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**SHORT-TERM RESPONSE OF THE SLOW GROWING SEAGRASS
POSIDONIA OCEANICA TO SIMULATED ANCHOR IMPACT**

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Key-words: anchoring, clonal integration, disturbance, *Posidonia oceanica*, seagrass.

Abstract

Experimental evaluations about the impact of anchors of small vessels have previously shown that each anchoring can on average damage up to 6 shoots of *Posidonia oceanica*, removing small amount of biomass and, at the same time, interrupting continuity among shoots. The aim of the paper was to investigate the response of *P. oceanica* to different damage intensity at two levels of substrata compactness. Three treatments were considered: Control (no damage); Low damage (simulated anchor damage by 3 strokes of a hoe); High damage (6 strokes). Disturbance was higher where the substratum was highly penetrable and after one year significant variation was observed among treatments for both the number of leaves per shoot and shoot density. Conversely, the number of leaves per shoot by the end of the study was similar among all treatment combinations, suggesting that this was the only phenological feature that recovered, and probably the only result that through clonal integration could be achieved. These data strongly highlighted the role that anchoring might have on the slow growing seagrass *P. oceanica* meadow.

INTRODUCTION

World-wide seagrass loss has been reported from a variety of both direct and indirect human-induced influences (Hemminga & Duarte, 2000). Among all the causes, some are responsible for large scale seagrass decline, such as eutrophication, industrial runoff, oil spillage, dredging (*e.g.* Short, Ibelings & den Hartog, 1988; Preen, Lee Long & Coles, 1995). Conversely, propellers and anchors only cause small gaps within the seagrass meadow, by removal of plant biomass depending on anchor size and type (Porcher, 1984; Walker, Lukatelich, Bastyan & McComb, 1989; Creed & Amado Filho, 1999; Rasheed, 1999; Francour, Ganteaume & Poulain, 1999; Milazzo, Badalamenti, Ceccherelli & Chemello, 2004).

Because loss of seagrasses within meadows has been quite well documented, information about the recovery capacity of seagrasses to all forms of disturbance have become particularly important, so that proper evaluations on the effective impact could be done. For example, seagrass recovery to physical damage was quite unknown until recently. Overall, recovery seems to be mostly due to vegetative propagation and, therefore, recovery time seems highly variable, being dependent upon species growth rate. For example, Creed and Amado Filho (1999) found that *Halodule wrightii* (Ascherson) recovered from 0.25 m² gaps in 9 months, while Rasheed (1999) found *Zostera capricorni* (Ascherson) recovered after 12 months from the same clearing plot size.

However, data for seagrass recovery are particularly needed for slow growing species (Meehan & West 2000). Zieman (1976) and Williams (1988) provided data for recovery of *Thalassia testudinum* (Banks ex Konig) which seems a much slower recovery

species with only minimal colonisation of anchor scars occurring after 7 months. A recent paper by González-Correa, Bayle, Sánchez-Lizaso, Valle, Sánchez-Jerez & Ruiz (2005) has highlighted the recovery capacity of *Posidonia oceanica* (L., Delile) to illegal otter trawling, although the performance of this seagrass seems much faster at control sites where meadows are much denser and resuspension of sediments was lower. Other predictions on the recovery rate to mechanical disturbances could only be done indirectly on the basis of data on population dynamics, seasonal and annual growth, and productivity of all seagrasses (*e.g.* Marbà, Cebrià, Enriquez & Duarte, 1996; Marbà, Duarte, Cebrià, Gallegos, Olesen & Sand-Jansen 1996; Vermaat, Agawin, Duarte, Fortes, Marbà & Uri, 1995; Pergent-Martini, Rico-Raimondo & Pergent, 1994; Alcoverro, Duarte & Romero, 1995; Marbà & Duarte, 1997).

Due to the spatial extent of *Posidonia oceanica* meadows and its high productivity, it is a major component of the Mediterranean coastal system (Buia, Zupo & Mazzella, 1992; Pergent, Rico-Raimondo & Pergent-Martini, 1997), where it plays a very important role sheltering understoried and speciose assemblages (*e.g.* Macpherson, Biagi, Francour, Garcia-Rubies, Harmelin & Harmelin-Vivien et al., 1997; Guidetti, 2000). *P. oceanica* ranks among the longest-lived seagrasses (Duarte 1991) exhibiting very slow growth rate (Cebrian, Duarte, Marbà & Enríquez, 1997).

Experimental evaluations of the impact of anchors have suggested that *Posidonia oceanica* is differently affected by anchors depending on the anchor model used (4 kg in weight) (Milazzo, Badalamenti, Ceccherelli & Chemello, 2004). In particular, the use of the Hall type anchor has a lower impact than Danforth and Folding grapnel anchors. Each complete anchoring cycle (*e.g.* anchor fall, dragging/lock-in and weighing) can uproot or

break on average up to 6 shoots and create discontinuity among shoots within the meadows.

In this paper we examine the short-term response of *Posidonia oceanica* to simulated anchoring. By experimentally scarring the meadow we reproduced the damage provoked by a Folding grapnel anchor, which was found to be the highest impacting anchor (Milazzo, Badalamenti, Ceccherelli & Chemello, 2004). The aim of the paper was to estimate *P. oceanica* changes in above ground biomass after being damaged: the response to different damage intensity at two levels of substrata compactness was evaluated. Substratum compactness, and thus penetration, was evaluated as a possible important source of variation in damage intensity. In particular, the lower the compactness the higher is the probability expected for rhizomes to be uprooted. The higher the compactness the lower should be the number of shoots uprooted.

MATERIALS AND METHODS

The study was carried out in a shallow (2 m depth) *Posidonia oceanica* meadow located at Stagnone, (37° 52' 32"N and 12° 26' 17"E) along the western coast of Sicily, which is a particularly sheltered and little frequented site. Within this meadow 12 areas (about 3 × 3 m in size) were chosen so that 6 were on substratum highly penetrable and the other 6 on substratum which was less penetrable. Penetration of the substratum was quantified by using a penetrometer such as that used by Francour, Ganteaume & Poulain (1999). A 2 m long, 8 mm diameter rod was placed perpendicularly to the bottom and a 5 kg weight was dropped 50 cm on to a stop in the middle of the rod. The impact of the

weight hitting the stop provided a constant force to drive the rod into the rhizome mat. This measurement was repeated randomly at the site 20 times to define areas of different compactness. Weak substratum compactness refers to a mean penetration depth of 26.6 cm (± 2.1 SD), while high compactness refers to a mean penetration of 9.2 cm (± 1.9 SD). At the high penetration areas a higher shoot density and proportion of orthotropic shoots was found, while at low penetration areas a higher sediment accumulation was observed. In each of these areas 3 replicated experimental units (40×40 cm in size) were randomly selected and marked at the corners with plastic stakes (35 cm in length). Within these areas anchor damage was experimentally simulated by using a garden hoe. It was impractical to use a real anchor because it was not possible to throw an anchor several times in the same experimental area. The three treatments considered are: Control (no simulated anchor damage); Low damage (3 strokes); High damage (6 strokes). Strokes were given as simulation of the damage caused by folding grapnel anchor (Milazzo et al., 2004); the hoe was drawn through the sediment face on so that it ploughed like an anchor. The three treatments were randomly assigned to the six areas within each compactness group, so that two areas belonged to the same compactness $\times$ damage combination of treatments. All the 36 experimental units established occurred within a 120 m² area.

Damage treatments were carried out on December 2000 and recovery after damage was evaluated by sampling every month until November 2001, as we believed that it was a feasible time period to detect a response of the plant. The response variables considered were plant leaf number per shoot, leaf length and shoot density, as they are the common phenological variables to evaluating the aboveground biomass performance of this

seagrass. They were all evaluated in the field: shoot density was estimated by counting the number of shoots in each experimental unit, while leaf length (the length of the longest leaf in the shoot) and leaf number (adult, intermediate and juvenile leaves in the shoot) were estimated by choosing two shoots at random within each unit.

A multifactorial analysis of variance was performed on data taken at the last sampling time for each response variable. 'Penetration' (2 levels) and 'damage' (3 levels) were treated as fixed and orthogonal, while 'area' (2 levels) as random nested in their interaction. For leaf length and number 'experimental unit' (3 levels) was also considered and treated as random nested in area. Cochran's test was performed for all analyses to check for homogeneity of variances. Post-hoc multiple comparisons were done using SNK test (Underwood, 1997).

RESULTS AND DISCUSSION

Estimation in the field of the biomass of *Posidonia oceanica* removed or broken due to strokes of the hoe, because it was not possible to collect fragments of leaves in the water column. However, the impact on shoot density was estimated by counting the shoots in each unit before and right after damaging: at the low and high damage treatment 11.16 (± 1.44) and 13.80 (± 2.24), 7.00 (± 0.68) and 11.3 (± 1.33) were the mean number of shoots ($\pm$ SE) removed per plot, at weak and high compactness, respectively (Fig.1). The mean number of shoots removed in the experiment at low damage and weak compactness was equivalent to about the effect of one anchoring cycle, while in the other three combinations of treatment the mean number of shoots removed was equivalent to about two anchoring cycles.

Each response variable analysed in this study has given a specific result to the experimental damage of the plants. For example, after one year, leaf number per shoot was not affected by damaging (Fig 2a and b, Table 1). Although differences among treatments were evident after a few months, especially at high penetration of substratum (Fig 2b), analysis performed using November 2001 data did not detect significant differences. The patterns found suggest that plant recovery in leaf number per shoot occurred during the study period.

Conversely, leaf length was still affected by experimental damage even after one year (Table 1 and Fig 2c and d). In fact, analysis of data has detected that the shortest size was found at a high level of damage, while leaf length in control and medium damaged plots were similar (SNK test, Table 1). Although no analysis has been performed on previous sampling times, differences among treatments could have changed through time being more evident in the latter period at low penetration condition (Fig. 2c). Evident temporal variations were found through the study period at both types of substratum, according to patterns already described extensively (Pergent-Martini, Rico-Raimondo & Pergent, 1994; Alcoverro, Duarte & Romero, 1995; Marbà, Cebrià, Enríquez & Duarte, 1996; Cebrià, Duarte, Marbà & Enríquez, 1997).

Overall, among the three response variables, *P. oceanica* shoot density was the most affected by experimental damaging especially where the substratum was highly penetrable (Table 1 and Fig 2e and f). A significant interaction between substratum penetration and damage intensity was found. In fact, at low penetration of substratum, high and medium levels of damage had a similar effect on shoot density but one year after the damage were similar to the controls. Conversely, at high penetration three very different shoot densities

occurred among levels of damage intensity. Possibly, where substratum is highly compact (low penetration) *P. oceanica* was better able to resist the simulated anchor strokes and a lower number of shoots were uprooted, compared to the low compactness (high penetration) areas. This could be corroborated by the similarity in mean shoot density among levels of damage at low penetration, rather than at high penetration, in December 2000. In fact, data taken before and right after damaging estimated mean values of 11.16 and 7.0 shoots removed where damage was low, while 13.83 and 11.33 where damage was high, at high and low compactness, respectively (Fig.1). However, although at high compactness the initial shoot density was apparently very similar among the three treatments, it was very evident that through time differences among them increased. Although the hoeing of low penetration substrate could not uproot but only initially damage shoots, the detachment of shoots occurred later over a period of three months. Nevertheless, this finding might indicate a different impact of simulated anchors on *Posidonia oceanica* shoot density depending on the substratum penetration. Unfortunately, the effect of Time×Damage×Substratum penetration interaction could not be estimated because the assumption of independence of data by time would have not been met. However, graphical inspection could easily bring further evidence to the model that differences found at the end of the study are basically due to the different impact and not to a different recovery pattern. We believe that different features that are likely to occur at the two substratum types, such as sediment mobility and nutrient availability for roots, did not affect recovery but are merely affecting natural spatial variability of *P. oceanica* shoot density independently of the impact (e.g. Balestri, Cinelli & Lardicci, 2003).

In summary, damage caused by folding grapnel anchors, although considered small

scale damage, can create an impact on *Posidonia oceanica*, particularly to shoot density, from which it is very difficult to recover in the short-term. This result was not unexpected given the slow pattern of growth demonstrated in several studies for this species (Pergent-Martini, Rico-Raimondo & Pergent, 1994; Alcoverro, Duarte & Romero, 1995; Vermaat, Agawin, Duarte, Fortes, Marbà & Uri, 1995; Marbà, Cebrià, Enriquez & Duarte, 1996; Marbà, Duarte, Cebrià, Gallegos, Olesen & Sand-Jansen 1996; Marbà & Duarte, 1998). Moreover, as the holes created by the anchors and other impacts can take a long time to be re-colonised, the possible increase in sediment mobility due to holes could be a further indirect cause of disturbance affecting recovery through time. Conversely, the most interesting result could be that the number of leaves per shoot was the only response variable recovered within one year, probably due to resource reallocation through modules. Overall, this gives good estimates on the quantitative and qualitative short-term response of *P. oceanica* suggesting that biomass allocation after a stress is addressed in terms of the number of leaves per shoot.

Consideration should be given to the possible influence of clonal integration, among shoots of *Posidonia oceanica*. Indeed, anchor damage may be responsible for module discontinuity, through breakage of rhizomes, besides biomass removal, through both uprooting shoots and breakage of leaves (apex up to portions of several centimetres in length). For the findings about the importance of nutrient mobility through modules collected for *P. oceanica* and other seagrasses (*e.g.* Libes & Boudouresque, 1987; Nielsen & Pedersen, 2000; Marbà, Hemminga, Mateo, Duarte, Mass, Terrados et al., 2002) and stressed by Kendrick, Duarte & Marbà (2005), module interruption could deplete efforts from neighbouring shoots of the same clone. So, whether interruption among shoots

occurs, leaf production rate could be decreased and not be able to cope with the leaves' removal within 1 year. This could be the reason for the low leaf length of shoots at highly impacted plots, even at the end of the study.

Unfortunately, in this experiment, treatments have been attributed to several areas within a *P. oceanica* meadow regardless of clonality, and whether shoots occurring in the same plot belong to the same clone remains unknown. Also, identification of clone dimension at the study site could help to interpret the very high recruitment rate of shoots (e.g. Vermaat, Agawin, Duarte, Fortes, Marbà & Uri, 1995; Marbà, Cebrià, Enriquez & Duarte, 1996) observed at controls. This finding could lead to the possibility that biomass removal of a clonal individual could off set by an increase growth in other 'communicating' portions. However, only properly designed experiments could investigate the influence of clonality on recovery from damage, estimating the distances to which integration among shoots could occur in the field.

Overall, the results obtained within this study strongly highlighted the role that anchor activity, even by small vessels, might have on *P. oceanica* meadow representing a small-scale but affective cause of degradation. The frequency of anchoring at some visited sites, in terms of number of boats and extension of the visitation period, could make this chronic disturbance very stressful to the growth of the seagrass over several years.

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Legends

Fig. 1 *Posidonia oceanica*. Mean ($\pm$ SE) shoot density (n=6) before and after hoeing at low and high substratum penetrability for control, medium and high damage.

Fig. 2 *Posidonia oceanica*. Temporal changes in mean ($\pm$ SE) number per shoot (n=12), longest leaf length per shoot (n=12) and shoot density (n=6) during the study period at low and high substratum penetrability.

Table 1 Analysis of variance on *P. oceanica* shoot density, longest leaf length and leaf number per shoot with two different types of substratum penetration (high vs. low) one year after performing damage intensity (control, medium and high) at the two experimental areas. Shoot density was estimated in each experimental units (n=3), while leaf length and number were estimated in duplicate in each experimental unit (n=2).

shoot density						leaf length			leaf number			
Source of variation	df	MS	<i>F</i>	<i>P</i>	df	MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>	
Penetration	=P	1	342.2		1	2.07	1.86	0.2213	0.34	0.41	0.5457	
Damage	=D	2	1667.6		2	12.68	11.43	0.0090	0.59	0.70	0.5309	
P × D		2	299.2	5.32	0.0166*	2	0.31	0.28	0.7674	0.26	0.31	0.7435
Area (P × D)	=A	6	68.1	1.23	0.3270	6	1.11	0.16	0.9838	0.84	2.18	0.0809
Ex.unit.(A(P × D))		24			24	6.73	1.45	0.1511	0.39	0.34	0.9966	
Residual		36	55.5		36	4.63			1.15			
*on Residual												
Cochran's test												
C=0.3383 ns				C=0.2430 ns				C=0.1084 ns				

Table 1A

SNK test on the interaction term P × D (Table 1) for *Posidonia oceanica* shoot density:

(i) low (Lp) and high (Hp) substratum penetration; (ii) damage intensity, control, medium and high. SE=3.04 df=24.

(i) Penetration		(ii) Damage	
Damage		Penetration	
Control	Hp>Lp	Hp	Control>Medium>High
Medium	Hp>Lp	Lp	Control>Medium=High
High	Hp=Lp		

SNK test on damage factor for *Posidonia oceanica* leaf length. SE=0.21 df=6

Control(31.16)=Medium(30.63)>High(29.72)

Fig 1:

Fig 2: