial relationships between meadow structure (e.g., patchiness, plant coverage, shoot density, morphological features of the canopy) and faunal distribution and abundance (Scipione *et al*., 1996), while a single manipulative experiment, related to *Cymodocea nodosa* meadows, is available on this subject (Connolly and Butler, 1996). This aspect is critical to recognize the possible "cascade effect" in ecosystem functioning resulting from seagrass meadow regression, impact and loss. This problem is particularly relevant in *Posidonia oceanica* beds, which hosts some of the richest faunal assemblages of coastal Mediterranean ecosystems.

In this work we present a first approach to study the relationships between *P. oceanica* meadow structure and faunal abundance at a small spatial scale by means of the "Kriging" technique, considering a peculiar group of detritivores, the borer polychaetes. These organisms, in fact, are strictly associated with seagrass shoots, since they inhabit the scales (remains of the old leaves which persist on the rhizomes), and are widespread in meadows under various conditions and in different seasons (Gambi, 2002, Gambi *et al*., 2005).

Previous studies at the meadow (Gambi, 2002) and at the regional (Gambi *et al.*, 2005) scales revealed a significantly higher occurrence and frequency of these organisms in deep stands (25-28 m) characterized by a much lower shoot density than shallow stands. A significant negative correlation, although with a low coefficient value, was found between borer frequencies and shoot density, expressed both as absolute values and as the categories proposed by Pergent-Martini and Pergent (1996) according to depth (Gambi *et al.*, 2005). Therefore, a general negative correlation between polychaete borer abundance and shoot density was our initial expected pattern.

Study site, material and methods

The studied bed is the continuous meadow off Lacco Ameno (Ischia island, Gulf of Naples, Tyrrhenian Sea) where *Posidonia oceanica* extends at depths between 0.5 and 32 m, and has been extensively studied in past years (Zupo *et al.*, 2006). In this meadow, an extensive survey of shoot density and bottom morphology was performed in summer 1992, using a grid of 105 sampling points (nodes of the grid; Zupo *et al.*, 2006a). The study revealed a strong anisotropy in the shoot density structure, with various nest-like structures of density increasing towards the centre (type A) and a single nest-like structure with decreasing density towards the centre (type B), resulting in complex patterns which were closely related to the bottom topography (Zupo *et al.*, 2006a). A similar survey was repeated in May 2000 using 21 sampling points, within the 2.5 m to 29 m depth range (Fig. 1), selected on the basis of the first survey, by corresponding to the nest-like structures, to test spatial changes over time (Zupo *et al.*, 2006b). In each of the 21 grid- points, along with measurements of depth, %

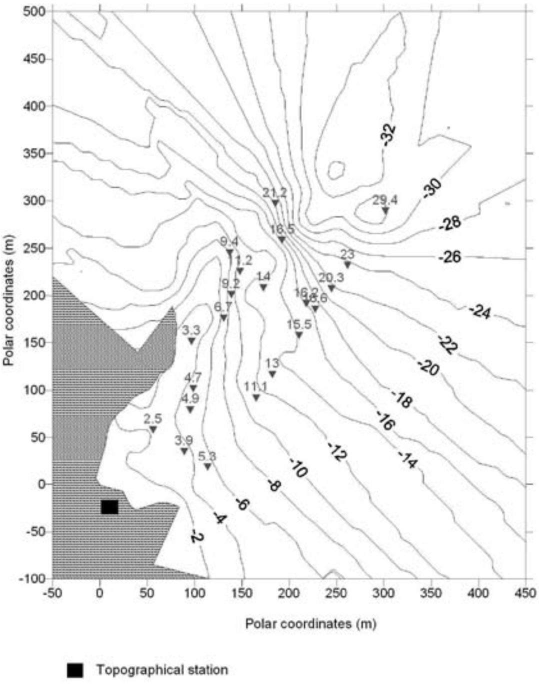

Fig. 1 - Location of the 21 grid-points sampled in the 2000 Kriging survey of the *Posidonia oceanica* bed located off Lacco Ameno. Numbers indicate depths in meters.

bed coverage and shoot density (two replicates using a 40 x 40 cm quadrat) was evaluated, and from 18 to 25 (mean 20) orthotropic (vertical) rhizomes were sampled. The analyses included plant morphology and lepidochronology, genetic structure (Migliaccio *et al.*, 2005) and occurrence of borers. For each sampling point, the frequency of borers, expressed as IB index, was calculated as the % of rhizomes with animals over the total rhizome sampled (Gambi, 2002). The IB values were analysed by the Kriging technique using Surfer ver. 8.01 software (Golden Software; Zupo *et al.*, 2006b). Kriging is a geostatistical technique that allows for stochastic reconstruction of natural trends based on variograms. It can also model theoretical spatial grids based on a reduced set of experimental data, and permits testing and modelling of the spatial structure of natural objects or processes.

Results and discussion

The shoot density pattern in the year 2000 was generally consistent with that from the 1992 survey, although the actual values of density measured in 2000 were generally lower than in 1992, especially in the shallow stand (Zupo *et al.*, 2006b). In both surveys, shoot density decreased with increasing depth (Table 1,

Tab. 1 - Main variables measured in the 21 grid sampling points indicated in Fig. 1.

sample points	X coord.	Y coord.	depth (m)	n. of shoots sampled	mean density n. shoot m^2	n. borer polychaetes	polychaete IB index (%)
I-7	56.07	57.88	2.5	18	631.2	1	5.5
G-17	96.58	151.47	3.3	19	468.7	2	10.5
O-7	88.95	35.1	3.9	20	581.2	10	50
L-13	98.46	101.51	4.7	24	446.9	4	12.5
M-11	95.29	79.37	4.9	25	546.9	6	24
R-7	113.61	18.02	5.3	20	306.2	4	10
G-20	113.67	176.13	6.7	19	334.4	7	26.3
G-23	130.76	200.79	9.2	21	321.8	6	23.8
E-27	137.1	245.06	9.4	18	200	2	11.1
R-16	164.87	91.99	11.1	20	356.2	4	10
G-26	147.84	225.45	11.2	20	343.7	10	50
R-19	181.95	116.65	13	20	190.6	6	20
L-26	172.5	208.36	14	20	475	8	25
R-24	210.43	157.75	15.5	24	331.2	15	46
O-26	197.16	191.28	16.2	19	137.5	5	21
G-30	170.62	258.33	16.5	20	275	8	35
P-26	205.38	185.58	16.6	22	343.7	7	27.3
R-30	244.6	207.07	20.3	22	178.1	8	32
F-34	185.19	296.9	21.2	21	132.5	9	23.8
R-33	261.69	231.73	23	20	200	5	25
R-40	301.56	289.26	29.4	19	87.5	3	10.5

Fig. 2a). The nest-like structures were still present, although with a lower resolution, due to the reduced number of grid points considered (21 *vs* 105). However, in the 2000 survey, only nest-like structures of the A type, characterized by shoot density increasing towards the centre, were recorded.

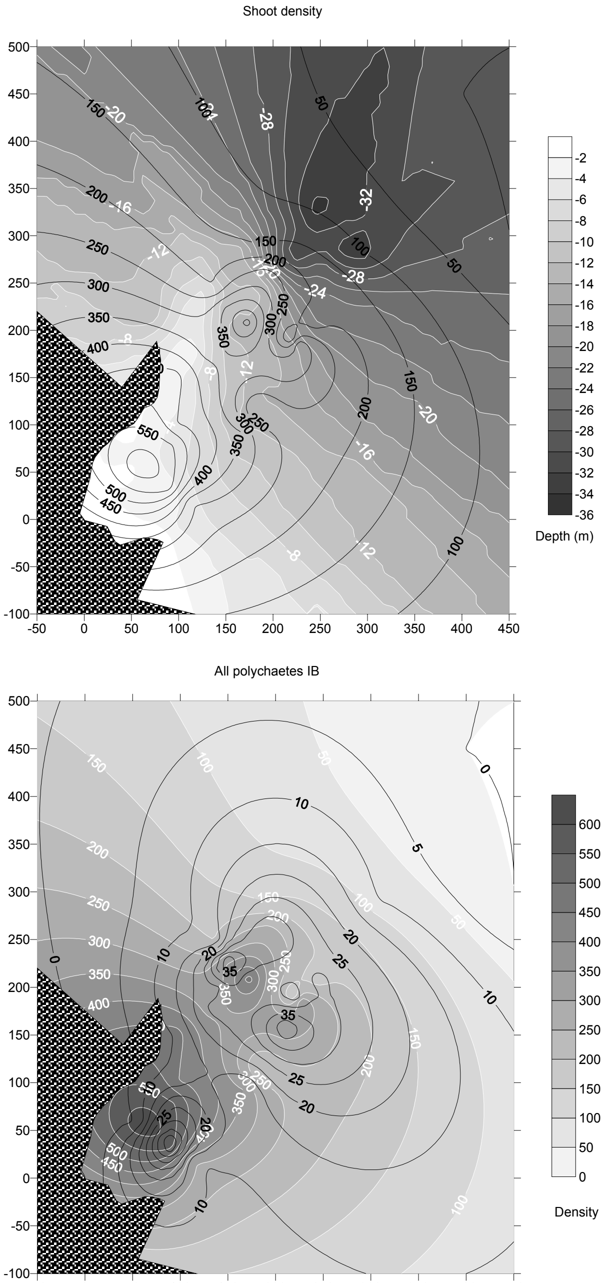

Fig. 2 - a) Isolines of distribution of shoot density *vs* depth (grey pattern). b) Isolines of borer frequency (IB index) *vs* shoot density (grey pattern).

The borer analysis revealed the occurrence of polychaetes in all the sampling points with a total of 130 specimens belonging to 4 species of Eunicidae: *Lysidice collaris* (65%), *L. ninetta* (18%), *Nematonereis unicornis* and *Marphysa fallax* (3.8% and 2.3%, respectively).

The distribution of their frequencies (IB index) revealed the presence of nest-like structures, with values increasing towards the centre, corresponding quite well to the same nest-like pattern of shoot density, except for a slight divergence in the shallow stand (Fig. 2b). Borer frequency values were quite comparable in each nest-like structure, regardless of depth or absolute shoot density, although they were slightly higher in deep nest-like structures compared to the shallow one (max. IB 35% in the centre of the shallow stand and 49% in the centre of the deep stand) (Fig. 2b). This finding suggests a lack of direct correlation between absolute values of shoot density and borer frequency.

The general nest-like structures of the studied *Posidonia* bed off Lacco Ameno are conserved over time (1992 *vs* 2000). This first approach demonstrates that the small scale distribution of polychaete borers in the *P. oceanica* meadow off Lacco Ameno is not uniform, but is organised in a complex spatial structure which is consistent with that observed for shoot density. Possible factors affecting this pattern may involve biotic aspects, such as reduced competition among species and individuals, increased resource availability or other ecological processes.

The spatial pattern exhibited by borer polychaetes, studied at this scale, seems to contrast with what has previously been observed at a different spatial scale (along depth in single meadows and along Sardinia coast), where polychaetes were more frequent in deep stands having lower shoot densities. The contrasting results, when comparing different spatial scales, stress once again the importance of this factor, but could be biased by the confounding effect between shoot density and other factors related to depth, which may favour an increase of polychaete frequency at a larger scale.

Future perspective of our work will be focused at repeating the survey in time, increasing the number of samples in the grid, and testing the patterns in meadows at different depths and with different structure (patchy *vs* continuous) as well as using other model organisms showing different relations with the host plant (e.g., vagile or sessile epiphytes).

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E. Giovannetti, M. Montefalcone, C.N. Bianchi, C. Morri, G. Albertelli

DipTeRis, Dipartimento per lo Studio del Territorio e delle sue Risorse
Università di Genova, Corso Europa, 26-16132 Genova, Italia

STRUCTURAL VARIABILITY OF THE EPIPHYTIC COMMUNITY IN A *POSIDONIA OCEANICA* MEADOW (LIGURIAN SEA, NW MEDITERRANEAN)

Abstract

Several factors influence the epiphytic assemblage of Posidonia oceanica *(L.) Delile (Monocotyledones, Posidoniaceae) leaves. The aim of this study was to investigate the epiphytic community structure in the* P. oceanica *meadow of Prelo cove (Ligurian Sea, NW Mediterranean) along a yearly cycle and a depth gradient. Sampling was carried out monthly from September 2003 to August 2004 at three different depths: 4, 9 and 14 m. Each month, 10 randomly selected* P. oceanica *shoots were collected by SCUBA diving in each station. Percentage cover of the most representative taxa was estimated and subjected to multivariate analysis in order to detect seasonal changes in the epiphytic assemblage along the bathymetric gradient. In all stations, the epiphytic assemblage exhibited cyclic variability, and two major phases were distinguished: an autumnal one dominated by* Hydrolithon-Pneophyllum *spp, and a spring-early summer one in which the algae were flanked by* Electra posidoniae. *A poorer but more various assemblage, with the appearance of the erect hydrozoans and bryozoans, was found in the deep station.*

Key-words**:** Posidonia oceanica*, epiphytic assemblage, depth gradient, seasonal cycle, Mediterranean Sea.*

Introduction

The most important seagrass in the Mediterranean Sea is *Posidonia oceanica* (L.) Delile, an endemic species that forms wide meadows. This plant hosts on its leaves and rizhomes a characteristic epiphytic community. Several factors influence the epiphytic assemblage, such as the cyclic phenology of the host plant, hydrodynamic forces, light intensity and anthropogenic disturbances (Mazzella *et al.*, 1989; Dalla Via *et al.*, 1998; Piazzi *et al.*, 2004; Montefalcone *et al.*, 2006).

In this work the epiphytic community structure of *P. oceanica* leaves was investigated in a yearly cycle and along a depth gradient in order to describe its spatial and temporal variation.

Materials and methods

The study was carried out in the *Posidonia oceanica* meadow of Prelo cove (Ligurian Sea, NW Mediterranean). Along a depth transect (directed perpendicularly to the shoreline, 270°N), three stations were identified: 4 m (44°20’12” N, 009°13’33” E), 9 m (44°20’11” N, 009°13’36” E) and 14 m (44°20’11” N, 009°13’39” E), where 10 randomly selected *P. oceanica* shoots were collected by SCUBA diving monthly, from September 2003 to August 2004 (December data are missing). In the laboratory both sides of each *P. oceanica* leaf blade were analysed using a binocular stereomicroscope to estimate the percentage cover of the following taxa: *Hydrolithon-Pneophyllum* spp., *Myrionema orbiculare*, *Giraudia sphacelarioides*, *Electra posidoniae*, *Aetea truncata*, *Fenestrulina johannae*, *Plumularia obliqua*, *Sertularia perpusilla*. All the other organisms were grouped in: Other

Bryozoans, Serpulids, Other Animals and Other Algae. The cover data per shoot were analysed with multivariate techniques (ANOSIM, MDS, SIMPER) in order to detect spatial and temporal variability of the epiphytic community structure.

Results

Two-way crossed analysis ANOSIM test revealed clear differences in the epiphytic community structure among depths (R = 0.456; P = 0.1 %) and months (R = 0.692; P = 0.1 %); the month factor determined the major dissimilarity. The MDS ordination showed, at three depths, a cyclic seasonal variability of the epiphytic community structure (Fig. 1). The vicinity of the ending point (8= August 2004) to the starting point (9= September 2003) was greater at 14 m than at the shallow and the intermediate stations.

Moreover the MDS ordination showed, at all stations, differences in dispersion (variability) of the epiphytic assemblage in each month, with the greatest dispersion observed in the autumn-winter months.

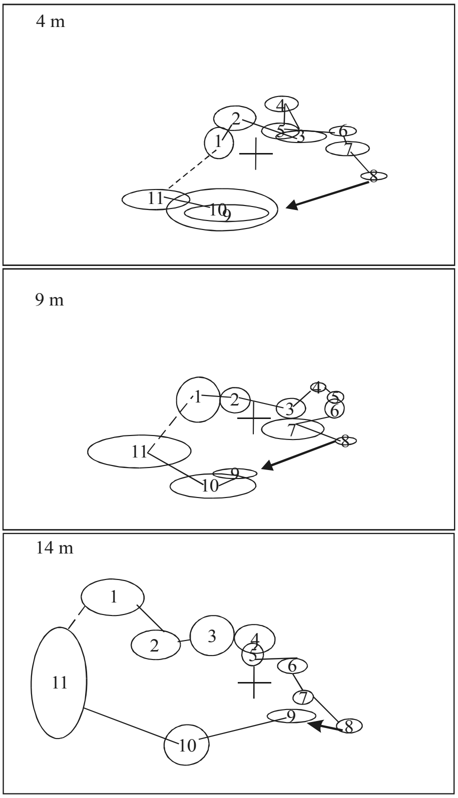

Fig. 1 - MDS ordination comparing the epiphytic community structure at the three stations (4 m, 9 m, 14 m) along a yearly cycle. The three depths have been plotted separately (Stress = 0.14). The numbers indicate the months: from September 2003 (9) to August 2004 (8). December data are missing (dashed lines). The centre of the ellipses corresponds to monthly means, ellipses depict 95 % confidence intervals. Arrows indicate the tendency to close the cycle. The cross indicates the centre of the axes.

SIMPER, applying the usual cut-off of a cumulative % similarity of 80 %, identified a total of 8 taxa as important in characterizing the epiphytic community structure (Fig. 2). The remaining 4 taxa (namely *Myrionema orbiculare*, *Giraudia sphacelarioides*, *Fenestrulina johannae* and Other Algae) played a non-significant role.

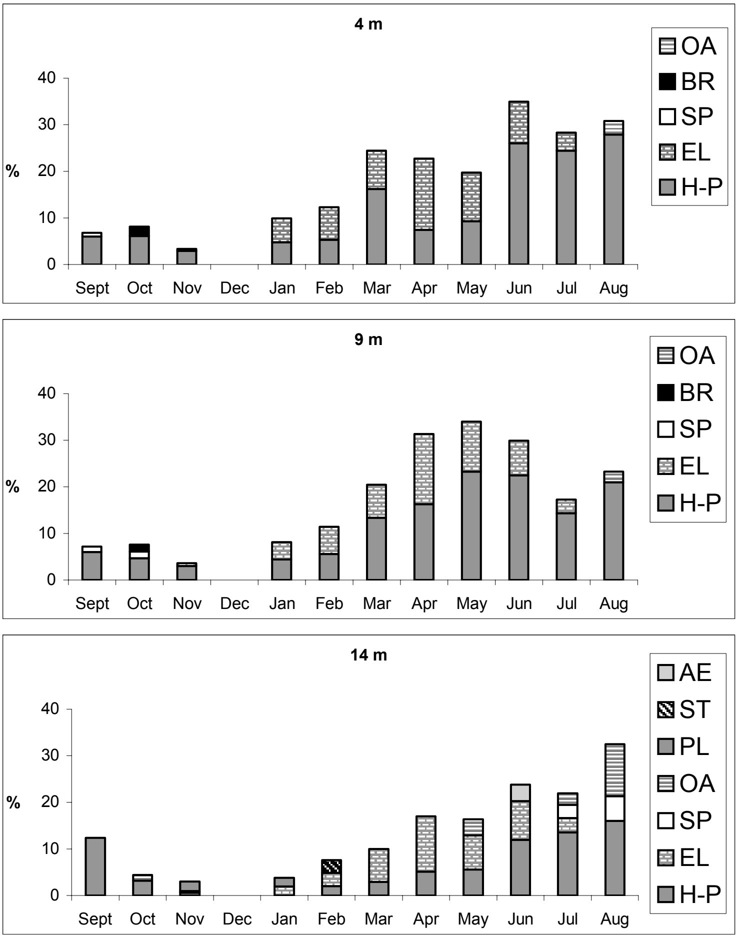

Fig. 2 - The mean percentage cover of the major epiphytes, determined through SIMPER, along a yearly cycle at three depths (4 m, 9 m, 14 m). H-P = *Hydrolithon-Pneophyllum* spp, EL = *Electra posidoniae*, SP = Serpulids, BR = Other Bryozoans, OA = Other Animals, PL = *Plumularia obliqua*, ST = *Sertularia perpusilla,* AE = *Aetea truncata*.

Hydrolithon-Pneophyllum spp., the main epiphytes at all stations, were present all year round. Apart the autumn months, these epiphytes were flanked by *Electra posidoniae* that, sometimes, exhibited the major percentage cover. At the deep station a poorer but more various community was found than at the shallow and the intermediate stations, with also the appearance of *Aetea truncata*, *Sertularia perpusilla*, and *Plumularia obliqua*.

Discussion

The epiphytic community structure was mainly influenced by seasonal variability. The great environmental variability at the two shallower stations was reflected by the more open seasonal cycles compared with the one at the deep station, implying in turn a colonisation delay between shallow and deep meadow (Mazzella *et al.*, 1986). Possible relationships with size and ages of leaves remain to be investigated.

The depth factor as compared to the month factor had a lower influence on the epiphytic assemblage, because of the small bathymetric gap among the stations. The appearance of erect hydrozoans and bryozoans at the deep station should be correlated to the lower water movement there as compared to the shallower stations (Piraino and Morri, 1990; Balduzzi *et al.*, 1991) and to the lesser development of *Hydrolithon-Pneophyllum* spp (Giovannetti, 2004), leaving greater space to other epiphytes.

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I. GUALA[1-2], S. SIMEONE[1-3], M.C. BUIA[2], S. FLAGELLA[2], M. BAROLI[1], G. DE FALCO[1]

[1]IMC – International Marine Centre, Loc. Sa Mardini, 09072 Torregrande (Oristano, Italy).
[2]Stazione Zoologica 'A. Dohrn', Laboratorio di Ecologia del benthos, 80077 Ischia (Naples, Italy).
[3]Dip.to Ecologia e Sviluppo Economico Sostenibile, Università della Tuscia, Viterbo (Italy).

POSIDONIA OCEANICA 'BANQUETTE' REMOVAL: ENVIRONMENTAL IMPACT AND MANAGEMENT IMPLICATIONS

Abstract

The removal of beach-cast Posidonia oceanica *seagrass litter, called 'banquettes', was analyzed on the island of Sardinia (western Mediterranean) in order to quantify this practice on a broad scale, to evaluate the potential impacts on the beach geomorphology and the ecological implications for coastal ecosystems. 'Banquette' removal resulted to be a widespread practice applied on 44 beaches (out of 116), along 114 km of shoreline out of the 289 km analyzed; in the year 2004 the total amount removed was 106,180 m*^{3}*, mainly in low energy beaches. Meadow leaf production was assessed in 5 localities which collectively account for about 70% of* P. oceanica *removed from Sardinian beaches; the loss of biomass due to the removal varied between 1.8 and 14.9% of meadow production. The main consequences of leaf material removal are the loss of sediment and the permanent depletion of biogenic elements from the shore. Management measures are suggested in order to minimize the possible effects on the dynamics of shoreline and the growth in front of the meadows.*

Key-words: Posidonia oceanica*, 'banquettes', Sardinia, beach cleaning, leaf production.*

Introduction

Seagrass meadows represent extremely productive systems in coastal areas all over the world (Buia *et al.*, 2000; Duarte, 2002). Most of the production is due to the aboveground compartment (i.e. leaves) (Romero *et al.*, 1992; Pergent-Martini *et al.*, 1994) of which only a small amount is consumed *in situ*; most of the leaf material becomes litter, that can be decomposed within the meadow, exported to other ecosystems or which accumulates on adjacent shorelines (Walker *et al.*, 2001).

Posidonia oceanica (L.) Delile (Potamogetonaceae) loses leaves mainly in autumn (Romero *et al.*, 1992; Chessa *et al.*, 2000) and beach-cast litter can be found in coastal areas where extensive seagrass meadows occur, forming deposits known as 'banquettes' up to 2 m in height (Boudouresque and Meinesz, 1982). 'Banquettes' are often removed because they are believed to reduce the touristic value of beaches (Mateo *et al.*, 2003; Duarte, 2004).

'Banquettes' may affect beach profile trapping high amounts of sediment and reducing its movement (Chessa *et al.*, 2000); as a consequence 'banquette' removal could influence the beach sediment budget. On the other hand their removal could play an important role in the nutrient budget of the meadows, as the leaf litter is the main source of biogenic elements (Romero *et al.*, 1992).

In this work, removal of *P. oceanica* 'banquettes' and related management practices (i.e., frequency and removal techniques) were quantified at a regional scale on the island of Sardinia (Italy, Western Mediterranean). The relationship between 'banquette' removal and beach characteristics, as well as the amount of sediment subtracted from beaches during the cleaning operations, were estimated in order to evaluate the impact of ‘banquette’ removal on the beach sediment budget. Moreover, the mass balance between meadow leaf production and the amount of ‘banquettes’ removed were evaluated for five localities which together accounted for about 70% of the leaf material removed from Sardinian beaches. The nutrient export represented by the removal of ‘banquettes’ was assessed in order to evaluate the permanent loss of carbon, nitrogen and phosphorous for *P. oceanica* meadows and coastal ecosystems.

Materials and methods

Sardinia region has a coastal length of 1,896 km (Fig. 1) of which 289 km, corresponding to 116 beaches, were investigated. Data on deposition and removal of ‘banquettes’ were collected from the technical services departments of coastal municipalities by means of a questionnaire.

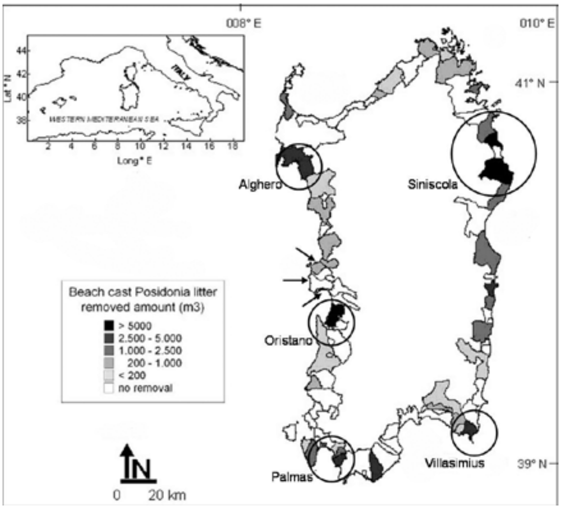

Fig. 1 - Map of Sardinia region showing the amounts of beach-cast *Posidonia* litter removed. Arrows indicate the three experimental sites used to estimate the sediment content in ‘banquettes’; circles indicate the five locations where meadow primary production was assessed .

The amount of removed sediment trapped in the ‘banquettes’ was estimated in three localities on the western Sardinian coast. ‘Banquette’ samples were collected using a box (20 x 20 x 20 cm) and the sediment was separated from the leaves by wet sieving and then weighed.

Multivariate Factor Analysis (Dal Cin and Simeoni, 1996) was applied on morphodynamic variables (Atzeni *et al.*, 2004) and ‘banquette’ removal data from 30 beaches (Table 1).

Meadow shoot density was calculated for 15 sites in the neighbourhood of the five selected localities (Fig. 1) and 30 orthotropic rhizomes were sampled for lepidochronological analysis. Primary leaf production per m^2 was calculated according to Pergent-Martini *et al.* (1994). Meadow production was calculated multiplying leaf production and meadow surface area obtained in the framework of the “Mappatura delle prateria di *Posidonia oceanica* lungo le coste della Sardegna e delle piccole isole circostanti” (Buia M.C., unpublished data).

Results

In 2004 'banquettes' were cleared from 44 of the 116 beaches studied and the total removed material amounted to 106,180 m^3. The frequency of removal was generally once a year (26 beaches out of 44); 8 beaches were cleared twice and 10 three or more times a year. The removal operations were generally carried out using heavy machinery such as bulldozers and excavators (25 beaches). Removal was carried out by hand on 6 beaches and by specialised beach-cleaning machines on 13 beaches.

The sediment content in the 'banquette' samples showed a normal distribution with a mean value of 68.1 kg m^{-3} (C.I. ± 95% 50.6 – 85.7 kg m^{-3}; n= 50). From the Factor Analysis, three factors explained 71.9% of total variance (Table 1). Factor 1 (38.8%) grouped energy variables with those related to 'banquette' removal, highlighting that low energy beaches had a higher amount of removed 'banquettes' for unit beach length. Factor 2 (16.5%) grouped variables related to beach morphology and Factor 3 (16.6%) grouped variables related to sediment texture.

Tab. 1 - Score coefficient and explained variance resulting from Factor Analysis of beach parameters. Bold coefficients are significant.

Variable		Factor 1	Factor 2	Factor 3
Energy variables				
$\mathbf{E_F}$ - Mean energy flux	GN m m^{-1}	**0.86**	0.19	0.02
$\mathbf{E_L}$ - Longshore Energy (from left direction)	W m^{-1}	**0.81**	0.17	0.05
$\mathbf{E_R}$ - Longshore Energy (from right direction)	W m^{-1}	**0.66**	0.31	-0.25
$\mathbf{E_N}$ - Net energy flux	W m^{-1}	**0.77**	0.13	0.14
Beach morphology variables				
$\mathbf{B_L}$ - Beach lengths	m	0.22	-0.04	**0.81**
$\mathbf{B_W}$ - Backshore width	m	0.01	-0.01	**0.87**
Sediment texture variables				
$\mathbf{S_E}$ - Emerged beach sediment grain size	mm	0.04	**0.84**	-0.16
$\mathbf{S_S}$ - Submerged beach sediment grains size	mm	0.15	**0.86**	0.12
'Banquette' removal variables				
$\mathbf{R_V}$ - Removed volume per beach length unit	m^3 m^{-1}	**-0.83**	0.13	-0.18
$\mathbf{R_S}$ - Average decrease of emerged beach elevation	mm	**-0.83**	0.11	-0.30
Explained variance (Total 71.9%)		**38.8%**	**16.5%**	**16.6%**

Leaf production ranged from 226.1 g DW m^{-2} $year^{-1}$ in the Gulf of Palmas to 528.0 g DW m^{-2} $year^{-1}$ at Villasimius with values comparable to those recorded for other Mediterranean areas (Pergent-Martini *et al*., 1994). Beach and meadow features, litter removed and carbon and nutrient losses for the 5 locations studied are given in Table 2.

Conclusion

'Banquettes' were removed from 40% of the analyzed beaches, indicating that this practice is common along the coast of Sardinia.

The findings of this study allow a preliminary evaluation of the environmental impact of 'banquette' removal on coastal geomorphology. 'Banquette' removal is mainly carried out on low energy beaches. ‘Banquette clearing’ and the concurrent sediment removal may lead to substantial changes in beach morphology, including possible shore erosion following storm events. Post-storm beach recovery on low energy beaches occurs at a slow rate (Jackson *et al.*, 2002) and consequently the impact of ‘banquette’ removal before a storm event could have an effect on beach morphology for prolonged period.

In addition, ‘banquettes’ represent a temporary sink of biogenic elements for the seagrass ecosystems (Mateo *et al.*, 2003), and their removal causes a permanent loss of C, N and P. Nutrient depletion was extremely variable among five locations studied. The highest N and P losses (respectively 2.3-5.4% and 0.6-1.2% of the annual requirement of the plant as estimated by Romero *et al.*, 1992; Mateo and Romero, 1997; Gacia *et al.*, 2002) was found at Villasimius. The relevance of this nutrient loss should be investigated further.

In conclusion, the removal of ‘banquettes’ during winter and spring should be discouraged to avoid shore erosion after storm events. Removal during summer period should be subject to an Environmental Impact Assessment procedure, to minimize the impact of removal (i.e. avoiding heavy machineries) and to suggest possible mitigation measures.

Tab. 2 - Beach and meadow features in the studied localities.

Location		**Alghero**	**Oristano**	**Palmas**	**Siniscola**	**Villasimius**
Covered beach length	km	2.0	0.9	6.0	8.6	0.7
Mean meadow density	shoots m^{-2}	302.1	311.3	241.0	314.6	465.4
Meadow surface	km^2	10.3	85.1	75.5	59.0	4.3
Annual leaf production	g DW $m^{-2}y^{-1}$	253.9	325.4	226.1	231.7	528.0
	tons DW $meadow^{-1}y^{-1}$	2605.1	27701.5	17077.2	13671.1	2281.1
Litter removed	tons DW	171.9	586.1	304.8	2031.9	156.3
Loss of biomass	% of leaf production	6.6	2.1	1.8	14.9	6.9
Loss of biogenic elements	kg DW C $km^{-1}y^{-1}$	33612.1	254637.3	19861.7	92380.0	94019.9
	kg DW N $km^{-1}y^{-1}$	679.1	5144.8	401.3	1866.5	1899.6
	kg DW P $km^{-1}y^{-1}$	18.1	136.8	10.7	49.6	50.5

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M. LORENTI

Laboratorio di Ecologia del Benthos, Stazione Zoologica 'A. Dohrn' di Napoli,
Punta S. Pietro, 80077 Ischia (Naples), Italy

ISOPOD CRUSTACEANS INSIDE MEDITERRANEAN SEAGRASSES: BORERS, MINERS AND INTRUDERS

Abstract

The presence of isopods burrowing into dead sheaths of Posidonia oceanica *and belonging to a species in the family Limnoriidae* (Limnoria mazzellae) *has been recorded mostly around Italian coasts. I here review a number of unpublished records from several Mediterranean locations. The occurrence of another limnoriid species burrowing into living leaves of* Cymodocea nodosa *is also reported. Among cryptic invertebrates inhabiting burrows made by both limnoriids and polychaetes inside* P. oceanica *sheaths, isopods of probable exotic origin belonging to the anthurid genus* Mesanthura*, a new record for the Western Mediterranean Sea, have been collected near the harbour of Taranto (Italy). The role of isopods living in tissues of Mediterranean seagrasses is discussed.*

Key-words*: Isopoda, Limnoriidae, seagrass borers, Mediterranean Sea.*

Introduction

Isopod crustaceans constitute morphologically and functionally diversified taxocoenes within mesofauna associated with seagrasses. A number of species belonging to the family Limnoriidae form part of the specialised guild of small animals burrowing into seagrass tissues (Gambi *et al.*, 2003). The presence of isopod borers has been systematically recorded from inside dead leaf sheaths (scales) that are still attached to the stem of the Mediterranean endemic seagrass *Posidonia oceanica* since the mid-1990s. Examined specimens were eventually assigned to the new species *Limnoria mazzellae,* described by Cookson and Lorenti (2001), which seems to date to be strictly associated with *P. oceanica*. All extant published records of *L. mazzellae* are from Italian insular and peninsular coasts (e.g. Gambi *et al.*, 1997; Cookson and Lorenti, 2001; Di Maida *et al.*, 2003). I here report the finding of new material consisting of both animals and burrowed sheaths from a number of collections of *P. oceanica* shoots around the Mediterranean Sea. Empty burrows made by polychaete and isopod borers in *P. oceanica* dead sheaths are often occupied by small cryptic invertebrates such as non-borer polychaetes, sipunculids, tanaids and harpacticoid copepods. The presence of an interesting species of anthurid isopod within this opportunistic assemblage is here reported.

Another category of isopod burrowers, described from Australia and originally designated as "miners" by Brearley and Walker (1995) based on their functional similarity to terrestrial leaf mining insects, includes forms which excavate long linear burrows into seagrass leaves. Traces of linear burrowing into leaves of the other dominant Mediterranean seagrass *Cymodocea nodosa* were observed at two locations around the island of Ischia (Gulf of Naples, Italy) and are here attributed to the activity of limnoriid isopods.

Material and methods

Collections of *P. oceanica* stems performed between 2000 and 2004 from Turkey (Gulf of Edremit; M. De Stefano *legit*), Malta (Cirkewwa; J. A. Borg *legit*), Italy (Vada, Tuscany; L. Piazzi *legit* and San Pietro Island, Taranto; G. Costantino *legit*) and Spain (Medas Islands; M.A. Mateo *legit*) were examined for the presence of isopods in dead sheaths, or of burrowing traces attributable to them. The index of traces (IT, % of stems bearing burrowing traces) and the index of borers (IB, % of stems hosting animals) were then calculated (Gambi *et al.*, 2000).

Shoots of *C. nodosa* bearing traces of burrowing along the leaf axis were collected at two locations on the north-eastern coast of the island of Ischia (Gulf of Naples, Italy). Of the limnoriid specimens taken from burrows, two were kept in aquaria in order to observe their feeding behaviour. A first attempt to quantify the proportion of shoots infested by limnoriids was made by collecting three cores (diameter 21.5 cm) within the *C. nodosa* meadow at one location (Castello d'Ischia); shoot counts within each core were reported per square meter.

Results

New records of *L. mazzellae* and of traces attributable to the species are reported in Tab. 1. In samples from Vada and Medas Is. only traces attributable to *L. mazzellae* were found.

Tab. 1 - Records of *L. mazzellae* and of traces attributable to the species. IT = index of traces. IB = index of borer occurrence.

	No. of stems examined	IT	IB
Turkey (Gulf of Edremit, 5 m depth)	18	22 %	11 %
Malta (Cirkewwa, 15 to 20 m depth)	43	14 %	9 %
Italy (Vada, Tuscany, 15 m depth)	162	32 %	-
Spain (Medas Islands, 10 m depth)	63	14 %	-

In *P. oceanica* stems collected off the island of San Pietro, near the offshore limit of the harbour of Taranto (Ionian Sea, Italy), two isopods assignable to the genus *Mesanthura* Barnard (Isopoda: Anthuridea) were found inside the empty burrows made by eunicid polychaetes into dead sheaths.

Tab. 2 - Estimates of infestation by *Limnoria* sp. in a *C. nodosa* stand. IT = index of traces.

	Estimated shoot density (shoots m^{-2})	IT	Estimated no. of infested shoots m^{-2}
Core 1	935	12 %	110
Core 2	1017	5 %	55
Core 3	578	52 %	302

Limnoriids from the leaves of *C. nodosa* were found to belong to a possibly undescribed *Limnoria* species, henceforth designated as *Limnoria* sp. They excavate linear burrows inside the leaf blade tissue which often reach leaf bases. As observed in the laboratory, *Limnoria* sp. actively grazed on living leaf tissue. A first estimate of grazing speed yielded a mean value of ca. 2 mm per day. Sampling of *C. nodosa* shoots at Castello d'Ischia yielded the results reported in Tab. 2.

Discussion

The new records of *L. mazzellae* support the close association between this species and *P. oceanica* over most of the longitudinal distribution range of the seagrass. It is highly probable that more surveys may fill many gaps in the distribution map of *L. mazzellae*. Limnoriids found in *C. nodosa* belong to a species different from *L. mazzellae*. They show many similarities both in morphology and behaviour with *Limnoria simulata*, a burrower in tissues of the seagrass *Thalassia testudinum* in the Caribbean (Gambi *et al.*, 2003). It seems unlikely that their presence is a local phenomenon restricted to the island of Ischia. Host specificity would occur for both species, an issue of particular interest in marine plant-animal interaction.

Limnoriids are typical mesograzers in that search for food and shelter are combined in their behaviour. They play a possibly important role as direct consumers of either decaying or living tissues, as has been shown with Australian and Caribbean species. *L. mazzellae* can consume fresh detrital tissue (debris) in proportion up to ca. 11 % of the whole sheath mass (Gambi *et al.*, 2000). The association of limnoriid isopods with *C. nodosa*, if confirmed on a wider distribution range, may have important implications for the ecology of this seagrass species, also in view of the relatively high proportion of shoots which result to be infested on preliminary estimate. While the consumption of living tissue has only exceptionally been recorded for *L. mazzellae*, grazing of living leaves is actively performed by *Limnoria* sp. It has to be determined whether the consumption of living tissue can impair the growth of grazed *Cymodocea* leaves.

Isopods are also components of the assemblage which exploits *P. oceanica* sheaths as a biotic cryptic habitat. Interestingly, the same species of *Mesanthura* (still undetermined) which has been found in stems from the island of San Pietro has been recently recorded from the fouling community in the harbours of Salerno and Taranto (Southern Italy) (Lorenti, pers. obs.). A number of features are suggestive of an exotic origin of the *taxon* found, also in view of the lack of previous records of the genus *Mesanthura* from Italian waters, and, in general, from the Western Mediterranean Sea. This raises questions about the ecological role of cryptic biotic shelters, including dead sheaths burrows, in providing habitats for introduced species.

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K. Mezali[1-2], V. Zupo[3], P. Francour[4]

[1]Département des Sciences de la Mer et des Ressources Halieutiques, Faculté des Sciences
Université de Mostaganem. Rue Hocine Hamadou. BP 300 Mostaganem (27000) (Algeria).
[2]Faculté des Sciences Biologiques, Université des Sciences et de la Technologie Houari Boumediene,
BP 32 El Alia, Bab Ezzouar / Alger (16000) (Algeria).
[3]Laboratorio di Ecologia del Benthos – Stazione Zoologica "A. Dohrn" (Naples, Italy).
[4] Laboratoire environnement marin littoral. Université de Nice- Sophia Antipolis, Faculté des Sciences,
Parc Valrose, 061108 Nice cedex 2 (France),

POPULATION DYNAMICS OF *HOLOTHURIA (HOLOTHURIA) TUBULOSA* AND *HOLOTHURIA (LESSONOTHURIA) POLII* OF AN ALGERIAN *POSIDONIA OCEANICA* MEADOW

Abstract

Deposit-feeder holothurians represent a major component of Posidonia oceanica *(L.) Delile ecosystems. They actively contribute to the turnover of organic matter by ingesting materials in the detritus layer. The evolution of the biomass/density ratio was investigated for two aspidochirotid species* Holothuria (H.) tubulosa *(Gmelin, 1978) and* Holothuria (L.) polii *(Rowe, 1969). Quantitative samples were seasonally collected from March 2001 to February 2002, at 3 m depth in two Algerian contiguous shallow stations. The data collected in each season were statistically compared between stations, species and seasons. A significant difference between species was demonstrated in both stations. However, for both species, the biomass/density ratios exhibited a maximum in summer and a minimum in fall. The minimum value of the biomass/density ratio may be interpreted as an indication of recruitment. The mean abundance of* Holothuria (L.) polii *was significantly lower in the polluted station than in the unpolluted station. The data collected confirm the importance of* Holothuria (L.) polii *as an indicator of the pollution.*

Key-words: *echinoderms, population dynamics, seagrass, pollution, Algerian basin.*

Introduction

Holothurians are conspicuous component of *Posidonia oceanica* meadows of the Mediterranean Sea, where they are reported as generally large sized and rather abundant (Harmelin *et al.,* 1980; Francour, 1990; Coulon and Jangoux, 1993). They play an important role in the detritus food chain (Zupo and Fresi, 1984) and are implied in the recycling of organic matter by ingestion of sediment (Massin, 1982; Francour, 1990). Few studies were devoted to their life cycle (Massin and Jangoux, 1976; Azzolina and Harmelin, 1989; Francour, 1989, 1990, 1997; Bulteel *et al.*, 1992; Mezali, 1998; Despalatovic *et al.,* 2004). The literature contains mainly punctual estimations of the density (Parenzan, 1963; Crump, 1965; Massin and Jangoux, 1976; Gustato and Villari, 1979; Harmelin *et al.*, 1980, 1981; Azzolina and Harmelin, 1989; Francour, 1989, 1990). However, few data are available in the literature on the recruitment process (see Francour, 1990). The main objective of this study was to follow population dynamics to assess recruitment. In addition, the status of *H. (H.) tubulosa* and *H. (L.) polii* as bioindicator of pollution (see Harmelin *et al.*, 1981) is discussed.

Materials and methods

Two contiguous stations were chosen. The first was located in Sidi Fredj peninsula (Fig. 1); it is unpolluted and characterized by a dense *Posidonia oceanica* meadow (467.28 shoots/ m^2) (Mezali, 1998). The second station (Tamentfoust) is a semi-closed area. Its water is generally calm and well protected from the dominant East and North winds. This station is influenced by two river flows (wadi El Hamiz and wadi El Harrach), which drain untreated domestic and industrial waste waters (Mezali, unpubl. data). The seagrass meadows present in these areas are degraded and in a state of regression (Semroud, 1993). Holothurians were investigated by scuba divers. Monthly counting and measuring were carried out during one year (March 2001 to February 2002) and gathered by season (spring, summer, fall and winter). The method of quadrats was used to evaluate the density of each species. Each quadrat consisted of a metal frame (1m x 1m) subdivided in four areas (50cm x 50cm each) (Azzolina and Harmelin, 1989). In each season, 60 quadrats were randomly sampled at each station. Individuals present in each quadrat were identified, measured and counted and the mean densities of species evaluated. In order to obtain a fast and non-destructive estimation of the biomass, we established relationship between the contracted length (CL in mm) and the wet weight (WW in g) of the individual (Francour, 1990): $WW= 0.0013\ CL^{2.1665}$, $WW= 0.0005\ CL^{2.4035}$ respectively for *H. (H.) tubulosa* and *H. (L.) polii* at the Sidi Fredj station (Mezali, 2001); and $WW= 0.0020\ CL^{2.0353}$, $WW= 0.0013\ CL^{2.1674}$ respectively for *H. (H.) tubulosa* and *H. (L.) polii* at the Tamentfoust station (Mezali, unpubl. data). The biomass/density ratio and the contracted length were then calculated and seasonally followed. Differences in mean values between stations, species and seasons have been carried out by ANOVA. If the null hypothesis was rejected, the differences between samples have been sought by Student-Newman-Keuls test.

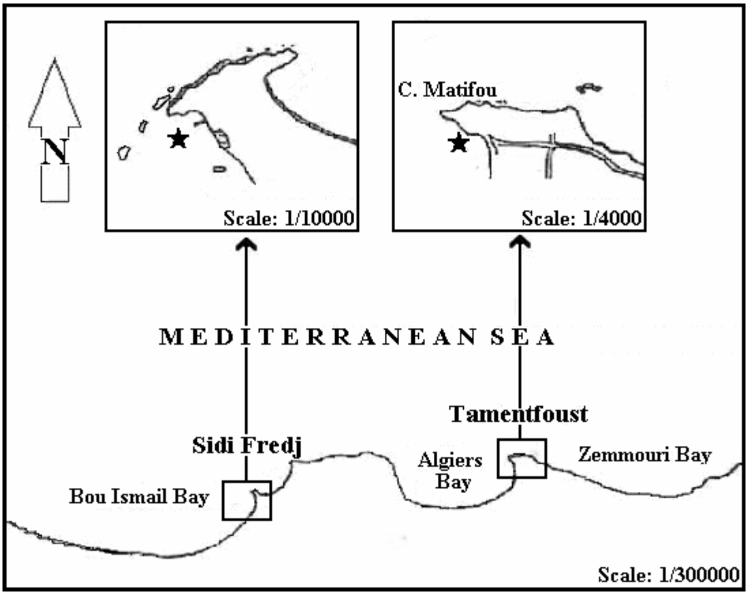

Fig. 1 - The studied areas and their localization in Algerian coast.

Results

Holothuria (H.) tubulosa was the dominant species in both stations ($p < 0.05$; Fig. 2). This species was also more abundant than *H. (L.) polii* in both stations ($p < 0.05$; Fig. 2). Seasonal mean of *H. (H.) tubulosa* biomass/density ratio was maximum in summer (39.89 and 215.07g/ind.) respectively for Sidi Fredj and Tamentfoust ($p < 0.05$; Fig. 3). For *H. (L.) polii* this ratio was maximum in summer (90.36 g/ind.) for Sidi Fredj station and in autumn for Tamentfoust station ($p < 0.05$; Fig. 3). In contrast, mean ratios were significantly lower in autumn than during the warm season, except for *H. (L.) polii* in Tamentfoust with a minimum in spring (all $p < 0.05$; Fig. 3).

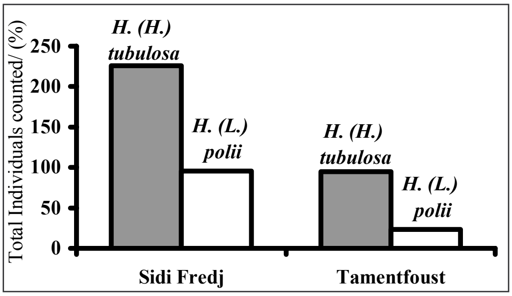

Fig. 2 - Total abundance of holothurians in the studied stations.

Discussion

According to Harmelin *et al.*, (1981) holothurian species are very sensitive to pollution, and can be considered as good benthic bioindicators of pollution. The differences recorded between Sidi Fredj, an unpolluted station, and Tamentfoust, a polluted station, confirm the conclusions of Harmelin *et al.* (1981). Moreover, *H. (H.) tubulosa* was always more abundant than *H. (L.) polii* in both stations, and the decrease of total abundance (Fig. 2) was less important for *H. (H.) tubulosa* than *H. (L.) polii*. As a result, both species demonstrated to be sensitive to pollution and *H. (H.) tubulosa* may be considered more resistant to pollution than *H. (L.) polii*. However, this last hypothesis has to be confirmed by experiments.

The few data available in the literature on the reproduction of the two species indicate that the spawning occurs generally in summer (Hyman, 1955; Azzolina and Harmelin, 1989; Despalatovic *et al.,* 2004). The larval lifespan seems to be rather important (Rutherford, 1973), and the recruitment begins in April for *H. (H.) tubulosa* and in August for *H. (L.) polii* (Francour, 1990; Mezali and Semroud, 1997). The growth is fast during the first months (Rutherford, 1973; Francour, 1990).

The highest values recorded in summer indicate migration of adult individuals towards the shallow part of *Posidonia oceanica* meadows for reproduction during this period, when the water temperature increases (22 °C; Mezali, unpubl. data). So, this period corresponds to the spawning in summer reported in the literature. The minimum value obtained in autumn could be due to recruitment of juveniles, as reported by Francour (1990). The numerous young individuals, ranging 20-50 mm (contracted length), counted during fall in the two stations confirm recruitment at the end of the summer. The values of the biomass/density ratio generally increased from fall to winter, and could be related to the rapid growth of young individuals.

The low biomass/density ratios recorded in summer for *H. (L.) polii* of Tamentfoust area (Fig. 3) could be partly related to the behaviour of *H. (L.) polii* against light (low tolerance to the high irradiance during July-August). *H. (L.) polii* generally merge with the substrate (homotypy) and/or cover its body with a sand layer (Francour, 1990). This made difficult their observations and sampling in this area which was characterised by higher turbidity (Semroud, 1993). This protection allows this species for more marked diurnal activity (Crump, 1965; Massin and Jangoux, 1976). The effects of pollution could also explain this low value. In fact, the low dissolved oxygen concentration associated with the increase of water temperature (24 °C) during summer causes proliferation of pathogenic micro-organisms and thus mass mortalities in this species (Mezali, unpubl. data).

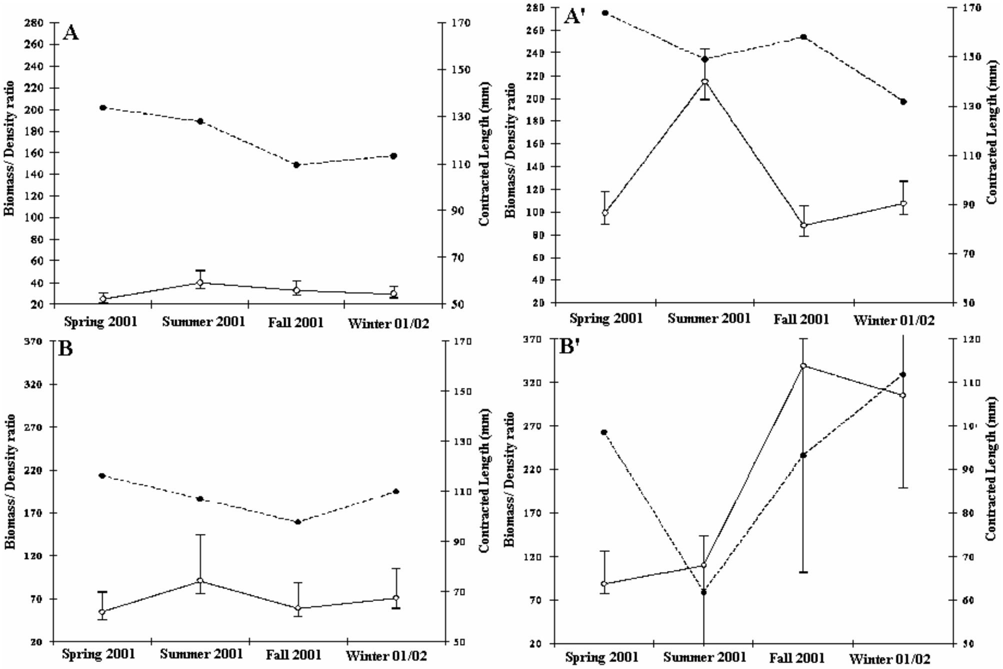

Fig. 3 - Seasonal trend of biomass/density ratio and contracted length of the two sampled holothurians. **A**: *H. (H.) tubulosa* of Sidi Fredj; **A'**: *H. (H.) tubulosa* of Tamentfoust; **B**: *H. (L.) polii* of Sidi Fredj; **B'**: *H. (L.) polii* of Tamentfoust —○—: Biomass/Density; --●--: Contracted length.

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U. Nesti[1], D. Balata[2], L. Piazzi[2], F. Cinelli[2].

[1]ICRAM Central Institute of Applied Marine Research
[2]Dipartimento di Biologia Università di Pisa

TWO DIFFERENT APPROACHES TO DETECT SPATIAL VARIABILITY OF EPIPHYTIC ASSEMBLAGES OF *POSIDONIA OCEANICA* (L.) DELILE: USE OF SPECIES AND MORPHOLOGICAL GROUPS.

Abstract

The aim of this paper is to give a contribution to the knowledge of epiphyte assemblages of Posidonia oceanica *leaves. Two pristine islands of NW Mediterranean Sea were investigated and two different methodological approaches (species vs morphological groups) were compared. Results showed that both rhizome and leaf epiphyte assemblages of* P. oceanica *are characterized by a high variability at small scale, among plots few meters apart. Moreover, the same patterns were detected by using both species and morphological groups as descriptors.*

Key-words: *epiphytes,* Posidonia oceanica*, morphological groups, spatial variability, Mediterranean Sea.*

Introduction

Epiphytic assemblages of seagrasses play a key role in marine habitats, highly contributing in biodiversity and production of coastal systems and constituting an important food resource for many invertebrates and fishes (Borowitzka and Lethbridge, 1989; Moncreiff *et al*., 1989). In the Mediterranean Sea, the most common seagrass is the endemic *Posidonia oceanica* (L.) Delile that constitutes wide meadows between 0 and 40 m. Epiphytic assemblages of *P. oceanica* are widely studied but the different methods used make it difficult to use meadows as biological indicators. Moreover, only few meadows around little islands have been investigated (Mazzella *et al*., 1989).

The aim of this paper is to give a contribution to the knowledge of epiphyte assemblages of *P. oceanica* leaves. Two pristine islands of NW Mediterranean Sea were investigated and two different methodological approaches (species vs. morphological groups/phyla) were compared.

Material and methods

The study was carried out around two small islands of the Tuscany Archipelago (north-western Mediterranean Sea): Gorgona and Pianosa (Fig. 1) during the months of July and August 2005.

A hierarchical sampling design was used to test for differences in spatial variability at four different spatial scales: islands (km apart), locations (300-400 m apart), sites (100-200 m apart), plots (10-20 m apart). Five vertical shoots 10s of cm apart were collected in each plot by SCUBA diving, at 10 m depth. Animal and macroalgal organisms were identified at level of species and the abundance of each species was expressed as percentage cover. Moreover, macroalgae were lumped in the following morphological groups: crustose algae, filamentous algae, foliose algae, articulated algae, corticated-terete algae (Steneck and Dethier 1994), while animals were lumped in phyla.

Multivariate analysis of variance based on permutations (PERMANOVA) was used to test the hypothesis that epiphytes show different patterns of variation in composition and in abundance of species and morphological groups/phyla in relation to spatial scales (Anderson, 2001). Bray-Curtis dissimilarity was calculated using untransformed data. Both for rhizome and leaf epiphytes, the analysis consisted in a 4-way model with Island (2 levels), Location (2 levels), Site (2 levels) and Plot (5 levels) as random and nested factors.

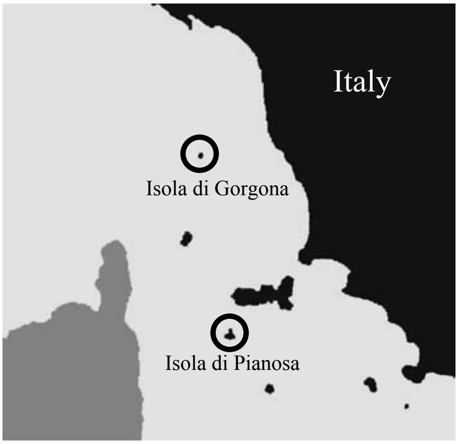

Fig. 1. Localisation of the sampling areas

Results

A total of 46 macroalgae (5 Chlorophyta, 10 Pheophyta, 31 Rhodophyta); 44 sessile animals (5 Porifera, 7 Cnidarians, 27 Bryozoans, 1 Polichaetes, 4 Ascidians); and 5 Foraminifera were found.

The structure of the epiphytic assemblages of leaves appeared similar in all the meadows: encrusting algae and Bryozoans dominate everywhere while the other forms are widespread with low cover. The most common species for the leaf assemblages were among calcareous algae *Pneophyllum fragile* Kützing (mean coverage 41.16; ± 4.82 mean ± SE) and among bryozoans *Electra posidoniae* Gautier, 1954 (mean coverage 9.18 ± 0.78; mean ± SE) and *Aetea truncata* Landsborough, 1852 (mean coverage 3.51 ± 0.38; mean ± SE).

The structure of the epiphytic assemblages of rhizomes was characterized mainly by Bryozoans *Beania hirtissima* Heller (mean coverage 14.25 ± 0.50 mean ± SE), *Aetea truncata* Landsborough, 1852 (mean coverage 13.75 ± 0.48 mean ± SE); *Scrupocellaria reptans* (Linnaeus, 1758) (mean coverage 10.25 ± 0.48 mean ± SE); *Idmonea serpens* (Linnaeus, 1758) (mean coverage 15.75 ± 0.48 mean ± SE), by the green alga *Flabellia petiolata* (Turra) Nizamuddin (mean coverage 18.50 ± 0.43 mean ± SE), and by the Hydrozoans *Obelia geniculata* (Linnaeus, 1758) (mean coverage 23.0 ± 0,40 mean ± SE).

PERMANOVA showed for leaf epiphyte assemblages a significant variability at the smallest scale examined, both for species/samples matrices and for morphological groups/phyla-samples matrices (Tab. 1). Rhizome assemblages showed a significant variability at the smaller spatial scales, between sites and plots, both for species-samples matrices and for morphological groups/phyla-samples matrices (Tab. 2).

Tab. 1 - Results of PERMANOVA analysis on leaf epiphytes. Significant factors are in bold.

		Species			**Morphological Groups/Phyla**		
Source	df	MS	PseudoF	P perm	MS	Pseudo F	P perm
Island = Is	1	12463.81	6.21	0.315	12399.87	43.23	0.315
Location (Is) = Lo (Is)	2	2006.32	0.82	0.563	286.86	0.13	0.992
Site (Lo(Is)) = Si (Lo(Is))	4	2458.76	0.97	0.488	2150.56	1.00	0.451
Plot (Si(Lo(Is))) = Pl(Si(Lo(Is)))	16	2533.69	3.19	**0.001**	2155.55	3.22	**0.001**
Residual	96	793.91			669.86		
Total	119						

Tab. 2 - Results of PERMANOVA analysis on rhizome epiphytes. Significant factors are in bold.

		Species			**Morphological Groups/phyla**		
Source	df	MS	PseudoF	P perm	MS	Pseudo F	P perm
Island = Is	1	9197.28	0.63	1.001	4554.70	0.44	1.001
Location (Is) = Lo (Is)	2	14566.02	0.89	0.523	10273.34	0.72	0.597
Site (Lo(Is)) = Si (Lo(Is))	4	16333.85	2.28	**0.001**	14187.55	2.38	**0.001**
Plot (Si(Lo(Is))) = Pl(Si(Lo(Is)))	16	7160.00	4.34	**0.001**	5968.75	4.16	**0.001**
Residual	96	1649.73			1435.27		
Total	119						

Discussion

Results of the present study showed that both rhizome and leaf epiphyte assemblages of *Posidonia oceanica* are characterized by a high variability at small scale, among plots few meters apart. Moreover, the same patterns were detected by using both species and morphological groups as descriptors.

A high variability at small scale has been widely reported for seagrass leaf epiphytes (Piazzi *et al.*, 2004; Vanderklift and Lavery, 2000), on the contrary, no data are available for spatial variability of rhizome assemblages. The same patterns of small-scale spatial variability detected in leaf and rhizome assemblages show that *P. oceanica* meadows, despite their apparent homogeneity, are ecosystems with high variability that determine a patch distribution of the organisms. Small-scale variability of epiphytes could be related to local characteristics of the meadows;

in fact, differences in shoot density, canopy and distance from meadow boundaries cause small-scale variability of hydrodynamic flow and light irradiance that can determine a patch distribution of organisms.

The obtaining of similar patterns of variability by using species and morphological groups as descriptors represents an interesting result of the present study. The use of morphological groups instead of species in monitoring surveys and impact evaluation studies allows to decrease the time necessary to sample analysis and to examine a higher number of samples; moreover, morphological groups could give information about environmental characteristics (Steneck and Dethier, 1994). Results of this study, if confirmed by further investigations, show that morphological groups can be used to describe patterns of variability of *P. oceanica* epiphytes assemblages.

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M. Polifrone, A. Abelardo-González, V. Quevedo-Díaz

Departamento de Biología, Universidad de Las Palmas de Gran Canaria, Campus Universitario de Tafira, 35017 Las Palmas, Spain.

FISH COMMUNITY AND GRAZING PRESSURE ON MEADOWS OF *CYMODOCEA NODOSA* (UCRIA) ASCHERSON IN GRAN CANARIA (CANARY ISLANDS, SPAIN)

Abstract

The presence and abundance of fish species associated to Cymodocea nodosa *meadows were compared at three study sites in Gran Canaria island. Differences were observed in three habitats (rocks, sand and seagrass meadow) during two seasons (winter and summer) using a hierarchical sampling design. These data were referred to the shoot density, the length of blades and the bites on* C. nodosa *(Magnoliophyta) leaves to establish a possible relationship with the fish abundance.*

Key-words**:** Cymodocea nodosa*, fish communities, biodiversity indexes, Gran Canaria, Canary Islands.*

Introduction

Seagrasses occupy the intertidal and subtidal regions of subartic, temperate and tropical waters (den Hartog, 1970; Larkum *et al.*, 1989) and their ecological importance is mainly based on their structural stability, the nutrient recycle, the effects on the coast, the food and shelter offered to many organisms, the high productivity, and they also represent a nursery area for many fish species of commercial interest (Wood *et al.*, 1969). The aim of this study was to compare the ichtyofauna and the abundance of species in *Cymodocea nodosa* (Ucria) Ascherson meadows in Gran Canaria (Canary Islands, Spain) according to different spatial and temporal scales.

Materials and methods

The three study sites, Tufia (**T**), Risco Verde (**RV**) and Playa del Cabrón (**EC**), were located in the eastern part of Gran Canaria island. The *Cymodorea nodosa* meadow at **T** had the area with less extension (about 1250 m^2), followed by the one at **EC** (about 12000 m^2) and at **RV** (about 15000 m^2). The abundance of species and individuals have been obtained with observations in winter and summer 2005-2006 along six transects of 50m at three areas (the meadow, the sandy and rocky area) within the three localities, following the methods described by Kingshford and Battershill (1998). At **RV** the sandy area has been observed only during summer, due to the bad weather in winter. The shoot density/m^2 and the number of bites on the leaves of *C. nodosa* were also considered to determine a possible relation with the fish abundance.

Results

The richness of species and the biological diversity were very similar in the three localities (**T:** 30 species, Shannon Index H'= 2.31; **EC:** 32 species, Shannon Index H' = 2.39; **RV**: 29 species, Shannon Index H' = 1.98), although the sandy area of **RV** in winter has not been analysed. The Pielou's index (J) was lower at **RV** (0.56) than at **T** (0.71) and **EC** (0.71), indicating that the individuals were not equally distributed inside each species. In winter, **T** showed the lowest richness and homogeneity of species in the meadow, while in summer the diversity and evenness of species were higher in this area (Table 1). On the whole, at **T** no differences have been found between the two seasons for all considered indexes. In winter, at **EC** the sandy area showed the lowest species richness and the highest diversity with the presence of a few dominant species (*Trachinus draco*, *Spondyliosoma cantharus* and *Xyrichthys novacula*), while in summer the most abundant species in this area were *Trachinus draco*, typical of sandy habitats, and *Abudefduf luridus*, typical of rocky habitats. At **RV** there was a considerable difference in the results obtained in the two seasons, mostly due to the lack of information about the sandy area in winter. On the other hand at both the meadow and rocky areas the species richness and the diversity index were higher than in winter, and it was possible to evidence the dominance of a few species, such as *Diplodus annularis*, *Spondyliosoma cantharus*, *Sparisoma cretense* (the latter species was constituted mostly by juveniles). The abundance of fish species in the meadow was higher in summer (28 species) than in winter (26 species), following an opposite trend in regard to the number of bites on the leaves, being these ones more abundant in winter than in summer, with the exception of **T** where they are more abundant in summer. Only in **EC** the number of bites of fishes increased with the number of *C. nodosa* shoots$\cdot$ m^2.

Tab. 1 - Biodiversity indexes of the icthyofauna of *C. nodosa* meadow, sandy and rocky areas at three localities in Gran Canaria island during winter and summer 2005-2006.

		Winter			**Summer**		
		Species richness	**Shannon-Wiener Index**	**Pielou Index**	**Species richness**	**Shannon-Wiener Index**	**Pielou Index**
Tufia	**Meadow**	7	1..20	0.61	10	1.88	0.82
	Sand	14	1.71	0.65	12	1.19	0.48
	Rocks	17	1.60	0.57	16	1.88	0.68
	Total	**25**	**2.01**	**0.62**	**25**	**2.25**	**0.70**
El Cabrón	**Meadow**	18	1.25	0.43	10	1.62	0.71
	Sand	15	2.15	0.79	9	1.75	0.79
	Rocks	18	1.73	0.60	26	1.32	0.43
	Total	**28**	**2.16**	**0.65**	**27**	**1.98**	**0.57**
Risco Verde	**Meadow**	13	1.43	0.56	14	2.22	0.84
	Sand	-	-	-	11	2.11	0,88
	Rocks	18	1.81	0.63	25	1.76	0.54
	Total	**22**	**1.50**	**0.49**	**29**	**1.96**	**0.58**

Conclusions

Comparing the different areas within each locality it was possible to observe a higher richness of species in the rocky area, and some coincidence of species between the sandy and the meadow areas. Nevertheless, some species typical of rocky habitats have been found inside of the meadows in their juvenile stages. In conformity with studies of classic phenology of the *C. nodosa* meadows of Gran Canaria islands (Polifrone, 2005), the present study showed a clear relation between stability and maturity of the meadows with the abundance and diversity of fish species. Despite it has been observed a reduced anthropogenic impact in all areas, the higher extension of the meadows of **RV** reflects a higher degree of diversity of species than the other study sites. The number of species at the prairie of **T** was proportional to smaller dimensions of the study site, but the data set showed a high diversity for this locality on the whole. No clear relationship has been found between the fish abundance, the density of *C. nodosa* and the number of bites on its leaves.

Acknowledgments

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M. Vetere, N. Nurra, R. Mussat Sartor, D. Pessani

Dip.to di Biologia Animale e dell'Uomo, Laboratorio di Biologia Marina,
Via Accademia Albertina, 13, 10123 Torino (Italy)

THE MOLLUSCAN COMMUNITY OF THE FOLIAR STRATUM OF *POSIDONIA OCEANICA* (L.) DELILE MEADOW FROM PUNTA MANARA (EASTERN LIGURIAN SEA). ZONATION PATTERN IN RELATION TO DEPTH AND TIME

Abstract

The aim of this paper is to provide additional data on mollusc populations associated to the foliar stratum of a Posidonia oceanica *meadows off Punta Manara (Eastern Ligurian Sea), and discuss their structure and zonation pattern in relation to depth and time. Samples were collected along a depth gradient every two metres from 5 to 23 m depth in May and September 1985 and March 1986. A total of 15,056 gastropod and bivalve molluscs belonging to 87 species have been determined. The DCA analysis showed that samples collected in May showed a higher discontinuity respect to the others and are clearly ordered according to depth. As a whole, the trends showed by molluscs in the studied bed and periods seems to be related somehow to the dynamics of leaf growth and canopy dynamics of the meadow.*

Key-words*: Molluscs, Posidonia community structure, zonation pattern, time, depth gradient, Ligurian Sea.*

Introduction

Gastropod and bivalve molluscs are among the most studied animals associated to *Posidonia oceanica* meadows, especially in the leaf canopy (Gambi *et al.*, 1992). Previous studies on distribution pattern of the mollusc fauna in *Posidonia* meadows have elaborated a zonation model correlated to depth and largely attributable to changes of various factors, mainly the hydrodynamic conditions (Idato *et al.*, 1983). The zonation pattern, albeit with some qualitative-quantitative changes, has been also identified in meadows with different exposure (Russo *et al.*, 1983-84), according to nycthemeral migrations (Russo *et al.*, 1984), and during time (Russo *et al.*, 1984; 1991; Gambi *et al.*, 1992). Different meadows, however, showed different species composition and abundance pattern at different depths and periods, as well as at large regional scale (Russo and Terlizzi, 1998) although a "*core stock*" of common species could be identified (Russo *et al.*, 1985; 1991).

The aim of this paper is to provide additional data on mollusc populations associated to the foliar stratum of a *P. oceanica* meadows off Punta Manara (Eastern Ligurian Sea), and discuss their structure and zonation pattern in relation to depth and time.

Material and methods

The research was conducted in the *P. oceanica* bed located along the eastern rocky side of Point Manara. The meadows extends for approx. 16 hectares. On the upper rocky limit (5 m) the mean density is 509 shoots/m^2; in the intermediate station (13-17 m) the bed grows on a "*matte*" and the mean density is 477 shoots/m²; the lower limit (21-23 m) is regressive and the bed is settled on a muddy-sand, the mean density is 216 shoots/m². Sampling was carried out by SCUBA diving using the standardized hand-towed net technique (Russo *et al.*, 1985). Samples were collected along a depth gradient every two metres from 5 to 23 m depth in May and September 1985 and March 1986. For each depth and sampling period, three hand-towed net sample were taken; for each sample Shannon-Wiener diversity index (H') was calculated. A multivariate analysis was performed using the Detrended Correspondance Analysis (DCA). The data (abundances) from the three sampling periods were arranged in a matrix (39 species x 29 samples), excluding from the analysis the species occurring only once (*singletons*, 26) and those with abundance < 4 in order to reduce variability due to rare or occasional species (Gambi *et al.*, 1982).

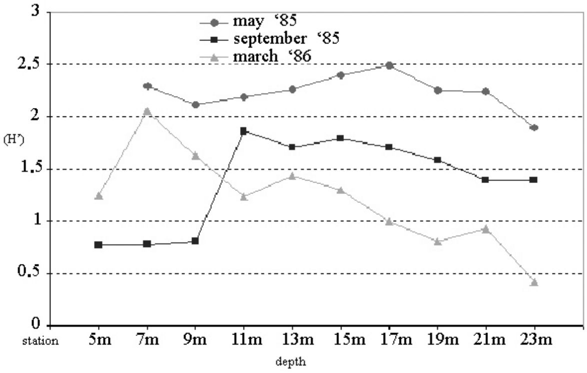


Fig. 1 - Diversity index (H'): temporal variations

Results

A total of 15,056 gastropod and bivalve molluscs belonging to 87 species have been determined, while 14,955 specimens and 39 species (37 gastropods and 2 bivalves) were considered for the DCA analysis. The trend for species diversity (H') in relation to depth in the three sampling periods considered is shown in Fig. 1. Sample from May, when generally leaf growth is high and the epiphytic community is well-developed, showed the highest values at depths between 13 and 17 m, and in the shallow station (5 m) where the bed is settled on a hard substrate. In September, the shallow stations show values of diversity much lower than the intermediate and deep stations. In contrast, in March at the upper limit, the highest values are recorded. The DCA pattern, on the space of the first two factors, shows station points ordination according to depth along F1, and to sampling periods along F2 (Fig. 2). The samples of May (A) show a higher disjunction and discontinuity respect to the others and are clearly ordered according to depth along F1. In September (B) the position of the shallow samples is closer to

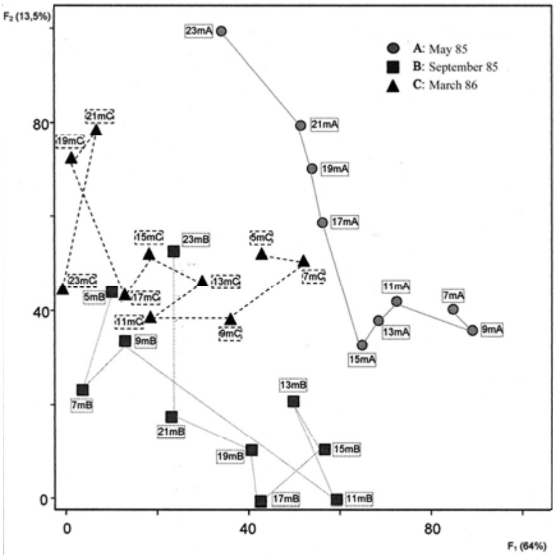
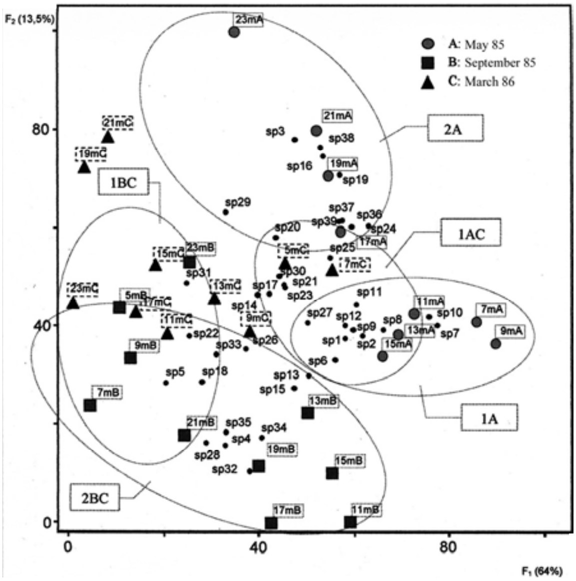


Fig. 2 - DCA model of station points



1	*Jujubinus exasperatus*	14	*Pusillina incospicua*	27	*Curveulima devians*
2	*Calliostoma conulum*	15	*Pusillina radiata*	28	*Muricopsis cristata*
3	*Smaragdia viridis*	16	*Alvania cancellata*	29	*Ocinebrina aciculata*
4	*Tricolia pullus*	17	*Alvania discors*	30	*Chauvetia brunnea*
5	*Tricolia tenuis*	18	*Alvania lineata*	31	*Mitrella minor*
6	*Barleeia unifasciata*	19	*Alvania cimex*	32	*Nassarius incrassatus*
7	*Crisilla semistriata*	20	*Alvania geryonia*	33	*Raphitoma linearis*
8	*Rissoa guerinii*	21	*Bittium jadertinum*	34	*Weinkauffia turgidula*
9	*Rissoa auriscalpium*	22	*Bittium latreilli*	35	*Odostomia acuta*
10	*Rissoa variabilis*	23	*Bittium reticulatum*	36	*Nassarius pygmaeus*
11	*Rissoa ventricosa*	24	*Cerithiopsis minima*	37	*Raphitoma pupoides*
12	*Rissoa violacea*	25	*Cerithiopsis tubercolaris*	38	*Musculus discors*
13	*Pusillina philippi*	26	*Triphoridae (*ind.)	39	*Hiatella arctica*

Fig. 3 - Joint plot of station points and species points: seasonal variations

the deep samples than to the intermediate ones. In March (C), the zonation according to depth is recognisable only in the shallow stand. The subjective impression of "*joint plot*" (specie *plus* samples) (Fig. 3) allow for the identification of five groups of mollusc species. Groups 1A and 2A represent May samples. Group 1A is represented by the exclusive *Crisilla semistriata* species, and by two trochids and five rissoids which, in the stations associated to this group (from 7 to 15 m depth), display values of abundance higher than in other samples. Group 2A associated to May, is formed by seven species typically related with deep samples and by four species which show here greater values of abundance. Group 1AC contains a set of species which are common to the intermediate stations from May, with the shallow stations from March. No species are exclusive to this group. Groups 1BC e 2BC correlate the intermediate March and September samples. 1BC includes the shallowest stations and the deepest ones from September, with the intermediate ones from March and it is characterised by extremely high abundances of *Bittium latreilli*. 2BC included all the September samples with two intermediate stations (-11 and -17 m) from March. 11 species are present, among which six are exclusive.

Discussion and conclusions

The mollusc population of the foliar stratum in the *Posidonia* meadow at Punta Manara, shows differences both in species composition and in the zonation pattern among the different sampling periods considered. Both the diversity and the multivariate analysis indicate that the populations in May are better-structured. The ordination pattern in particular confirms that the main factor which explains population distribution in May is the depth gradient and its related factors. The mollusc community in this period is largely formed by species of the *Rissoa* genus which prevail over *Pusillina* and *Alvania* ones. The principal "stock" is represented by 25 species accounting for 98.13% of quantitative dominance. Ten of these species can be considered as the principal nucleus of the community, or "*core stock*", (Russo *et al*., 1991) (4 *Rissoa: R. guerinii, R. violacea, R. auriscalpium*, *R. variabilis,* 3 *Pusillina*: *P. philippi, P. radiata, P. incospicua* and 3 *Bittium*: *B. latreilli, B. reticulatum, B. jadertinum*). The community in September is characterized by the high dominance of *Bittium latreilli*, a species often observed in high densities in seagrass meadows (Russo *et al*., 2002). In March the mollusc populations are still dominated by *Bittium latreilli* (with greater adult presence) and also by with cerithids, as well as by some species that were absent or less abundant in September. This trend occurred mainly in the shallower part of the meadow.

The dynamics showed by molluscs in the studied bed and periods seems to be related somehow to the dynamics of leaf growth and canopy dynamics in the meadow (although data on canopy phenology and epiphyte colonization are not measured and direct relationships need to be still evaluated), as shown in other studies (Scipione *et al*., 1996). However, also the life history of some species (e.g., *Bittium latreilli*), seems to play a relevant role in the observed dynamics.

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R. Zakhama-Sraieb, Y-R. Sghaier, F. Charfi-Cheikhrouha

U. R. de Biologie Animale et Systématique Evolutive, Faculté des Sciences de Tunis,
Campus Universitaire, 2092 Manar II, Tunisie.

IS AMPHIPOD DIVERSITY RELATED TO THE QUALITY OF *POSIDONIA OCEANICA* BEDS?

Abstract

The Amphipod fauna (Crustacea, Peracarida) associated with the seagrass Posidonia oceanica *was studied on four localities in the eastern Tunisian coast exposed to increasing levels of anthropogenic influence (Mahdia, Hergla, Monastir and El Kantaoui) using diversity indexes and statistical analysis. At each locality, three sites were sampled along a depth gradient at 2, 5 and 10 m. To study associated amphipods,* Posidonia *shoots were collected in three replicates at each station using a quadrate (30*30 cm) by SCUBA diving. Thirty-seven amphipod species were identified with an important abundance particularly in Mahdia sites. The data analysis revealed a clear relationship between amphipod diversity and epiphyte biomass. A correlation was also found between amphipod population diversity and the quality of* Posidonia *meadows. Amphipod population structure appears to be more stable in* Posidonia *meadows in good health. No particular groups of amphipod species were specifically linked to the degree of degradation of* Posidonia *meadows. However,* Apocorophium acutum, Ampithoe helleri *and* Ampithoe ramondi *were more frequent in degraded meadows. These species can be proposed as bioindicators of the bad quality of* Posidonia oceanica *meadows.*

Key words: *Amphipods,* Posidonia oceanica*, diversity, Tunisian eastern coasts.*

Introduction

Posidonia oceanica is the most abundant and widely distributed seagrass species along the Tunisian coast. *P. oceanica* meadows constitute a very important habitat for benthic communities. They provide habitat, protection and trophic resources for many species, particularly crustaceans amphipods which are one of the most abundant groups associated to this seagrass (Chessa *et al.*, 1983). *P. oceanica* has been used as a bioindicator of coastal water quality (Pergent *et al.*, 1995), and also amphipods have been studied to assess the coastal water pollution (Bellan Santini, 1980; 1981; Guerra Garcia and Garcia Gomez, 2001). However few studies, focusing on the relationship between associated Amphipods and *P. oceanica* meadow quality, have yet been developed (Diviacco, 1988; Scipione, 1998).

Materials and methods

The study area was located in the eastern Tunisian coasts in the Gulf of Hammamet (Fig. 1). Four localities were chosen (Mahdia, Hergla, Monastir and El Kantaoui) where *Posidonia* meadows are exposed to an increasing level of human impact. Fieldwork was carried out in October 2004. At each locality, three stations were sampled at 2, 5 and 10 m depth. A study of bed and plant features of the four localities was carried out in order to evaluate *P. oceanica* meadow status and the result was presented in Sghaier *et al.* (2006, this volume).

To sample the amphipods associated to *Posidonia* we used a quadrate of 30 cm side and 25 cm height. The quadrate was placed in each *Posidonia* meadow.

The shoots were pulled up and collected in a bag (net size 0.3 mm). A total of 9 samples were sampled in each localities, 3 replicates for each depth.

P. oceanica shoots collected were washed with freshwater over a 0.5 mm sieve. Retained amphipods were sorted, identified and counted.

The species abundance data were analysed through cluster analysis using Bray-Curtis similarity index (fourth root transformation). Difference between amphipod density averages was tested using ANOVA analysis. The regression analysis was performed using non-parametric Spearman correlation to reveal an eventual relationship between *Posidonia* meadows features and amphipods diversity.

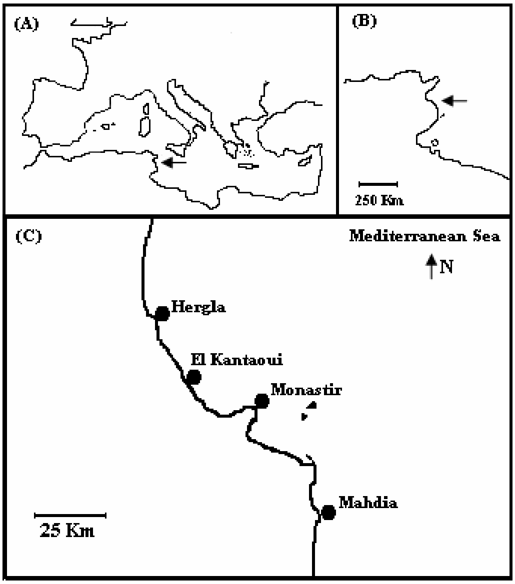

Fig. 1 - Sampling localities in the Tunisian eastern coast

Results

A total of 37 species of amphipods were identified (Tab. 1); among them *Ampelisca unidentata, A. rubella, Ampithoe helleri, Leptocheirus guttatus, Ericthonius punctatus, Elasmopons brasiliensis* and *Maera inaequipes* are particularly frequent representing 70.4% of the total of specimens. These species exhibited a large distribution, occurring in almost all the samples.

The amphipods in El Kantaoui stations, the most disturbed particularly in A1 station (-2m depth), are dominated by *A. helleri*, *Ampelisca ramondi*, *E. punctatus* and *Pseudoprotella phasma*. Whereas, *Ampelisca unidentata*, *A. rubella*, *E. punctatus* and *E. brasiliensis* are more frequent at Mahdia stations (D1, D2 and D3). In term of density, *A. helleri* and *A. ramondi* showed the highest densities at El Kantaoui probably related to the high epiphyte biomass recorded in these sites (A1 and A2). On the other hand, *A. rubella*, *A. unidentata* and *Ampelisca spinipes* occurred mainly at Mahdia characterized by clean and the transparent water conditions.

Density of amphipods per m^2, species richness (total and average), diversity index (Shannon-Weaver) and Evenness were shown in Tab. 2. The highest value of amphipods density was recorded in Mahdia; (ANOVA test; $p<0.001$). The

Tab. 1 - Average density of amphipod species at El Kantaoui (A), Monastir (B), Hergla (C) and Mahdia (D). Standard deviations are given in parentheses

		A1	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**	**D1**	**D2**	**D3**
1.	*Ampelisca rubella* A. Costa, 1864	-	55.56 (19.25)	-	44.44 (19.25)	3.70 (6.42)	162.96 (33.95)	66.67 (22.22)	51.85 (25.66)	44.44 (11.11)	162.96 (16.97)	122.22 (22.22)	137.04 (33.95)
2.	*Ampelisca spinipes* Boeck, 1861	7.41 (6.42)	7.41 (6.42)	-	3.70 (6.42)	7.41 (12.83)	-	7.41 (6.42)	14.81 (6.42)	3.70 (6.42)	22.22 (11.11)	22.22 (19.25)	29.63 (16.97)
3.	*Ampelisca unidentata* (Schellenberg, 1936)	7.41 (6.42)	51.85 (16.97)	18.52 (6.42)	40.74 (27.96)	37.04 (6.42)	170.37 (65.11)	129.63 (16.97)	77.78 (48.43)	162.96 (23.13)	177.78 (22.22)	196.30 (61.20)	151.85 (12.83)
4.	*Amphilochus neapolitanus* Della Valle, 1893	-	7.41 (6.42)	-	-	11.11 (11.11)	-	-	3.70 (6.42)	7.41 (6.42)	-	-	-
5.	*Peltocoxa marioni* Catta, 1875	-	14.81 (6.42)	-	-		22.22 (11.11)	-	-	14.81 (16.97)	-	-	-
6.	*Ampithoe helleri* G. Karaman, 1975	677.78 (55.56)	140.74 (16.97)	-	59.26 (33.95)	-	-	111.11 (40.06)	51.85 (6.42)	3.70 (6.42)	14.81 (16.97)	29.63 (12.83)	11.11 (0.00)
7.	*Ampithoe ramondi* Audouin, 1826	148.15 (16.97)	7.41 (6.42)	3.70 (6.42)	62.96 (6.42)	-	7.41 (12.83)	18.52 (16.97)	-	-	18.52 (12.83)	14.81 (6.42)	22.22 (11.11)
8.	*Aora gracilis* (Bate, 1857)	7.41 (6.42)	-	-	-	25.93 (16.97)	18.52 (6.42)	18.52 (12.83)	22.22 (11.11)	3.70 (6.42)	22.22 (11.11)	25.93 (6.42)	29.63 (12.83)
9.	*Lembos* sp	-	-	-	51.85 (16.97)	-	-	-	-	-	-	-	-
10.	*Lembos websteri* Bate, 1857	7.41 (6.42)	-	-	-	-	-	-	-	-	-	-	-
11.	*Leptocheirus guttatus* (Grube, 1864)	18.52 (6.42)	11.11 (0.00)	22.22 (11.11)	55.56 (11.11)	40.74 (16.97)	137.04 (39.02)	18.52 (16.97)	77.78 (11.11)	185.19 (16.97)	10 (29.40)	114.81 (23.13)	118.52 (16.97)
12.	*Tethylembos viguieri* (Chevreux, 1911)	-	-	-	-	-	-	-	-	-	22.22 (11.11)	18.52 (6.42)	11.11 (11.11)
13.	*Apocorophium acutum* (Chevreux, 1908)	14.81 (6.42)	-	-	-	-	-	-	-	-	-	-	-
14.	*Dexamine spiniventris* (A. Costa, 1853)	-	25.93 (6.42)	-	66.67 (11.11)	-	137.04 (27.96)	14.81 (16.97)	3.70 (6.42)	11.11 (11.11)	25.93 (6.42)	25.93 (12.83)	37.04 (6.42)
15.	*Dexamine spinosa* (Montagu, 1813)	-	-	-	-	7.41 (12.83)	-	14.81 (6.42)	11.11 (0.00)	3.70 (6.42)	-	-	-
16.	*Tritaeta gibbosa* (Bate, 1862)	-	11.11 (11.11)	-	-	-	59.26 (23.13)	44.44 (11.11)	48.15 (16.97)	-	-	-	-
17.	*Hyale comptonyx* (Heller, 1866)	51.85 (16.97)	44.44 (29.40)	-	77.78 (48.43)	-	-	-	-	-	-	-	-
18.	*Parahyale aquilina* (A. Costa, 1857)	14.81 (6.42)	25.93 (6.42)	-	-	-	-	7.41 (12.83)	-	-	-	-	-

Tab. 1. *(Continued)*

	A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3
19. Iphimedia sp.	-	-	11.11 (0.00)	-	-	-	-	-	-	-	-	-
20. Gammaropsis ostroumoui (Sowinsky, 1898)	-	-	-	51.85 (23.13)	-	-	-	-	-	-	-	-
21. Ericthonius punctatus (Bate, 1857)	237.04 (23.13)	103.70 (12.83)	22.22 (11.11)	181.48 (16.97)	55.56 (11.11)	74.07 (25.66)	140.74 (16.97)	233.33 (72.86)	51.85 (27.96)	214.81 (16.97)	248.15 (63.18)	218.52 (46.26)
22. Leucothoe spinicarpa (Abildgaard, 1789)	22.22 (11.11)	3.70 (6.42)	-	-	14.81 (6.42)	-	14.81 (6.42)	11.11 (0.00)	44.44 (22.22)	-	-	-
23. Leucothoe venetiarum Giordani-Soika, 1950	-	-	-	-	-	-	-	-	-	-	-	7.41 (12.83)
24. Lysianassa costae Milne Edwards, 1830	7.41 (6.42)	29.63 (6.42)	7.41 (6.42)	-	-	-	7.41 (6.42)	14.81 (16.97)	14.81 (6.42)	-	-	-
25. Lysianassa sp1	-	-	-	-	-	155.56 (22.22)	-	-	-	-	-	-
26. Lysianassa sp2	-	-	-	-	-	148.15 (16.97)	-	-	-	-	-	-
27. Orchomene humilis (A. Costa, 1853)	-	11.11 (11.11)	-	-	-	-	7.41 (6.42)	44.44 (11.11)	22.22 (19.25)	-	-	-
28. Lepidepecreum longicorne Bate & Westwood, 1861	-	18.52 (6.42)	-	-	-	-	-	-	-	-	-	-
29. Socarnes filicornis Heller, 1866	-	-	-	-	-	-	-	-	-	14.81 (6.42)	18.52 (12.83)	14.81 (12.83)
30. Elasmopus brasiliensis (Dana, 1855)	-	-	-	-	-	-	40.74 (35.72)	3.70 (6.42)	55.56 (19.25)	159.26 (16.97)	155.56 (22.22)	20 (44.44)
31. Elasmopus pocillimanus (Bate, 1862)	-	25.93 (6.42)	-	3.70 (6.42)	14.81 (6.42)	18.52 (6.42)	-	-	-	-	-	-
32. Maera hirondellei Chevreux, 1900	33.33 (11.11)	3.70 (6.42)	7.41 (6.42)	-	-	-	25.93 (6.42)	7.41 (12.83)	-	25.93 (12.83)	37.04 (6.42)	44.44 (19.25)
33. Maera inaequipes A.Costa, 1857	59.26 (6.42)	129.63 (12.83)	11.11 (11.11)	103.70 (25.66)	92.59 (27.96)	248.15 (39.02)	10 (29.40)	103.70 (16.97)	222.22 (11.11)	103.70 (35.72)	111.11 (29.40)	114.81 (16.97)
34. Metaphoxus simplex (Bate, 1857)	14.81 (12.83)	7.41 (6.42)	-	-	-	-	3.70 (6.42)	-	18.52 (12.83)	92.59 (6.42)	114.81 (23.13)	77.78 (11.11)
35. Caprella liparotensis (Haller, 1880)	-	-	-	-	-	-	25.93 (25.66)	125.93 (23.13)	25.93 (6.42)	-	-	-
36. Caprella sp.	-	-	-	11.11 (11.11)	3.70 (6.42)	11.11 (11.11)	-	-	-	-	-	-
37. Pseudoprotella phasma (Montagu, 1804)	11.11 (11.11)	40.74 (6.42)	66.67 (11.11)	-	-	-	-	-	-	33.33 (11.11)	25.93 (6.42)	44.44 (11.11)

total species richness, unlike the average richness, increases in relation with the disturbance level of the various studied sites. Diversity index (H') and Evenness (J') are lowest in El Kantaoui compared with the others sites.

Tab. 2 - Density, Specific Richness, Diversity index of Amphipod populating in studied sites.

	El Kantaoui	Monastir	Hergla	Mahdia
Density n. ind/m-^{2}	762.96(333.59)	833. 33(304.80)	874.07(42.17)	1254.32(52.02)
Species Richness total	26	22	22	17
Species Richness average	13.55(5.24)	11.44(2.00)	15.11(2.14)	15.77(0.66)
Diversity H'	3.35	3.80	3.68	3.49
Evenness J'	0.71	0.85	0.82	0.85

The cluster analysis, using the similarity matrix on the average density of amphipods species, shows two distinct groups of stations (Fig. 2). The first group includes El Kantaoui stations (A1, A2 and A3), characterised by the lowest values of shoot density and shoot size, and where the *Posidonia oceanica* meadow can be qualified on in bad-poor ecological status (Sghaier *et al*., 2006). The second group can be split into three subgroups with different similarity levels (from 45% to 90%); the first subgroup includes Mahdia stations (D1, D2 and D3) characterised by a clean water and the highest values of shoot density and shoot size. The second (C1, C2 and C3) and the third subgroups (B1, B2 and B3), located respectively at Hergla and Monastir, are characterised by medium values of shoot density and shoot size. The *Posidonia* meadows are qualified in these two subgroups, having a moderate status (Sghaier *et al*., 2006).

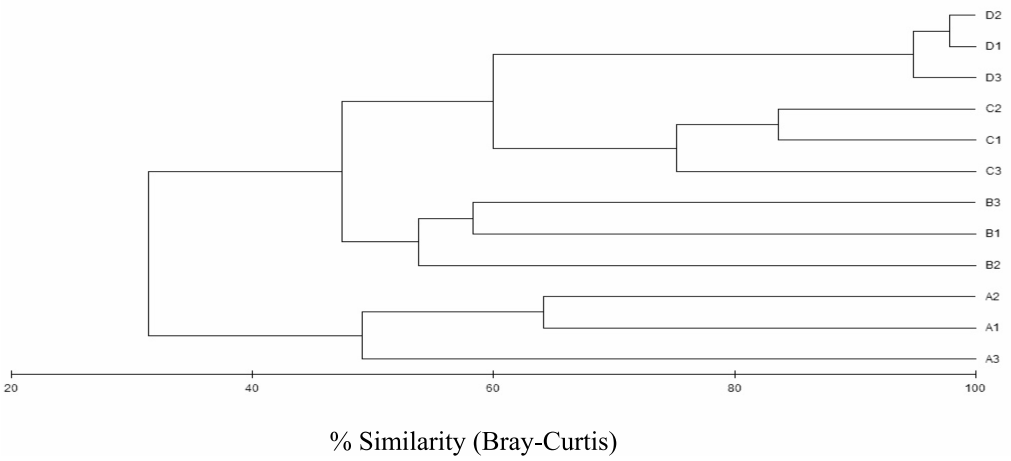

Fig. 2 - Dendrogramme of similarity between the stations of the four localities based on the average density of Amphipods species (A: El Kantaoui, B: Monastir, C: Hergla, D: Mahdia)

The relationships between amphipods and the *P. oceanica* meadow features are shown in Fig. 3. A significant relationship was observed (significant correlation,

$p<0.05$) between the *Posidonia* shoot density and amphipods density (r= 0.94) and also between the epiphytic biomass (mg/cm^2) and the species richness (r= 0.98).

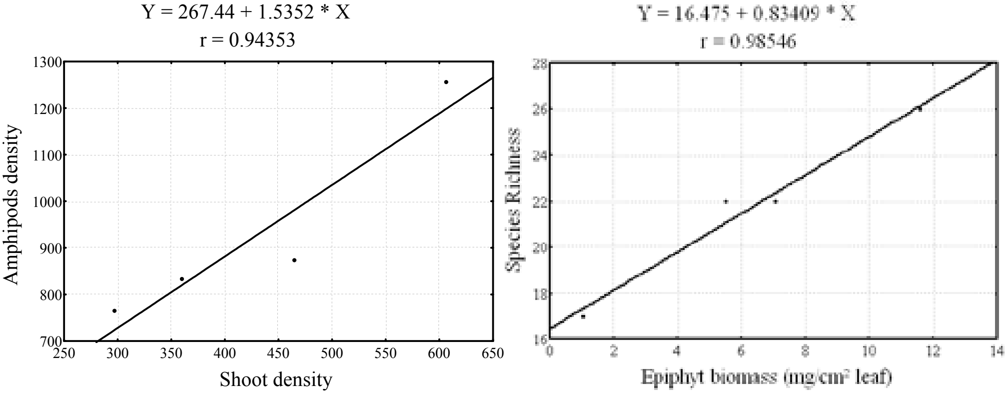

Fig. 3 - Linear regression: Shoot density and Amphipod density (left), Epiphyte biomass and Species Richness (right).

Discussion and conclusions

During this study, a relatively high number of amphipod species have been recorded in Eastern Tunisian *P. oceanica* meadow (37 species) when compared with other sites in Mediterranean coast; 22, 25, 28 and 34 species were identified respectively in *Posidonia* meadow in Alicante coast (Spain) by Sanchez-Jerez *et al.* (1999), in Porto Conte (Sardinia) by Scipione (1998), in Apulia coast (Italy) by Diviacco (1988) and in Punta Vico, Lacco Ameno, Island of Ischia (Italy) by Scipione and Fresi (1984). Differences of number of amphipods species between the various beds in the Mediterranean can be attributed to the sampling method (e.g., Scipione, 1998, used the hand-net method to collect amphipods from the *Posidonia* leaf stratum).

Structure and diversity of amphipods was influenced by the features of *Posidonia* meadows, wich are affected by anthropogenic impact. This is shown in particular by the decrease of amphipod density, average species richness and diversity indexes in the most perturbed sites (e.g. El-Kantaoui). The decrease of number of specimens, when pollution increases, was already observed by Bellan-Santini (1980).

The cluster analysis was in accordance with the classification of meadow status, showing the distinctiveness of El Kantaoui, the most disturbed locality. Furthermore, the highest similarity between stations was recorded in Mahdia indicating more homogeneous amphipod populations (Fig. 2).

The present study suggests that the amphipods are good bioindicator of *Posidonia* meadows quality. The presence/absence and frequency of some species can be considered to underscore the quality of the meadow. *E. punctatus* was the most abundant and frequent species in our samples. It was present in almost all stations with the highest densities in Mahdia. This is also the case of *A. unidentata* and *L. guttatus*. These filter-feeding species (Grassé, 1999) are more abundant and frequent in the low disturbance stations. On the contrary, the grazer species *A. helleri* was present in almost all the stations exhibiting highest density at El Kantaoui; this species consumes epiphytic algae and seems related to photophilic algae more than to the *Posidonia* leaves.

L. websteri, *C. acutum*, *Iphimedia* sp. and *L. longicorne* are found only in El Kantaoui; *C. acutum*, a tube-builder and filter-feeding Amphipod (Grassé, 1999), is a typical species of harbour and silty environments (Conradi and Lopez-Gonzalez, 2001).

This study underscores that amphipods community inhabiting the seagrass *Posidonia* meadow constitutes a useful tool to assess the health status of *Posidonia oceanica* and indirectly the coastal water quality.

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SECTION 3

MONITORING, MANAGEMENT AND RESTORATION OF SEAGRASSES

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P.L.A. Erftemeijer

WL Delft Hydraulics, P.O. Box 177, 2600 MH Delft, The Netherlands

MANAGING THE EFFECTS OF DREDGING ON SEAGRASSES IN THE MEDITERRANEAN SEA

Abstract

A concise review of the impacts of dredging and disposal of dredged material on seagrasses is presented, with a special emphasis on the Mediterranean Sea. The critical threshold of seagrasses for turbidity and sedimentation are discussed, along with four case studies of dredging impacts on seagrasses in the Mediterranean Sea (accounting for the loss of an estimated 1,450 ha). The paper concludes with an overview of mitigating measures and management options (including site-specific dredging criteria) that can be applied to control and minimise the impacts of dredging on seagrasses in the Mediterranean.

Key-words**:** *dredging impacts, Mediterranean Sea, regulation and mitigation, review, seagrasses.*

Introduction

The need for dredging to deepen and maintain navigation channels and harbour entrances is eminent in many ports of the world and indeed in the Mediterranean. In some areas, commercial sand extraction is meeting an ever-increasing demand for sand for construction, beach nourishment and land reclamation. The excavation, transportation and disposal of soft-bottom material may, however, lead to adverse impacts on the marine environment, especially when dredging or disposal is done in the vicinity of sensitive marine environments, such as coral reefs and seagrass beds. Seagrasses develop highly productive meadows that fulfil important ecological and economic functions in the coastal zone, particularly in sustaining fisheries (Jackson *et al.*, 2001), stabilising sediments and preventing coastal erosion (Scoffin, 1979; Fonseca, 1989). The loss of seagrass over the past two decades due to human impacts worldwide is estimated to be in the order of 33,000 km^2, based on an extrapolation of known losses and a global seagrass area estimate of 177,000 km^2 (Green and Short, 2003). For the Mediterranean, no specific estimate of the extent of seagrass loss exists, but numerous cases of deterioration and loss have been documented (Procaccini *et al.*, 2003; Lipkin *et al.*, 2003). Five species of seagrass occur in the Mediterranean Sea, the most common and widespread of which is the endemic *Posidonia oceanica*, estimated to cover between 2.5 and 5.5 million hectares (Buia *et al.*, 2000). The primary cause of seagrass degradation and loss worldwide is reduction in water clarity, both from increased turbidity and increased nutrient loading (Short and Wyllie-Echeverria, 1996). In many cases, dredging operations have directly or indirectly contributed to the loss of seagrass vegetation.

Environmental impacts of dredging

The potential impacts of dredging and disposal operations on seagrasses include the physical removal or burial of the vegetation and the indirect effects of temporary increases in turbidity and sedimentation. In some cases, dredging may be associated with the release of pollutants and nutrients from the sediment, a reduction in dissolved oxygen levels and secondary effects from hydrographic changes. Changes in turbidity and sedimentation associated with dredging will only result in adverse environmental effects when significantly larger than the natural variation of turbidity levels and sedimentation rates in the area (Orpin *et al.*, 2004). Such natural variability can sometimes be substantial and may be caused by factors such as storms, river discharges and wind-induced wave actions, further exacerbated by anthropogenic perturbations such as commercial shipping, bottom fishing and eutrophication. There are, however, numerous reports of sub-lethal and lethal effects on seagrass meadows due to prolonged exposure to high turbidity and siltation associated with dredging activities (Erftemeijer and Lewis, 2006). The degree of environmental impacts caused by dredging and disposal depends on the quantity and frequency of dredging, methodology of dredging and disposal, physical dimensions and water depth of the dredging location, grain-size composition and degree of contamination of the dredged material, background water quality, seasonal variations in weather conditions, and the species composition of the seagrass bed and its proximity relative to the dredging or disposal site.

Critical thresholds of seagrasses for turbidity

The primary effect of increased turbidity on seagrasses is a reduction in the amount of light available for photosynthesis. The tolerance of seagrasses to low light conditions depends on their minimum light requirements. The minimum light requirements (expressed as % of surface irradiance) reported in literature for the five seagrass species that occur in the Mediterranean Sea vary considerably: 2% of SI for *Zostera noltii*, 3% of SI for *Halophila stipulacea*, 7.3 to 10.2 % of SI for *Cymodocea nodosa*, 4.4 to 16 % of SI for *Posidonia oceanica*, and 11 to 37 % of SI for *Zostera marina* (Erftemeijer and Lewis, 2006). Part of the variation reported in the literature is caused by differences in methodology used to derive at these figures. The length of time that different species can survive at low light levels is also of importance. Laboratory and field experiments have shown that most seagrass species can survive in light intensities below their minimum requirements for some period of time ranging from two weeks to several months, depending on their carbohydrate storage capacity (Erftemeijer and Lewis, 2006).

Critical thresholds of seagrasses for sedimentation

Several studies have documented deterioration of seagrass meadows by smothering due to excessive sedimentation. The consequences of enhanced sedimentation for the seagrass plant depend on several factors such as the depth of burial and the life history of the species involved. The values reported in literature for maximum allowable sedimentation rates for the five seagrass species that occur in the Mediterranean Sea range from 2 cm year^{-1} for *Zostera noltii* to 5 cm year^{-1} for *Cymodocea nodosa* and *Posidonia oceanica* (Erftemeijer and Lewis, 2006). An indication of the duration that seagrasses can tolerate high rates of sedimentation was revealed by field experiments in Spain. Artificial burial of the seagrass *Posidonia oceanica* with as much as 15 cm of sediment caused 100% mortality after 200 – 300 days (Manzanera *et al.*, 1995). Sudden burial of *Cymodocea nodosa* with 5 cm of sediment resulted in 90% mortality after 35 days, although some individual shoots of this species were able to survive burial as great as 6 cm (Marba and Duarte, 1994). Mills and Fonseca (2003) observed >50% mortality of *Zostera marina* in field burial treatments of 4 cm for 24 days. Plants buried with 16 cm of sediment experienced 100% mortality.

Seagrass recovery

Despite the known causes of widespread seagrass loss, few studies documented post-disturbance recovery rates of seagrasses. The paucity of data on the rate and extent of recovery relates to the lack of data from long-term monitoring programmes and because many seagrass meadows have either failed or taken many years to recover following stress from declining water quality (Short and Willie-Echeverria, 1996). In shallow areas affected by small-scale disturbances (e.g. propeller scars), recovery can occur within weeks to months (Rasheed, 1999). Recovery of subtidal seagrass meadows from large-scale disturbance has been shown to take 2-4 years (Preen *et al.*, 1995) or more than 5 years (Sheridan, 2004), provided that water quality conditions return to their original state. The ability of seagrasses to endure and recover from periods of reduced light differs between species. Smaller fast-growing (opportunistic) species do not endure long once environmental conditions are beyond that to which they can adapt, but they tend to recolonise more quickly following an impact. Larger slow-growing (climax) species tend to be more resilient because they have substantial reserves that can be mobilised to sustain the plant temporarily during periods of reduced light. If, however, the impact persists to the point where these plants have depleted all their reserves, they die and recovery is unlikely or at best slow (Erftemeijer and Lewis, 2006).

Case studies from the Mediterranean Sea

There are few quantitative reports documenting the impact of dredging on seagrasses in the Mediterranean Sea. Intensive literature search yielded 4 documented cases, together accounting for the loss of an estimated 1,450 ha of seagrass vegetation, which indicates that the scale of damage can be substantial. Pasqualini *et al.* (1999) reported an almost complete disappearance of *Posidonia oceanica* beds in the far end of the Gulf of Porto-Vecchio, Corsica (France) due to dredging and port construction for Porto-Vecchio's commercial port during the 1970s. De Falco *et al.* (2000) documented a substantial loss (about 800 ha) of *Posidonia oceanica* meadows in the Gulf of Oristano, Sardinia (Italy) as a result of channel dredging and commercial port construction during the 1970s. Dredging and filling operations for the construction of a gas pipeline between Italy and Tunisia in 1993 caused the loss of 150 ha of seagrass (*Posidonia oceanica*) at Capo Feto, SW Sicily (Italy) and disturbed the surrounding seagrass meadows by the indirect effects of subsequent pulsed siltation (Di Carlo *et al.*, 2004). Gambi *et al.* (2005) described the loss of about 4 ha of about *Posidonia oceanica* off Ischia island, Gulf of Naples (Italy) due to sand extraction for the filling of a beach in 2002.

Mitigating measures

A range of management options and mitigating measures may be applied to avoid, minimise or mitigate the effects of dredging on seagrasses. Such measures may include: confined land-disposal, turbidity thresholds, seasonal restrictions, minimising the duration of dredging, limiting over-dredge quantities, establishment of no-spud zones, prohibiting dredging near dense seagrass areas, monitoring of turbidity and seagrasses, stopping the dredging when turbidity thresholds are exceeded, and banning the use of certain dredging methods (e.g. hydraulic clam dredging, which was banned in parts of the USA to prevent damage to seagrasses). Impact prediction through turbidity plume- and habitat modelling prior to the issuing of a permit and subsequent monitoring during the dredging operation have proven to be successful tools in minimising environmental impacts of dredging on seagrasses (Erftemeijer and Lewis, 2006). Salvage of seagrasses for use in transplantation efforts (Lewis, 1987) and protection of seagrass areas with silt screens (Sabol *et al.*, 2005) are sometimes considered, with limited success.

Regulation of dredging

Both dredging and disposal operations are increasingly regulated more strictly with regards to their environmental impacts. The simple copying, however, of dredging criteria used for regulating dredging in seagrass areas elsewhere in the world for use in the Mediterranean has no scientific basis. This could either prove fatal to the seagrass ecosystem or lead to exaggerated limitations resulting in unnecessary costs and delays in the dredging operations. When developing dredging criteria, one should acknowledge that seagrasses tolerate periods of naturally high turbidity and can withstand some increase in the frequency of turbid events. In areas that experience large natural fluctuations in background turbidity, such resilience is likely to be greater than in areas where such fluctuations are minimal. Whilst dredging may cause an above average elevation of turbidity levels over seagrass, this may in many instances be within the long-term background range for the area and short-lived when compared to frequent and naturally occurring events such as significant rainfall (wet season) or storms. It can therefore not be assumed that transitory dredge plumes in excess of an area's average levels would necessarily cause significant impacts on seagrasses. Nevertheless, meaningful regulations and control measures based on a proper impact assessment, along with application of relevant mitigating measures and a relevant monitoring programme will all help in preventing or minimising the adverse environmental impacts on Mediterranean seagrasses. The extra costs incurred in such efforts are probably negligible in comparison with the costs of seagrass restoration programmes, which are often small in scale and appear to have limited success.

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G. Casazza[1], C. Lopez Royo[1,2], C. Silvestri[1]

[1]APAT, Agency for Environmental Protection and Technical Services, via Vitaliano Brancati 47, Roma, Italy
[2]University of Corsica – Faculty of Science, EqEL, France

SEAGRASSES AS KEY COASTAL ECOSYSTEMS: AN OVERVIEW OF THE RECENT EU WFD REQUIREMENTS AND CURRENT APPLICATIONS

Abstract

In the last years biological monitoring, to assess the quality of aquatic ecosystems, has increased in importance and in effort. Seagrasses, as key element of coastal ecosystems, are gaining importance as bioindicators. The innovative EU Water Framework Directive (WFD)(Directive 2000/60/EC) for the protection of all surface waters, clearly underlines the importance of these ecosystems. This paper analyses the Directive requirements regarding seagrass, in coastal waters, providing information on the use of seagrass data for classification within the Mediterranean ecoregion. Posidonia oceanica *has been selected as the promising species to be tested for classification of coastal waters, due to the past and present amount of data and research available in the Mediterranean. The classification systems proposed so far, and currently under development and test, are compared and discussed. Moreover, comparison and considerations on some other tentative classification systems, based on different seagrasses in other European ecoregions (North East Atlantic, Baltic Sea) are analysed.*

Key-words: *Seagrasses, Bioindicator, Water policy, Ecosystems, Coastal waters, Classification systems.*

Introduction

The importance of seagrass ecosystems in the coastal environment has increasingly raised in the last decades, not only within the scientific community but, particularly within management and administrations, and therefore among policy makers. Legal measures for the protection of seagrasses, in general and for some species in particular, are being developed together with legal "technical" requirements for seagrass monitoring, control and analysis (Borum and Greeve, 2004). Marine plants as key component of the ecology of coastal waters have been legally clearly stated with the publication of the recent EU Water Framework Directive (Directive 2000/60/EC), for the protection of all surface waters (i.e. rivers, lakes, transitional and coastal waters), that is pushing all Member States (MS) towards an innovative view of their water management. The Directive introduces the concept of ecological status of water bodies as an expression of the quality of structure and functioning of aquatic ecosystems associated with surface waters; the main objective of the Directive is to achieve good water status, for all the European waters, by 2015. A key issue is the mandatory use of organisms (bioindicators) for the assessment and evaluation of the ecological status. Groups of organisms (Biological Quality Elements, BQE: phytoplankton, macroalgae and angiosperms and benthic invertebrate fauna) are therefore defined as basic quality elements to be used for the evaluation of ecological status, i.e. classification. Seagrass are marine angiosperms that can be used as monitoring elements because they are very sensitive to changes in their environment, and specifically to human impacts (Short and Wyllie-Echeverria, 1996). Works on seagrass responses to specific impacts offer a wide array of variables to assess seagrass ecological status, therefore these organisms can be excellent bioindicators (Pergent *et al.,* 1995, Romero *et al.*, 2005, Pergent-Martini *et al.*, 2005). The Directive requirements for coastal waters classification can be found in Casazza *et al.* (2004; 2005), where specifications for the application of the Directive to the Mediterranean ecoregions was detailed. Work within the Mediterranean Geographic Intercalibration Group: MED-GIG, of the WFD implementation was described; four subgroups, according to the different BQEs required for classification, were established. This paper analyses the current use of seagrass data for classification of Mediterranean coastal waters addressing to more specific papers for the classification systems proposed. Moreover, other tentative classification systems, based on different seagrasses in other European ecoregion (North East Atlantic and Baltic sea) are discussed.

Results and discussion

In the Mediterranean Sea, a great amount of research has been dedicated to *Posidonia oceanica*, an endemic key species that plays a major ecological, sedimentary and economic role (Buia *et al.*, 2004). Therefore, *P. oceanica* has been considered as representative of the angiosperm subgroup, due to its unique characteristics within the Mediterranean ecosystem. This provides a common and strong scientific background, allowing a particular approach to the WFD requirements, approach which goes beyond the indications given by the Directive: indeed classification systems will not and cannot be limited to composition and abundance, in order to obtain ecological relevance.

Common monitoring parameters and methodologies, representative of the status of the seagrass beds, such as meadow shoot density, rhizome growth type (horizontal, plagiotropic *vs*vertical, orthotropic rhizomes) and shoot size, are shared by most of the Med countries. Tentative classification systems for coastal waters based on *P. oceanica* are under study and development. Therefore the agreed working strategy, within the MED-GIG, is that each MS develops its own classification system; these classification systems are then cross-tested by exchanging data in order to verify their results. Spain, Italy, France and Malta are at the moment developing classification systems. Spain (Catalunya) has developed POMI (Romero *et al.*, 2005), Italy is refining its “Posware” system (Buia *et al.*, 2005; Silvestre *et al.*, this volume) and France and Malta are working on their data. The different classification systems are similar in concept: they are based on the choice of selected descriptors, the descriptors’ data is then elaborated through statistical analysis, which eventually leads to classification of the *P. oceanica* meadow, in one of the five classes required by the WFD: high, good, moderate, poor, bad. Although the classification systems are similar in concept, they differ on many aspects, such as the choice of descriptors, the data analysis methods and the Reference Conditions (Table 1). The different descriptors were selected using different criteria. The Italian classification system, attempts to reduce error by excluding descriptors that are subject to seasonal variability, thus descriptors related to the plant morphology or to the coastal geomorphology were chosen. POMI, on the other hand, uses additional meadow descriptors and has selected a number of plant physiological descriptors. The use of the latter is debated as, although ecologically relevant, they considerably increase the complexity of sampling and analytical methods. The statistical analysis differ substantially. The Italian classification system uses Autoclass C for data elaboration: all data are loaded in Autoclass

C, which elaborates them in a probabilistic way (datamining technique). Results are visualised with Miner 3D producing a 3-Dimensional graph that relates the different clusters with the 5 ecological classes required by the WFD. POMI uses the Principal Component Analysis PCA for data elaboration, using a correlation matrix, in which descriptors are either positively or negatively correlated to the 1st component. The position of the scores along the quality axis, divided into 5 equal parts, gives the ecological status of a *P. oceanica* meadow.

Tab. 1 - *P. oceanica* descriptors, data analysis methods and reference conditions used in the classification methods under development.

COUNTRIES	DESCRIPTORS	DEPTH	ANALYSIS	REFERENCE CONDITIONS
FRANCE	Shoot density Lower limit depth Type of lower limit Cover Rhizome growth type Baring Leaf production Rhizome elongation	lower limit 15 m	Under study	
ITALY	Shoot density Depth Rhizome production Rhizome elongation Leaf production Width of longest intermediate leaf	0-15 m 16-29 m > 30 m	Autoclass C & Miner 3D	Existing meadow (best one)
MALTA	Shoot density Shoot Foliar surface Shoot biomass	9-12 m	PCA	Existing meadow
SPAIN Catalunya	Shoot density Cover Shoot Foliar surface Rhizome growth type Leaf necrosis N content (rhizomes, leaves) Epiphyte Nitrogen content P content (rhizomes, leaves) Total non-structural carbohydrates in rhizomes Nitrogen isotopic ratio in rhizomes and leaves Sulfur isotopic ratio in rhizomes Trace metals in rhizomes (Fe, Zn, Mn, Cu, Ni, Pb, Cd, Cr)	15 m	PCA	Virtual meadow (best existing values for each parameter)
SPAIN Valencia	Shoot density Shoot Foliar surface % dead matte Baring	13-17 m	PCA	Virtual meadow

Work is still proceeding both within the MED-GIG and MSs. Though the different metrics and classification systems used, common agreement has been reached on some important points. Reference conditions, which are the basis of the classification systems, have to be defined at sub-ecoregional level and according to meadow depths. They can be considered as existing sites, where pristine areas are present, as in "Posware", or virtual sites, using the best existing values for each parameter (POMI). Different statistical approaches, used for the data analysis do not seem to affect substantially the general agreement among classification methods. Moreover, the critical and delicate issue of class boundaries definition has been approached. Through a joint exercise, *P. oceanica* experts have established a common agreement on the "good/moderate" boundary i.e. defining the Ecological Quality Rate (EQR) value for this limit. This, in fact, represents the most critical and important boundary as it identifies the limit between having to "take action" (legally required) and improve the water quality or not. Another important conclusion of the group was the identification of two common metrics that are used by all the Mediterranean participants: shoot density and shoot size. As the methods for measuring these parameters are quite spread and widely used the group suggested to initiate a standardization process; this will facilitate data exchange and comparability and it could be exported very usefully to all Mediterranean countries.

Other European ecoregions are applying the WFD requirements for angiosperms according to their peculiar situations and particular ecosystems. Not many countries have data and/or consider angiosperms as relevant quality elements for their coastal waters classification. Within the North East Atlantic, UK applies the WFD normative definitions for angiosperms with a very pragmatic approach to existing seagrass species (i.e. *Zostera* spp). Taxonomic composition (presence of disturbance-sensitive taxa) and abundance (determined by seagrass shoot density and spatial extent) are evaluated using 3 indexes that have been developed to meet the monitoring requirements (UK Technical Advisory Group, 2006). By combining the assessment indices (use of a mean score) ecological status classes are assigned for angiosperms. These have to be further compared with indices deriving from other BQEs. Within the Baltic Sea, angiosperms are not considered as relevant BQE due to low salinity and, in some coastal types, to exposure and substrate features. Denmark is the only country developing a classification approach using angiosperms (Krause-Jensen *et al.*, 2005). Based on a large historic data set on depth limits of *Zostera marina* (eelgrass) obtained by a national survey in 1901, the study characterises reference levels and compares them with the actual depth limits, considering differences in water salinity and meadows depth. These Northern European systems differ substantially from the Mediterranean approaches, not providing an immediate comparison. Nevertheless, in the proceeding of the WFD implementation useful methodological approaches could be exchanged and compare for mutual benefits.

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A. Cossu, F. Ragazzola, S. Demelas

Dipartimento di Botanica ed Ecologia vegetale; Università di Sassari, via Muroni 25, 07100 Sassari (Italy)

DISTRIBUTION AND ECOLOGICAL CONDITIONS OF *POSIDONIA OCEANICA* (L.) DELILE MEADOWS IN LA MADDALENA NATIONAL PARK (SARDINIA)

Abstract

The La Maddalena Archipelago became National Park in 1996 and together with the southern part of Corsica it forms the Bocche di Bonifacio International Park. Posidonia oceanica *beds widely distributed in the area play an important role in dividing La Maddalena National Park (MNP) into zones with different level of protection in order to manage and coordinate the human activities inside the park. The regression of* P. oceanica *meadows has become generalised around much of theArchipelago coastline.The most common anthropogenic factors in the decline of P. oceanica habitats in near-shore coastal areas are eutrophication and boat anchoring.*

Key-words: Posidonia oceanica*, distribution, La Maddalena National Park, Tyrrhenian Sea.*

Introduction

La Maddalena Archipelago is located in the North-East coast of Sardinia, and consists of seven large islands, with a surface area larger than 100 ha and a high numbers of smaller islets. The surface area of the entire archipelago is about 51 km^2 with 200 km of jagged coast. The seabeds are characterized by wide erosion platforms with a granitic-metamorphic lithology derived from a Paleozoic substratum.

La Maddalena Archipelago became National Park in 1996 and, together with the southern part of Corsica, forms the Bocche di Bonifacio International Park, which represents the first international marine reserve in Europe.

La Maddalena National Park is divided into zones with different levels of protection. Each of these zones has different restrictions related to the exploitation and human use of the marine environment. MA zone is a marine area of high natural interest where the access to tourists is strictly regulated, while the MB is a marine area of high natural interest where the access to tourists is permitted. Finally, a no-take area is present where only scientific research is permitted.

The *Posidonia oceanica* bed is the main biocoenosis of the coastal area, creating an almost continuous belt around the 60 islands of the Archipelago and with the presence also of "rècif-barrières" in shallow waters (Cossu and Gazale, 1997).

The distribution and the structure of the *Posidonia oceanica* beds along the Island have played an important role in dividing La Maddalena National Park into zones.

Materials and methods

From the biocenotic map of the area obtained from previous investigations (Cossu and Gazale, 1997) 82 sampling sites were chosen. In the sampling sites estimates of *P. oceanica* shoot density was measured in 1600 cm^2 quadrate areas (5 replicates in each site). In each site, 5 orthotropic shoots of *P. oceanica* were collected for the analysis of plant phenology (Giraud, 1977). The shoots were collected 50-100 m apart from each other, and the geographic coordinates were registered with a GPS.

Results

The survey of the area revelaed that *P. oceanica* meadows settle on sandy and rocky substrata down to 38 meters depth and are distributed around the coastal area of the 60 islands of the Archipelago, for a total area of 40 km^2 (Table 1).

Tab. 1 - Distribution of the *P. oceanica* meadows (hectares) in different area of La Maddalena National Park.

meadows (h)	Maddalena, Caprera, S. Stefano	Razzoli, S. Maria, Budelli, Barrettini	Spargi	Bisce	Nibani	Mortorio	Tot.
Rocky beds	42	513	202				757
Sandy beds	1646	433	271	345	99	408	3201
Semi-praires	29	21					50
With *C. proliera*	31						31
Total	1748	967	473	345	99	408	4040

The area of *P. oceanica* in state of degradation is about 50 ha and it is located south of La Maddalena Island near the harbour, near the town and Passo Ceca di Morto, and between Santa Maria e Budelli Islands (Table 1). According to Giraud (1977) the results show that 23.1% are dense meadows (class II), 28.2 % are sparse meadows (Class III), considered in a transition state, 38.5 % are very sparse meadows (Class IV) which usually refers to meadows that are in regression or in the process of colonizing the environment. And the last 10.2 % are semi-prairies (Class V), meadows most prone to regression (Giani, 2001).

This classification however, it is not correlated to depth, therefore another classification was used (Pergent *et al.,* 1995), where shoot density is related to depth. Fig. 1 shows how shoots density changes with depth in each site, and how the meadows are healthier in the deeper stands; in fact 33% of the meadows that are very disturbed (abnormal density, AD) are mostly located between 1 and 10 meters depth, 20% are disturbed (low density, LD) and 47% are prairies in equilibrium (normal density ND). The shallow waters are highly frequented by boaters. Several studies have assessed the impact of anchoring on *P. oceanica,* demostrating that there may be a direct adverse effect on meadow cover and shoot density (Porcher, 1984; Francour *et al.,* 1999). Major damage to *P. oceanica* seems to be caused by dragging anchors and scraping anchor chains along the bottom with consequent removal of plants and even pieces of “matte” (Milazzo *et al.*, 2004).

In the sampling sites, the anchor impact was highlighted by the presence of chain drills and sandy patches within the *P. oceanica* meadow

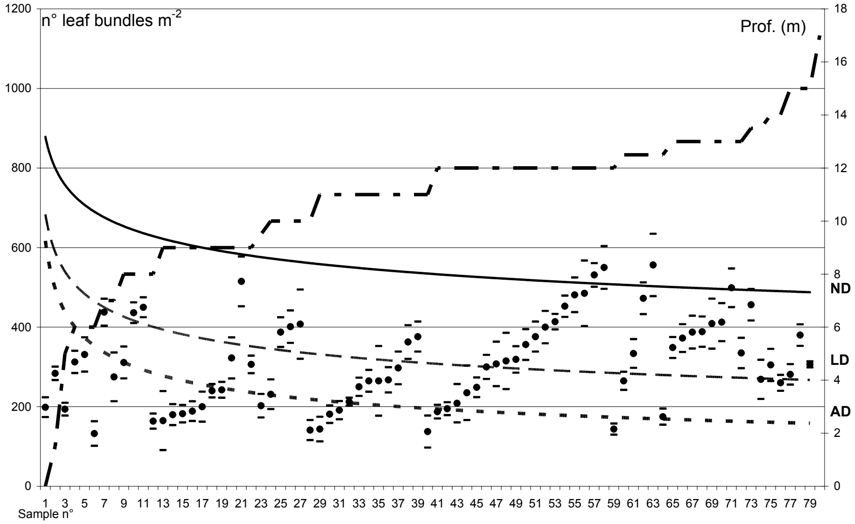

Fig. 1 - Mean shoot densities (•) with their s.d. (=) in relation to depth (— -). AD (- - -) = very disturbed meadows; abnormal density. LD (---) = disturbed meadows; low density. ND (–) = meadows in equilibrium; normal density.

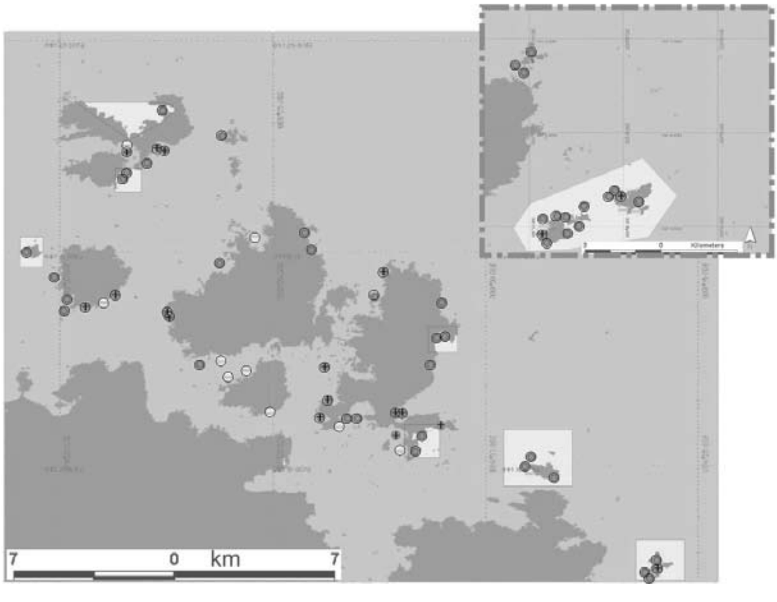

Fig. 2 - Location of the sampling site. (+) = very disturbed meadows; (=) = disturbed meadows; (O) = meadows in equilibrium; clear areas = no-take zones. On the up right there is Mortorio and Mortoriotto Islands, located in the south part of the park.

Fig. 2 represents the overview of the park area and of the *P. oceanica* meadows. The most important meadows in equilibrium, where a high shoot density was found, are inside the no-take zones. There are same exceptions, such as the area in front of the pink beach off Budelli Island. Six years ago this beach became an integral reserve to protect the pink sand (derived from the foraminifer associated to Posidonia, *Miniacina miniacea*). Recent studies (Ragazzola *et al.*, 2005) show how the *P. oceanica* meadow is still in a regression state, and the shoot density data show lower values compared to the calculation made during a previous study in 1999-2000 (Giani, 2001). The small islands of Soffi, Mortorio and Mortoriotto, located in the southern part of the park, have some *Posidonia* meadows in a degraded state. As recorded in front of the pink beach, signs of chain drill in the seabed and sandy patches were discovered, despite the fact that the Ente Parco created a buoy camp.

Conclusion

The major cause of the *P. oceanica* regression in La Maddalena National Park comes from dragging anchors and scraping anchor chains of recreational boats. The shallow water area near the town of La Maddalena shows a *Posidonietum* affected by the urban activities (eutrophication) and the ferries transit. In order to contain the damage in protected areas, some different strategies can be proposed: the identification of sandy areas where the boat anchoring can be permitted, the creation of new buoy camps in the area of high boat frequency, and the implementation of a self regulatory approach based on educating and informing boaters on the respect of *Posidonia* meadows and on the correct anchor type to use.

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G. Costantino, F. Mastrototaro, R. Carlucci, A. Matarrese, M. Panza

Dipartimento di Zoologia - Università degli Studi
Via Orabona, 4 - 70125 – Bari (Italy)

FIRST DATA ON MEADOW STRUCTURE OF A LARGE *POSIDONIA OCEANICA* BED ALONG THE SOUTHERN TYRRHENIAN COAST (MEDITERRANEAN SEA)

Abstract

On the east side of the Gulf of Policastro (Southern Tyrrhenian Sea – Italy) a large Posidonia oceanica *(L.) Delile meadow spreads in NNW-SSE direction for about 14 km. This meadow covers about 550 ha with a width ranging from 150 to 700 m. Along its upper limit the bed shows a patchy distribution and a mean shoot density ranging from 399 to 514 shoots m*$^{-2}$*. The investigated meadow appears to be relatively homogeneous both in the core and in the lower limit showing densities from 185 to 345 shoot m*$^{-2}$ *and from 128 to 278 shoots m*$^{-2}$*, respectively. The bio-ecological analysis indicates a good status of health and a satisfactory degree of conservation. In particular, the morphological and lepidochronological data collected seem to be comparable to those reported for other pristine meadows of the Mediterranean Sea.*

Key-words: Posidonia oceanica*, morphology, lepidochronology, Southern Tyrrhenian Sea.*

Introduction

A large *Posidonia oceanica* meadow, distributed on the coast off the Basilicata region (Gulf of Policastro - Southern Tyrrhenian Sea, Italy), spreads in NNW-SSE direction for 14 km. A first map of this meadow was drawn during a survey and technical report (scale 1:25.000) (Ministero Marina Mercantile, 1991), but its bio-ecological features were not analyzed in details.

Recently, the morphology and the ecology of this meadow have been studied in the framework of a research program supported by the Italian Ministry of the Environment and the Territory (MIA).

In this paper, data on morphological and lepidochronological features of this large *P. oceanica* meadow are reported, as well as an estimate of its health condition.

Materials and methods

The survey was carried out during June 2002. Five line transects (T1-T5) perpendicular to the coast, and spaced at about 2.5-4 km along the coast were considered. For each transect, 3 stations were investigated by two SCUBA divers, corresponding to the upper limit (U), the middle zone (M) and the lower limit (L) of the meadow, for a total of 15 sampling stations (Fig. 1). Five replicates of shoots counting were carried out in each station using a 40x40 cm quadrate. Cover estimates (%) were conducted by each diver separately, considering a round area with 5 m radius on the bed (Buia *et al.*, 2004). For each station, twenty orthotropic (vertical) shoots were collected from each station (300 shoots in total). Ten shoots were used for morphological and biometrical analyses (Giraud, 1977; Pergent and Pergent-Martini, 1988). Other 10 shoots were used for the lepidochronological analysis (Pergent, 1990; Pergent and Pergent-Martini, 1991; Pergent-Martini and

Pergent, 1994). Production data were calculated per each meadow zone (upper limit, middle zone and lower limit), pooling the 10 shoots of each transect (T1-T5), for a total of 50 shoots per zone. Measures of the last 3 lepidochronological years of the rhizome were excluded from the analysis (Peirano, 2002; Buia *et al.*, 2004). The leaf production values (LP = g d.w. shoot^{-1} year^{-1}) concerning the years 2000 and 2001 were calculated (Pergent and Pergent-Martini, 1991; Buia *et al.*, 1992; 2004). A matrix of morphological parameters (density, cover, leaf length, leaf surface per shoot, coefficient "A", LAI) was compiled using average data of each station. Ordination of the sampled stations was performed by means of the non metric multidimensional scaling (nMDS), based on the Euclidean distance of the raw data, (PRIMER 5 software). The ANalysis Of SIMilarities (ANOSIM) was applied to test the differences between the groups of stations identified by the nMDS (Clarke and Warwick, 2001).

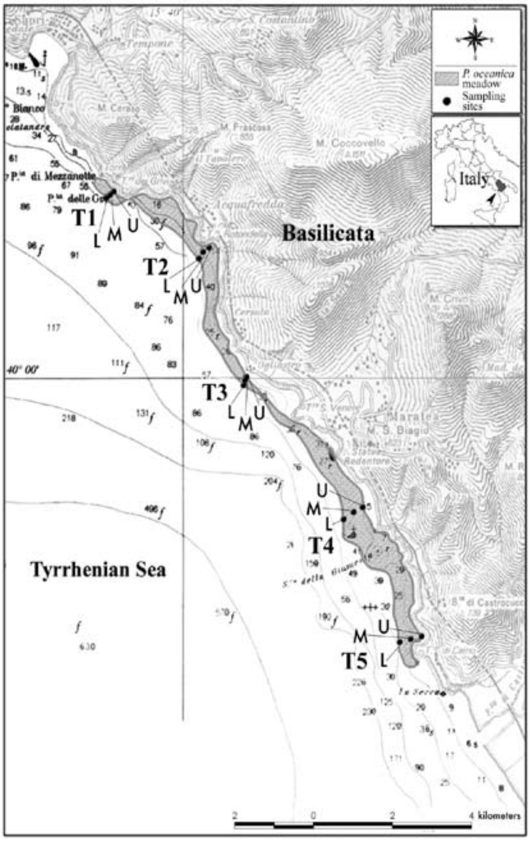

Fig. 1 - Map of *P. oceanica* bed along the Basilicata region coast reporting sampling sites.

Results

The studied meadow covers an area of about 550 ha, colonizing a seabed from 150 to 700 m wide and generally sloping around 5-10%. The upper limits were generally located at 6-8 m depth and the lower limits at 26-30 m. The former presented a patchy distribution along the coast, showing a variable cover percentage ranging from 35 to 80%, and a mean shoot density ranging from 399 to 514 shoots m^{-2}. On the contrary, both in the core and along the lower limits, the cover percentage appeared homogeneous (80-100%) and the mean shoot density ranged from 185 to 345 shoots m^{-2} in the middle zone and from 128 to 278 shoots m^{-2} in the lower limits. At the lower limits of the meadow two different typologies were observed: a clear-cut limit on coarse-sandy sediment (T1, T2 and T3) and an erosive limit sometimes characterized by a “matte” about 2 m high (T4 and T5).

Considering the morphological parameters (Tab. 1), the average number of leaves per shoot ranged between 5.3 and 6.0 in the upper limits, between 5.2 and 6.0 in the middle zone and between 5.1 and 6.6 in the lower limits. The mean total length of intermediate leaves ranged between 29.7 cm and 45.6 cm in the upper limits, between 31.6 cm and 68.3 cm in the middle zone and between 34.8 cm and 43.2 in the lower limits. The mean total length of adult leaves ranged between 41.5 cm and 50.0 cm in the upper limits, between 30.1 cm and 68.2 cm in the middle zone and between 30.2 cm and 41.9 in the lower limits.

Tab. 1 - Ecological and morphological data (mean ± s.d.) recorded by sampling site.

transect	meadow zone	depth (m)	shoot density (no. m^{-2})	cover (%)	no. leaves per shoot	intermediate leaves total length (cm)	adult leaves total length (cm)	leaf surface (cm^2 shoot^{-1})	intermediate leaves coeff. "A" (%)	adult leaves coeff. "A" (%)	LAI (m^2 m^{-2})
1	U	7	455 ± 70	35	5.6 ± 1.3	43.5 ± 17.2	44.8 ± 14.3	161.3 ± 49.6	0	24	7.3
	M	20	305 ± 53	80	5.2 ± 0.6	39.7 ± 12.9	37.1 ± 10.5	158.6 ± 32.8	5	11	4.8
	L	30	194 ± 22	100	5.8 ± 0.8	42.6 ± 14.2	41.9 ± 15.3	184.0 ± 53.3	0	4	3.6
2	U	8	514 ± 81	35	6.0 ± 0.9	45.6 ± 15.2	45.2 ± 15.1	194.4 ± 68.4	0	0	10.0
	M	19	264 ± 21	90	5.6 ± 1.0	68.3 ± 13.2	68.2 ± 22.0	281.5 ± 89.1	0	3	7.4
	L	29	198 ± 32	100	5.9 ± 0.7	42.7 ± 11.9	39.1 ± 14.3	178.4 ± 44.1	0	10	3.5
3	U	6	431 ± 61	35	5.4 ± 1.3	29.7 ± 18.8	42.3 ± 16.0	136.9 ± 61.9	20	25	5.9
	M	16	301 ± 43	100	5.6 ± 1.1	46.4 ± 11.5	41.2 ± 13.5	158.0 ± 42.4	0	4	4.8
	L	29	278 ± 67	100	5.1 ± 1.4	35.3 ± 13.8	30.2 ± 12.7	113.3 ± 49.7	6	9	3.1
4	U	7	399 ± 50	40	5.3 ± 0.8	36.2 ± 13.3	41.5 ± 12.4	138.0 ± 57.4	0	15	5.5
	M	16	345 ± 44	90	6.0 ± 1.3	31.6 ± 14.7	35.0 ± 14.3	138.1 ± 62.3	0	6	4.8
	L	28	258 ± 26	100	6.6 ± 0.5	43.2 ± 14.0	37.8 ± 14.4	188.9 ± 34.0	0	9	4.9
5	U	7	433 ± 107	80	6.0 ± 1.2	45.0 ± 27.0	50.0 ± 19.5	202.9 ± 96.7	0	3	8.8
	M	22	185 ± 7	90	5.6 ± 1.0	35.0 ± 9.0	30.1 ± 11.0	112.1 ± 38.6	0	4	2.1
	L	26	128 ± 25	80	5.7 ± 0.8	34.8 ± 9.4	33.9 ± 11.7	137.4 ± 40.4	0	4	1.8

The leaf surface per shoot showed mean values ranging from 136.9 cm^2 and 202.9 cm^2 in shallower stands and achieving values between 112.1 cm^2 and 281.5

cm^2 in the core of the meadow (16-22 m). Moreover, mean values between 113.3 cm^2 and 188.9 cm^2 were recorded for shoots sampled at the lower limit. In accordance with the shoot density data, the average Leaf Area Index (LAI) value calculated for the shallower stands of the meadow (7.5±1.9 m^2 m^{-2}) was higher than that observed in the middle zone (4.8±1.9 m^2 m^{-2}) and at the lower limit (3.4±1.1 m^2 m^{-2}). In addition, the percentage of adult and intermediate leaves with broken apices (coefficient "A"), generally showed low mean values in each of the stations (adult leaves: max 25%; intermediate leaves: max 20%).

The ordination of the sampling sites by nMDS (Fig. 2) showed significant differences between the upper limit, middle zone and lower limit (ANOSIM test R=0.636; p=0.001). Moreover, the pair-wise test showed significant differences between the upper limit and lower limit (R=0.99; p=0.008) and between the upper limit and the middle zone (R=0.71; p=0.008). Conversely, the middle zone and lower limit were statistically not separable (R=0.09; p=0.22). The nMDS analysis showed clear differences between the shallower stands (upper limit) and those settled both in the core and near the lower limits, suggesting the influence of depth on the structure and morphology of the meadow, as significant ecological factor.

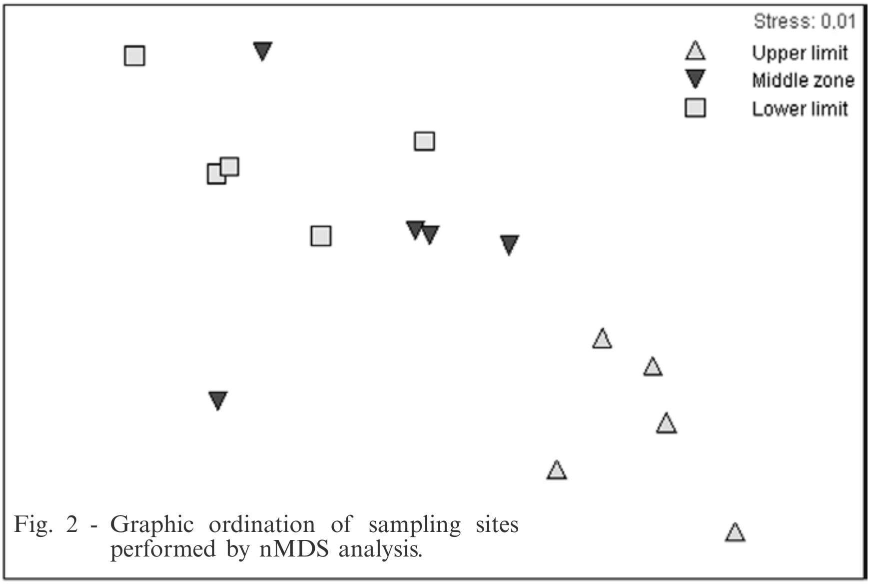

Fig. 2 - Graphic ordination of sampling sites performed by nMDS analysis.

Sampled rhizomes aged between 7 and 9 years. Concerning the production (Tab. 2), the LFR (no. of leaves shoot^{-1} year^{-1}) showed an average value ranging from 6.1 to 6.3 in each investigated zone. The rhizome elongation rate (RhER = cm shoot^{-1} year^{-1}) showed an average value of 0.6 cm in the upper limit, with a slight increase (0.8 cm) and a clear decrease (0.5 cm shoot^{-1} year^{-1}) observed in the middle zone and in the lower limit, respectively. The rhizome production (RhP = mg d.w. shoot^{-1} year^{-1}) presented the same bathymetric pattern, showing an average value of 46.1 mg in the upper limit, 53.6 mg in the middle zone and 35.4 mg in the lower limit.

Regarding the leaf production, values of 1.01 g d.w. shoot^{-1} year^{-1} in the upper limit, 0.84 g d.w. shoot^{-1} year^{-1} in the middle zone and 0.67 g d.w. shoot^{-1} year^{-1} in the lower limit were estimated in 2001. Quite similar values were estimated in 2000 (0.98 g d.w. shoot^{-1} year^{-1} in the upper limit, 0.90 g d.w. shoot^{-1} year^{-1} in the middle zone and 0.67 g d.w. shoot^{-1} year^{-1} in the lower limit).

Tab. 2 - Age of rhizomes and production data (mean ± s.d.) distinct by meadow zone.

	MEADOW ZONE		
	upper limit	middle zone	lower limit
mean age of rhizomes (years)	7.2 ± 3.8	9.0 ± 3.6	8.4 ± 3.3
LFR (n. leaves shoot^{-1} year^{-1})	6.3 ± 1.1	6.1 ± 0.9	6.1 ± 1.0
RhER (cm rhizome^{-1} year^{-1})	0.6 ± 0.3	0.8 ± 0.4	0.5 ± 0.2
RhP (mg d.w. rhizome^{-1} year^{-1})	46.1 ± 14.6	53.6 ± 15.9	35.4 ± 9.5
Leaf Production in 2000 (g d.w. shoot^{-1})	0.98	0.90	0.67
Leaf Production in 2001 (g d.w. shoot^{-1})	1.01	0.84	0.67

Discussion and conclusions

Data reported in this paper describe the investigated seagrass meadow in terms of bio-ecological features. The nMDS analysed showed quite clear differences between the shallower stands, settled along the upper limit, with those in the meadow core and in the lower limit, confirming the influence of depth as ecological factor driving the overall structure and morphology of *P. oceanica*. In addition, the along-shore (NNW-SSE) analysis showed a general homogeneity, indicating the absence of important ecological gradients along the coast.

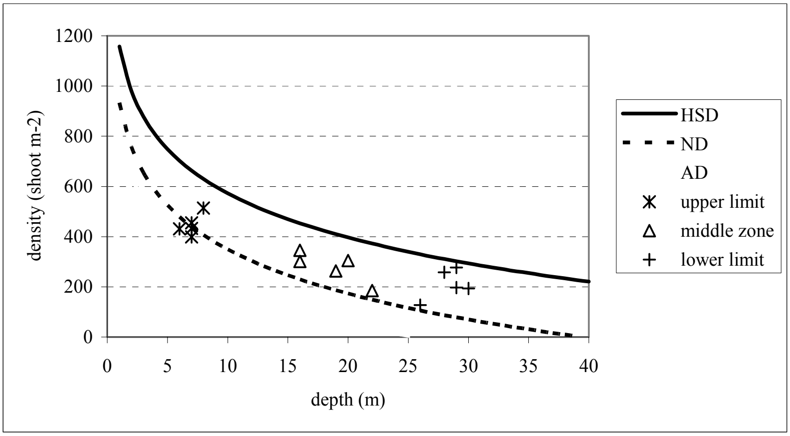

Fig. 3 - Classification of meadow density per sampling sites, using a graphic elaboration based on the theoretical values proposed by Pergent-Martini *et al.*, 1999. (AD: abnormal density limit; ND: normal density limit; HSD: higher sub-normal density limit).

The morphological and lepidochronological data, collected for the *P. oceanica* meadow settled in this area of Southern Tyrrhenian Sea, seem comparable to those reported for other pristine meadows of the Mediterranean Sea (Pergent and Pergent-Martini, 1990; Semroud *et al.*, 1990; Pergent-Martini *et al.*, 1994; Mostafa and Halim, 1995; Calvo *et al.*, 1995; Ballesta *et al.*, 2000; Guidetti *et al.*, 2000). That condition seems to be effective even considering the recent criteria proposed by Pergent-Martini *et al.* (1999) in order to estimate the health state and the conservation of a seagrass bed. In fact, the density values recorded in almost all the sampled stations fall in the range of "normal density" (ND) and in particular, in the lower limit, almost all the density values fall near the higher sub-normal limit (HSD) (Fig. 3).

Moreover, the mean values calculated for both the LFR and the RhER are in the normal-subnormal range (Pergent-Martini *et al.*, 1999). The lower limits were always recorded in a depth range of 25-30 m, confirming a persistent condition of transparency of the water column over the investigated meadow. The general good state of these *P. oceanica* meadows is consistent with the very low urbanization of the coast and the fact that in the area fishing is not allowed inshore (Rende *et al.*, 2005).

Acknowledgments

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S. Debono[1], J.A. Borg[2]

[1] Nature Protection Unit, Malta Environment and Planning Authority, Floriana, Malta
[2] Department of Biology, University of Malta, Msida, Malta

USE OF *POSIDONIA OCEANICA* AS A BIOINDICATOR OF ECOLOGICAL STATUS FOR MALTESE COASTAL WATERS

Abstract

Data on Posidonia oceanica *attributes from Maltese Coastal waters are available from a number of research studies and environmental monitoring programmes. As part of Malta's obligations for implementing the European Union's Water Framework Directive, the Malta Environment and Planning Authority participated in an intercalibration exercise, involving Mediterranean countries that are also EU member states, aimed at ensuring comparability of different methods for the classification of coastal waters using* P. oceanica *as bioindicator. Data on* P. oceanica *attributes (shoot area, shoot biomass and shoot density), collected during the period 1999 to 2004 from 19 sites in Malta and Gozo, were analysed using Analysis of Variance and Principal Component Analysis to identify differences in values of seagrass attributes between the different sites surveyed. The output from the statistical analyses was used to derive values of the Ecological Quality Ratio (EQR) for the five status classes (high, good, moderate, poor or bad). Of the 19 sites considered, 2 sites were classified as having 'high' status, 14 sites had 'good' status, 2 sites had 'moderate' status, and 1 site had 'poor' status. None of the sites were classified as having 'bad' status; however, this was attributed to the lack of* P. oceanica *data from localities that represented highly degraded coastal areas. The results obtained are discussed in the light of knowledge of the environmental characteristics of the sites surveyed, and recommendations for development of a national method for assessing and classifying the ecological status Maltese coastal water, based on a larger data set and inclusion of additional seagrass descriptors, are proposed.*

Key-words: *Ecological status classification, environmental monitoring, Maltese Islands*, Posidonia oceanica*, Water Framework Directive.*

Introduction

The Water Framework Directive (WFD), adopted by the European Union in 2000, obliges EU member States to assess and monitor the ecological status of water bodies across Europe through the use of Biological Quality Elements (BQEs), and to classify the waters in one of five status classes: *high, good, moderate, poor or bad*. In the Mediterranean region, the endemic seagrass *Posidonia oceanica* was selected (amongst others) as a BQE for marine coastal waters, owing to its widespread distribution and sensitivity to disturbance (Pergent-Martini *et al*., 2005). As part of the its Common Implementation Strategy (CIS), the EU also requires close collaboration between different member states to ensure European-wide comparability of methods used to classify the water bodies. To achieve this, EU member states were required to undertake an intercalibration exercise. In the meantime, no procedure had yet been adopted in Malta to assess and monitor the ecological status of coastal waters around the Maltese Islands. The aim of the present work was to test the Maltese method for classifying coastal waters, using a given set of *P. oceanica* descriptors, as established by the Geographical Intercalibration Group for the Mediterranean (Med GIG).

Materials and methods

Studies of *P. oceanica* undertaken between 1999 and 2004 were reviewed for availability of data on three seagrass attributes, namely shoot density, shoot area and shoot biomass, identified by the Med GIG for the intercalibration exercise. To avoid potential confounding effects, data was only selected from studies in which sampling was carried out within a given sampling period (spring or summer) and from a narrow depth range (9 m - 12 m). Using these selection criteria, data on *P. oceanica* morphometric descriptors available from a total of 23 coastal sites around the Maltese Islands (see Fig. 1) were selected for the analysis.

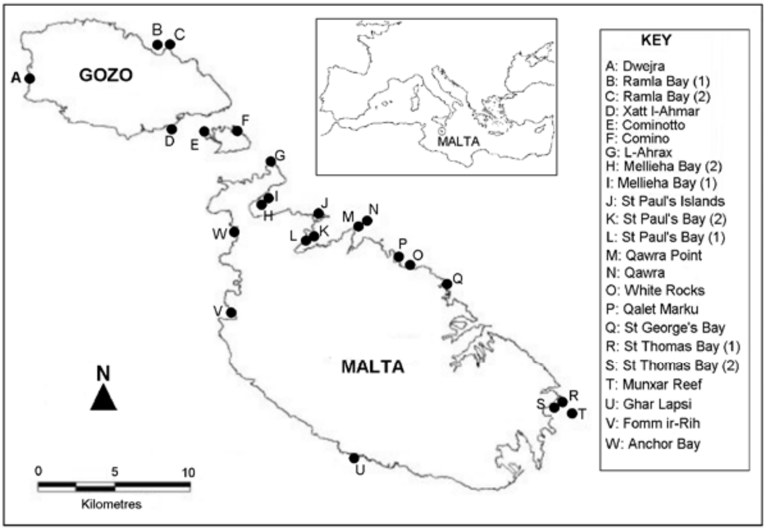

Fig. 1 - Map of the Maltese Islands showing the locations from where *P. oceanica* data used in the present work, had been collected.

The available data were first analysed using Analysis of Variance (ANOVA) to test for significant differences in the three *P. oceanica* attributes between different sites. In the analysis, data for the *P. oceanica* attributes, which are known to exhibit seasonal variation (shoot area and shoot biomass; e.g. see Borg and Schembri, 1995), were analysed separately by season; while data for meadow density, which does not exhibit seasonal variation (e.g. Buia *et al.*, 2000) was analysed collectively. Where significant differences were detected by the ANOVA, post hoc Student-Neuman-Keuls (SNK) tests were carried out to identify the source of difference. Data on shoot density and shoot area collected in summer were then analysed using Principal Components Analysis (PCA) to test for grouping of sites (see Romero *et al.*, 2005). It was not possible to include shoot biomass in the multivariate analysis, since data for this attribute had been collected in spring. The results from ANOVA and PCA, together with data of the physico-chemical characteristics of the sites (Axiak, 2004), were then used to categorise the 19 different sites into the five status classes. Values of the Ecological Quality Ratio (EQR) were then calculated, using the equation below:

$$EQR = \left(\frac{Y_1}{X_1} + \frac{Y_2}{X_2} + ... + \frac{Y_n}{X_n} \right) \times \left(\frac{1}{n} \right)$$

Where: X_1 = maximum mean value of the first *P. oceanica* attribute recorded from the reference condition; Y_1 = mean value of the first *P. oceanica* attribute recorded from any other condition being compared with the reference; X_n = maximum mean value of the nth *P. oceanica* attribute recorded from the reference condition; Y_n = mean value of the nth *P. oceanica* attribute recorded from any other condition being compared with the reference; n = number of different *P. oceanica* attributes used.

Results

The results of one-way ANOVA indicated significant differences for the three *P. oceanica* attributes between the different sites considered. Results of PCA using data of the two *P. oceanica* attributes from 19 sites, together with the estimated EQR values for the respective sites, enabled the setting of class boundaries (Fig. 2) and classification of the sites as shown in Table 1. The highest mean values of shoot area and shoot density were recorded from Ramla Bay. These were used as reference conditions for the estimation of EQRs, from which the final classification of sites was produced.

Tab. 1 - Proposed classification and estimated EQR values for the 19 sites. The site labels are the same ones used in Fig. 1.

Status	**Site**		**EQR**
High	B	Ramla (1)	**0.89**
	C	Ramla (2)	**0.82**
Good	H	Mellieha Bay (1)	**0.70**
	I	Mellieha Bay (2)	**0.66**
	P	Qalet Marku	**0.73**
	E	Cominotto	**0.72**
	F	Comino	**0.72**
	U	Ghar Lapsi	**0.74**
	M	Qawra Point	**0.75**
	D	Xatt l-Ahmar	**0.76**
	V	Fomm ir-Rih	**0.69**
	G	l-Ahrax	**0.69**
	A	Dwejra	**0.62**
	O	White Rocks	**0.66**
	J	St Paul's Islands	**0.65**
	R	St Thomas Bay (1)	**0.64**
Moderate	S	St Thomas Bay (2)	**0.54**
	Q	St George's Bay	**0.53**
Poor	W	Anchor Bay	**0.45**
Bad		N/A	**N/A**

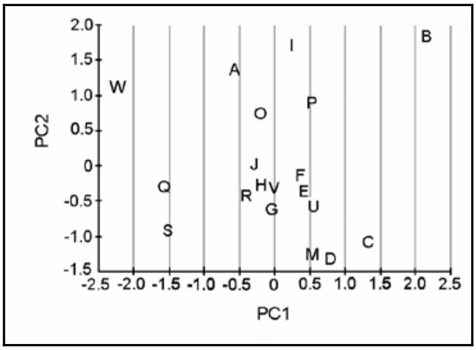

Fig. 2 - PCA ordination of the 19 sites using data of shoot area and shoot density (see Figure 1 for interpretation of labels used). Percentage variation explained by PC1 and PC2 = 50.7% and 49.3% respectively.

Most of the sites considered in the present study were classified as having 'good' status, two sites were classified in each of the 'high' and 'moderate' status classes, and a single site was classified as having 'poor' status. None of the 19 sites was classified as having 'bad' status.

Discussion and conclusions

The present work was entirely based on *P. oceanica* data that was available from local studies held in the past. Although several studies on the morphology of *P. oceanica* have been carried out in the Maltese Islands, relatively few qualified for use in the present study since the data had to fit the preset criteria (i.e. water depth at the sampling stations, sampling period and timescale of data, amongst others). The present results show that it is possible to use univariate and multivariate analysis to group sites based on values of *P. oceanica* attributes for the setting of status class boundaries as required by the WFD. There is also corroboration between the resulting classification and information on physico-chemical characteristics for the respective sites. For example, nutrient levels in the water column are higher (Axiak, 2004) at sites that classified as having 'poor' and 'moderate' status, hence showing that the various status classes are indicative of anthropogenic pressure.

Present results showed that most of the sites were classified as having 'good' status, whereas few sites classified as having 'poor', 'high' or 'moderate' status, and no site classified as having 'bad' status. The use of data for only two *P. oceanica* attributes, together with the small number of sites representing the 'high', 'moderate', 'poor' and 'bad' status classes in the statistical analyses may render the analyses insufficiently robust, and as such the present work should be considered as preliminary. Therefore inclusion of additional *P. oceanica* attributes (e.g. percentage plagiotropic to orthotropic rhizomes, percentage necrosis of leaves, and shoot epiphyte biomass) and sites in the analysis, would render the procedure sufficiently robust for assessing and monitoring the ecological status of Maltese coastal waters.

Acknowledgments

We are grateful to the Authors of works from which data were considered for the present study.

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G. Di Carlo[1], F. Badalamenti[1], A. Terlizzi[2]

[1]Laboratorio di Ecologia Marina, IAMC-CNR,
Via G. da Verrazzano 17, 91014, Castellammare del Golfo (TP), Italy
[2]Laboratorio di Zoologia e Biologia Marina, Dipartimento Scienze e Tecnologie Biologiche ed Ambientali,
Università di Lecce, CoNISMa, 73100 Lecce, Italy

RECRUITMENT OF *POSIDONIA OCEANICA* ON RUBBLE MOUNDS: SUBSTRATUM EFFECTS ON BIOMASS PARTITIONING AND LEAF MORPHOLOGY

Abstract

Large and small scale impacts on seagrass meadows are increasing globally. In this study, the morphological response of Posidonia oceanica *after natural vegetative recruitment onto a disturbed site was investigated. Leaf length was measured as well as biomass partitioning. Leaf length was found to differ between the rubble field and the matte. A similar pattern was found for biomass allocation. This study provides new insights on the development process of* P. oceanica *in response to physical disturbance. Evidences presented indicate which morphological characteristics occur after seagrass settlement on unconsolidated substrates, compared to those found in matte environment.*

Key words**:** *rubble mounds, Posidonia oceanica, vegetative fragments, biomass, Mediterranean Sea.*

Introduction

Increased attention to the function of seagrass ecosystems in the coastal area has provided evidence that seagrass meadows are experiencing a worldwide decline because of human disturbance (Short and Wyllie-Echeverria, 1996). In the Mediterranean Sea, anthropogenic pressure is causing a widespread decline of coastal habitats (Green and Short, 2003). Thus, there is concern about the loss of seagrass ecosystem functions and efforts are being focused on protecting seagrass habitats. *Posidonia oceanica* (L.) Delile, ranks among the longest lived seagrasses (Duarte, 1991). Due to the plasticity of rhizome growth modules, *P. oceanica* has the potential to adapt its form and function to the immediate environment (Marbà and Duarte, 1998). Thus, changes in distribution patterns, homogeneity of cover, shoot density, biomass partitioning and dynamic features of *P. oceanica* can be used to obtain an integrated response to disturbances (Marbà *et al.*, 1996; Badalamenti *et al.*, 2006). At Capo Feto, (SW Sicily, Italy) a monospecific *P. oceanica* meadow was severely damaged by a dredge-fill operation (Badalamenti *et al.*, 2006), but water conditions were not altered (i.e. light penetration). The dredged trench was back-filled with rubble to form a series of rubble mounds on the seabed (Di Carlo *et al.*, 2005). This study investigates the morphological features of *P. oceanica* following the establishment of fragments of the plant on the rubble mounds in order to understand the link between substratum disturbance and the morphological features of *P. oceanica* as well as to explore seagrass

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[1] Author present address: National Ocean Service, NOAA/NOS/NCOS, Center for Coastal Fisheries and Habitat Research, 101 Pivers Island Road Beaufort, North Carolina 28516-9722, USA

biomass partitioning at the very early stage of seagrass recruitment in disturbed habitats.

Material and Methods

The study was conducted in June 2001 at Capo Feto (SW Mediterranean, Italy, Fig. 1). Samples were collected from depths of 5, 10 and 15 m, for each of three locations on rubble mounds (crest, side and valley) and from an adjacent pristine bed (matte). Twenty randomly chosen shoots were collected for each combination of location × depth.

Seagrass for biomass determinations was collected using stainless steel quadrats (40 × 40 cm) and scooping out the belowground parts as deep as 20 cm into the substratum.

For each location (crest, side, valley and matte), biomass was sampled at 3 random sites chosen at 5 and 10 m depth. Within each site, six independent replicates were collected.

Fig. 1 - Study area at Capo Feto (Sicily, Italy).

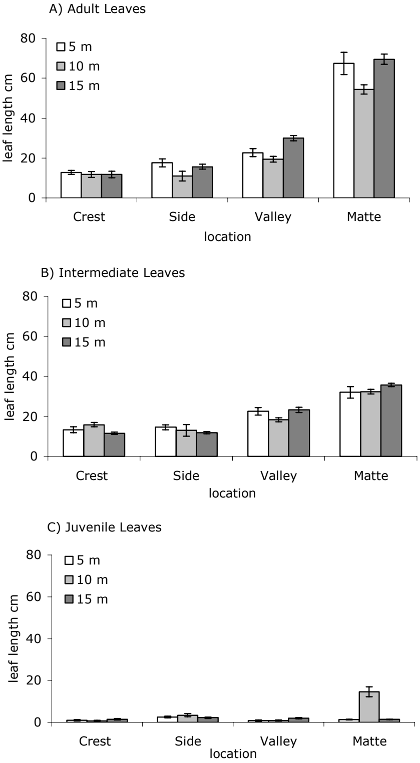

Fig. 2 - Seagrass leaf length on the crest, side, valley and matte at different depths (5, 10 and 15 m). Bars = SE

The leaves were then separated into the various categories (Adult A, Intermediate I, Juvenile J) as described by Giraud (1979). For each leaf, total length and width were measured as well as sheath and 'brown tissue' length. Only results for the most significant parameter (leaf length) are presented here. For biomass determinations, the samples were subdivided into two compartments: above- (leaf) and below-ground organs (rhizomes + roots). Samples were dried to constant weight at 60 °C.

Analysis of Variance (ANOVA) was used to test for differences in leaf features and biomass allocation between seagrass on rubble mounds and seagrass growing on matte, and for differences between rubble locations.

Results

Leaf morphology

The results revealed a significant difference in leaf length between rubble locations (crest, side and valley) (Fig. 2). For mean leaf length of J leaves and leaf surface area of J leaves, such differences were consistent at all depths (5, 10 and 15 m).

The results indicated that mean values

of leaf length were lower for the crest and side of the mounds with respect to the valleys (i.e. C=S<V, Fig. 2). ANOVA tests showed that within each level of the factor depth, differences between rubble locations were often not significant for 5 and 10 m. However, whenever significant differences were found, the valleys accounted for the highest mean values. The pattern of C=S<V was always observed at 15 m.

Significant differences in leaf length (A, I, J) were also detected between shoots collected on the matte and shoots collected on rubble locations (R-v-M) (Fig. 2). In all cases, significantly lower mean values were found on rubble locations with respect to the matte (rubble < matte).

Biomass Partitioning

The analysis of biomass partitioning indicated that differences between crest, side and valley varied with depth and biomass compartment (Fig. 3). There was no significant difference between average biomass values for plants living on rubble mounds and plants living on matte (Fig. 3). Root biomass did not significantly differ between crest, side and matte at both 5 and 20 m, and the valley values were the highest for root biomass (C=S=M<V). A similar pattern was recorded at 10 m depth for rhizome biomass. At 5 m depth all locations differed significantly in rhizome biomass with crest accounting for the lowest values and matte for the highest (C<S<V<M). The C=S=V<M pattern was consistent at both 5 and 10 m depth.

At the matte location, the above- and below-ground compartments had mean values of 56.7% and 43.3% of total biomass at 5 m and 46.1% and 42.5% of total biomass at 10 m (Fig. 4). The rubble field presented a biomass allocation skewed towards the below-ground biomass (averages of 98.2% and 95.0% respectively at 5 and 10 m depth), with low values found for the above-ground biomass (averages of 1.8% and 5.0%) (Fig. 4).

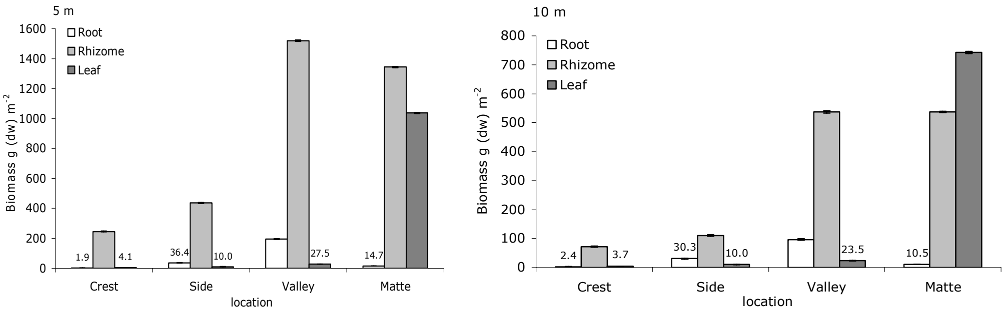

Fig. 3 - Biomass allocation to different plant compartments at different locations (crest, side, valley and matte) at 5 m and at 10 m depth. In each case n = 6, bars equal standard error. Numbers on graph show values of biomass < 40 g dw m^{-2}.

Discussion

The results indicated reduced leaf lengths for *P. oceanica* on the rubble substratum compared to the matte. This suggests that substratum type and sediment characteristics can influence morphological and growth variables of seagrasses

(Marbà and Duarte, 1997; Mills and Fonseca, 2003). Within the rubble field leaf lengths also varied between plants living on crests, sides and valleys. *P. oceanica* recruited on crests and sides displayed smaller leaf lengths than seagrass established in valleys. Such variations might be related to the different physical processes occurring in the two microhabitats (crest *vs* valleys) described by Di Carlo *et al.* (2005). Significant differences in biomass allocation were found between crests, sides and valleys. *P. oceanica* on crests lacked roots and had a low leaf biomass. Seagrass established in valleys had high root and low leaf biomass.

If these values are compared to those for the matte, it can be inferred that in valleys plants allocate more resources to root growth, suggesting that only those plants with better anchorage may survive in the unconsolidated rubble substratum. Similar data have been reported by other authors (Hemminga and Duarte, 2000) who pointed out the structural importance of belowground organs in mechanically anchoring the plants in the substratum.

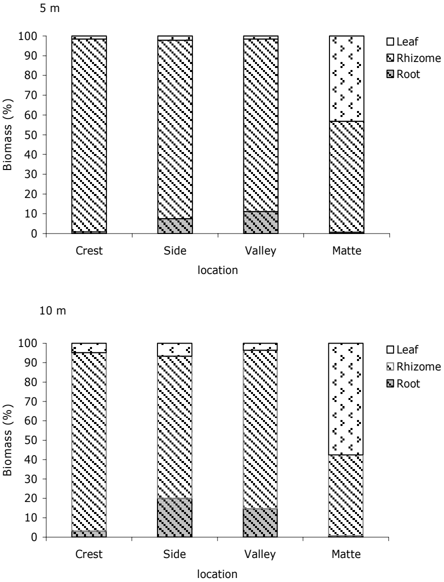

Fig. 4 - Percentage contribution of the above (leaves) and belowground (roots + rhizomes) compartments of *P. oceanica* at different locations.

The work conducted at Capo Feto shows the very early stage of the development of a *P. oceanica* seagrass bed on a disturbed marine environment (i.e. rubble field) and the following main conclusions result: (1) *P. oceanica* leaf length varies in relationship to substratum type (rubble *vs* matte); (2) biomass allocation is determined by substratum type as the need for anchorage increases root development; and (3) above-ground biomass is reduced where the presence of an adequate sediment layer to support seagrass growth is limiting.

We suggest the importance of our results in mitigation and restoration activities of *P. oceanica* meadow in disturbed environments, where water conditions have not been altered, and in forecasting seagrass response to substratum disturbance.

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S. Flagella, I. Borriello, M.C. Gambi, M.C. Buia

Stazione Zoologica 'A. Dohrn', Laboratorio di Ecologia del benthos, 80077 Ischia (Naples, Italy).

RESPONSES OF *POSIDONIA OCEANICA* TO ENVIRONMENTAL DISTURBANCE

Abstract

Structural and functional changes in two meadows (Cava and Maronti) off the island of Ischia (Gulf of Naples, Italy) were monitored since 1996 by means of lepidochronology. No differences in mean shoot density were recorded while two abnormal increases in the rhizome growth rate were evident for the years 2000 and 2001 in the shallowest stands of the Cava bed, and for the year 2002 in the Maronti bed. These high values in the rhizome production could be related to two different kinds of disturbance events. In fact, in late December 1999 a violent sea storm affected the western sector of the island of Ischia where the Cava bed is located: as an effect, a large amount of leaves and rhizomes were beached to build 'banquettes' up to 180 cm in height. In March 2002, dredging for sand extraction activities (related to a beach nourishment) were carried out off the Maronti Bay, in an area largely overlapping with the distribution of the P. oceanica *bed: post impact observations revealed the destruction of about 4 hectares of the meadow. In spite of the different nature of the two disturbances (natural and anthropogenic), a variation in the sedimentation rate may have induced a similar plant response in both beds: a significant increase in the rhizome growth rate. Temporal occurrence between growth anomalies and the impact events seems to testify the capacity of* P. oceanica *to respond to variations of environmental conditions.*

Key-words: *ecosystem disturbance, primary production, coastal zone management, monitoring Posidonia oceanica.*

Introduction

The most complex and widespread ecosystem in the Mediterranean phytal zone is formed by *Posidonia oceanica* meadows (Procaccini *et al*., 2003); this seagrass is a well recognized bio-indicator for ecological research applied to the conservation of littoral ecosystems (Ferrat *et al*., 2003; Marbà *et al*., 1996; Pergent *et al*., 1995).

As the regression of *Posidonia* beds is evident in many parts of the Mediterranean Sea due to disturbances of different nature (Cancemi *et al*., 2003; Gonzàlez-Correa, 2005; Guidetti, 2001), changes in meadows density and in the plant growth patterns have been often used to obtain an integrated response to such disturbances (Cavazza *et al.,* 2000; Marbà and Duarte, 1997; Peirano *et al*., 2005). In fact, by using ageing reconstruction technique (lepidochronology, Pergent, 1990) it is possible to point out time variations of growth referable to environmental changes (Buia *et al*., 2005; Pergent-Martini *et al*., 1994).

Variations in structural and functional features in two meadows off the island of Ischia (Gulf of Naples, Italy) were studied in order to evaluate the response of the plant to environmental disturbances.

Materials and methods

Two meadows off the island of Ischia (Gulf of Naples, Italy), Cava and Maronti, both situated at about 600 m far from the coast (Fig. 1) and submitted to high hydrodynamic conditions were monitored since 1997 and 2001, respectively.

Cava meadow, located on the western part of the island, extends from 10 to 30 m depth and grows on coarse sandy and rocky substraum. Its lower limit is both progressive and erosive. A 'matte' 50 cm high is present. Two stations, at 11 m and at 28 m depth, were considered.

In the south part of the island in front of the Maronti 'pocket' beach, the *Posidonia* meadow extends from 16 to 20 m depth settled on sandy substratum, and with a lower limit mostly erosive and clear-cut. The 'matte' is over 200 cm high. A single station, between 15 and 18 m depth, was sampled.

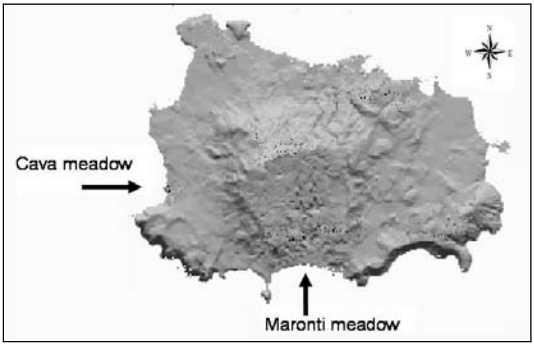

Fig. 1 - Map of the Ischia Island with location of the two meadows studied.

Shoot density evaluations were performed on 40 x 40 cm quadrates (10 counts) since 1997 in the Cava meadow, and since 2001 in the Maronti meadow.

A total of 80 vertical shoots were randomly collected in 2005 in each station of the two meadows for estimates of past rhizome production (mg y^{-1} and mm y^{-1}) by means of the lepidochronology technique (Pergent, 1990). The analysis of variance (ANOVA) was performed to evaluate inter-annual differences in the production.

Results

During monitoring years, shoot density did not change significantly both in the Cava meadow (shallow *vs* deep stands) and in the Maronti meadow (Fig. 2): all the density values, in fact, corresponded to the Normal Density class (ND) according to Pergent et *al.* (1995).

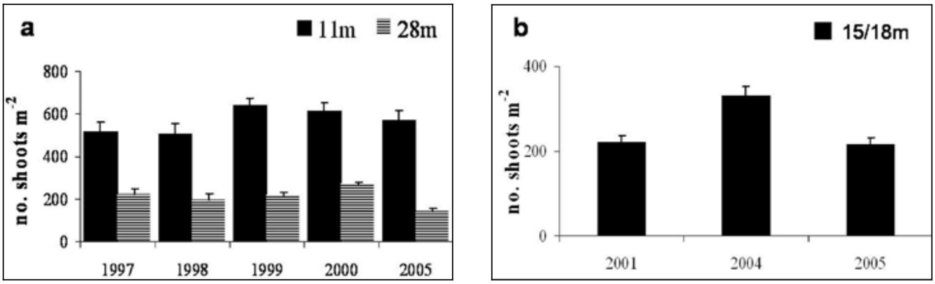

Fig. 2 - Density (no. shoots m^{-2}) in Cava (a) and Maronti (b) meadows.

Rhizome production pattern was estimated from 1996 to 2002. In the Cava meadow the production was significantly different in respect to time (Table 1). In particular, a higher production was evident in the year 2000 and 2001 for the rhizome compartment.

In the Maronti meadow the production was significantly different only for the rhizome elongation (Table 2) and in particular an anomalous high value was recorded in 2002.

Tab. 1 - One way ANOVA for rhizome production parameters in the Cava meadow (*p< 0.05; n.s. not significant).

	df	rhizome growth			rhizome elongation		
		MS	F	p	MS	F	p
1-DEPTH	1	71449.78	31.36	*	771.8336	19.87	*
2-TIME	6	11000.65	4.83	*	121.3442	3.12	*
1x2	6	4826.042	2.12	n.s.	50.35142	1.3	n.s.
Error	56						

Tab. 2 - One way ANOVA for rhizome production parameters in the Maronti meadow (*p<0.01; n.s. not significant).

	df	rhizome growth			rhizome elongation		
		MS	F	p	MS	F	p
1-TIME	6	2120.48	1.168	n.s.	41.34	3.67	*
Error	63						

Discussion and conclusions

The higher production values recorded in Cava meadow in 2000 - 2001 could be related to sea storm of exceptional severity which occurred in December 1999, and that particularly affected the western sector of Ischia Island, where the Cava meadow is located (Zucco, 2003). This storm was characterized by a low barometric pressure down to 970 millibars, a wind speed of 50 miles per hour, and waves up to 10 meters high. As an effect, a large amount of leaves and rhizomes were cast up onto the beach in front of the Cava meadow and formed “banquettes” up to 180 cm high. In addition, sand waves of about 50 cm high were evident inside the meadow, especially at its lower limit (Gambi and Iacono, personal observations).

In the Maronti meadow a high growth rate, expressed as elongation in mm, was recorded in 2002 corresponding to dredging operations (March 2002) which were related to the Maronti beach nourishment activities. The sand extraction activity (authorized as an urgent civil protection action for the security of the coast) was conducted in an area largely overlapping with the meadow distribution. Post–impact observations revealed the destruction of about 4 hectares of the meadow (Gambi *et al.*, 2005).

Temporal occurrence between growth anomalies and the impact events seems to testify the capacity of *P. oceanica* to respond to variations of environmental conditions.

In spite of the different nature of the two disturbances (natural and anthropogenic), a variation in the sedimentation and burial rate may have induced a similar plant response in both beds: a significant increase in the rhizome growth rate.

Nevertheless, there is an evident imbalance in the growth rate recorded in the Maronti meadow where rhizome production is shifted towards an increase in length rather than in weight. A higher increase in rhizome elongation in comparison to rhizome growth may reduce, on the long term, the resistance of the hypogeal compartment and impair its anchoring function.

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I. Gattorna, P. Panzalis, A. Navone., R. Cattaneo-Vietti, M. Montefalcone,
C. Morri, C.N. Bianchi

Dip.Te.Ris., Università di Genova, Corso Europa 26, 16132 Genova (Italy).

PRELIMINARY SURVEY OF THE HEALTH STATE OF THE *POSIDONIA OCEANICA* BED AT SPALMATORE DI TERRA, TAVOLARA-PUNTA CODA CAVALLO MARINE PROTECTED AREA (SARDINIA, WESTERN MEDITERRANEAN)

Abstract

This study was set up in Spalmatore di Terra within Tavolara-Punta Coda Cavallo Marine Protected Area (Sardinia) in order to determine the preliminary health state of the Posidonia oceanica *meadow. This meadow is subject, especially during summer, to the impact caused to boat anchoring, for this reason three depth zones were identified (shallow, intermediate, and deep), where boats of different sizes moor with different types of anchors. Health status of the meadow was estimated using the Conservation Index (CI), which relates the cover of living* P. oceanica *to that of dead matte, and the Fragmentation Index (FI), which measures the heterogeneity within the meadow. The shallow and deep zones exhibited significantly lower values of CI than the intermediate zone and, conversely, they showed higher values of FI than the intermediate zone.*

Key-words: Posidonia oceanica*, boat anchoring, Conservation Index, Fragmentation Index, Marine Protected Area.*

Introduction

Posidonia oceanica (L.) Delile is a marine phanerogam endemic to the Mediterranean Sea. This species forms extensive seagrass meadows from the surface to more than 30-40 m depth. *P. oceanica* is the only seagrass that forms a "matte", i.e. a terrace constituted by live and dead intertwined rhizomes, together with the sediment which fills the interstices (Molinier *et al.,* 1952). Decline of *P. oceanica* has been attributed to both natural and anthropogenic disturbances.

The aim of the present study was to evaluate the health state of a *P. oceanica* meadow that is subject, especially during summer, to the impact caused to boat anchoring. Among human impacts on the bed, in fact, boat anchoring has been identified as one of the most important direct causes of small scale degradation of seagrass meadows (Francour *et al.,* 1999), which not only provokes the baring of the matte, taking shoots away, but may eventually lead to erosion of the matte itself. Intensity of damage was likely to be connected with the anchor type, which is linked in turn to the size of the boat. The health state of the meadow was estimated using two synthetic environmental indices: the first, "Conservation Index" (CI), was proposed by Moreno *et al.* (2001) and reviewed by Montefalcone *et al.* (2006); the second is a new one, defined as the "Fragmentation Index" (FI).

Materials and methods

The study was carried out at Spalmatore di Terra, located in Tavolara island within Tavolara-Punta Coda Cavallo Marine Protected Area (North-East Sardinia, Italy) (Fig. 1). Spalmatore di Terra is highly frequented by boaters during summer.

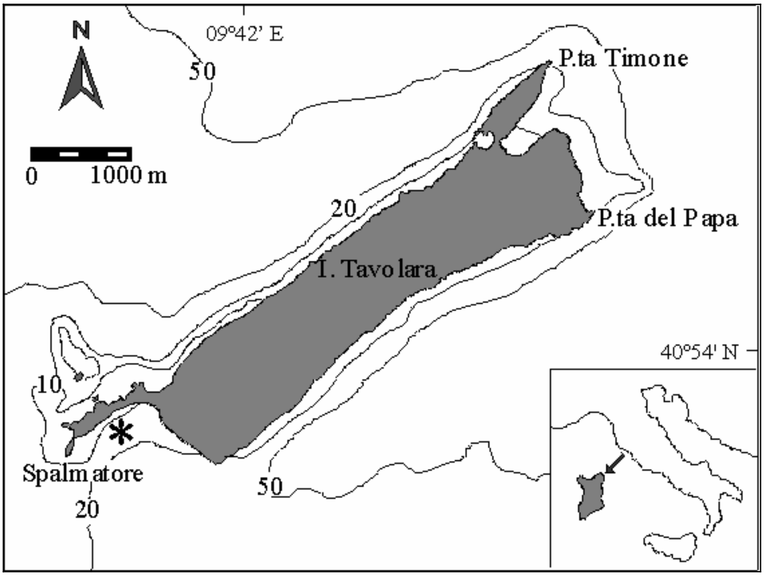

Fig. 1 - Tavolara - Punta Coda Cavallo Marine Protected Area. The location of the study area is also reported (*).

The investigation of the *P. oceanica* meadow was carry out in summer 2005 using underwater surveys by SCUBA diving in order to obtain data on the percentage cover of living *P. oceanica*, dead matte, and sand. Three depth zones were identified in which boats of different sizes anchored utilising different types of anchors: shallow zone (0-5 m), intermediate zone (5-10 m) and deep zone (10-15 m). In each zone, four sites were recognized, spaced about 200 m from each other and 10 replicated cover values were estimated within each site. In the shallow zone the anchored boats were less than 6 m long and mostly utilised Folding grapnel and Danforth anchors. In the intermediate zone the boats had a total length between 6 and 16 m and used Danforth, Bruce, Cqr and Hall anchors. In the deep zone the anchored boats were more than 16 m long and used Hall anchors.

Based on the percentage cover data collected, two different indices were calculated: the Conservation Index (CI) and the Fragmentation Index (FI). CI (Moreno *et al.*, 2001; Montefalcone *et al.*, 2006) is expressed by the formula:

$$CI = L / (L+D)$$

where L is the percentage cover of live *Posidonia oceanica* and D is the percentage cover of dead matte. The index ranges between 0 (minimum state of conservation) and 1 (maximum state of conservation).

FI is an index that has been taken as indicative of the matte erosion, leading to the appearance of sandy channels and patches within the meadow. This index is expressed by the formula:

$$FI = 1-\Sigma p_i^2$$

where $p_i = x_i/100$ and x_i is the percentage cover of different parameters considered: living *P. oceanica*, dead matte and sand. The index ranges from 0 (minimum state of fragmentation) to 1 (maximum state of fragmentation).

The resulting values of both CI and FI were assigned to four intervals following the procedure suggested by Montefalcone *et al.* (2006):

1. CI or FI values < (x-½s)
2. CI or FI values from (x-½s) to x excluded
3. CI or FI values from x included to (x+½s)
4. CI or FI values > (x+½s)

where x is the mean and s the standard deviation of the index.

CI and FI data were analysed using a two-way ANOVA (Underwood, 1997), with "zone" (three levels, orthogonal) and "site" (four levels, nested in zone). Cochran's test was used to check for the homogeneity of variances. When appropriate, Student-Newman-Keuls' (SNK) test was employed to separate means ($\alpha = 0.05$).

Results

Surveys in 2005 showed that in August, the month of maximal frequentation, the number of boats anchored at Spalmatore di Terra reached 1772; 62% of the total number of boats anchored in the shallow zone, 36.9% in the intermediate zone and 1.2% in the deep zone.

The cover of living *P. oceanica* was not uniform along the three depth zones. In the shallow zone, where the meadow upper limit was at 0.8 m depth, living *P. oceanica* cover exhibited the lowest values, whereas sand showed the highest values. On the contrary, the intermediate zone showed the highest values of living *P. oceanica* and the lowest values of dead matte. The deep zone is characterized by a mosaic of living *P. oceanica* and dead matte, both with high values of percentage cover (Fig. 2).

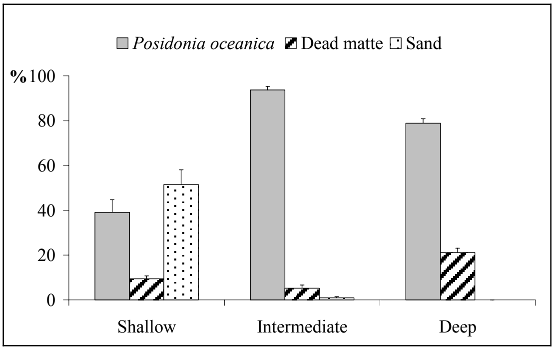

Fig. 1 - Percentage cover of *Posidonia oceanica*, dead matte and sand in the different depth zones (mean ± s.e.)

The values of the Conservation and Fragmentation Indices were different among depth zones ($F_{2,108} = 15.73$, $p<0.001$ for CI; $F_{2,108} = 47.26$, $p<0.001$ for the FI; Tab. 1), whereas no differences were observed among sites. The SNK results showed that the intermediate zone was different compared to the shallow and the deep zone.

Tab. 1 - Two-way ANOVA on the CI and FI

		CI			FI	
Source of Variation	Df	MS	F	df	MS	F
Zone: Zo	2	1.1027	15.73***	2	1.2268	47.26***
Site: Si (Zo)	9	1.0701	1.43	9	1.0260	0.54
Residual	108	0.0490		108	0.0479	
Transformation		None			None	
Cochran's test		C=0.17 ns			C=0.13 ns	

CI ranged from 0.5 to 1. CI values were divided into four intervals, representing distinct states of conservation of *P. oceanica* meadow:

1) advanced degree of regression (CI< 0.76)
2) impacted meadow (CI between 0.76 and 0.84)
3) low to moderate conservation status (CI between 0.84 and 0.91)
4) high state of conservation (CI> 0.91)

The shallow and the deep zones were characterized by an impacted meadow (class 2), conversely the intermediate zone exhibited a high state of conservation (class 4).

FI ranged from 0 to 0.56. Similarly to CI, FI values were divided into four intervals, illustrating four different degrees of fragmentation:

1) little or no fragmentation (FI<0 .14)
2) low fragmentation (FI between 0.14 and 0.21)
3) moderate fragmentation (FI between 0.21 and 0.29)
4) high fragmentation (FI>0.29)

The shallow and the deep zones showed a moderate status of fragmentation (class 3), on the contrary the intermediate zone is characterized by little or no fragmentation (class 1).

Discussion

These preliminary results suggest that the *P. oceanica* meadow investigated is probably damaged to some extent by the impact of boat anchoring. However, since we don't know the "natural" degree of meadow fragmentation and patchiness in the studied area, inferences about anchor impact have to be considered with caution. We can hypothesize that in the shallow zone, characterized by great number of anchored boats, the very low conservation status and the moderately high values of fragmentation can be favoured by the use of most penetrating anchor type (e.g. Folding Grapnel) (Milazzo *et al.,* 2004). In the deep zone, with low values of CI and the high values of FI, the possible impact may be due to largest boats with heaviest and biggest anchors (Francour *et al.,* 1999).

Further investigations and field experiments, with reference control areas, are needed to explore the actual entity and consequences of the anchor mechanical damage on the *Posidonia* meadow in this marine protected area.

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Y.A. GENEID, H.H. EL-HADY

National Institute of Oceanography and Fisheries, Egypt

DISTRIBUTION, BIOMASS AND BIOCHEMICAL CONTENTS OF THE SEAGRASS SPECIES OF LAKE BARDAWIL, MEDITERRANEAN SEA, EGYPT.

Abstract

Lake Bardawil is a large lagoon on the Eastern Mediterranean coast, north Sinai, Egypt. It harbors large meadows of seagrasses that have not been studied since 1977. The distribution and biomass of these seagrasses are reported here, in addition to their biochemical and chlorophyll a contents, from spring 2003 to winter 2004. The seagrasses were collected from five stations, covering almost the whole area of the lake, at a depth of around 2 m. Two seagrass species were recorded: Ruppia cirrhosa and Cymodocea nodosa. *Seasonal variations were detected in the biomass of both species. Ruppia cirrhosa's biomass was higher in most stations in the summer, while that of* Cymodocea nodosa *was higher in spring. Moreover, the biochemical composition showed significant variations over time. Total protein contents were higher in winter in both species, while in the summer the contents of carbohydrates and chlorophyll a+b were higher in both seagrass species.*

Key-words: *Seagrasses, distribution, biochemical contents, Mediterranean Sea.*

Introduction

Information concerning seagrasses of the Mediterranean Sinai coast is very scarce. Furthermore, the seagrasses of Lake Bardawil, Eastern Mediterranean coast, north Sinai, have not been studied since 1977, when Y. Lipkin surveyed the seagrass vegetation of Sinai. He reported that *Ruppia cirrhosa* occupied almost the whole western third of the lagoon, but may disappear completely during severe winters (Lipkin, 1977). Lake Bardawil is an important fishing area for many species that use the seagrass beds as nursery grounds. In the present work, the seasonal variations in the distribution and biomass of these plants were studied. Because of their high productivity, the seagrasses of Lake Bardawil were also analyzed for their biochemical composition (total proteins, total lipids and total carbohydrates).

Study area

Lake Bardawil is a shallow hyper-saline lagoon located along the northern shore of the Sinai Peninsula between longitudes 32^0 40' and 33^0 30' E and latitudes 31^0 03' and 31^0 14' N. The lagoon, which is a natural depression, is separated from the Mediterranean Sea by a long, 300-1000 m wide, arrow-shaped sand bar. It has been described as a wetland of major international importance since it is a major bottleneck for migrant water birds passing through the Eastern Mediterranean region where wetlands are scarce (Meinnger and Atta, 1990). Human impact on Bardawil lagoon is minimal due to the unexploited surrounding area, thus it is considered one of the cleanest water mass in the region (Varty *et al.,* 1990). The lagoon extends for about 90 km and has a maximal width of 22 km. The lake is very shallow (from 0.5 to 2 m deep), and thus warms up very quickly during summers and cools easily during winters. Three openings (Boughaz) connect the lagoon to the sea. Two of these are man-made (the western Boughaz I and the Middle Eastern Boughaz II), while the third one is natural (eastern Boughaz III at the Zaranik protectorate). The main water supply of the lagoon comes from the Mediterranean Sea, and flows constantly through these three openings (Ibrahim *et al.*, 1987).

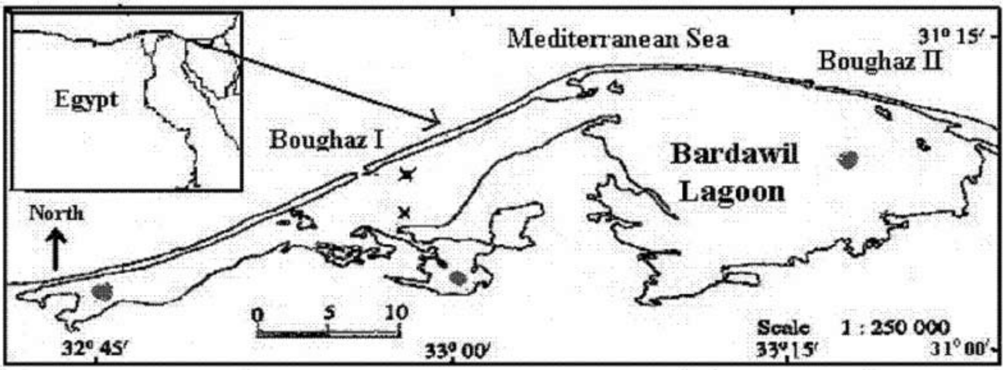

Fig. 1 - Map of seagrass sampling sites in Lake Bardawil. Dotes are the *Ruppia* sites while cross are the *Cymodocea* sites.

Material and methods

Seagrass plants were sampled from five sites representing most of the lake (Fig.1). Skin diving was used to collect the plant materials. At each site, a 25x25 cm metal quadrate was used in the collection of plants (both above and underground parts). Five quadrates were sampled from each locality. The plants were washed in running water, oven dried at 60°C to a constant weight, and the biomass was determined (g dry wt/m^2). The dried seagrasses were then ground. Total protein contents of the dried plants were determined using the Biuret method (David and Hazel, 1993). Total carbohydrates and lipids contents were measured using the phenol sulphoric acid method (Dubois *et al.*, 1956) and sulphophos phovanillin procedure (SPV) (Chabral and Castellano, 1961), respectively. Chlorophyll a+b contents were determined by the method of Golterman and Clymo (1971).

Tab. 1 - Mean biomass (g dry w/m^2) of *Ruppia cirrhosa*

Season / Station	spring	summer	autumn	winter
RI	97.04	178.344	85.985	67.2
RII	108.379	98.304	75.436	75.82
RIII	129.75	222.832	97.616	105.52

Results

Biomass and distribution of the seagrasses: Two seagrass species were identified and collected from Lake Bardawil between spring 2003 and winter 2004. *Ruppia*

Tab. 2 - Mean biomass (g dry w/m^2) of *Cymodocea nodosa*

Season / Station	spring	summer	autumn	winter
CI	274.396	359.448	173.488	98.35
CII	164.921	236.16	117.104	106.72

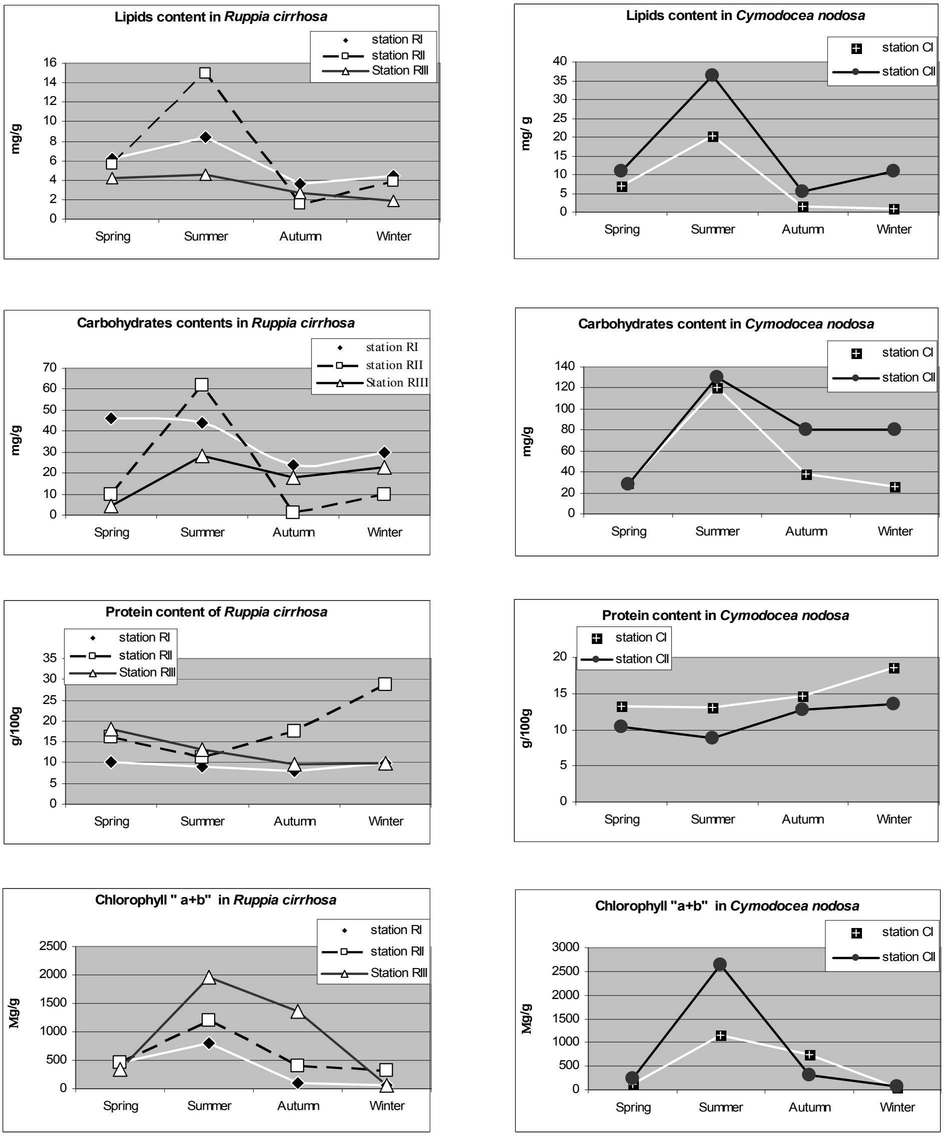

Fig. 2 - Lipids, carbohydrates, protein and Chlorophyll “a+b” readings in *Ruppia cirrhosa* and *Cymodocea nodosa* during study period.

cirrhosa was found at three localities, RI in the eastern part of the lagoon, RII in its middle and RIII at its western part. The densest vegetation of *Ruppia* occurred at the western part of the lagoon. The second species, *Cymodocea nodosa,* which formed a small, scattered yet dense vegetation (CI, CII), occurred mainly in the middle part of the lake near its western opening (Boughaz I). Tables 1 and 2 summarize the mean biomass of *Ruppia cirrhosa* and *Cymodocea nodosa*, respectively, during the period of study. The mean biomass of both seagrass species was higher in the summer at all stations except RII, where it was higher in the spring.

Biochemical composition: Fig. 2 shows the biochemical composition of the two seagrass species during the study period. Lipid contents of both species were high in the summer and low in the autumn. The same pattern was detected for carbohydrate contents, while protein contents showed a different distribution over time; its highest value was detected during the winter for both seagrasses. Chlorophyll a+b contents of the two recorded seagrass species were highest in the summer and lowest in the winter.

Discussion

The distribution, biomass and biochemical composition of the seagrass species found in Lake Bardawil, Eastern Mediterranean, Egypt were reported**.** Seagrasses were collected from five stations covering almost all of the lagoon area. Two species were identified: *Ruppia cirrhosa* and *Cymodocea nodosa.* The latter species was not recorded in the study made by Lipkin in 1977. He reported *Ruppia cirrhosa* as being the only member of the plant community inhabiting Bardawil Lagoon below 0.4 m depth.

In the present study, *Cymodocea nodosa* was recorded and observed to grow in small scattered but dense populations in the middle part of the lake near its opening to the Mediterranean Sea, from which plants may have entered the lake and began to inhabit it. Lipkin also stated that *Ruppia cirrhosa* occupied the western third of the lagoon, while the eastern part was clear of any macrophytic growth due to anaerobic conditions on the bottom. During this study, spread aggregations of *Ruppia cirrhosa* were found in some localities of the eastern part of the lake, which could be explained by the improved water and sediment conditions in this area after the new man-made openings (Boughaz) connected the lagoon to the sea.

The biochemical content analysis of *Ruppia cirrhosa* and C*ymodocea nodosa* revealed that both species had the same pattern of distribution for lipids, carbohydrates and protein over the year. Lipid and carbohydrate contents were higher during the summer, which coincides with Gobert *et al.* (1995) who stated that both lipids and total carbohydrates of *Posidonia oceanica* leaves increased in the summer. Protien levels, on the other hand, increased during winter, which also agrees with Gobert *et al.* (1995) who reported that protein contents in leaves of *Posidonia oceanica* were 11% in the winter and 7.7% during the other seasons. These readings may resulted due to the natural cycle of the plant (with different uptake, translocation in the different part of the plant: rhizome, leave, roots) regarding the seasons which probably regarding temperature and light (Gobert S., pers. comm.).

During summer, both seagrass plants were observed to be covered with a thick layer of epiphytic algae. The same observation was also been described by

Lipkin (1977). This might explain the higher chlorophyll $a+b$ contents during the summer season in compensation for a reduced photosynthetic irradiance reaching the epiphyte covered leaves.

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S. Gobert, G. Lepoint, R. Biondo, J-M. Bouquegneau

Mare Center, Oceanology, University of Liège, Sart Tilman, B6c, B-4000 Liège, Belgium

IN SITU SAMPLING OF PORE WATERS FROM SEAGRASS MEADOWS

Abstract

A routine and low cost method to collect pore water in a seagrass meadow in situ and underwater is here proposed. The method consists of direct-suction filtered sampling allowing the determination of nutrient concentrations in the sampled water. Data ranges of nutrient (NO_2^-*+*NO_3^-*;* $HPO_4^{\ 2-}$ *and* NH_4^+*) concentrations in a* P. oceanica *meadow are also discussed.*

Key-words: *seagrass, pore water, nutrient, Mediterranean Sea.*

Introduction

Bottom sediment of seagrass meadows is a particular environment: it provides the substrate for plant anchoring and furnishes a part of required nutrients. The presence of phanerogams modifies the granulometry, the composition and the physico-chemical properties of this compartment (Gobert *et al.,* 2003). *Posidonia oceanica* (L.) Delile colonizes large area in the Mediterranean Sea from the surface to about 40 m depth. The study of the sediment characteristics in this ecosystem is not easy and necessitates underwater work; these types of investigation are relatively a few (Terrados *et al.,* 1999; Hebert and Morse, 2003, Lopez and Duarte, 2004). Here we propose a routine and cost effective method to collect pore water in seagrass meadows.

Material and methods

The measurements have been made in Calvi Bay (Corsica, France) near the research station STARESO. The sampling system (Fig. 1) is composed of a PVC syringe of 5 ml. One three-ways tap is screwed on the syringe. A filter (GF/C) (inserted in modified support: Millex (Millipore®) + Pall4320®) is installed on the tap. The support has been modified to permit the suction and the ejection of water through the filter avoiding ripping of the filter. Four needles (i.e. the needle system), arranged in square (3 cm²), of 10 cm long (0.05 mm ID) (stainless steel) are connected to the filter support.

The watertighness and gastightness have been tested in laboratory and *in situ*. The sampler system has been installed *in situ,* maintained in suction position and enclosed in a bell jar with fluoresceine. The contamination of the sampled pore water with coloured column water has been controlled by spectrophotometry (490 nm) (Test 1). At laboratory, the sampler system filled with N_2 saturated seawater was successively incubated during 1 hour in N_2 saturated seawater (SN_2-SWN_2), in O_2 saturated seawater (SN_2-SWO_2) and in atmospheric air (SN_2-AO_2). After that, the sampling system filled with O_2 saturated seawater was incubated in O_2 saturated seawater (SWO_2-SWO_2) or in N_2 saturated seawater (SWO_2-SWN_2) during 1 hour. O_2 concentrations were measured in the seawater of the sampler at the end of each experiment (Test 2).

Nitrogen (NH_4^+ and NO_2^-+NO_3^-) and phosphate (HPO_4^{2-}) concentrations in the pore water were analysed with an autoanalyser (SKALAR) by the classical method for an automated system adapted for oligothophic seawater (detection limits: 0.1, 0.04 and 0.05 µM for ammonium, nitrite+nitrate and phosphate respectively).

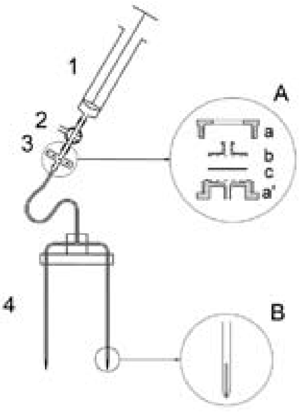

Fig. 1 - Design of the sampler. 1: syringe, 2: tap, 3: support and filter, 4: needle system; A: detail of support and filter (a and a': Pall filter holder modified, b: 25 mm Millex filter unit, c: GF/C); B: detail of the needle (0.5 mm-10 mm).

Results

The pore water sampled with the system installed inside the bell jar do not contain significant traces of fluoresceine (<2%) (Test 1). The results (Fig. 2) show that the O_2 concentrations (1.4 mg O_2 L^{-1}) in the seawater saturated in N_2 inside the sampler do not vary when placed in seawater saturated in O_2 or when placed in atmospheric air. They also show that the O_2 concentrations (8.5 mg O_2 L^{-1}) of saturated O_2 seawater enclosed in the sampler do not present any significant variations after one hour of incubation in N_2 saturated seawater (Test 2).

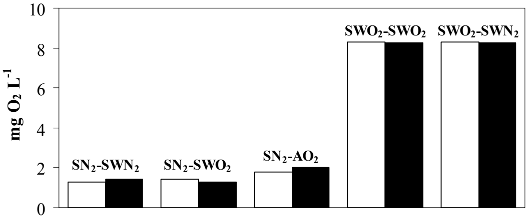

Fig. 2 - Oxygen concentrations into the sampler at T_0 (white bars) and after one hour of incubation (black bars).

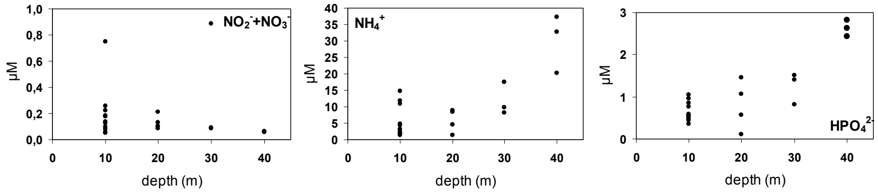

Fig. 3 - Nutrient concentrations (µM) in pore waters sampled at 10 cm depth in *P. oceanica* meadow (Calvi Bay) from 10 to 40 m depth.

Discussion and conclusions

The tests 1 and 2 have demonstrated the water tightness and gas tightness of our system which allows to sample interstitial water.

Sediment nutrient concentrations exhibit a broad spatial variation at all depths (Fig. 3). In spite of this broad range of values, NH_4^+ and HPO_4^{2-} pore water concentrations show a significant increase along the depth transect (0.89 and 0.92 for the NH_4^+ and HPO_4^{2-} respectively).

Tab. 1 - Pore water nutrient concentrations (µM) in *P. oceanica* meadows (data, range or mean ± standard deviation).

	NH_4^+	$NO_2^-+NO_3^-$	N Total	HPO_4^{2-}	Methods	References
Italy	158 -332	-	-	-	Core	Kraemer et al., 1997
Spain	-	-	235±73	11±4	Core	Lopez and Duarte, 2004
			479±481	23±28	Core	Alcoverro et al., 1995
			219-862	1-60	Core	Lopez et al., 1995
France	40-120	4.2±1.7	-	-	Core	Pergent-Martini et al., 1995
South Corsica	1.8			1.7	Succion	Cancemi et al., 2003
Calvi, Corsica	5.5	0.5	-	10	Core	Caschetto et al., 1980
	15	3	-	-	Core	Jorgensen et al., 1981

Literature data show that nutrient concentrations in sediment pore waters of *P. oceanica* meadows display strong seasonal (i.e. Pergent-Martini *et al.*, 1995, Lopez *et al.*, 1995) and spatial (local to regional) variations (Tab. 1). The nutrient pore water concentrations are generally lower in winter, decrease with increasing seagrass density and regardless of the sediment layer examined (Pergent-Martini *et al.*, 1995, Caschetto *et al.*, 1980, Kraemer *et al.*, 1997).

Our results show that the Calvi area presents very low nutrient pore water concentrations in comparison with other Mediterranean sites (Tab. 1). The seawaters along Corsican coasts are characterised by an oligotrophic status but our pore water concentrations fit better with values encountered in tropical seagrass sediment (Holmer *et al.*, 2001).

The increasing of NH_4^+ and HPO_4^{2-} concentrations observed in this work, along the bathymetric gradient could be related to the decrease of the shoot density with depth (Bay, 1984). Vertical distribution of nutrients in pore water sediment is influenced by the presence of *P. oceanica* meadow and by the physico-chemical processes which vary according depth. The meadow density (number of shoot. m^2, above and belowground biomasses), light, temperature and water movements decrease with increasing depth (Bay, 1984; Gobert *et al.*, 2003). On local and annual time scales, *P. oceanica* production is directly related to bacterial activity, while on seasonal scales, *P. oceanica* and bacteria metabolism are inversely related because of competition for inorganic nutrients (Danovaro *et al.,* 1994; Lopez *et al.,* 1995). Sediment phosphate and ammonium concentrations higher at deeper depths could be attributed to the lower utilisation of the pool of nutrients because of light reduction and could also be related to the decrease in growth and biomass of *P. oceanica* shoots. In order to understand these nutrient variations, more experiments and measurements 1) on the short term daily variations in relation with changes of biomass and communities of bacteria (Canon *et al.,* 1998) and 2) on the effect of metabolism changes of aboveground, belowground and of the connection between these two compartments along the bathymetric transect must be realised in *P. oceanica* meadows.

This paper shows that the proposed sampling system is adapted to obtain pore waters for the determination of nutrient in the sediment of *P.oceanica* meadows. This system permits *in situ*, rapid and low cost samples. The results obtained confirm that the studied area presents the characteristics of an oligotrophic zone with low levels in nitrogen and phosphorus concentrations. The spatial distribution of the nutrient concentrations in the meadow probably reflects the large influence of the plants on the pore water characteristics.

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M. Gosselin, J-M. Bouquegneau, G. Pergent, C. Pergent-Martini, S. Gobert

Mare center, Oceanology, University of Liège, Sart-Tilman, B6, 4000 Liège, Belgium.

USE OF *POSIDONIA OCEANICA* SHEATHS AS A BIOMONITOR OF METAL POLLUTION

Abstract

Dead sheaths of Posidonia oceanica *have been studied to gain knowledge on the quantitative aspects of metal pollution in order to use it as biomonitor. After leaf decay, metal variations in the environment can occur and change the concentrations in the sheaths through physicochemical processes. Thus sheaths metal concentrations can deviate from their initial values and no longer reflect the original environmental conditions. To shed light on a potential metal temporal deviation in dead sheaths, data of six trace metals (Cr, Ni, Cu, Zn, Cd, Pb) have been compared in living leaves collected in Calvi bay (Corsica) in 1993, 1994, 2003 and 2004 and in dead sheaths sampled in 2002 which gave, after retrodating, the metal concentrations between 1992 and 2002. Results showed that Cr and Pb concentrations were significantly higher in sheaths than in leaves while concentrations of Ni, Cd and Zn presented higher values in leaves than in sheaths. On the other hand for Cu, no significant differences were revealed between the dead and living tissues. From these results, dead sheaths could be considered as a good biomonitor for Cu environmental concentrations.*

Key-words**:** Posidonia oceanica*, monitoring, trace metals, sheaths, N-W Corsica.*

Introduction

The endemic seagrass *Posidonia oceanica* (L.) Delile forms meadows that are crucial to the health and function of coastal Mediterranean ecosystems. Over the last 30 years, frequent alterations and regressions of these meadows have been noted (e.g., Peres and Picard, 1975; Meinesz *et al*., 1991). These alterations are often linked to human activities such as tourism, pollution by urban and industrial centers, and coastal facilities (Guidetti *et al*., 2002). Biological pollution in the Mediterranean Sea (Boudouresque and Verlaque, 2002; Piazzi and Cinelli, 2000) increases the vulnerability of *P. oceanica* systems (Ceccherelli and Cinelli, 1999). Because of the importance of this key macrophyte, *P. oceanica* is now considered a threatened species requiring protection and is listed in the 'Habitats Directive' of the European Community (Platini, 2000; UNEP, 1990).

P. oceanica has been recognized as an effective tool for investigating the coastal environment because it has a widespread distribution around the Mediterranean basin, is fixed on the bottom, and is sensitive to both pollution and human activities. Markert *et al*. (2003) define a "bioindicator" as an organism that contains information about the quality of the environment and a "biomonitor" as an organism that contains quantitative information regarding the quality of the environment. Previous studies suggest that *P. oceanica* can be used as a bioindicator (Ferrat *et al*., 2003; Pergent-Martini and Pergent, 2000). In addition, lepidochronology (Guidetti and Fabiano, 2000; Pergent *et al*., 1989), phenology (Pergent and Pergent-Martini, 1988), and tissue contents (Campanella *et al*., 2001; Warnau *et al*., 1995) can be used to examine historical changes in the environment.

Using lepidochronology, it is possible to use the dead sheaths from *P. oceanica* to examine the history of metal concentrations in the environment over several decades. The sheath is the basal part of the leaf that remains attached to the rhizome after abscission of the leave apex (blade). The technique of lepidochronology, which is analogous to dendrochronology, is derived from the life cycle of the phanerogam sheaths, which have an annual periodicity with a maximum and a minimum thickness. Cyclical patterns, therefore, allow each sheath to be assigned a chronological date. In this way, it is possible to measure metal concentrations in each sheath and recreate the temporal history of metal concentrations in the environment (Pergent-Martini and Pergent, 2000).

The aim of this work was to attempt to use *P. oceanica* as a biomonitor by comparing, over the last 10 years, trace metal contents measured in leaves with results found in sheaths at Calvi.

Materials and methods

Study sites

We sampled shoots of *P. oceanica* just offshore from the city of Calvi, in a meadow situated in the Revellata Bay next to the STARESO research station.

Sampling for trace metal measurements in living tissues

Ten orthotropic shoots were collected from February 1988 to December 2004 (February and May 1988; March, June, September, and December 1993; February, April, and June 1994; November 2003; and December 2004). In the laboratory, epiphytes were removed with a shard of glass to avoid metal contamination. Leaves from samples collected between 1988 and 1994 were lyophilized and digested using a double boiler, whereas samples collected in 2003 and 2004 were subjected to microwave digestion. For the samples collected between 1988 and 1994, the levels of Cr, Ni, Cu, Zn, Cd, and Pb were measured with an inductively coupled plasma-atomic emission spectrometer (ARL-3510). Recovery ranges with certified materials were 88 ± 1.5% to 99 ± 1% for these metals. Samples collected from 2003 and 2004 were analyzed by a certified laboratory (Institut Malvoz-Laboratoire Santé et Cadre de Vie, Liege, Belgium) with an inductively coupled plasma-mass spectrometer (Elan DCR II). A set of certified Material samples (DORM-2, National Research Council, Institute for National Measurement Standards, Ont; Canada) spiked with grade concentration of metals was analyzed to ensure the accuracy of metals. The metal concentrations were expressed as $\mu g.g_{DW}^{-1}$.

Sampling for trace metal measurements in dated sheaths

Fifteen orthotropic rhizomes were collected during November 2001 and February 2002 to measure the trace metal levels. The orthotropic rhizomes were dissected for lepidochronological analysis (Pergent *et al.*, 1989). The sheaths of three shoots were pooled according to lepidochronological year, pulverized, and lyophilized. A portion of the sample (155 mg) was digested with a mixture of 1 ml of concentrated HNO_3, 0.1 ml of H_2O_2, and 0.25 ml of deionized distilled H_2O. The mixture was placed in a Teflon bomb and microwaved for 5 min at 300 W, 30 s at 600 W, and 4 min at 250 W. Concentrations of Cr, Ni, Cu, Zn, As, Se, Cd, and Pb were measured in sheaths using an inductively coupled plasma-mass spectrometer and are expressed in $\mu g.g_{DW}^{-1}$.

Statistical analysis

To compare relative metal concentrations between dated sheaths and living leaves at Calvi, a Mann-Whitney U Test was used. The results were considered statistically significant at $P \leq 0.01$.

Results

Comparison of the dead and living tissue of *P. oceanica* shoots at Calvi showed that the concentrations were significantly different (Fig. 1). Cr and Pb concentrations were significantly higher in sheaths than in leaves, whereas concentrations of Ni, Cd, and Zn were higher in leaves than in sheaths.

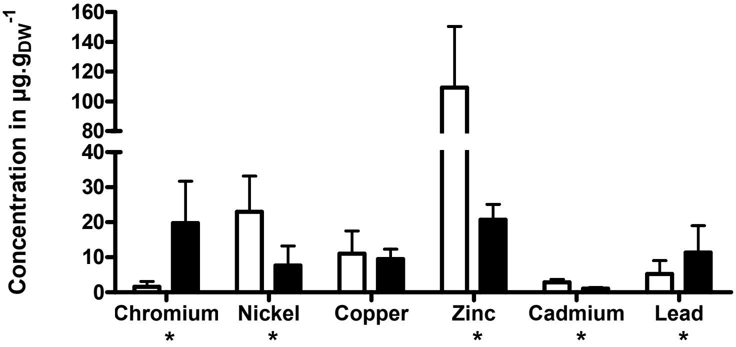

Fig. 1 - Mean metal concentrations in different tissues of *P. oceanica*. Concentrations (mean ± standard deviations) were expressed in µg g_{DW}^{-1} for each metal studied in leaves (white bars) and in sheaths (black bars). Significantly different samples are marked as * ($p< 0.01$).

Discussion and conclusions

These different concentrations could be the result of 1) methodological factors, in that we compared dead sheaths and living leaves, even though living leaves are equivalent to living sheaths plus living blades; and 2) physicochemical factors, in that during decay, dead sheaths desorb Ni, Cd, and Zn and adsorb Cr and Pb. Our results do not support the conclusions of previous studies (Pergent-Martini and Pergent, 2000) that compared metal concentrations in living leaves and dead sheaths. For *P. oceanica*, the previous studies found that Cr, Ni, Cu, Zn, Cd, and Pb are present at higher concentrations in living leaves (aboveground compartment) than in dated sheaths. The difference can be explained by the fact that our study focused on the sheath degradation process rather than the leaf accumulation process.

Moreover, in other data from the Adriatic Sea, Kljaković-Gaśpić *et al*. (2004) showed that the oldest sheaths and the dead sheaths of *P. oceanica* exhibited similar Cd and Pb concentrations. This difference could be due to the process used to measure metals in the living tissues. Our results correspond to a mean concentration measured from one entire shoot, and it has been demonstrated that leaf aging causes a dilution effect (Pergent-Martini, 1998). Thus, the extent of the change in concentration can be minimized (Ni, Zn, and Cd) or maximized (Cr and Pb) by the variation in the metal concentration, which increases with leaf age.

We suggest that future studies should 1) rigorously examine the metal concentrations along a leaf age gradient from the same shoot, and 2) compare trace metal concentrations in dead sheaths with measures in living sheaths and not in the whole living leaves. Although many of the metals did not follow the expected patterns, our results suggest that Cu concentrations were the same in both living leaves and dated sheaths. Therefore, data on Cu for dead sheaths could be used to reconstruct the original leaf concentrations. Gagnon (1998) noted that mean Cu concentrations in plants were between 5 and 20 $\mu g.g_{DW}^{-1}$, which correspond with data found in *P. oceanica* leaves and sheaths (Fig. 1) and confirm the good health status of north Corsica meadows.

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E.L. Jackson, S.C. Glanfield, J. Delacruz, O. Langmead, C. Arvanitidis,
M.J. Attrill, H. Tyler-Walters, L. Mee

Marine Biology and Ecology Research Centre, University of Plymouth, Plymouth, UK

AN OBJECTIVE APPROACH TO DEFINING LOSS OF SEAGRASS IN THE MEDITERRANEAN: CAUSAL CHAIN ANALYSIS AND SCENARIO PREDICTIONS

Abstract

The primary causes of loss and degradation of Mediterranean seagrass beds were objectively examined within a Driver Pressure State-Change Impact Response (DPSIR) framework as part of a larger EU project on European Lifestyles and Marine Ecosystems (ELME). The specific study presented here summarises the information on loss of seagrass habitat in the Mediterranean over the last 100 years (albeit most losses occurred in the last 30 years). Data gathering was carried out via three main methods; searching published material, an online questionnaire and via direct contact with key organisations and experts. We estimated total losses of 44 625 ha of seagrass over the last 100 years. The evidence gathered on the causes of loss (assigned based on frequency of occurrence and area weighted scales of loss) of seagrass habitat is used to produce Bayesian belief network (BBN) models to forecast the impact of changes in social and economic drivers and policy.

Key-words: *Bayesian Belief Networks, Posidonia, Mediterranean Sea.*

Introduction

Marine ecosystems possess great intrinsic value as reserves of biological diversity and are vital providers of goods and services to humanity. However, they are often disregarded during economic and social development. Europe's four sea areas; the Baltic, NE Atlantic, Mediterranean and Black Sea have suffered to differing degrees from eutrophication, chemical pollution, unsustainable fisheries and physical destruction of habitats closely connected with human lifestyles. Bringing marine ecosystems into policies for sustainable development requires better information on the causal connections between human pressures and the changing state of the systems. This is particularly important at a time when the European Community is expanding, re-examining its agricultural and chemical policies, implementing a new fisheries policy and exploring new ways to protect marine systems. The project ELME (European Lifestyles and Marine Ecosystems) aims to enhance our understanding of causality, forecast the impacts of divergent development scenarios and inform evolving Community policies.

In this paper we report on the work being carried out by ELME on seagrass beds, one of the priority habitats identified for the Mediterranean. ELME assesses the causes of historical seagrass habitat loss in the Mediterranean and the evidence gathered is used to produce Bayesian belief network (BBN) models to forecast the impact of changes in social and economic drivers and policy, on seagrass habitats.

A fringe of seagrass (primarily *Posidonia oceanica*) is found on almost all coasts of the Mediterranean (Lipkin *et al.*, 2003; Procaccini *et al.*, 2003). They play a crucial role in stabilising sediments, maintaining water quality, protecting sedimentary shores from erosion and represent the most important fish production areas in the Mediterranean. All seagrass species are considered under the broad habitat type of seagrass beds in this study. Modelling state change and causes of loss and degradation was carried out on *Posidonia oceanica* alone due to variability between species.

Materials and methods

Initial investigations into long-term studies of seagrass loss in the Mediterranean unearthed long-term data series for only a few isolated regions for example Marseille (Boudouresque *et al.*, 2000), Spain (Marba *et al.*, 1996), Hammamet Gulf, Tunisia (Ben Mustapha and El Abed, 2001) and Croatia (Zavodnik and Jaklin, 1990). Due to temporal spatial mismatches the decision was taken to adopt a metadata analysis approach to populate the causal models. Data gathering was carried out via three main methods; searching published material, an online questionnaire and via direct contact with key organisations and experts. Individual records were given a spatial reference point and specific details were entered regarding dates, amount and causes of seagrass loss.

Using a method outlined by Green and Short (2003), a size frequency distribution of seagrass losses (irrespective of cause) was calculated using the information on losses that had been gathered (between 1900 and 2004). The assumption was then made that losses had occurred for other beds across the Mediterranean at a similar frequency distribution.

ELME aims not only to estimate the loss or degradation of seagrass habitats, but also to identify the associated causes (direct and indirect). To cope with instances in which multiple pressures occurred or where the quantification of loss/ degradation was not available we adopted a method to weight the observed ‘state change’ whilst addressing various spatial and temporal scales, the permanency of the effect and/or recovery potential once the pressure was removed. A set of ranks or scales were adapted from similar approaches used by OSPAR (OSPAR, 2003) and *MarLIN* Biology and Sensitivity Key Information Sub-programme (Hiscock and Tyler-Walters, 2006). The primary causes of loss and degradation were assigned based on frequency of occurrence and area weighted scales, which incorporates measures of the amount of seagrass loss, decline and degradation; recovery potential; evidence/ confidence; and marine natural heritage importance. For the numerically dominant pressures and those that dominated based on area of habitat lost, the specific relationships and thresholds between habitat loss and pressure were researched to feed into the modelling process.

The BBN Model was parameterised (i.e. probabilities calculated) using data standardised for time to account for ‘intensity’ and the number of reports. To account for the fact that the data arise from different sampling locations in the Mediterranean, we have assumed that ‘since we are building a model for the whole sea, the data should be treated as representative replicates’ (*i.e.* sampling locations are representative of the Mediterranean). Data for each pressure or state change indicator has been predefined into a bin class that denotes the state/condition of the indicator for example data for *Caulerpa* are expressed as present or absent, data for mechanical disturbance are expressed as high, medium, low *etc*.

Expert knowledge/opinion was used to derive probabilities for indicators in which no data are available.

Results

Over 180 data sources both from published works, questionnaires, unpublished reports (particularly from past projects) and personal communications were used to compile the information on seagrass habitat loss. This report summaries the information on loss of habitats in the Mediterranean over the last 100 years (but primarily in the last 30 years). A searchable summary of the database is available on the ELME website (www.elme-eu.org).

We estimated total losses of 44625 ha of seagrass over the last 100 years, which on average equates to more than 1 ha of seagrass lost per day (although the rate is increasing). Although this estimate does not consider recovery of the habitat it is likely to be an under estimation since much of the habitat remains unmapped and unstudied, particularly for Libya which is thought to have some of the largest expanses of unmapped seagrass in the Mediterranean.

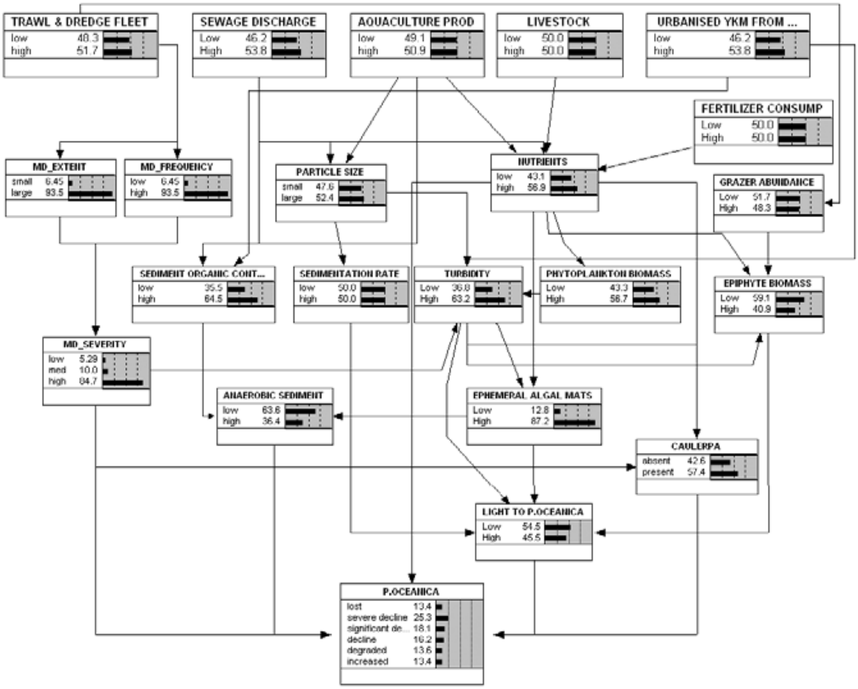

Fig. 1 - Graphical representation of the Bayesian belief network for *P. oceanica* loss and degradation. *P. oceanica* has six states [lost, severe decline, significant ("signif") decline, decline, degraded and increased]. Each box represents a named variable and the numbers aligned with the name of each state express the belief (probability) of that state as a percentage. Causal relationships are indicated with arrows. SED ORG = Sediment organic content, PHYTOPLANK = Phytoplankton Biomass, MD = Mechanical disturbance.

The causes of loss of seagrass habitat (Pressures) and the activities creating these pressures (Drivers) showed patterns over time. There is a decreasing trend in the loss of seagrass due to deposition of physical material since the 1930s. Nutrient enrichment as a pressure on the seagrass showed a peak in the 1980s then a gradual decline between the 1980s and 1990s. The loss of seagrass due to mechanical disturbance (mainly from trawling and anchor/mooring damage) showed a gradual increase and there was an increase in the relative number of reports of losses due to introduced organisms (primarily *Caulerpa*) since the 1980s (Guillen *et al.*, 1994; Ceccherelli and Cinelli, 1997). The most numerically dominant activity causing past losses throughout the Mediterranean was input of urban sewage via deposition of physical material (increased turbidity of the water column), increases in plant nutrients and chemical oceanographic change (e.g. increased BOD). However, indications are that this pressure has been decreasing in frequency since the 1970s, perhaps due to improved treatment. Therefore the relative contribution of primary, secondary and tertiary treatment will have different implications for seagrass loss and is taken into account in the scenario outcomes. The second most frequently observed pressure is from the fishing sector (trawling). Trawling removes biological and physical resources and mechanically disturbs the seagrass beds. There has been a gradually increasing trend in the loss of seagrass due to trawling, despite bans in the vicinity of seagrass beds in many parts of the Mediterranean. Other activities that are of growing concern are aquaculture (fish cages), tourism and shipping (port construction, anchoring).

In addition to assessing past patterns of seagrass loss, these data have been used to develop BBN models to predict levels of seagrass loss and habitat degradation under different combinations of pressures (Fig. 1). These models use an indicator-based approach for both the pressures and the environmental and biological responses, and explicitly represent causal relationships between them as well as the uncertainty surrounding these relationships.

Discussion

Taken in isolation many of the pathways of loss identified during the course of the study are already considered established links, however the graphical structure explicitly represents cause-and-effect assumptions between variables that may be obscured by other approaches. These assumptions allow the complex causal chain linking management actions to ecological consequences to be factored into a sequence of conditional relationships. This model represents the first step towards a scenario analysis. By aligning the temporal patterns of the losses and direct causes with changing patterns in Drivers (sectoral activities and policy) future probabilities of loss and degradation of seagrass habitats can be forecast. Scenarios will be based on plausible future development patterns, policy options and likely social changes at European Community level. The outputs of this study, in terms of transdisciplinary scenario models, are intended to provide scientific support for the implementation of the ecosystem approach in Europe and hopefully will assist in policy decisions that minimise the destruction of these sensitive ecosystems and associated loss of biodiversity.

Acknowledgements

This study was part of the EU funded Sixth Framework Project ELME (European Lifestyles and Marine Ecosystems). The authors would like to thank all seagrass experts across the Mediterranean, who answered our calls for information and data, we are only sorry that there are too many of you to mention within these 4 pages!

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R. Lasagna, M. Montefalcone, C.N. Bianchi, C. Morri, G. Albertelli

Dip.Te.Ris., Dipartimento per lo studio del Territorio e delle sue Risorse
Università di Genova, Corso Europa, 26-16132 Genova, Italy

MORPHOLOGY OF A *POSIDONIA OCEANICA* MEADOW UNDER ALTERED SEDIMENTARY BUDGET

Abstract

The construction of a small jetty in Prelo cove (Ligurian Sea, NW Mediterranean) involved cutting in the Posidonia oceanica *(L.) Delile (Monocotyledonae, Posidoniaceae) matte terrace, modifying the rip current path and altering the sedimentary budget of the cove. The aim of this study was to investigate how the sedimentary regime influenced the meadow morphology. Distribution, cover and other morphological features of the meadow were described.* P. oceanica *was patchy distributed between 0.5 and 15 m depth, covering a total area of about 4.8 ha. Three classes of living* P. oceanica *cover were distinguished: high cover (> 65%), low cover (65% to 15%) and single shoots (< 15%). Non vegetated areas fell into four types: dead matte, sandy channels, sandy patches and beyond the lower limit of the meadow. Regressive sharp limit was present in the central area of the meadow and regressive shaded limit at the extremities.*

Key-words: Posidonia oceanica*, sediment, meadow morphology, Ligurian Sea.*

Introduction

The general decline that *Posidonia oceanica* meadows are undergoing has been attributed to two main causes linked to human impacts: coastal constructions and increased turbidity (Ruiz and Romero, 2003). The former affects the currents (Meinesz *et al*., 1991) and influences the sedimentary budget (Albertelli *et al*., 1996; Góngora-González *et al*., 1996; Cavazza *et al*., 2000); the latter, decreasing light penetration, may cause regression of the deep limit or, at higher rate, even stifle the plants (Góngora-González *et al*., 1996). Concomitant causes may be ascribed to the presence of sewage discharges or to trawling activities (Pérès and Picard, 1975), as well as to climate fluctuations (Peirano and Bianchi, 1997; Peirano *et al*., 2005). In order to protect the *P. oceanica* meadows, they were listed as a priority habitat on Annex I of the EC Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Flora and Fauna (EEC, 1992).

A general regression trend, due to various human activities, has been observed as a common feature of all Ligurian meadows (Peirano *et al*., 2005). Peirano and Bianchi (1997) focused on the deficiency and discontinuity of quantitative data on the present status of the Ligurian *P. oceanica* meadows.

In the first decades of the XIX century the construction of a small jetty at Prelo cove, in the Eastern Ligurian Riviera, required the cut of the *P. oceanica* fringing reef. This cut, causing modifications in both coastal and meadow morphologies, might have involved the reinforcement of the rip current and have altered the sedimentary budget (Lasagna, 2004). The aim of this work was to describe the present day morphology of the *P. oceanica* meadow after the fringing reef cut and the supposed alteration in sedimentary budget.

Materials and methods

Prelo is a small cove (about 8 ha) of the Ligurian coast sited between Rapallo and Santa Margherita Ligure, in proximity of Portofino Marine Protected Area (Ligurian Sea, NW Mediterranean). The cove is sheltered by winds and by waves (Danovaro *et al.*, 1994) and is characterized by a rocky coast, as typical in Eastern Liguria, and by a small beach. At the beginning of the XIX century, this cove underwent different anthropogenic impacts such as the construction of a jetty, used by pleasure craft, and of a small promenade.

Direct observations by scuba diving were conducted between September 2003 and April 2004. A total of 11 underwater transects were described, each about 300 m long. These transects were stretched perpendicularly to the coast from the upper limit of the meadow to the living *Posidonia oceanica* lower limit and, when present, as far as the end of dead matte areas. During each dive the following data of the *P. oceanica* meadow were recorded on a PVC slate, every 10 m: depth, features of the meadow, percentage cover by living *P. oceanica*, presence of intermatte channels, dead matte areas, and sandy patches. As recommended by Buia *et al.* (2004), the percentage cover by living *P. oceanica* and by dead matte was estimated by eye by two divers independently at about 1 m upon the bottom. Three classes of living *P. oceanica* cover were distinguished: high cover (> 65 %), low cover (65 % to 15 %) and single shoots (< 15 %). Lower limit morphologies were described according to Montefalcone *et al.* (2006a). Shoot density was measured using a quadrat (40 x 40 cm) in three replicated counts at selected sites (in correspondence of upper limit, lower limit and in an intermediate zone) along each transect. Shoot density values were classified according to Giraud (1977) and Pergent *et al.* (1995).

Data recorded during field activities were elaborated in profiles and in a thematic map of meadow morphology.

Results

Posidonia oceanica meadow, ranging from 0.5 m to 15 m depth, extended on a total surface of nearly 4.8 ha, which was about 60 % of the total surface of the sea bottom of Prelo cove. *P. oceanica* developed on rock at the extremities of the cove, while either on sand or on matte within the cove.

A homogeneous and high *P. oceanica* cover (about 80-90 %) characterized the shallow portion of the meadow (1 m to 6 m), with average density value indicating a dense meadow according to Giraud (1977) and a lower sub-normal density according to Pergent *et al.* (1995). At the sides of the jetty a residual fringing reef was observed; on the contrary, in front of the jetty an intermatte erosional channel crossed the meadow to end in a large central sandy patch.

A heterogeneous cover, between 15 % and 80 % (from low to high cover), characterized the intermediate portion of the meadow (6 m to 11 m) and its continuity was also interrupted by dead matte areas, caused by pleasure boats anchoring (Montefalcone *et al.*, 2006b), and by small sandy patches. Moreover, the average density value indicated a dense meadow according to Giraud (1977) and lower sub-normal density according to Pergent *et al.* (1995).

The lower limit of the meadow reached the maximum depth of 15 m. Regressive shaded limits were found in the northern and the southern extremities of the cove (Fig. 1A); these limits showed a living *P. oceanica* cover gradually decreasing with depth, from 65 % to 0 %, and dead matte areas beyond the lower limit itself. In the northern extremity the dead matte area showed an extension of about 100 m, in the southern extremity of about 20 m. In correspondence of these limits the average density value indicated a sparse meadow (Giraud, 1977) and lower sub-normal density according to Pergent *et al.* (1995). Regressive sharp limit was found in the central area of the cove (Fig. 1B); this limit showed a living *P. oceanica* cover passing abruptly from 80 % to 0 % with depth, and ending with dead matte areas having an extension of about 40 m. In correspondence of this limit the average density value indicated a sparse meadow (Giraud, 1977) and normal density according to Pergent *et al.* (1995). Between the regressive shaded limits and the regressive sharp limit we described regressive sharp limits with patches, which passed abruptly from a *P. oceanica* cover of about 50 % to zero, and showing isolated patches of living *P. oceanica* and dead matte areas beyond the lower limit of the meadow (Fig. 1C). In correspondence of this limit the average density value indicated a very sparse meadow (Giraud, 1977) and lower sub-normal density according to Pergent *et al.* (1995).

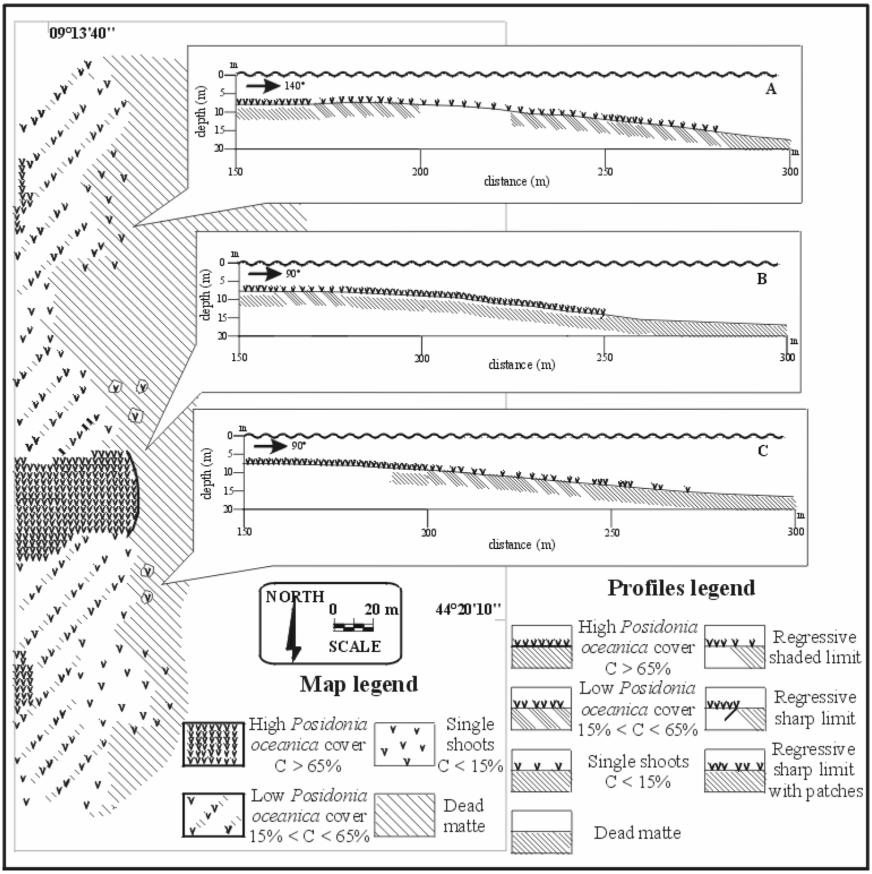

Fig. 1 - A detail of the *Posidonia oceanica* meadow map with the profiles showing different morphologies of lower limits: A = regressive shaded limit, B = regressive sharp limit, C = regressive sharp limit with patches.

Conclusions

A general resemblance between the present distribution of *Posidonia oceanica* and that reported 10 years ago by Bianchi and Peirano (1995) was observed. Some differences can be easily explained by differences in scale and mapping method. Lack of older data prevents any definite conclusion about the importance and magnitude of the presumed alteration in the sedimentary budget. Sediment data by Lasagna (2004) and the present observations on morphology, however, provide suggestive evidence that such an alteration has been dramatic. The occurrence of erosional patterns at shallow depths (i.e. sandy patches and intermatte channel) was spatially related to the presence of the small jetty. The enlarged path of the rip current moved and settled fine sediment in correspondence of the lower limits, causing their extensive regression (as showed by the occurrence of buried dead matte areas and low density values). Lower limit regression was more evident in the northern area because of greater erosion of the matte along the path of the rip current.

On the whole, the present-day *P. oceanica* meadow showed a mediocre state of health with alternation of high and low cover. The meadow resulted sparse according to Giraud (1977) and disturbed (with lower sub-normal densities) according to Pergent *et al*. (1995).

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N. Mayot[1,2], C-F. Boudouresque[1], E. Charbonnel[3]

[1]UMR DIMAR CNRS Parc scientifique et technologique de Luminy Case 901
13288 Marseille cedex 9 France
[2]LEML EA 3156 Université de Nice Sophia Antipolis, Faculté des sciences,
Parc Valrose 06108 Nice Cedex 2 France
[3]GIS Posidonie Parc scientifique et technologique de Luminy Case 901 13288 Marseille cedex 9 France

CHANGES OVER TIME OF SHOOT DENSITY OF THE MEDITERRANEAN SEAGRASS *POSIDONIA OCEANICA* AT ITS DEPTH LIMIT

Abstract

Posidonia oceanica *is routinely used as a biological indicator of coastal water quality. Shoot density is one of the most widely used parameters for characterizing the vitality of* P. oceanica *meadows. Here, we analyse the data from a long term (1985-2005) monitoring programme of the shoot density at the meadow depth limit at 19 sites situated along the Provence and French Riviera coasts (France). Study sites feature contrasting meadow margin dynamics (i.e. regression, stability or progression). Margin progression is associated with high shoot density (> 175 shoots/m²), whatever the depth (16 through 30 m). In contrast, margin withdrawal is associated with lower density (< 175 shoots/m², 16-38 m depth). Shoot density is significantly different between regressive and progressive meadow margin. This result confirms that shoot density is a good indicator of P. oceanica meadow dynamics at its depth limit. In the long term, shoot density exhibited a significant regime shift in the early 1990s, when mean density dropped. This density decline is consistent with the regression trend of the depth limit of most P. oceanica meadows, observed in the north-western Mediterranean basin. Given the overall improvement of coastal water quality in this area since the 1970s, the causes of these phenomena remain unclear.*

Key-words: Posidonia oceanica*, shoot density, long term monitoring, regression, regime shift.*

Introduction

Posidonia oceanica (Linnaeus) Delile is a marine Magnoliophyta (Viridiplantae) endemic to the Mediterranean. It thrives between the sea surface and 30-40 m depth where it forms extensive meadows (Molinier and Picard, 1952). These meadows constitute a key ecosystem which plays a major ecological role in Mediterranean coastal areas (Cebrian and Duarte, 2001; Pergent, *et al.*, 1994). A wide range of parameters were used for characterizing the vitality of *P. oceanica* and for monitoring the meadow. Shoot density is considered as a good descriptor of meadow vitality and is the most widely used for studying meadow dynamics (Pergent-Martini, *et al.*, 2005). Shoot density is sensitive to depth, or to various disturbances that could affect the *P. oceanica* meadow such as human activities (Balestri *et al.*, 2004; González-Correa *et al.*, 2005; Pergent *et al.*, 1999; Ruiz and Romero, 2003). The efficiency of this descriptor for characterizing meadow vitality and the link between shoot density and dynamic of the meadow (*i.e.* progression or regression) has not previously been studied.

Here, we analysed a 20-year data set for shoot density at the lower limit of the *P. oceanica* meadow along the Provence and French Riviera coast (North-Western Mediterranean Sea). We aimed to answer the following questions: is shoot density an efficient descriptor for monitoring the *P. oceanica* meadow? What kinetic pattern does shoot density present over the long term?

Materials and methods

Nineteen *Posidonia oceanica* meadows, at their deep limit (from 16 m down to 38 m depth) situated along the Provence and French Riviera coast were studied from 1984 to 2004 (Fig. 1). Study sites feature contrasting environmental conditions (*i.e.* exposed or sheltered, distance from harbour facilities and outfall of previously untreated sewage water) and different meadow limit dynamics (*i.e.* progression, stability or regression). The 19 sites were randomly distributed within 3 sets. Each year, the sites of one set were visited, so that each site was studied (by scuba-diving) every 3 years, a time interval consistent with the slow growth rate of *P. oceanica* plagiotropic (creeping) rhizomes. Shoot density was measured by counting the number of shoots in a 20 cm x 20 cm frame (30 replicates per site) (Pergent-Martini *et al.*, 2005; Pergent *et al.*, 1995). Due to weather conditions, some data are missing. Statistical analysis was based on ANOVA (Statistica v6.0, Statsoft®) and a regime shift detection algorithm (Rodionov and Overland, 2005; Rodionov, 2004). The parameters used for this algorithm are: probability= 0.05; cutoff length= 5; Huber weight parameters= 10.

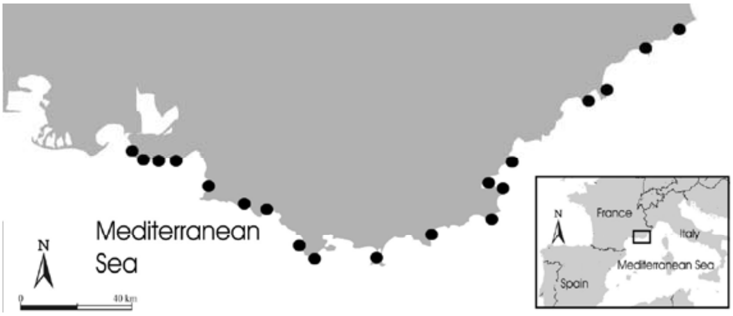

Fig. 1 - Study sites from West to East (depth in m): Martigues (20), Carro (22), Carry-le-Rouet (30), Niolon (23), Marseilles-Prado (26), Cassis (24), La Ciotat (33), Le Brusc (31), Toulon-Cap Sicié (27), Giens (32), Bormes-les-Mimosas (38), Cavalaire (36), Saint Tropez (37), Grimaud (22), Saint Aygulf (18), Golfe Juan (31), Antibes (21), Villefranche (16), Eze (32).

Results

Our data were first analysed without considering the temporal variation. Nearly all the meadow limits deeper than 27 m exhibit regression whatever the shoot density or the 3-year period (Fig. 2). Shoot density is higher (usually > 175 shoots.m^{-2}) where the meadow limit is in progression, whatever the depth (Fig. 2), than in meadows whose limit is in regression (usually < 175 shoots·m^{-2}) (ANOVA: F=180.3156***, p<0.0001). The mean annual shoot density exhibits a significant regime shift for the year 1990 (Fig. 3). Before the shift, the mean annual shoot density was above 160 shoots·m^{-2} whereas after the shift, the mean annual shoot density declined to 125 shoots·m^{-2}.

Discussion

The fact that shoot density differs between meadows with depth limits in regression or in progression, whatever the depth, confirms that shoot density is a good indicator for *Posidonia oceanica* meadow dynamics at its depth limit. So, shoot density appears to be a good descriptor for the vitality of deep meadow. Shoot density in relation to depth could predict a regressive or a progressive trend for the meadow limit studied, at least at the depth limit. For these reasons, shoot density appears to be an efficient tool for monitoring the *P. oceanica* meadow.

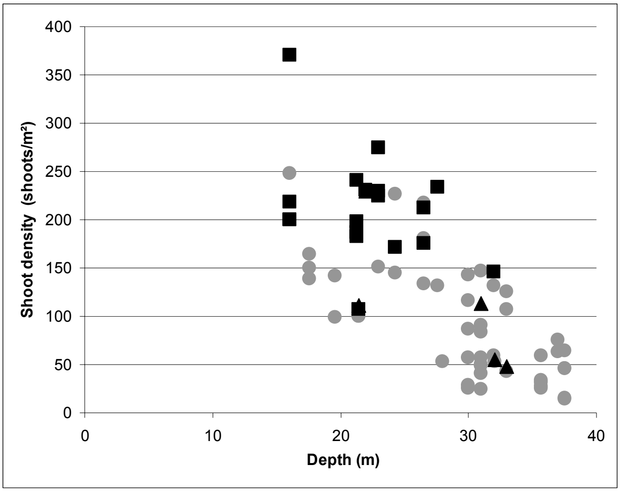

Fig. 2 - Shoot density at depth limit of *Posidonia oceanica* meadow. Black squares represent progressive depth limit, grey circles regressive depth limit and black triangle steady depth limit.

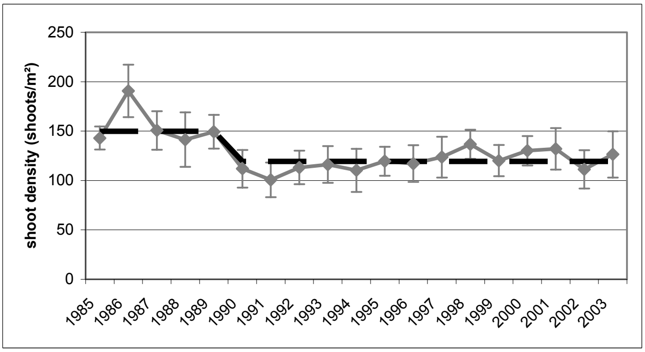

Fig. 3 - Grey line: mean shoot density (mean number of shoots by m² by year) including all study sites from 1985 to 2003, vertical line corresponds to standard error. Black discontinued line: Regime shift detection algorithm (probability= 0.05; cutoff length= 5; Huber weight parameters= 10).

A significant regime shift in shoot density occurred in 1990. This decline in the mean shoot density of deep *P. oceanica* meadows was unexpected in the context of the overall improvement in coastal water quality since the 1970s, with the setting up of sewage treatment plants: the percentage of urban sewage undergoing treatment in the study area (Provence and French Riviera) was 10 %, 18 %, 40 %

and 95 % in 1970, 1980, 1990 and 2000, respectively (Agence de l'Eau Rhône Méditerranée Corse: http://rdb.eaurmc.fr). It appears more than ever that the observed decline in shoot density at our study sites could not be easily related to a direct human nuisance such as sea discharge, coastal development or invasive species. This decline in shoot density of the deep meadow should also be interpreted in the context of the general withdrawal of the deep *P. oceanica* meadow limit (Boudouresque *et al.*, 2000). This withdrawal affects 13 of our 19 study sites as it does a large part of the North-western Mediterranean Sea. We might suppose that both these phenomena (decline in shoot density and general withdrawal of the deep limit) are linked and have a single cause. This cause remains unclear, all the more so as the shallow limit of the meadow was not affected by this withdrawal and presents a progressive trend. Various hypotheses might explain this decline in shoot density: natural variation of growth pattern (*e.g.* structural dead matte: the natural phenomenon of the appearance of dead matte after the "natural" death of an orthotropic rhizome); direct or indirect impact of variation in certain environmental parameters (*e.g.* temperature).

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C. Mifsud[1], F. Cinelli[2], S. Acunto[2], D. Balata[2], E. Lanfranco[3], S.A. Micallef[3],
L. Piazzi[2], D.T. Stevens[3], S. Calvo[4]

[1]Nature Protection Unit, Environment Protection Directorate, Malta Environment and Planning Authority,
P.O. Box 200, Valletta, CMR 01 Malta
[2]Department of Biology, University of Pisa, via Volta, 6 – 56121 Pisa (Italy)
[3]Department of Biology, University of Malta, Valletta, VLT07 Malta
[4]Department of Botany, University of Palermo, via Archirafi – 92100 Palermo (Italy)

THE DISTRIBUTION AND STATE OF HEALTH OF *POSIDONIA OCEANICA* (L.) DELILE MEADOWS ALONG THE MALTESE TERRITORIAL WATERS

Abstract

The results of a cartographic assessment of the Posidonia oceanica *meadows around the Maltese Islands carried out between spring and summer 2002 are presented here. Another objective of this baseline survey was to determine the state of health of the meadows. A comparison was also made between some results of this survey and those obtained by previous studies.* P. oceanica *colonizes 55.06 km*^{2} *of sea bottom, representing approximately the 1.36% of Maltese territorial waters.* P. oceanica *meadows surrounding the Maltese Islands appear to be in a good state of health: in certain areas the depth limit of the meadows is greater than 40 m, primary production is normally high and shoot density and LAI values are comparable to those observed along the coasts of Sicily. However, considerable meadow regression has been observed in some areas, even prior to the 2002.*

Key-words**:** Posidonia ocanica*, cartography, phenology, Maltese Islands.*

Introduction

The meadows constituted by the seagrass *Posidonia oceanica* (L.) Delile are considered one of the most important ecological habitat of Mediterranean coastal systems (Mazzella *et al.*, 1992; Pergent *et al.*, 1999: Buia *et al.*, 2000) and have recently been defined as a priority habitat[1] in Annex 1 of the EU Habitats Directive [Natura 2000 Code 1120]. A lot of studies were recently carried out in the whole Mediterranean in order to determine the extent and the characteristics of the meadows (Meinesz *et al.*, 1978, 1988; Colantoni *et al.*, 1982; Falconetti and Meinesz, 1989; Calvo *et al.,* 1993; Pasqualini *et al.*, 1998; Piazzi *et al.*, 2000, 2001). Around the Maltese Islands, only fragmentary information existed on distribution and ecology of *P. oceanica* meadows before 1995 (Drew and Jupp, 1976). But during the last 10 years a good number of surveys were performed improving the knowledge of the *P. oceanica* biological and ecological characteristics and of the assemblages associated to the meadows, moreover, some studies also evaluated the impacts of human activities (Micallef, 1996, Borg 1995; Borg and Schembri, 1995a, 1995b; Borg *et al.*, 1997; Pirotta and Schembri 1997a, 1997b; Dimech *et al.*, 2000, 2002).

Because of the requirements of the EU Habitats Directive, in 2002, the Maltese Government, through the Department of Environment and the Planning

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[1] *Priority habitat types* are defined in article 1(d) of the Habitats Directive as, natural habitat types in danger of disappearance, which are present in the territory of Member States [MS] and for the conservation of which the MS have particular responsibility in view of the proportion of their natural range which falls in their territory.

Authority, funded an extensive survey in the territorial waters of Malta in relation to the extent and characteristics of *P. oceanica* meadows. The survey was contracted and awarded to G.A.S. s.r.l. (Geological Assistance and Services of Bologna, Italy). The aim of this survey was to map the geographic extent of the meadows and the spatial distribution of the morphotypes and to provide details regarding their state of health.

The present paper reports the main results of this survey. Moreover, parts of the results are compared with some previous studies, in order to evaluate any recent changes in the characteristics of the meadows.

Materials and methods

Malta consists of an archipelago of three main inhabited islands and a number of small uninhabited islets and rocks. The northeast facing seaboards of both Malta and Gozo tend to be gently sloping while the western and southern coastlines are characterised by steep cliffs. The baseline survey was carried out within the whole of the Maltese Islands' territorial waters (12 nautical miles from the coast, including Hurds bank, which is an 'offshore' reef less then 50 metres deep) up to a maximum depth of 50m, the latter being considered as the depth limit of the *Posidonia oceanica* meadows. The survey was carried out in spring and summer 2002.

Geophysical survey

Surface positioning throughout the fieldwork was provided by differential GPS (DGPS), whilst underwater positioning was provided using a Simrad HPR 410P hydroAcoustic position reference system. The Navigation system is the software NavPro (Navigator Professional), which is manages two-way serial connections with the external sensors: DGPS, gyrocompass, echosounder, Multibeam, Side Scan Sonar, HPR. Fixing/logging interval of the vessel position was set every 20 metres for echosounder acquisition and every 25 metres for SSS acquisition. The automatic acquisition of navigation data allowed the processing of the track plot charts, containing all the profiles performed in the areas and the relevant fixes. Navigation data were converted into ascii format following QA/QC Manual procedures and prepared for CAD software. The interpretation of the recorded data was performed on charts having a scale of 1:10000; the geo-referenced coastline labelled with the main toponyms was drawn over these charts.The bathymetric data was acquired in continuous mode using a Navisound 210 precision hydrographic echosounder. Side scan sonar equipment KLEIN System 2000 was used in order to provide details of seabed features along the surveyed area. The position of the tow fish was determined acoustically and interfaced with the navigation data to ensure correlation between geophysical data sets. Side Scan Sonar data were acquired simultaneously with the bathymetric ones. Range settings of 100 m and 150 m were used, depending on water depth and survey line spacing.

Biological sampling and data analysis

In order to study the state of health of the beds, fifteen locations around the Maltese Islands were randomly selected and sampled by SCUBA divers [refer to table 1]. In each location, three sites a 100 m apart were chosen and in each site, three areas a few metres apart were sampled. The sampling area was 0.25 m² at 10 m water depth. The depth chosen is the mean depth of distribution of *P. oceanica*, also reported in similar studies (RTI, 2002, 2004). In each sampling area, shoot density (number of shoots per square metre) was assessed and five vertical shoots were collected for phenological and lepidochronological studies. Samples were preserved in a 4% formalin seawater solution for laboratory observations. A total of 1080 shoots were gathered. In the laboratory, the following phenological variables were calculated: mean number of leaves, mean leaf length and width per shoot and Leaf Area Index (mean leaf surface per shoot/2 × mean shoot density). Coefficient 'A' was also determined.

Primary production of rhizomes of the last five years (1997-2001) was obtained by lepidochronological analysis as described by Pergent *et al*. (1989). Scale thickness minima and maxima were determined using cross sections, localised at 10-12 mm above the base of the scale. The positions of floral stalk remains, inserted between the scales, were also recorded. The biometry of living leaves (foliar shoots) was determined using the technique of Giraud (1979). Data were analyzed by a 3-way ANOVA with Location (km apart, 15 levels), Site (100m apart, 3 levels) nested in Location and Area (m apart, 3 levels) nested in site considered as random factors; homogeneity of variances was checked before analysis by Cochran C test and data were transformed when necessary (Underwood, 1977).

Tab. 1 - *Posidonia oceanica* was sampled in the following sites [M,C and G stand respectively for Malta, Comino and Gozo Islands]

M1: St. Paul Islands	M6: Gharlapsi	C1: Cominotto
M2: Mellieha Bay	M7:Munxar Reef	C2: St. Marija Bay
M3: L-Ahrax	M8:St./ George's Bay	G1: Ramla Bay
M4: Anchor Bay	M9:Qalet Marku	G2: Dwejra
M5: Fomm ir-rih	M10:Qawra Point	G3: Xatt l-Ahmar

Results

Meadows Distribution and Morphology (Fig. 1)

Around the Island of Malta, a dense *Posidonia oceanica* meadow on matte grows in the NE region between Saint Paul's Islands and Qawra Point and a dense meadow interrupted at times by sand and by the seagrass *Cymodocea nodosa* is present between Qawra Point and Ras il-Qrejten. A relatively dense *P. oceanica* meadow characterizes the seafloor from Ras il-Qrejten to Saint Julian's bay; here a matte height greater than 2m is common, with erosion channels and areas colonized by *C. nodosa* and by the invasive Chlorophyta *Caulerpa racemosa* (Forsskal) C. Agardh. Rare patches of *P. oceanica* grow on rocks between Saint Julian's bay and Żonqor point. The meadow reappears at Munxar reef, colonising sand and rocky bottom but matte is also present. Scattered patches of *P. oceanica* characterize the sandy and rocky bottom from Xrobb l-Għagin to Bengħajsa Point and close to Għar Lapsi there are many patches of *P. oceanica* settled on rock. Further north-west *P. oceanica* reappears between Fomm ir-Riħ and Anchor bay, with patches growing on sand and rock. Around the Island of Gozo, *P. oceanica* is widely present along the north-eastern coast. Further west and up to the South-

East *Posidonia* is located in two small zones: one at Dwejra (with rare patches) and another just outside the Mgarr harbour, where it grows at a depth of 8-9m and then covers the gently sloping face with a dense meadow at the deeper end. The channels between Gozo and Comino and Comino and Malta are covered by a dense *P. oceanica* growing on matte. The the meadow further continues close to the Maltese coast and St. Paul's Islands.

Phenological and Lepidochronological analysis (Table 2)

The meadow density generally exceeds 400 shoots/m^2, with the exception of the two localities Munxar (M7)and St. George's Bay (M8). The highest densities

Tab. 2 - Mean phenological and lepidochronological descriptors

Meadow	Dens.	LAI	Mean growth Rate (mm/rhizome/year +-s.d.)		Mean Rhizome Pr.production rate (mgdw/rhizome/year +-s.d.)	
M1	494.7	12.87	9.6	3.0	83.8	35.7
M2	482.9	13.22	8.9	3.2	69.3	40.4
M3	471.1	13.77	9.3	3.7	71.1	52.9
M4	435.1	6.29	4.4	1.7	33.8	17.9
M5	503.6	14.20	10.5	2.9	84.7	29.4
M6	519.6	16.3	17.3	4.9	144.9	56.9
M7	331.1	8.23	6.1	2.0	57.4	26.0
M8	369.3	8.33	5.4	1.9	49.4	31.0
M9	627.6	16.14	12.6	4.8	116.8	64.0
M10	461.3	15.89	8.2	3.0	64.2	26.1
G1	541.8	19.47	11.3	2.9	95.8	30.8
G2	583.1	11.55	8.9	3.9	52.7	26.6
G3	468.9	16.4	15.8	5.9	172.0	87.0
C1	517.8	15.6	13.7	5.0	112.5	55.6
C2	532.9	15.45	10.4	2.3	84.6	31.3

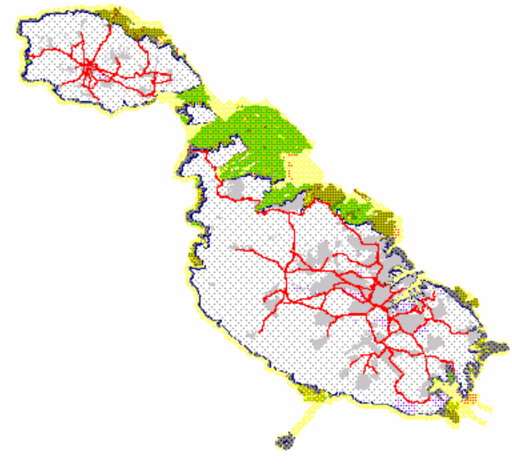

Fig. 1 - Distribution of *Posidonia oceanica* meadows around the Maltese archipelago, as recorded by GAS s.r.l. in 2002

occur at Qalet Marku (M9), Dwejra (G2), Ramla (G1), St. Marija (C2) and Ghar Lapsi (M6). LAI is highest at Ramla Bay (G1), Xatt l-Ahmar (G3) and Ghar Lapsi (M6) and lowest at Anchor bay (M4). Mean growth rate is highest at Ghar Lapsi (M6) and Xatt l-Ahmar (G3). The lowest mean growth rate is at Anchor bay (M4). Mean rhizome production is highest at Xatt l-Ahmar (G3) and Ghar Lapsi (M6) and lowest at Anchor bay (M4).

Discussion

Results of *Posidonia oceanica* mapping show that meadows are widespread around the coasts of the Maltese Archipelago principally distributed on rock but wide matte structure are also present. These results are in agreement with those of previous data about *P. oceanica* meadows of Maltese Islands (Borg and Schembri, 2002). The percentage cover is variable; several meadows appear continuous with no significant patches (M1, M2, M3, M6, M7, M8, M9, M10, see Table 1); while the meadows around Gozo (G1,G2,G3) and Comino (C1, C2) and some Maltese meadows (M4 and M5) show a patch structure. Changes in meadow architecture had been observed in some areas, even prior to 2002 (Dimech *et al.*, 2002) and some meadows have regressed or have been extirpated altogether (Borg and Schembri, 1995b; Schembri, 1995). The lepidochronological and phenological descriptors indicated that the meadows around the Maltese islands were generally healthy. Density values recorded for the various meadows belong to the normal density class (Pergent *et al.* 1995) except for Munxar Reef that was identified as sub-normal class, with a value of 331 shoots/m^2. Such a low density, at the Munxar reef, may be due to the high currents prevalent in the area, the geomorphology of the seabed and adjacent coast [which is of the more friable middle globigerina limestone type (Mifsud, 1995)] and the nearby tuna pen, which may all be causing a greater physical stress on this meadow. However, most of the meadows show quite high densities e.g. at Qalet Marku (M9), Dwejra (G2), Ramla (G1), St. Marija (C2) and Ghar Lapsi (M6). LAI is frequently over 10 m^2/m^2 and quite comparable to values observed along the coasts of Sicily (Calvo *et al.*, 1995). The meadows with the higher densities, also generally show higher LAI e.g. Ramla Bay (G1) and Ghar Lapsi (M6). The latter also show the highest mean growth rate and one of the higher mean rhizome 1° production. LAI values, mean growth and mean rhizome primary production are lowest in Anchor bay (M4). Such low values may be due to the sewage outfall present in the area. A comparison with previous shoot density data, for the locality of Anchor Bay done in 1995 (Micallef, 1996) showed higher values than those reported in the present survey, while for certain areas, the latter are in agreement with most of the recent studies (Borg and Schembri, 1995, Borg *et al.*, 2004).

Mean primary production estimates of orthotropic rhizomes of *P. oceanica* are consistent with the values reported in literature (Pergent-Martini and Pergent, 1994). However, 25% of the meadows have values of primary production that exceed those present in the Mediterranean Sea (Pergent-Martini and Pergent, 1994) and show signs of a high degree of meadow vitality within the temporal range of investigation.

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M. Montefalcone[1], C.N. Bianchi[1], C. Morri[1], A. Peirano[2], G. Albertelli[1]

[1]DipTeRis, University of Genoa, Corso Europa 26, 16132 Genoa, Italy
[2]ENEA, Marine Environment Research Centre, Santa Teresa, C.P. 224, 19100 La Spezia, Italy

LOWER LIMIT TYPOLOGY AND FUNCTIONING OF SIX *POSIDONIA OCEANICA* MEADOWS IN THE LIGURIAN SEA (NW MEDITERRANEAN)

Abstract

The relationships between lower limit typology (i.e. depth and morphology) and functioning (i.e. phenological and lepidochronological parameters) of six Posidonia oceanica *(Angiospermae, Posidoniaceae) meadows were investigated in 2002 along 300 km of the Ligurian coast (NW Mediterranean Sea). Shoot density of* P. oceanica *was measured and shoots were sampled for morphological analysis of the leaves and for lepidochronological analysis of the rhizomes. Seagrass functioning at the lower limit in the six meadows was compared (2-way ANOVA) through shoot density, number of leaves, leaf surface, maximum length of adult leaves, annual productivity and growth rate of rhizomes. Lower limits were situated at different depths and most appeared in a regressive state. Multidimensional scaling on functional parameters ordered sites according to the depth of the lower limits, while no clear relationship with lower limit morphology was evident.*

Key-words: Posidonia oceanica*, lower limit, functional parameters, Ligurian Sea.*

Introduction

The meadows of *Posidonia oceanica* (L.) Delile, the most important seagrass in Mediterranean coastal waters, are seriously threatened (Marbà *et al.*, 1996). Their location in shallow coastal waters makes them sensitive to disturbance caused by human activity, e.g., marine coastal construction, pollution, anchoring, and trawling. This sensitivity makes *P. oceanica* an ideal "bioindicator" for evaluating and monitoring the quality of coastal waters (Pergent-Martini *et al.*, 2005).

Depth and morphology of the lower limit of *P. oceanica* meadows are largely used as descriptors of the state of health of the seagrass and for understanding regressive phenomena that are especially evident in correspondence of the lower limit itself.

The morphology of the lower limits was described by Meinesz and Laurent (1978), that introduced three types of healthy lower limits (type 1, 2, and 3), together with the corresponding three types of regressive limits. Pergent *et al.* (1995) identified the healthy limits as progressive, sharp, and erosive, and introduced a single typology of regressive limit. Recently, Montefalcone *et al.* (2006) distinguished two different morphologies of regressive limit: a "regressive shaded limit", with living cover gradually decreasing with depth; and a "regressive sharp limit", passing abruptly from high cover of living *P. oceanica* (> 50%) to dead matte. Montefalcone *et al.* (2006) did not utilise the term "progressive" (Pergent *et al.*, 1995), which should be implied by the "progressive" decrease of cover, because it semantically contrasts with the "regressive" state of the limit.

In 2002, within the framework of RIPO Project (CoNISMa and Ministry of the Environment), several lower limits of *Posidonia oceanica* meadows in the Ligurian Sea were investigated in order to evidence relationships among lower limits typologies and functioning.

Material and methods

Six deep *P. oceanica* meadows were studied along 300 km of the Ligurian coast (NW Mediterranean Sea) by scuba diving: Mortola (MO), off Capo Mortola (Imperia); Gallinara (GA), around Gallinara Island and facing the coast between Alassio and Albenga (Savona); Cogoleto-Arenzano (CA), between Cogoleto and Arenzano (Genoa); Pagana (PA), between Prelo and San Michele di Pagana (Genoa); Manara (MA), off Punta Manara (Genoa); Monterosso (MM), at Monterosso al Mare (La Spezia). A hierarchical sampling design was employed to assess differences among and within the meadows and to identify the scale (from hundred meters to kilometres) on which they showed the important variations.

In correspondence of the lower limit of each meadow (spaced 30 to 60 km from each other) two sites (1 and 2), approximately 300 m apart, were randomly selected. In each site, the depth of the lower limit was recorded, the morphology was described (according to Montefalcone *et al.*, 2006), shoot density of *P. oceanica* was measured in 10 replicated counts randomly chosen, and 10 orthotropic shoots were also sampled for morphological analysis of the leaves and for lepidochronological analysis of the rhizomes (Pergent *et al.*, 1995; Buia *et al.*, 2004). Seagrass functioning at the lower limit was compared through shoot density, number of leaves, leaf surface, maximum length of adult leaves, annual productivity and growth rate of rhizomes. Univariate analysis of variance (2-way ANOVA) was applied according to Underwood (1997). The model of the analysis consisted of two factors: Meadows (six levels, fixed), Sites (two levels, random and nested within meadows), with n = 10 observations per combination of factors levels. Prior to analysis, homogeneity of variance was tested by Cochran's test. Shoot density values were square-root transformed. If transformations did not produce homogeneous variances, ANOVA was nevertheless used after setting α to 0.01 in order to compensate for the increased likelihood of Type I error. When a treatment factor was significant, the differences between levels were determined using the Student-Newman-Keuls test (SNK test).

Finally, a multivariate analysis was applied to the functional parameters; data were normalized and the Bray-Curtis index used to construct the similarity matrix. A multidimensional scaling (MDS) was employed in order to assess the relationships among functioning and typology of lower limits.

Results and discussion

In Mortola meadow (Table 1), the deepest lower limit reached 25-26 m, which can be nearly considered as the maximum depth compatible with the growth of the plant in the moderately clear waters of the Ligurian Sea (Bianchi and Peirano, 1995). We distinguished a “natural progressive limit” in the site 1 and a “natural sharp limit” in the site 2. In all the other five meadows, the lower limits were shallower (from 14 to 22 m depth) and always showed signs of regression, with the occurrence of dead matte deeper than the deepest living *P. oceanica* shoots. Both sites of Gallinara meadow exhibited regressive shaded limits at 18 m depth. Cogoleto-Arenzano meadow had a regressive shaded limit at 17 m in the site 1 and a regressive sharp limit at 18 m in the site 2. In Pagana meadow, a regressive sharp limit was found at 14 m in the site 1, while in the site 2, at 14 m, the lower limit was located on a high matte terrace, with the meadow passing abruptly from a situation of high cover of living *P. oceanica* (> 50%) to dead matte. We called this morphology as "regressive erosive limit". Both Manara and Monterosso meadows exhibited regressive shaded limits at 21 to 23 m depth.

Shoot density values (Table 1) were mostly "normal" according to Pergent's classification (Pergent *et al.*, 1995), but in Gallinara meadow, where a lower subnormal density was found in both sites. Analysis of variance revealed significant differences ($p < 0.001$) in the shoot density among the six meadows, and no difference between the two sites of the same meadow, thus confirming the large-scale variability and the depth-dependence of this functional parameter. SNK test indicated no difference in the shoot density between Mortola and Gallinara lower limits, the former being deeper and healthy and the latter being shallower but in a regressive state, and between Cogoleto-Arenzano and Monterosso meadows, located at a similar depth. The phenological parameters (number of leaves, maximum length of adult leaves and leaf surface) and the lepidochronological parameters (annual productivity and rhizome growth rate) showed no differences among the meadows. However, significant differences ($p < 0.001$) were reported in the Sites factor for leaf surface, maximum length of adult leaves, annual productivity and growth rate of the rhizomes. The great variability of these parameters at the "Sites scale" could be the reason of the apparent "no variability" among the meadows, otherwise it may indicate that the environmental conditions at the lower limits are uniform on a basin-wide scale. For both leaf surface and maximum length of adult leaves, SNK test indicated significant differences ($p < 0.001$) between the sites 1 and 2 of Gallinara, Cogoleto-Arenzano and Monterosso meadows. In Cogoleto-Arenzano meadow the highest values of these parameters were recorded in correspondence of the regressive sharp limit. For both annual productivity and growth rate of the rhizomes, SNK test revealed significant differences ($p < 0.001$) between site 1 and 2 of Mortola and Cogoleto-Arenzano meadows, with higher values recorded in the sharp limits than in the shaded limits, and

Tab. 1 - Lower limit typologies and selected functional parameters in the six meadows

Sites	**Depth (m)**	**Lower limit morphology**	**Shoot density (shoots·m^{-2})**	**Leaf surface (cm^2·shoot^{-1})**	**Rhizome productivity (mg·year^{-1})**
MO1	25.1	Natural sharp	155.6 ± 52.9	294.3 ± 63.3	0.082 ± 0.042
MO2	26.1	Natural progressive	150.1 ± 37.5	235.1 ± 44.7	0.041 ± 0.016
GA1	19.1	Regressive shaded	148.1 ± 67.6	353.3 ± 58.7	0.057 ± 0.019
GA2	17.6	Regressive shaded	146.7 ± 59.9	199.7 ± 49.4	0.057 ± 0.018
CA1	17.6	Regressive shaded	279.4 ± 66.3	247.6 ± 58.5	0.064 ± 0.025
CA2	17.9	Regressive sharp	280.6 ± 41.7	347.8 ± 89.4	0.105 ± 0.045
PA1	14.0	Regressive sharp	331.9 ± 111.2	361.6 ± 101.8	0.037 ± 0.018
PA2	13.5	Regressive erosive	333.6 ± 63.9	386.7 ± 81.8	0.066 ± 0.037
MA1	20.3	Regressive shaded	190.0 ± 48.9	345.9 ± 91.9	0.122 ± 0.029
MA2	24.2	Regressive shaded	190.3 ± 32.1	332.5 ± 88.4	0.075 ± 0.042
MM1	20.2	Regressive shaded	262.5 ± 138.8	338.3 ± 47.6	0.073 ± 0.035
MM2	21.8	Regressive shaded	264.7 ± 94.5	236.3 ± 46.8	0.060 ± 0.023

between sites 1 and 2 of Pagana meadow, with higher values in the erosive limit than in the sharp limit.

MDS on functional parameters ordered sites according to the depth of their lower limits (Fig. 1). Functioning at the deepest Mortola lower limits resulted different from that at the intermediate-depth lower limits of Gallinara, Cogoleto-Arenzano, Manara, and Monterosso, and from that at the shallowest ones of Pagana. Most regressive shaded limits occupied a central position in the MDS ordination plane, while other regressive morphologies and the natural ones occupied opposite marginal positions.

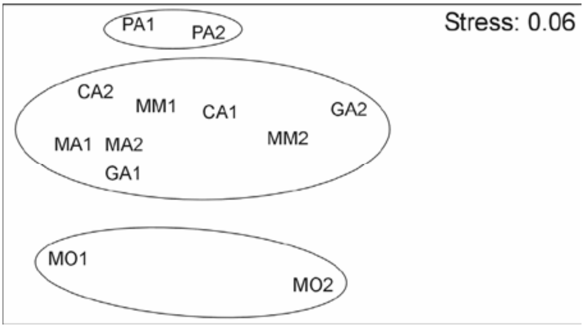

Fig. 1 - MDS ordination of functional parameters data in the six meadows

Conclusions

The regressive condition of the lower limits reported for the most of the meadows investigated confirmed the generalised regressive status of the Ligurian *P. oceanica* meadows observed with other approaches during previous large-scale analyses (Bianchi and Peirano, 1995; Peirano *et al.*, 2005). However, the shoot density values resulted as "normal" while the orthotropic rhizome annual productivities and growth were comparatively high, thus suggesting some potential for recovery (Pergent-Martini and Pergent, 1994). As a whole, functioning was related to lower limit typology, especially to their depth. When the lower limits of the two sites of the same meadow were different, erosive and sharp limits showed higher values of functional parameters than shaded ones. However, no univocal relationship between lower limit functioning and morphology was evident, and further studies of a greater number of cases, in different environmental situations, are necessary.

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M. Ruiz de la Rosa, N. García, M. Zarranz, P. Manent, R. Domínguez,
M. Grimón, G. Louzara, N. González

Grupo de Investigación del Bentos, Instituto Canario de Ciencias Marinas, 35200, Telde, Canary Islands (Spain).

PRELIMINARY RESULTS OF EXPERIMENTAL EVALUATION ABOUT DIFFERENT METHODS OF TRANSPLANTING *CYMODOCEA NODOSA* IN THE CANARY ISLANDS

Abstract

The purpose of this study was to evaluate different transplanting methods and assess the practicality and costs of transplanting seagrasses over large, multi-hectare areas. The study was carried out on Tenerife Island, in a Cymodocea nodosa *meadow threatened by expansion of harbour facilities. We evaluated four transplanting methods at a -12 m subtidal location: 1) sods of seagrass with the associated sediment and 2) biodegradable containers with small sods of seagrass inserted within, 3) sediment-free plants (shoots and rhizomes) entwined into a 3-ply line and inserted into the sediment, 4) sediment-free rhizomes entwined in a mesh fabric and attached to the bottom, all obtained from adjacent, undisturbed meadows. We calculated shoot density and planting unit survival over time and the amount of effort involved in each transplanting method and found that a substantial mortality of plants occurred within the first two months. After that time, vegetative shoot production exceeded losses and planting units expanded. We conclude that transplanted sods and biodegradable pots were the most cost-effective techniques of those tested and should be tested for larger scale restoration actions.*

Key- words*:* Cymodocea nodosa*, transplant, methods, cost-effectiveness, multi-hectare areas.*

Introduction

Worldwide, seagrass transplanting has been conducted for conservation purposes over the last half-century (see Calumpong *et al.*, 2001), carrying out successful transplanting experiences with different species (Fonseca *et al.*, 1994, 1998, Short *et al.*, 2000) to restore ecosystem functions. The first known seagrass restoration experiences date from 1947, when Addy published a Eelgrass planting guide. Since then, numerous restoring projects has been carry out in North-America, Oceania and Europe with temperate and subtropical seagrasses species (Fonseca *et al.*, 1998). However, for one of the dominant European seagrasses, *Posidonia oceanica,* there have been only sundry restoration experiments. Meinesz *et al*. (1993) and Piazzi *el al*. (1998) attempted to restore seagrass losses due to pollution and destructive fishing practices with *Posidonia oceanica*. Just recently, Curiel *et al*. (2003) performed the only published transplanting study known for *Cymodocea nodosa* in the Venice Lagoon. In the Canary Islands, *Cymodocea nodosa* is the most common and abundant seagrass specie (Afonso-Carrillo *et al*., 1980; Reyes, 1993; Tuya *et al*., 2002) and forms extensive meadows between 3 and 25 meters depth, in a area expected to undergo significant tourist development. Due to increases in human activity in coastal areas of the Canary Islands in the past decades, the seagrass beds appear to have undergone a great decline, and for this reason, in some Environment Impact Evaluations, *C. nodosa* transplanting is being considered as a tool to compensate for seagrass losses. However, as little is known regarding *C. nodosa* transplanting, the purposes of this study were to both evaluate different transplanting methods and assess the practicality and cost of transplanting seagrass over large, multi-hectare areas.

Study area, Material and methods

The study site was on Tenerife Island, in a nearly continuous (~ 80% cover) *C. nodosa* meadow of ~ 466.38 hectares at depths ranging between 5 and 20 m with an average shoot density of ~ 672 shoots/m^{-2}. The transplant site was located in the north end of the natural meadow (Fig. 1). The site was in a bare sand area at a depth similar to the adjacent meadow (~ 12 m depth) absent of anthropogenic threats, and somehow protected from storms and sand movement effects. The donor site was located in what appeared to be the densest area of the nearby natural meadow (Fig. 1).

In October 2004 we established two replicate transplant stations (A, B). Each station had four 5 x 5 m cells; each cell was planted using a different method. Four transplanting methods were evaluated, two substrate-intact methods and two bare rhizome methods. The substrate-intact methods were (1) Sods (plugs of seagrass with the associated sediment) and (2) biodegradable containers (small seagrass sods with sediment inserted within a biodegradable peat pot); the rhizome methods were (3) line (sediment free plants tied in a 5 m line and inserted into the sediment) and (4) mesh (sediment free plants tied in a 1 m^2 mesh fabric and attached to the bottom).

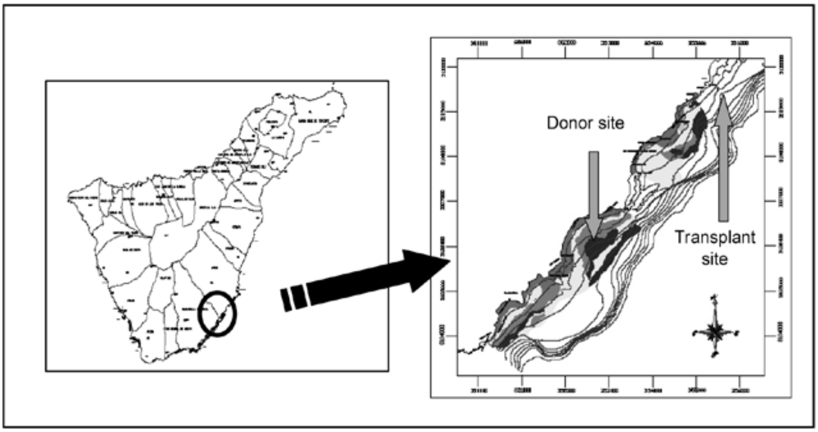

Fig. 1 - Map of Tenerife island with location of the study site

The sods and rhizomes were harvested by SCUBA divers using shovels. Plants were then kept (24-48 h) in four tanks with a closed circuit water system while the transplanting methods were prepared. The number of planting units (PU) was variable according to the method. The sod method used 30 PU station^{-1} while the biodegradable container method used 10 PU station^{-1}; both methods had a similar size PU (20 cm^2). In the Line method we tied between 35-40 rhizomes of 15-20 cm each into the 5 m line and we planted 10 lines station^{-1}. In the Mesh method, we tied 40 rhizomes of 15-20 cm in each mesh, and we transplanted 10 mesh per station.

We monitored survival rate (%) of the plants and the total shoots density from August 2004 to February 2006. We used a non-parametric test (Mann-Whitney U Test) to detect differences among the substrate-intact and rhizome methods.

Cost effectiveness was evaluated by recording time required for harvesting, PU preparation and time required for planting (*sensu* Fonseca *et al.*, 1994). However, because for shoots for the sediment free methods the amount harvested prior to fabrication could not be estimated, only the harvest of substrate-intact methods were timed.

Results

Planting unit survival and shoot density

In the first month, the shoot density remained stable for all four methods, after this period, the density decreased to ~50% of the original values (Table 1a, Fig. 2). Survival of individual PU followed the same pattern, declining after the 2nd month. From the 2nd month until the 6th month, shoot density and planting unit survival were stable. The difference between methods (substrate-intact and rhizome methods) started to be discernable after the first 6 months; PU survival and shoot density remained stable for the associated sediment methods but decreased for the rhizome methods (Fig. 3). After the 6th month, PU survival in the sediment associated methods was around 40-50%, and shoot density ~40 shoots m^{-2}.

Planting unit survival and shoot density for the rhizome methods decreased steadily until the 15th month to ~8 shoots m^{-2} and 5 % survival (Table 1c-1d).

Tab. 1 - Parameters recorded: a) and b) Density of shoots (m^2) and survival at the beggining, at the 6th month and the at15th month; c and d) Density of shoots and survival of the sediment methods and rhizome methods at the beggining, at the 6th month and at the 15th month.

a

	Shoots density/ m²			
	Transplant	**1st month**	**6th month**	**15th month**
Sods	114.4	102.7	55	43.2
Biodegradable container	69	64.9	37.7	37.7
Line	129.5	120.8	54	4.3
Mesh	190	178.1	83.7	12.2

b

	Survival %			
	Transplant	**1st month**	**6th month**	**15th month**
Sods	100	89,77	48,08	37,76
Biodegradable container	100	94,06	54,64	54,64
Line	100	93,28	41,70	3,32
Mesh	100	93,74	44,05	6,42

c

	Shoots density/ m²			
	Transplant	**1st month**	**6th month**	**15th month**
Sediment associated methods	91.7	83.8	46.35	40.45
Rhizome methods	159.75	149.45	68.85	8.25

d

	Survival %			
	Transplant	**1st month**	**6th month**	**15th month**
Sediment associated methods	100	91,38	50,55	44,11
Rhizome methods	100	93,55	43,10	5,16

Among all the methods, the biodegradable container method had the highest survival values of ~54%. After 15 months there were significant differences in PU survival and density among the substrate-intact methods and the rhizome methods (Mann-Whitney U-Test). Substrate-intact methods had a 40% greater survival than rhizome methods.

Cost-effective evaluation of methods

Our evaluation of cost-effectiveness included the tasks of harvesting, fabrication and planting activities. The mesh technique was the most complicated method requiring the greatest amount of time because the preparation included bringing plants to and from the surface, extracting individual rhizomes and manually entwining them into the mesh. Similar costs were associated with the line method. In contrast, the effort expended for preparation of the biodegradable container was only the sod removal and insertion into the container, transport and its insertion into the bottom. In the sod method, even these manipulations were avoided as the extraction, transport and insertion of the sods were the only actions. Overall, the restoration techniques using substrate-intact methods required less effort than restoration with rhizome methods (Table 2).

Tab. 2 - Parameters recorded to evaluate cost- effectiveness of the methods.

Harvesting	(10 - 12 m depth)		
5 Divers		15 m^2	
3 Divings			
Methods preparing			
7 peoples			
6 days	(10 h day^{-1})		
	PU	hours	Total
Sods	60	0.1	6
Biodegradable container	20	0.5	10
Line	20	1	20
Mesh	20	1.25	25
Transplant	(12 m depth)		
5 divers	8 cells	25 m^2	
4 divings			

Discussion

Among the four methods examined, the substrate-intact methods show the greatest promise for larger-scale transplantation; however, the transplanting survival in this study was lower than that of Curiel *et al.* (2003) with the same species. However, in our experiment, plantings were probably exposed to greater hydrodynamic action than in the Venice study, which likely contributed to the lower PU survival. Based on survival and time requirements, the sod method was the most cost-effective. Although successful, subtidal *Cymodocea nodosa* transplanting has substantial room for improvement and even though the most cost-effective, we believe it needs increased efficiency to conduct work over large areas (including considerations of seeding techniques). These manual methods are very laborious (especially at these depths where SCUBA diving is required) and the costs can become very high. Hence, with these methods, we consider the transplant of multi-hectare areas to not be viable. We recommend that sub-hectare plantings could be attempted with these methods and that spacing and PU arrangement be further explored in an effort to optimize natural meadow recovery.

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F.T. Short[1], E.W. Koch[2], J.C. Creed[3], K.M. Magalhaes[4]

[1]Department of Natural Resources, University of New Hampshire, Jackson Estuarine Laboratory,
Durham, NH 03824, USA.
[2]University of Maryland, USA
[3]Universidade do Estado do Rio de Janeiro, Brazil
[4]Universidade Federal Rural de Pernambuco, Brazil

SEAGRASSNET MONITORING OF HABITAT CHANGE ACROSS THE AMERICAS

Abstract

SeagrassNet, a world wide seagrass monitoring program, is now established in 18 countries with 48 monitoring sites in Asia, Australia, North and South America, Africa and Europe. Standardized protocols for scientific monitoring have been developed and are successfully implemented by trained teams of in-country scientists and managers. Across the Americas, SeagrassNet is also documenting new species distributions in Belize, identifying seasonal dynamics across the region, and, disturbingly, documenting widespread seagrass declines. Across the Americas, seagrass decline is seen at five monitoring sites between 43° N and 18° S latitude. The seagrass loss at these sites results from multiple of sources including eutrophication and climate change. Now in year 5, SeagrassNet world wide is yielding a data stream that affirms the persistent but dynamic nature of seagrass habitat (www.SeagrassNet.com). Future challenges to the program include insuring long-term continuity of the data stream and adding new SeagrassNet sites for more comprehensive world wide coverage.

Key-words: *Seagrass, monitoring, anthropogenic impacts, global climate change, decline.*

Introduction

SeagrassNet is an expanding monitoring program that investigates and documents the status of seagrass resources worldwide and the threats to this important and imperiled marine ecosystem. The program started in 2001 in the Western Pacific and now includes 48 sites in 18 countries; a global monitoring protocol and web-based data reporting system have been established. Our ultimate aim is to preserve the valuable seagrass ecosystem by increasing scientific knowledge and public awareness of this threatened coastal resource.

Teams composed of scientists and managers from participating countries conduct synchronous quarterly monitoring of selected plant and environmental parameters to determine seagrass habitat status and trends. A lack of information exists on the status and health of seagrasses worldwide, particularly in less economically developed regions (Green and Short 2003). SeagrassNet's efforts to monitor known seagrass areas and to reconnoiter uncharted seagrasses (Short *et al*., 2006b) are important first steps in understanding and sustaining the seagrass resource. From Brazil to the U.S. to Vietnam and many Western Pacific island nations, SeagrassNet is collecting information with the goal of elevating awareness of seagrasses and providing a "global report card" on the health of seagrass coastal habitat.

Seagrasses are underwater flowering plants that often occur in vast meadows and provide nurseries, shelter, and food for a variety of commercially, recreationally, and ecologically important species (e.g., fish, sea turtle, dugong, manatee, seahorse, crustaceans). Seagrasses filter estuarine and coastal waters of nutrients, contaminants, and sediments and are closely linked to other community types: in the tropics to coral reef systems and mangrove forests, and in temperate waters to salt marshes, kelp forests, and oyster reefs (Green and Short, 2003; Larkum *et al.,* 2006). Existing at the interface of the land margin and the world's oceans, seagrasses are threatened by numerous anthropogenic impacts as well as global climate change (Short and Wyllie-Echeverria, 1996) .

Here, we present five case studies across the Americas, ranging from New Hampshire, USA to Abrolhos, Brazil, all based on SeagrassNet monitoring over the past 2-4 years. The sites include two north temperate and three tropical locations. Each of the five case studies presented here is a change analysis of a specific seagrass habitat with its particular seagrass species and other environmental components. As a group, the case studies encompass many of the impacts to seagrasses throughout the hemisphere and indeed world wide. In each case, it is the quarterly, repeated analysis of a series of specific locations across a seagrass meadow that provides early evidence of change in the seagrass environment.

Material and methods

SeagrassNet uses a standardized protocol for monitoring of seagrass throughout the world (Short *et al.*, 2005; www.SeagrassNet.org). The goal is to scientifically measure change in seagrass, assessing qualitative and quantitative change through measurements of distribution, species composition, and abundance. The methods are derived from standard, peer-reviewed sampling techniques (Short and Coles, 2001) and are based on a statistically valid sampling scheme (Burdick and Kendrick, 2001). Based on these protocols, the monitoring results are comparable worldwide and over time at a given site, and these assessments, made using repeated measures and quarterly monitoring, allow the detection of quantitative change in relatively short time periods (1 – 2 yrs). SeagrassNet is designed to monitor indicator sites over time and does not map seagrass distribution nor is it able, from a single monitoring site, to characterize region-wide trends. However, it does capture small increments of change within a specific meadow and allow comparisons in a regional or worldwide framework.

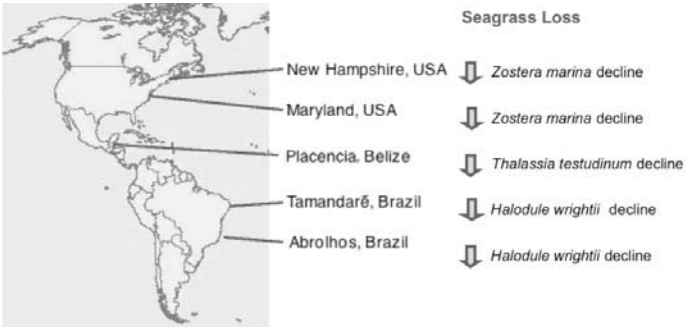

Fig. 1 - SeagrassNet sites across the Americas and associated declining seagrass species.

The SeagrassNet sites at the five locations each consisted of three fixed and parallel 50 m cross transects, the midpoints of these three cross transects representing a line perpendicular to the shore. Following the SeagrassNet protocol, the sites were chosen based on a set of parameters designed to locate a typical or representative seagrass bed for the area under consideration (Short *et al.*, 2005). Quarterly monitoring was done at twelve 0.25 m^2 quadrats placed at predetermined random locations along each cross transect. Distribution of seagrasses was determined by measuring the position of the meadow relative to the permanent cross transects over time. Species composition was measured along the cross transects, and seagrass abundance was determined by measuring the cover, canopy height, density, and biomass. Quarterly photographs were taken of each quadrat to create a permanent record of seagrass along each cross transect. Seagrass percent cover by species was visually estimated per quadrat using a photo guide. Canopy height was measured for the dominant seagrass species. Any evidence of grazing was identified. Biomass was collected with a 0.0035 m^2 core outside each quadrat at least 0.5 m from the quadrat but in an area of the same seagrass species and cover. The sediments were washed from the core sample and the plant material was separated into leaves, stems, and root-*plus*-rhizome. Epiphytes were scraped from the leaves and all plant parts were rinsed in fresh water, dried to constant weight (60 °C for 24 h) and weighed. Shoot density by species was measured from core samples by counting all shoots having intact meristems. Environmental data were obtained, including continuously measured water temperature (using an iButton® temperature data logger) and salinity.

Statistical analysis of case study results was performed using correlation analysis and least squares regression to identify trends over time. ANOVA was used for testing significant change at individual cross transects. Significance was determined at $p < 0.05$.

Tab. 1 - SeagrassNet sites across the Americas: locations, climate, species, environmental parameters, and monitoring results (Short *et al.*, 2006a).

Locations Climate	**Latitude Longitude**	**Seagrass species**	**Monitoring reveals**	**Cause of loss** Impact
New Hampshire, USA Temperate	43° 05′ N 70° 42′ W	*Zostera marina*	Declining cover, biomass, area, and density (at 9%/yr)	Climate change Increased grazing
Maryland, USA Temperate	38° 04′ N 75° 20′ W	*Zostera marina* *Ruppia maritima*	Declining cover	Eutrophication Algal overgrowth
Placencia, Belize Tropical	16° 38′ N, 88° 22′ W	*Thalassia testudinum* *Syringodium filiforme* *Halodule wrightii*	Declining cover and density (at 19%/yr)	Eutrophication Tourist development/ Increased nutrients
Tamandaré, Brazil Tropical	08° 47′ S 35° 07′ W	*Halodule wrightii* *Halophila decipiens*	Declining biomass, area, and density (at 5%/yr)	Climate change Storm frequency/ Shifting sediment
Abrolhos, Brazil Tropical	17° 58′ S 38° 43′ W	*Halodule wrightii*	Declining biomass, position shift, and density (at 11%/yr)	MPA Altered trophic cascade

Results

Five case studies from across the Americas (two sites in the USA, one in Belize and two in Brazil), demonstrate a common theme of seagrass decline (Short *et al.*, 2006a). These monitoring findings (Fig. 1), using a standard methodology, represent a latitudinal comparison across the Western Hemisphere and show clear evidence of seagrass loss. In two cases (Table 1), rapid loss of seagrass was due to eutrophication: Maryland (USA) and Placencia (Belize); in two cases, losses related to climate change issues: New Hampshire (USA) and Tamandaré (Brazil); and in one case, Abrolhos (Brazil), the loss is attributed to a complex trophic interaction resulting from the presence of a marine protected area. The greatest loss rate observed was in Placencia, Belize, where reduced water quality conditions resulted in a 19% shoot density decline per year (Table 1). In New Hampshire (USA) and Tamandaré (Brazil), where the declines were climate change related, the density loss per year was 9% and 5%, respectively (Table 1). And in Abrolhos, Brazil, the shift in seagrass bed distribution and variation in grazing intensity resulted in an 11% annual decline in seagrass density.

Discussion

Because the SeagrassNet methodology (Short *et al.*, 2005) was designed to provide documentation of seagrass change over time, with these monitoring results we can begin to make scientifically supported statements about the status of seagrass habitat and the extent of need for management action. World wide, we find seagrasses are relatively healthy in many locations, except where direct human impact occurs or where global change stresses plants. Across the Americas (Short *et al.*, 2006a), we see a general decline in seagrass health, particularly near centers of population expansion and rapidly increasing tourism, as evidenced by the *Zostera marina* decline in Maryland and the *Thalassia testudinum* loss in Placencia. In more complex ways, global climate change has indirectly resulted in the rapid decline of seagrass in New Hampshire from a change in Canada goose migration and increased grazing on *Zostera marina* (Rivers and Short, 2006), and a change in storm frequency and duration in Tamandaré that has led to increased sand wave activity that smothers and buries *Halodule wrightii*. The complex shift in trophic interaction and the cascade of predatory fish activity in Abrolhos has decreased the abundance and distribution of herbivorous fish such that the seagrass meadow is being overgrown by macroalgae while at the same time less grazing from reef-based fish allows the seagrass meadow to move closer to the reef.

The demonstrated declines in seagrass at all five of the seagrass monitoring sites in the study increase the level of concern regarding seagrass populations across the hemisphere. While these five sites are not representative of seagrass meadows in their regions or hemisphere-wide, they are indicator sites for each area and raise the level of concern for seagrasses throughout the Americas. SeagrassNet, through its comparative monitoring network, clearly demonstrates relatively short-term change in impacted areas. Humans, through commercial and subsistence fishing, as well as endangered fauna worldwide, depend on seagrasses for a living. Future SeagrassNet activities include expansion to new areas, including the Mediterranean, education of coastal managers, and synthesis of our findings in upcoming publications.

Acknowledgements

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F. Silvestre, G. Iacono, L. Tiberti, M.C. Buia

Stazione Zoologica 'A. Dohrn', Laboratorio di Ecologia del benthos, 80077 Ischia (Naples, Italy).

POSIDONIA OCEANICA DATA WAREHOUSING

Abstract

In the framework of different projects carried out at the Benthic Ecological Laboratory since 1976, a relevant amount of data on the seagrass Posidonia oceanica *(Magnoliophyta) were recorded. Due to their quantity and the variety of descriptors (both structural and functional, from molecular to system level, at different spatial and temporal scales), it was hard to store and manage these data with the classical techniques (e.g, Excel). As a consequence, it was necessary to develop a specific data warehousing system able to optimally support reporting and analysis activity on* P. oceanica *data. A relational database, performed in a hierarchical structure, was elaborated. Up to now it is formed by seven tables, corresponding to six levels of descriptor clustering, for a total of 250 variables and of 500,000 data. Appropriate data mining tools (softwares to detect trends, identify patterns and analyze data) were applied to a subset of data; 'Posware', a system for coastal waters classification by using P. oceanica as quality element according to WFD 2000/60/CE, is an example of the employment of the Posidonia data warehousing.*

Key-words*: relational database, data mining, data warehouse management system,* Posidonia monitoring.

Introduction

The monitoring of *Posidonia oceanica* (L.) Delile system is related to the gathering of data and information on the status of the plants and of associated communities over long time series. The amount of collected data depends on the temporal and spatial scales to which they are reported; in some cases they can be too large to be stored and analyzed by using traditional techniques (e.g. electronic spreadsheets). In addition, different typologies of data (categorical, numerical, alpha-numeric, etc.) used to describe different compartments of this complex system, can increase the effort of their management.

An effective tool to better manage the structural complexity of these data is the construction of a database which takes into account their different clustering pattern. Among databases, relational ones can optimally support the selection of target data and reporting and analysis activity. A data warehouse is a computer database that collects, integrates and stores an organization's data with the aim of producing accurate and timely management information and supporting data analysis (Fig. 1). Data warehousing is therefore the process by which, selecting a sub set of data (data mart) and analyzing them through data mining techniques, knowledge is discovered (Atzeni *et al*., 1999).

This work represents the first attempt to elaborate a data warehouse holding information on *Posidonia* system, in particular from those data coming from monitoring projects on large spatial and temporal scale.

Materials and methods

To elaborate a data warehouse that could fulfil the user's needs, it was fundamental to build an efficient database. As a consequence, a relational database was planned through three different phases: conceptual, logical and physical. The first phase was to evidence the variety of descriptors used to characterize *P. oceanica* system (physical, physiographical, structural and functional), their clustering in single groups in relation to the system's level that they represent (e.g. meadow, plant, leaf) and to define the relationships between them (e.g. corresponding to the hierarchical sampling design). The conceptual stage was then translated into a logical structure, according to the database management system selected (Access 2000, included in the Office package): each group corresponded to a single table in which the descriptors' format (e.g. number, text) and their attributes were indicated. At last, the links among tables set up the physical stage.

The next step was to import our data in the relational database through an 'encoding' process , that was from Excel spreadsheets to relational Access tables.

The right relationships among data of different related tables were checked by queries.

By using Visual Basic for Application (a Programming Language), the relational database was connected to the statistical software Autoclass C (performing a Baysian classification) (Cheesemann and Stutz, 1995) and to a multidimensional visual data analysis (Miner 3D). The connection with Arcview allowed reporting results of data mining on digital cartography (Fig. 1).

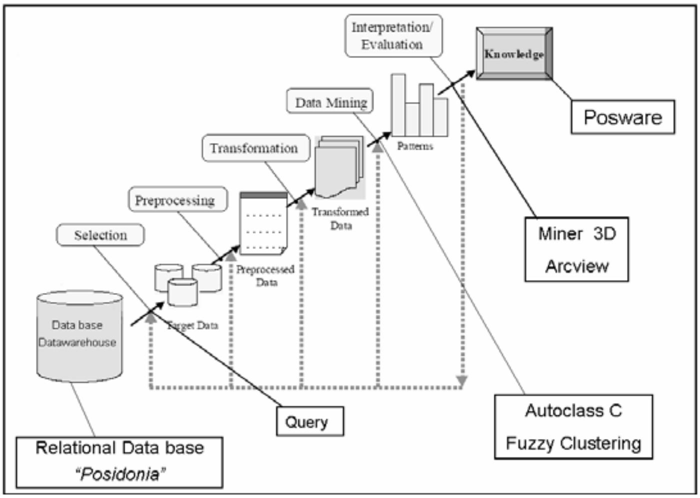

Fig. 1. A schematic diagram of data warehouse.

Results

The *P. oceanica* relational database has a hierarchical structure in which six levels can be recognized (Fig. 2). The highest two are referred to the geographical localization of the *P. oceanica* meadow; the others correspond to four tables in which descriptors are clustered according to meadow level (e.g. substratum, density, Leaf Area Index, molecular indexes), shoot level (e.g. shoot biomass, shoot leaf area), rhizome level (e.g. growth rate), and leaf level (e.g. morphometric features). An additional table on the epiphytic associated flora has been created.

Although at the Benthic Ecology Laboratory the researches carried out on *Posidonia oceanica* started in 1976 (Buia *et al.*, 2003; Procaccini *et al.,* 2003), up to now only data from projects carried out since 1988 have been imported (Iacono, 2003; Silvestre, 2003; Tiberti, 2003). A total of about 520,000 data, previously stored in 1700 tables, represents our *Posidonia* data set, described by 241 descriptors clustered in 7 tables.

It is worth to note that our relational database allocates only 6.5 Mbytes respect to 15.8 Mbytes allocated by neither normalized nor relational data bases.

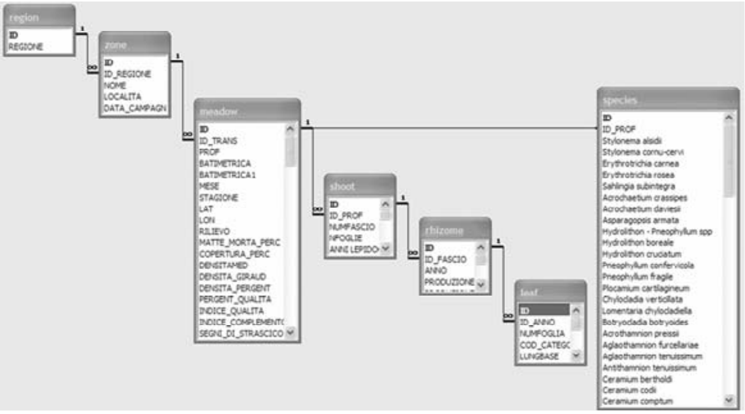

Fig. 2 - Hierarchical structure of *Posidonia* relational database.

A preliminary application of *Posidonia* data warehouse was 'Posware', a water body classification using *P. oceanica* as quality element, according to the WFD 2000/60 (Buia *et al.*, 2006). A data mart was obtained extracting by query all data referred to six descriptors: shoot density (nr. m^{-2}), annual rhizome growth rate (mg y^{-1}), annual rhizome elongation (mm y^{-1}), annual leaf formation (nr. leaf y^{-1}), leaf width (mm), depth (m). The table was then transformed in a "db2" file format and the software Autoclass C was used. A Fuzzy clustering technique (Zadeh, 1965; Zuccarello *et al.*, 1999) elaborated the results coming from the non

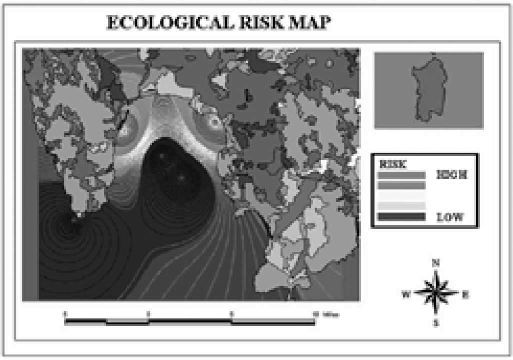

Fig. 3 - An example of a regression risk map for *P. oceanica* by using 'Posware' classification according to the WFD 2000/60/CE.

supervisioned analysis performed by Autoclass and an Index of water ecology, divided into five classes, was obtained. Introducing this Index as a new descriptor in the data set and connecting the software Arcview to the table, ecological risk maps of *Posidonia* status can be obtained (Fig. 3).

Conclusions

The relational database management system (RDBMS) elaborated for *Posidonia* seems to be a useful and dynamic tool to powerfully manage a large amount of data recorded in monitoring programmes, even if with different aims. This property is already clear, observing the memory allocation in the PC (6.5 MB against 15.8 MB) and the strong decrease in the total number of tables that should be contemporaneously open (7 against 1700) to analyze all the data. In particular, the addition of new descriptors will not change the structure of this hierarchical database, as they will constitute new fields in pre-existing tables or new tables to be linked to the rest.

Thanks to the Visual Basic Programming language supported by Access, the RDBMS can be directly linked to data sources (Excel spreadsheets), to data mining software (Autoclass C or Miner 3D), and finally to geographical support system (Arcview). This latter possibility will allow detecting year to year the *P. oceanica* status in relation to the increasing human impact on the coastal zones.

Acknowledgments

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R. Turk[1], L. Lipej[2]

[1] Institute of the Republic of Slovenia for Nature Conservation, Piran
[2] Marine Biology Station, Piran, Slovenia

RESEARCH ON SEAGRASSES OFF THE SLOVENIAN COAST (NORTHERN ADRIATIC) – STATE OF THE ART

Abstract

Four species of marine phanerogams, inhabiting the Adriatic Sea are also present in waters off the Slovenian coast – Posidonia oceanica, Cymodocea nodosa, Zostera marina *and* Z. noltii. *Their distribution in the Slovenian part of the Gulf of Trieste depends mainly of the geological structure of the coast, light conditions of the water column and urbanisation of the coastline. The presence of* P. oceanica *and* Z. marina *is limited while* C. nodosa *and* Z. noltii *are more widely distributed. At few localities all four species occur sympatrically.*

Key-words: *seagrass, distribution, Slovenia, Northern Adriatic Sea.*

Introduction

The Slovenian Sea covers the southern part of the Gulf of Trieste in the Northern Adriatic (Fig.1). The Gulf of Trieste is a shallow marine ecosystem. With few exceptions the depth does not exceed 25 m, however, only 10% of the Gulf is shallower than 10. Because of its shallowness and the freshwater inputs as well, the waters of the Gulf experience considerable temperature and salinity variations. Remarkable are also high tidal amplitudes. An important feature of the Gulf of Trieste as well as of the Slovenian coastal area is the intensive urbanisation which plays an important role as far as the state, the distribution and the conservation of seagrass meadows is concerned.

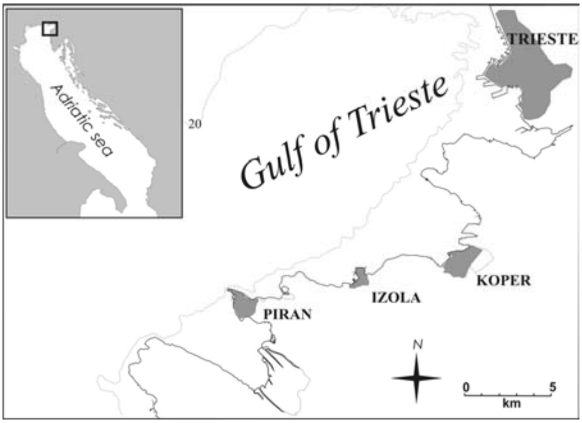

Fig. 1. The Gulf of Trieste and the investigated area of the Slovenian coast.

During the two last decades, various studies were done on different aspects of seagrass meadow distribution and biodiversity, especially on the meadow of *Posi-*

donia oceanica near Koper, including its genetic identity (Vukovič and Semroud, 1984; Vukovič and Turk, 1995; Turk and Vukovič, 1997; Ruggiero *et al.*, 2001, Turk *et al.*, 2002).

Materials and methods

The mapping of *Posidonia oceanica* meadow has been done using aerial photography and further image processing. The results of aerial photography were checked later on the field by SCUBA diving. On the basis of aerial photos and field data a map of the meadow was elaborated.

On the basis of a research, dealing with marine biodiversity in the Slovenian coast (Lipej *et al.*, 2004), carried out between 1998 and 2004, and taking account previous works on the subject, a preliminary assessment of the distribution of the seagrass species was made. The study was based on 63 vertical transects, investigated by the use of SCUBA equipment and accurately filmed with underwater camera (51 movies). Transects, although unevenly disposed, were planned in order to encompass the most characteristic parts of the coast. At the same time, an inventory of littoral fish assemblage, inhabiting *C. nodosa* meadows is going on from 1998.

Results and discussion

Four species of marine phanerogams, inhabiting the Adriatic Sea are also present in the Slovenian sea – *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina* and *Zostera noltii*. Their distribution in the Slovenian part of the Gulf of Trieste depends mainly of the geological structure of the coast, light conditions of the water column and urbanisation of the coastline. The results show that the seagrass meadows are present between the upper limit of the infralittoral belt (approx. 1 m depth) and the depth of 8 to 10 meters (Fig. 2). However, this pattern varies greatly due to the natural and geomorphological features of the coastline and to human influence, which are resulting in the higher sedimentation rate and reduced water transparency.

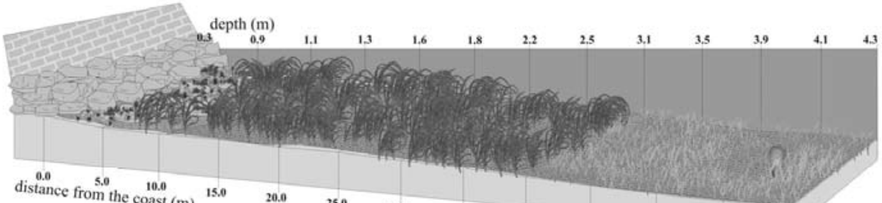

Fig. 2. Transects from the area with *P. oceanica*.

As regards to different species distributions, the results show a limited presence of *P. oceanica* and *Z. marina*, while *C. nodosa* and *Z. noltii* are more widely distributed. At few localities all four species occur sympatrically. However, due to the the limited coverage of soft bottom where light conditions are still satisfactory, even the two latter “common” species could be defined as rather endangered. This was shown also by a comparison between sites with different ecological conditions – the Strunjan Nature reserve and the inner part of the Gulf of Koper.

Apart from some shoots near Grado (Italy), it is very likely that at present the meadow of *P. oceanica* in the Gulf of Koper is the single meadow in the Gulf of Trieste and all along the western coast of the Istrian peninsula (Jaklin, pers. comm.). These first studies, which were done already in 1993, showed that the meadow starts close to the coastline and is approximately 1 km long, extending approx. 50 m offshore (Fig. 3). It has been shown that there are different microhabitat types in the meadow, providing a plethora of ecological niches for benthic invertebrates and fishes. Some cryptic and other peculiar fish species have been found in the area, as well, such as *Apletodon incognitus*, *Gobius roulei* and *Millerigobius macrocephalus*. The study on the genetic identity of *P. oceanica* showed that the meadow is formed by shoots sharing a single and completely homozygous genotype (Ruggiero *et al.*, 2001).

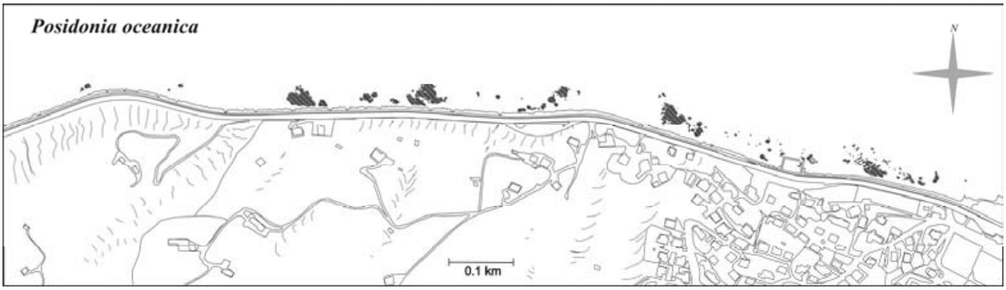

Fig. 3. Distribution of *P. oceanica* in the investigated area.

C. nodosa is the most widely distributed seagrass in the Slovenian sea. It occurs mainly on the muddy sands of the upper infralittoral, at depths ranging from 2 to approx. 10 m, all along the coast (Fig. 4). The results of the study on biodiversity (Lipej *et al.*, 2004) show that the seagrass meadows of *C. nodosa* in the Slovenian sea are of exceptional significance for numerous species of algae and animals, including some endangered species, such as the pen shell (*Pinna nobilis*) (Lipej et *al.,* 2006). Apart from its great importance for epifauna and epiflora, this natural habitat has many other important ecological implications.

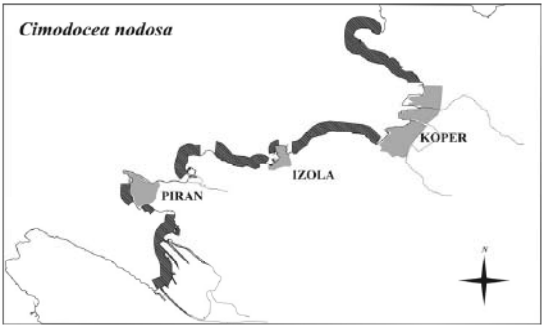

Fig. 4 - The distribution of *C. nodosa* along the Slovenian coast

Less data are available up to now for *Zostera marina* and *Z. noltii*. The former species occurs in form of small islets in the mouth of rivers, streams and lagoons. Apparently, the only true meadow in the Slovenian sea is known from the Bay of Koper (Fig. 5). *Zostera noltii* is usually found together with other seagrass species and only exceptionally forms monospecific meadows. According to the available data only a single monospecific meadow is known from the Moon Bay near Strunjan (Fig. 6).

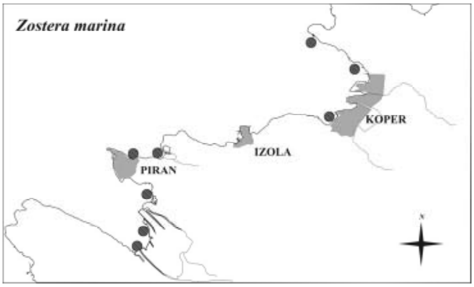

Fig. 5. Distribution of *Z. marina*.

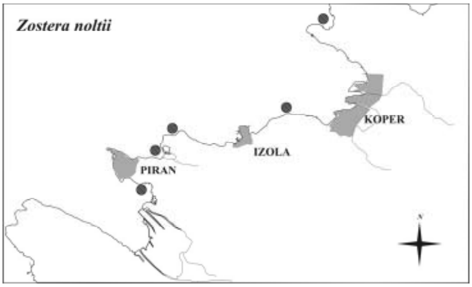

Fig. 6. Distribution of *Z. noltii*.

Conclusions

The present state of knowledge concerning the distribution of the four phanerogames present in the Slovenian coast is not yet satisfactory in terms of species and habitat types conservation. It is however important in terms of spatial planning and future development of various human activities. The available habitat for all four species is very limited due the geological structure of the coast, the light conditions on the sea bottom and the urbanisation of the coastline. Only *C. nodosa* appears to have a favourable status of conservation while the same can not be said for *P. oceanica*, *Z. marina* and *Z. noltii*.

More accurate results on the distribution of seagrasses in the Slovenian sea are expected in the forthcoming years. As part of the project Coastal Area Management Programme (CAMP) Slovenia, the whole coastal belt was covered with aerial photography and digital orto-photos. The latter will be the basis for the foreseen project on coastal habitat type cartography, that is supposed to start in 2006 and dealing in the first place with the three marine protected areas.

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G. Casazza[1], A.S. Heiskanen[2], S. Debono[3]

[1]APAT (Agency for Environmental Protection and Technical Services),
Via Vitaliano Brancati 48, 00144 Roma (Italy)
[2]European Commission, Joint Research Centre, Institute for Environment and Sustainability, TP 290
I-21020 Ispra (VA), Italy
[3] Malta Environment and Planning Authority, Nature Protection Unit,
St Francis Ravelin, Floriana, Malta, CMR 02

USE OF *POSIDONIA OCEANICA* AS A BIOINDICATOR OF WATER QUALITY IN RELATION TO THE IMPLEMENTATION OF THE WATER FRAMEWORK DIRECTIVE

Introduction

The EU Water Framework Directive (WFD) requires long-term sustainable management of water bodies and a high level of protection for the aquatic environment. The main objective of the WFD is to achieve good ecological status for all surface water bodies by 2015, thereby ensuring the conservation and proper functioning of aquatic ecosystems. The WFD requires monitoring and assessment of the ecological status using **biological quality elements (BQEs)**. Annex V of the WFD outlines two macrophyte-related attributes, with respect to the assessment of the ecological quality of coastal waters:

- Composition of aquatic flora
- Abundance of aquatic flora.

The specific status of a given water body should be defined depending on the presence and abundance of macroalgal and angiosperm taxa that are sensitive to disturbance. Marine Angiosperms, i.e. seagrasses, constitute ‘key’ species in shallow coastal water ecosystems, and represent one of the most diverse and productive habitats in the Mediterranean Sea. The state of health of *Posidonia oceanica* meadows is a good indicator of the ecological status of a particular coastal water body. Therefore, *P. oceanica* has been proposed as a BQE for Mediterranean coastal waters. Considerable work is being currently undertaken to develop classification tools and metrics that would allow use of *P. oceanica* as a BQE for WFD-compliant assessment of Mediterranean coastal waters. Work is aimed at developing harmonised seagrass metrics, and a common definition of reference conditions for *P. oceanica* meadows present in different parts of the Mediterranean.

The WFD also requires harmonisation of the understanding of ‘good’ ecological status and consistency in use of definitions with respect to the different quality elements described in Annex V of the Directive. Such work is currently being undertaken during an intercalibration exercise, and needs to be continued in order to develop harmonised targets for restoration of the ecological status of coastal waters, where required. Since *P. oceanica* is one of the promising bioindicators for the assessment, a joint effort is required to develop common seagrass metrics based on harmonised data sets collected from several coastal areas around the Mediterranean. The collaborative work also requires an assessment of the applicability of chosen metrics to the WFD requirements, with respect to identification of main processes that have adverse impact on the seagrass ecology.

Discussion

The Round Table was attended by a large number of participants and lasted for two hours. Discussion was focused on the application of *P. oceanica* as a bio-indicator for WFD-compliant assessment of the ecological status of Mediterranean coastal waters, on prospects of developing joint, harmonised *P. oceanica* metrics for classification of coastal waters. It was commonly acknowledged that successful application of any potential *P. oceanica* metrics for assessment of the ecosystem health of coastal waters, will require application of the current scientific knowledge on the functioning of the seagrass meadows as a key habitat, as well as development of further research on ecology, physiology, genetics, and population dynamics of *P. oceanica* and associated fauna and flora. The application of scientific knowledge on the impacts of the main pressures threatening this vulnerable ecosystem in the Mediterranean ecoregion is also of importance.

The round table was started with a brief introduction to the WFD requirements on ecological quality classification of surface waters in general, and the criteria applied in the development of harmonised classification for the Mediterranean coastal waters including some details on the application of the WFD classification criteria to angiosperms. Information on proceeding of the work for the Intercalibration exercise within Ecoregions was also provided, particularly for the Mediterranean Geographic Intercalibration Group (MED-GIG). Finally, detailed description of the activities of the *Posidonia*-subgroup in the MED-GIG was provided.

The proposed points of discussion were:

- ☐ Classification systems across Mediterranean Member States arise from the use of different metrics and/or different classification methods: discussion on the final goal of intercalibration, that is a common agreement on the comparability of the different methods.
- ☐ Two common metrics are used, at the moment, by 5 countries/regions: shoot density and shoot size (as shoot area or shoot biomass). Discussion on needs of standardization of methods and use of these parameters; importance of standardization especially for use by non-EU Mediterranean countries.
- ☐ Other potential parameters/methods that could be useful for this classification.
- ☐ Relationship between pressures and ecological quality status: discussion on which kind of effective/adequate correlation can be used.
- ☐ Reference conditions: discussion on their existence in the Mediterranean, and where these do not exist, how can reference values be applied/derived.
- ☐ Importance/need of applied research, overcrossing the EU and non-EU countries and possible ideas/proposals for foreseeing, together with EC, any specific direction for support/funding
- ☐ Possibilities of use of other seagrasses for the ecological classification purpose within the Mediterranean

The discussion was very lively and focused mainly on the information exchange between the scientists already involved in the WFD implementation within their

countries and the other Mediterranean seagrass experts.
The most important outputs were:

a) Widening of the knowledge on the WFD technical requirement and applications, and informing also the non-EU Mediterranean experts of the on-going implementation process in the EU.
b) General acceptance of the importance in developing and agreeing on harmonised monitoring methods and metrics for assessment of the status of the Mediterranean seagrass communities (development and application of simple and robust indicators, such as seagrass shoot density, shoot size, or cover, that are also used in the worldwide Seagrass monitoring network). However the use of other *P. oceanica* descriptors (such as percentage cover) indicative of mechanical damage and others describing the system (not just the species) should also be considered.
c) A good understanding of what is a pristine meadow needs to be developed. This can be obtained from historical data and to some extent from lepidochronological data.
d) Discussion about new methods ensued, such as the use of digital photography to assess physiological state, as is used for coral reefs; genetic studies; also consideration for the use of non-destructive methods for the general conservation of the meadows.
e) The scientific community has a very important role in developing such practical classification tools, that are based on scientific understanding and knowledge on the functioning of seagrass ecosystems, and which can be used by managers for monitoring and assessment of the coastal ecosystem status for the WFD purposes.
f) The legal framework of the WFD offers a great potential for the protection and improvement of the status of coastal marine ecosystem. The scientific and coastal manager communities need to work together to develop a common goal for protection of the most important coastal habitats.
d) The protection of the entire Mediterranean coastal ecosystems requires involvement of the experts and managers from the non-EU Mediterranean countries to join in the development of a common view on the ‘good’ ecological status of coastal waters, and to agree on standards and application of shared methodologies for monitoring and assessment.

With the contribution of Cecilia Silvestri and Cecilia Lopez y Royo (APAT, Roma)

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P.L.A. Erftemeijer[1], M.C. Gambi[2], C. Pergent-Martini[3]

[1]WL Delft Hydraulics, P.O. Box 177, 2600 MH Delft, The Netherlands
[2]Laboratorio di Ecologia del benthos- Stazione Zoologica "A. Dohrn", Napoli (Italy)
[3]Ecosystèmes littoraux, Université de Corse Pasquale Paoli, BP 52, 20250 Corte, France

CONSERVATION OF MEDITERRANEAN MARINE VEGETATION

Abstract

The Action Plan for the Conservation of Marine Vegetation in the Mediterranean, drawn up within the framework of the SPA Protocol of the 1995 Barcelona Convention, calls for the highest conservation efforts at national and international levels to ensure the conservation and prevent the loss and degradation of seagrass meadows and other key plant assemblages. This paper presents a summary of the results of a round-table discussion on this topic, held at the Mediterranean Seagrass Workshop (29th May – 4h June 2006) in Malta. The contributions and recommendations from 110 seagrass scientists and experts (mostly from the Mediterranean region) on priorities for management, research and education-awareness of Mediterranean seagrass resources are discussed.

Key-words: *Barcelona Convention, conservation, marine vegetation, Mediterranean Sea.*

Introduction

The Mediterranean Sea is home to over one thousand macroscopic marine vegetation species (including 6 species of seagrasses), with a high percentage of rare and/or endemic species. The crucial role of many of these species in maintaining the balance of marine ecosystems is evident. Although knowledge available on marine vegetation species in the Mediterranean is at present insufficient, and often fragmentary, it is obvious that these species are under increasing pressure, often anthropogenic in origin, in the Mediterranean, which engenders a degradation and decline of these species, observed in many Mediterranean regions (EEA, 2006).

The SPA Protocol of the 1995 Barcelona Convention recommends effective protection measures to safeguard elements of biological diversity in the Mediterranean Sea through action plans and technical inventory tools. One of these action plans specifically concerns marine vegetation and aims to ensure the conservation of marine vegetation and plant assemblages by implementing management and legal protection measures, prevent the loss and degradation of seagrass meadows and other key plant assemblages, and ensure the conservation of natural monuments such as *Posidonia oceanica* barrier reef formations, biogeochemical structures formed by Corallinales (e.g., *mäerl*) and *Cystoseira* spp. belts. To achieve these objectives, particular attention must be paid to 14 selected plant species that include three seagrass species (*Posidonia oceanica, Zostera marina* and *Zostera noltii*) all of which are worth of the highest conservation efforts at national and international levels (UNEP-RAC/SPA, 2000).

The Action Plan for the Conservation of Marine Vegetation in the Mediterranean includes the following recommendations: inventory of species and mapping of their distribution, detailed mapping of seagrass meadows, identification of threats, control of the impact made by watershed infrastructures on the marine environment, elaboration and implementation of appropriate legislation, establishment of marine vegetation monitoring networks, establishment of protected areas, and promote (regional) cooperation (UNEP-RAC/SPA, 2000).

Round-table discussion

This paper aims to summarize a round-table discussion on the topic of 'Conservation of Mediterranean Marine Vegetation', held during the Mediterranean Seagrass Workshop, 29th May – 4th June 2006, in Malta, attended by over 100 seagrass scientists and experts, including participants from 13 different countries around the Mediterranean basin.

As an introduction to this round-table session, Christine Pergent-Martini presented an overview of knowledge available concerning the mapping and monitoring of *Posidonia oceanica* in the Mediterranean, indicating large gaps in our present knowledge on the distribution, status and trends of this species and calling for greater regional information sharing.

Paul Erftemeijer discussed some of the management priorities (mapping and monitoring, marine protected areas and species protection, conservation action plans, EIA legislation and permits, networking and regional agreements, sustainable financing mechanisms), research priorities (natural dynamics and environmental variability, review sensitivity and tolerance to identify and fill knowledge gaps, exemplary quantitative impact studies of key threats, natural recovery and cost-effective restoration, evaluation of effectiveness of conservation measures, comparative regional studies and knowledge sharing), and education and awareness priorities (develop educational campaigns and materials, review, translate and disseminate scientific information to managers and stakeholders, develop best-practice guidelines and manuals, public participation in monitoring and restoration) that need to be addressed to improve the conservation of Mediterranean seagrasses.

Maria Cristina Gambi presented a short video-documentary on the extreme impact of dredging on a seagrass meadow in Ischia (Italy) as an example of the threats faced by seagrass meadows in the Mediterranean (Gambi *et al.*, 2005).

The discussion was given impetus by the subsequent presentation of the following statements and questions:

- education/awareness has greater potential than regulation (what role for scientists?)
- more research does not lead to better conservation (how to bridge the gap between science and management?)
- giving recommendations has no real impact (what initiatives can we take ourselves?)
- EU legislation will overtake national policies and plans (how can we harmonize?)

Management issues

It was recognised that there are a number of approaches to seagrass management, ranging from a reliance on strong and prescriptive (top-down) legislation and enforcement to a softer bottom-up approach that relies more on participation from stakeholders and local communities with a strong focus on education and awareness building. While the emphasis may differ between countries and cultures, it is probably more than likely that a mix of approaches will be the most effective in achieving conservation goals and objectives.

There was general consensus on the fact that legal measures/protection alone is not enough to safeguard seagrass resources in the Mediterranean. There appears to be a general lack of awareness and political will. Too many engineers and not enough scientists are involved in the planning and implementation of the various coastal developments that lie at the source of most of the threats to seagrasses.

There are some promising developments in a few cases (e.g., Spain), where the implementation of certain measures (e.g., anti-trawling reefs, improvements to water quality after building wastewater treatment facilities) have certainly improved the situation for the seagrass beds.

Several participants underscored the point that seagrass scientists (both as experts and as citizens) have a responsibility to act as ambassadors of seagrasses. In this role, seagrass scientists should act to ring the alarm bells when things go wrong, to pass information on worrisome trends and threats to managers and conservation groups (who can take legal action), and to sensitize decision makers and the public by producing popular scientific articles, issuing press releases on research findings and interacting with journalists.

Awareness and education issues

It was realised that there is a particularly low awareness on the values of seagrass resources among decision-makers and the general public in some of the less developed countries around the Mediterranean (e.g., Tunisia, Algeria), where marine conservation is given a low priority and the production of education and awareness materials is hampered by financial constraints.

There is a particular need to emphasize the economic aspects (especially in developing countries) of the role and function of seagrass resources and the costs of their degradation and loss. Unfortunately, it is often only the negative aspects (e.g., conflict between seagrass litter on beaches and tourism) that reach the media, ignoring the vital role of seagrass meadows in sustaining fisheries, stabilising sediments and reducing coastal erosion, and supporting a rich biodiversity.

The use of charismatic fauna associated with seagrass meadows (e.g., sea horses, octopus, pen shells, etc.) in educational campaigns was recommended in order to help making people appreciate the value of seagrass ecosystems in the Mediterranean.

Some excellent examples of educational materials on *Posidonia* meadows (e.g., video documentaries, brochures) have been developed in some Mediterranean countries (e.g., Croatia) which could be translated into other languages and made available for dissemination and use in other countries.

It was also recommended to learn from experiences gained in education and awareness campaigns for other important ecosystems, such as forests and coral reefs, where successful sensitization of the general public has resulted in public outcry over the degradation and destruction of these systems which have provoked a response from politicians and decision-makers.

Research issues

One of the major challenges that requires further scientific attention is to distinguish between impacts of human activities on seagrass meadows and natural dynamics caused by natural variation in environmental factors and processes, both in time and in space at various scales. Natural dynamics and environmental variability may obscure the effects from anthropogenic disturbances. There is a lack of information on the habitat requirements for continued seagrass viability and critical levels (area, density) required for sustaining seagrass habitat. There is insufficient knowledge on the resilience of seagrasses to environmental stresses, the ability of seagrasses to adapt to environmental changes, and the capacity of seagrasses to recover from loss or degradation and on realistic time-frames.

It would be particularly useful for managers, if scientists could carry out exemplary quantitative environmental impact studies on some of the key threats faced by seagrass meadows in the Mediterranean and to make this information widely available (in contrast to many descriptive EIA reports which are often inaccessible due to issues of confidentiality).

There is an urgent need for collaborative, interdisciplinary research into the issue of cumulative impacts of multiple developments and threats on meadows of *Posidonia oceanica* and other seagrass species.

There is also a need to carry out more research into the issue of economic valuation of seagrass resources, addressing the monetary value of the goods and services derived from seagrass meadows, and taking into account the high costs and limited success of efforts to restore seagrass beds.

Studies into the effectiveness of (past) management measures (including protected areas) in achieving conservation goals and objectives at various temporal and spatial scales is also considered a priority for future seagrass research in the Mediterranean region.

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