Universidade do Algarve

Faculdade de Ciências do Mar e do Ambiente

Impact of anthropogenic activities on the seagrass *Zostera noltii*

Tese para a obtenção do grau de doutor no ramo de Ciências do Mar, especialidade de Ecologia Marinha

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2007

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TÍTULO DA TESE:

Impacto das actividades de origem antropogénica na erva-marinha *Zostera noltii*

RESUMO: O declínio de ervas-marinhas é um fenómeno documentado à escala global, principalmente devido a distúrbios de origem antropogénica. Esta tese tem como objectivo avaliar o impacto das principais actividades antropogénicas que afectam a erva-marinha *Zostera noltii* na Ria Formosa. O distúrbio por nutrientes originado pela descarga de efluentes urbanos afecta claramente a estrutura da população, a morfologia das plantas e o conteúdo de N das folhas. As elevadas concentrações de amónia (158-663 µM) na estação mais próxima do efluente de descarga (270 m) revelou ser tóxica para esta espécie, reduzindo a sua biomassa e o tamanho das folhas e internós. Dois dos principais processos bióticos identificados pela análise multivariada nas populações de *Z. noltii*, i.e. o tamanho geral das plantas e a dinâmica da biomassa-densidade, estão significativamente correlacionados com os processos abióticos claramente relacionados com o efeito do efluente urbano, i.e. com os nutrientes e anóxia do sedimento e com o contraste entre a salinidade e a concentração de nutrientes na água, respectivamente. Os efeitos adversos do efluente urbano nas pradarias de *Z. noltii* da Ria Formosa parecem estar espacialmente restritos a áreas até 600 m de distância da descarga. As variações nas relações da biomassa-densidade reflectem os gradientes antropogénicos de nutrientes, descrevendo os parâmetros derivados da estrutura da população um padrão de variação com a disponibilidade de nutrientes. As estações impactadas por nutrientes apresentaram correlações significativas entre a biomassa e a densidade, enquanto que nas estações não-impactadas, os dados de biomassa-densidade não se correlacionaram. O marisqueio afectou negativamente as pradarias de *Z. noltii*, reduzindo significativamente a densidade de rebentos e a biomassa total. O marisqueio experimental revelou um impacto a curto prazo na densidade, que recuperou rapidamente para níveis controlo em um mês. A recuperação pode ocorrer mesmo que plantas com apenas 1 ou 2 módulos, incluindo o rebento apical, permaneçam no sedimento. O nível crítico de soterramento tolerado por esta espécie é extremamente baixo (entre +4 e +8 cm), devido ao seu pequeno tamanho e falta de rizomas verticais. Apesar desta ser uma espécie de crescimento rápido, a sua recuperação não ocorreu durante o período experimental (2 meses), mas plantas completamente soterradas sobreviveram em laboratório por 1-2 semanas. As ervas-marinhas mostraram ser extremamente sensíveis a alterações do nível do sedimento. A sua capacidade para resistir ao soterramento é significativamente dependente do seu tamanho. O comprimento das folhas e o diâmetro dos rizomas são as características que melhor predizem o impacto do soterramento nas ervas-marinhas. Os distúrbios antropogénicos estudados revelaram ser adversos para a erva-marinha *Z. noltii*, representado uma séria ameaça para esta espécie. No entanto, o seu rápido crescimento e elevada plasticidade permitem-lhe sustentar, até certo ponto, o distúrbio.

PALAVRAS-CHAVE: Nutrientes, Marisqueio, Soterramento, Erva-marinha, *Zostera noltii*,

Ria Formosa

TÍTULO DA TESE EM INGLÊS:

Impact of anthropogenic activities on the seagrass *Zostera noltii*

ABSTRACT: Seagrass declines have been reported worldwide, mostly as a consequence of anthropogenic disturbance. This thesis aims to assess the effects of main anthropogenic activities affecting the seagrass *Zostera noltii* in Ria Formosa lagoon. The nutrient enrichment originated by the urban wastewater discharge affected the population structure, the plant morphology and the leaf N content of *Z. noltii*. The high ammonium concentrations (158-663 µM) at the site closest (270 m) to the nutrient source showed toxic effects on this species by reducing total biomass and both leaf and internode size. Two of the main biotic processes revealed by the multivariate analysis operating within *Z. noltii* populations, i.e. the overall size of the plants and the biomass-density dynamics, were significantly correlated to the abiotic processes clearly related to the effects of the urban wastewater, i.e. to the nutrients and anoxia conditions of the sediment, and to the contrast between water column salinity and nutrient concentration, respectively. The adverse effects of the urban wastewater in *Z. noltii* meadows of Ria Formosa seem to be spatially restricted to areas up to 600 m distant to the WWTW discharge. Different biomass-density relationships were found along the nutrient disturbance gradients. Nutrient-disturbed stations showed significant correlations between biomass and density, whereas at nutrient-undisturbed stations the biomass-density data were uncorrelated. Population parameters derived from the population structure described a pattern of variation with the nutrient availability. Clam harvesting adversely affects *Z. noltii* meadows by significantly reducing shoot density and total biomass. Experimental harvest revealed a short-term impact on shoot density, which rapidly recovered to control levels during the following month. Recovery may occur even if plants with only 1 or 2 modules, including the apical shoot, remain on the sediment. The critical level of burial and erosion tolerated by *Z. noltii* is extremely low (between +4 and +8 cm), due to the small size of this species and lack of vertical rhizomes. Despite this being a fast-growing species, recovery did not occur within the experimental period (2 months), even though plants survive in laboratory for 1-2 weeks under complete burial. Seagrasses are highly vulnerable to changes of the sediment level. The capacity of seagrasses to resist sediment burial is strongly size-dependent. Leaf size and rhizome diameter are the best predictors of burial impact on seagrasses. The studied human-induced disturbances reveal to be detrimental for *Z. noltii*, representing a serious threat to this species. However, the species high growth and plasticity allows *Z. noltii* to sustain disturbance to a certain extent.

KEY-WORDS: Nutrients, Clam harvesting, Burial, Seagrass, *Zostera noltii*, Ria Formosa

Apoio financeiro da FCT e FSE no âmbito do

III Quadro Comunitário de Apoio

Bolsa de Investigação SFRH / BD / 2880 / 2000

financiada pelo POCTI – Formar e Qualificar –

Medida 1.1

AGRADECIMENTOS

Porque na vida nada se consegue sem a ajuda dos outros, a realização deste doutoramento não teria sido possível sem a contribuição de inúmeras pessoas que tanto a nível científico como pessoal permitiram a sua conclusão. Quero aqui expressar o meu mais sincero agradecimento a todos os que de alguma forma colaboraram neste trabalho. Um agradecimento especial:

À minha família: à minha mãe, por todo apoio, por todo o carinho e pela amizade; ao meu pai, por estar sempre presente em todos os momentos da minha vida. Aos meus avós, pela vossa amizade. E ao Sirius, amigo e companheiro sincero.

Ao meu orientador, Prof. Dr. Rui Santos, pelo apoio científico e orientação nesta tese, pelo apoio prestado ao longo destes longos anos de convivência, pela consideração, confiança e respeito demonstrados. Acima de tudo por acreditar em mim e no meu trabalho, mesmo quando eu própria duvidei.

Ao Prof. Dr. Carlos M. Duarte, que desde o início acompanhou o meu trabalho, pelo apoio, pela orientação científica e pelas ideias que permitiram clarificar muitas dúvidas e ver mais além. Pela oportunidade de ir ao IMEDEA (Mallorca) escrever um dos capítulos desta tese.

À Prof. Dr. Ester A. Serrão, pela colaboração, pelo apoio financeiro prestado pelo projecto M&MS que muito contribuiu para esta tese e pelas consultas de estatística.

A todos os que ajudaram no trabalho de campo (e sem nenhuma ordem em especial): à Alexandra Cunha, à Ana Alexandre, à Ana Quaresma, à Catarina Alves, à Cecile Godinho, ao João Silva, ao José António Bentes, ao Mestre Antero Fernandes, ao Pedro Morais, ao Pedro Neves e à Raquel Machás (com quem partilhei as duras amostragens da ETAR).

Ao Parque Natural da Ria Formosa, pela possibilidade de amostrar no Marim e pelo apoio logístico prestado nas amostragens da ETAR, e ao Sr. Sousa, proprietário da concessão do Marim, que prontamente disponibilizou uma área para o trabalho do impacto da actividade do marisqueio.

À FCT, pelo apoio financeiro concedido sob a forma de bolsa de doutoramento, sem a qual não teria sido possível realizar esta tese.

Aos que contribuíram para a revisão dos artigos incluídos nesta tese: à Raquel Machás, à Ana Alexandre, ao João Silva e aos revisores anónimos que em muito contribuíram para a melhoria dos artigos científicos publicados.

A todos os colegas do grupo ALGAE que sempre me apoiaram, pela vossa camaradagem e disponibilidade.

A tudo o que É!

CONTENTS

CHAPTER 1

General Introduction

1. General Introduction

1.1. Introduction to seagrasses

Seagrasses are marine angiosperms that occur in shallow coastal areas all over the world (Den Hartog 1970, Phillips & Meñez 1988 Hemminga & Duarte 2000). They are grouped into two families, namely Potamogetonaceae and Hydrocharitaceae, enclosing 12 genera and about 50 species (Hemminga & Duarte 2000). Most seagrass species grow on sandy to muddy sediments, where they develop extensive monospecific or multispecific meadows (Den Hartog 1970, Phillips & Meñez 1988, Hemminga & Duarte 2000). Seagrasses are clonal, rhizomatous plants, which grow through the regular addition of a basic set of modules. Each set of modules is composed by a piece of rhizome, a bundle of leaves attached to the rhizome (shoot), and a root system. Additionally, flowers and fruits may be also present, depending on the time of year (Hemminga & Duarte 2000). Seagrass meadows are subject to intense dynamics involving the continuous loss and replacement of modules, which maintain the dynamic equilibrium within a population (Tomlinson 1974, Duarte et al. 1994, Hemminga & Duarte 2000). If such equilibrium is disturbed (e.g. by natural or human-induced events), mortality will exceed recruitment and the population will be out of balance, resulting in meadow regression and seagrass loss (Duarte et al. 1994). However, seagrasses can tolerate moderate disturbance, as well as environmental heterogeneity, due to the plasticity of its architecture and growth strategy (Marbà & Duarte 1998, Marbà & Duarte 2003). Such plasticity allows plants to accommodate to a wide range of conditions, including the spatial variability of resources (Hemminga & Duarte 2000).

1.2. Ecological importance

Seagrasses are valuable components of marine ecosystems, being amongst the most valuable ecosystems in the biosphere (Phillips & Meñez 1988, Costanza et al. 1997, Duarte 2000, Hemminga & Duarte 2000). The ecological importance of their meadows extends beyond the areas where they develop, contributing to the overall functioning of the coastal systems (Hemminga & Duarte 2000). Seagrass meadows are highly productive ecosystems, among the most productive biomes on earth (Duarte & Chiscano 1999). They export about 24% of their net production to adjacent areas (Duarte & Cébrian 1996) and are responsible for about 15% of the carbon storage in the ocean (Duarte & Chiscano 1999), playing an important role in global carbon and nutrient cycling in the ocean (Hemminga & Duarte 2000). Seagrass meadows enhance the biodiversity and habitat diversity of coastal waters, and support the production of living marine resources. They constitute nursery and foraging areas for a number of resident and transient adult and juvenile species, including many commercially highly important fish and shellfish species (Duarte 2000, Hemminga & Duarte 2000).

Seagrasses improve the water quality by reducing the particle loads in the water and by absorbing the dissolved nutrients; and stabilize sediments by reducing the water current flows, promoting sedimentation and inhibiting sediment resuspension (Fonseca 1996, Duarte 2000, Hemminga & Duarte 2000). The ability of seagrasses to change the nearshore sedimentary processes result in shoreline protection from erosion and indirect contribution to the coastal dune formation (Fonseca 1996, Hemminga & Duarte 2000). The presence of seagrasses in coastal areas always benefit humans, directly or indirectly (Hemminga & Duarte 2000), due to the important functions and services they provide (Costanza et al. 1997).

1.3. Seagrass declines

Despite their ecological importance, seagrasses are a vulnerable resource, which may be easily lost face to environmental changes (Hemminga & Duarte 2000). Seagrass declines have been reported worldwide, whether natural or human-induced, both at local- and large-scales (Short & Wyllie-Echeverria 1996, Hemminga & Duarte 2000). Natural causes of seagrass loss include both geological (e.g. coastal erosion) and meteorological (e.g. hurricanes) events, and biological interactions (e.g. grazing, bioturbation and disease; Short & Wyllie-Echeverria 1996). Even though natural events have been responsible for seagrass losses, data compilation by Short & Wyllie-Echeverria (1996) and updated by Hemminga & Duarte (2000) suggests that more than 70% of the declines are attributed to anthropogenic disturbances. Human-induced disturbances include different events, such as the deterioration of water quality, mechanical damages and release of toxic compounds.

1.3.1. Deterioration of water quality

Urban and industrial development, as well as intensive agriculture, in proximity of coastal areas lead to the increase of nutrient inputs to the near shore and estuarine ecosystems all over the world. Eutrophication of coastal waters may follow the increased nutrient loading, promoting the deterioration of the water quality (Duarte 1995, Short & Wyllie-Echeverria 1996, Hemminga & Duarte 2000). Reduction of the water transparency decreases the light penetration into the water column, generating an important threat to seagrass survival (Short & Wyllie-Echeverria 1996). Although direct toxic effects of nutrients have been demonstrated for seagrasses (Burkholder et al. 1992, Van Katwijk et al. 1997, Brun et al. 2002), declines seem to be primarily related to the indirect effects associated with eutrophication, namely the responses of other primary producers to the increase of nutrients (Duarte 1995). Macroalgae, phytoplankton and epiphytes proliferate as nutrient inputs increase (Borum 1985, Tomasko & Lapointe 1991, Frankovich & Fourqurean 1997, Wear et al. 1999, Hauxwell et al. 2003), shading and suffocating seagrasses (Den Hartog 1994, Duarte 1995, Hemminga & Duarte 2000, Hauxwell et al. 2001). Moreover, eutrophication is often associated with the increased loading of sediments and organic matter, which leads to reduced conditions of sediments and bottom waters (Hemminga & Duarte 2000). Such anoxic conditions may negatively affect seagrasses (Terrados et al. 1999) and aggravate the negative effects of eutrophication.

1.3.2. Mechanical damages

Mechanical damages occur as a consequence of activities such as dredging, boating, and fishing practices. Such activities often result in considerable seagrass loss, due to injuries in seagrass shoots and rhizomes, and to the complete removal of plants from the sediment (Short & Wyllie-Echeverria 1996, Hemminga & Duarte 2000). Dredging and filling used in shipping channels maintenance or in coastal works directly impact seagrass meadows, resulting in plant removal from its habitat and consequent reductions of the vegetation cover (Onuf 1994, Short & Wyllie-Echeverria 1996, Burdick & Short 1999, Hemminga & Duarte 2000, Daby 2003, Ruiz & Romero 2003, Badalamenti et al. 2006). In addition, seagrass losses may be aggravated by the sediment burial and erosion occurring after disturbance as a consequence of the sediment redistribution (e.g. Ruiz & Romero 2003). The elevated water turbidity originated by such activities may also negatively affect seagrasses by limiting the light availability (Onuf 1994, Ruiz & Romero 2003, Eldridge et al. 2004, Badalamenti et al. 2006).

Boating activities may also impact seagrass meadows, especially as a consequence of boat moorings and propeller scars (Walker et al. 1989, Dawes et al. 1997, Creed & Amado Filho 1999, Milazzo et al. 2004). Its cumulative effects may, over time, result in severe reductions of seagrass cover and fragmentation of meadows (Short & Wyllie-Echeverria 1996). Fishing and mariculture practices disturb bottom sediments and damage plants, resulting in reductions of both shoot density and plant biomass, and of seagrass cover (Peterson et al. 1983, Fonseca et al. 1984, Peterson et al. 1987, De Jonge & De Jong 1992, Everett et al. 1995, Ardizzone et al. 2002, Boese 2002, Orth et al. 2002, Holmer et al. 2003, Neckles et al. 2005, Uhrin et al. 2005).

1.3.3. Toxic compounds

Seagrass declines related to human-induced releases of toxic compounds others than oil spills have not been identified so far (Hemminga & Duarte 2000). Several levels of seagrass response were reported after oil spills, ranging from no visible damages to plant loss and plant cover declines (Short & Wyllie-Echeverria 1996). Intertidal communities are the most vulnerable to oils spills, with the risk of being affected decreasing with increasing depth (Zieman et al. 1984). Even though seagrasses are able to bioaccumulate heavy metals (Brix et al. 1983, Nienhuis 1986, Schlacher-Hoenlinger & Schlacher 1998, Prange & Dennison 2000, Marin-Guirao et al. 2005), no evidence of serious adverse effects on seagrass meadows was reported (Short & Wyllie-Echeverria 1996, Hemminga & Duarte 2000). However, some species may show lower physiological performance when exposed to heavy metals (e.g. Macinnis-Ng & Ralph 2004).

1.3.4. Other threats

Other human-related activities may be potentially dangerous for seagrass ecosystems, even though large-scale declines have not been reported yet (Hemminga & Duarte 2000). Such threats include the invasion of exotic species (e.g. tropical green algae *Caulerpa taxifolia* in Mediterranean Sea, Meinesz & Hesse 1991) and the global climate changes (e.g. global warming and sea-level rise, Short & Neckles 1999).

The loss of seagrass ecosystems leads to the loss of functions and services they provide both in the ecological and economic perspective (Costanza et al. 1997, Duarte 2000). Seagrass loss due to disturbances may be a rapid process, but the recovery of seagrass meadows is likely to be slow or never occur (Duarte 1995, Hemminga & Duarte 2000). Hence, knowing the threats that may lead to seagrass declines is the best way to achieve seagrass conservation and recovery.

1.4. *Zostera noltii*

Zostera noltii Hornemann (Fig. 1.1) is a small seagrass species occurring along the Atlantic coasts of Europe, extending from southern Norway to Mauritania, and in the Mediterranean, Black, Caspian and Aral Seas (Fig. 1.2; Den Hartog 1970, Phillips & Meñez 1988). *Z. noltii* grows in intertidal and subtidal areas, withstanding exposure to air (Den Hartog 1970). The species have a high population dynamics characterised by a short life span of modules (Hemminga et al. 1999, Hemminga & Duarte 2000) and high shoot mortality and recruitment rates (Laugier et al. 1999). Leaf growth rate is also high, as well as shoot production, which represents much of the species production (Vermaat & Verhagen 1996). The fast rate of the horizontal rhizome elongation (68 cm year^{-1}) along with the species rhizome branching rate (2.62%) and angle (81º) leads to a compact space occupation (Marbà & Duarte 1998).

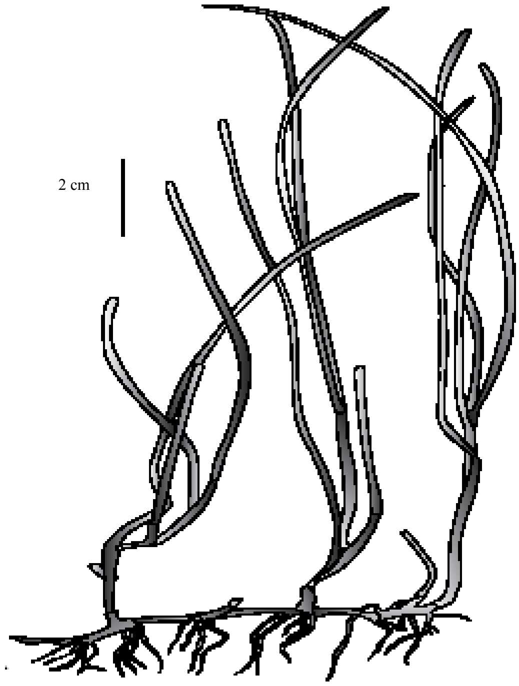

Fig. 1.1. Schematic representation of the seagrass *Zostera noltii* (from Borum & Greve 2004)

Z. noltii also reproduce sexually by the production of flowering shoots and seeds. Flowering of *Z. noltii* can extend from March to November (Hootsmans et al. 1987, Loques et al. 1988, Buia & Mazzella 1991, Curiel et al. 1996, Alexandre et al. 2005), but the flowering season may vary depending on factors such as temperature, day length, tidal amplitude and salinity (Hemminga & Duarte 2000). The flowering shoots comprise several inflorescences (spathes), each containing both the male and female flowers (monoecious species). The overall process of flowering and fruiting lasts approximately 47 days, during which the formation and maturation of the fruits is the most time consuming stage (27 days, Alexandre et al. 2006). Seeds are not likely to disperse far since they are negatively buoyant (Pettit 1984), but detached flowering shoots containing seeds may still add to the reproductive success of the species, depending to the fate of the shoots (i.e. decease or proceed maturation; Loques et al. 1988, Alexandre et al. 2006).

Observation of *Z. noltii* seedlings in the field is a rare event, suggesting that sexual reproduction may not be the main via for the species spreading (Hootsmans et al. 1987). However, the high genetic variability reported in recent genetic studies indicates that sexual reproduction plays an important role in *Z. noltii* populations of Ria Formosa and elsewhere (Coyer et al. 2004, Diekmann et al. 2005).

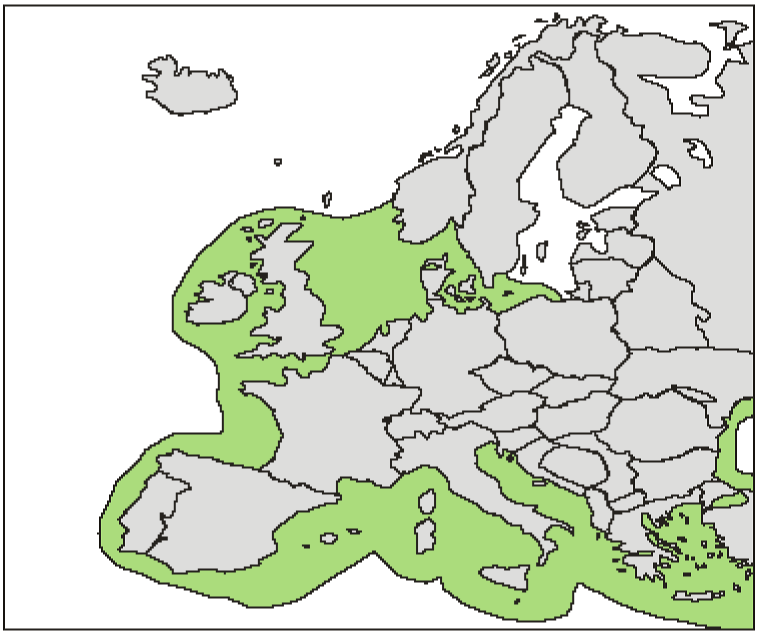

Fig. 1.2. Geographical distribution of the seagrass *Zostera noltii* in Europe (from Borum & Greve 2004)

The decline of the seagrass *Z. noltii* has been documented along its geographical distribution. The disappearance of *Z. noltii* meadows was reported in the Wadden Sea (De Jonge & De Jong 1992, Philippart & Dijkema 1995), in the southern coasts of Spain (e.g. Palmones River estuary; Niell et al. 1996, Hernández et al. 1997) and in the Portuguese coast (e.g. Mondego estuary; Oliveira & Cabeçadas 1996, Cardoso et al. 2004), mainly as a consequence of eutrophication. Mechanical damages primarily related to fisheries have also been shown to negatively impact this species, including declines in seagrass cover and failure of seagrass restoration in the Dutch Wadden Sea (De Jonge & De Jong 1992). Dredging activities were also reported to cause the decline of *Z. noltii* meadows in Ria de Aveiro lagoon, Portugal by inducing changes in sediment redistribution and water turbidity (Figueiredo da Silva et al. 2004). In Ria Formosa lagoon (southern Portugal), *Z. noltii* is the most abundant seagrass species, playing an important role in the lagoon productivity (Santos et al. 1994) and biodiversity, including economically important species, such as bivalves, cephalopods, crustaceans and fish (Ribeiro et al. 2006). The main anthropogenic disturbances threatening *Z. noltii* in Ria Formosa lagoon are the nutrient loads via urban effluents, both the clam harvesting activity and the creation of clam culture beds in intertidal areas, and the dredging activities related to the maintenance of navigation channels and to the artificial inlet relocation. Even though this system constitutes a Natural Park, the current status of this species in the lagoon is unknown, because of the lack of long-term monitoring studies in the past. Hence, the study of the effects of anthropogenic disturbances on this seagrass species assumes a major importance in order to prevent potential seagrass declines in Ria Formosa lagoon and elsewhere.

1.5. Objectives

The present thesis aims to assess the effects of main anthropogenic activities affecting the seagrass *Z. noltii* in Ria Formosa lagoon, specifically the effects of urban wastewater discharges, of clam harvesting activity, and of burial and erosion events. Understanding the effects these human-induced disturbances have on *Z. noltii* is the best way to contribute to the species conservation, to the development of adequate monitoring and management of its meadows and to the overall services provided by this seagrass to the nearby coastal area. Such knowledge may also be used to reveal and monitor the anthropogenic disturbances in coastal areas using *Z. noltii* as a biological indicator.

The specific objectives of this thesis were:

(i) to determine the relationships among the urban wastewater discharge and the population structure, plant morphology, nutrient content, epiphyte load and macroalgae abundance of *Z. noltii* meadows of Ria Formosa lagoon, along a gradient from a major urban wastewater discharge to the main navigation channel of the lagoon (Chapter 2);

(ii) to assess how the biomass-density relationships of the seagrass *Z. noltii* vary along anthropogenic nutrient gradients resulting from the discharge of Waste Water Treatment Works. This information could be interpreted as a useful biological indicator to monitor the nutrient loading of coastal systems (Chapter 3);

(iii) to (1) analyse the effects of the clam harvesting, as it is performed by local fisherman, on *Z. noltii* population density and biomass through the comparison of disturbed and undisturbed meadows; (2) test the effects of clam harvesting on the *Z. noltii* density and its recovery through *in situ* experimental manipulation; and (3) determine the effects of physical damage caused by clam harvesting technique in plant survival, growth and production, through the experimental manipulation of both rhizome and shoot fragmentation at different modular levels, i.e. altering the intact number of modular units (Chapter 4);

(iv) to assess (1) the effects of burial and erosion on the shoot density, plant morphology, carbon and nitrogen contents, and non-structural carbohydrates reserves of the seagrass *Z. noltii*, through the *in situ* experimental manipulation of the sediment level; and (2) the survivorship, longevity, production and growth of *Z. noltii* plants submitted to experimental burial in laboratory conditions (Chapter 5);

(v) to compile the available information on species-specific impacts of burial and erosion disturbance to test the hypotheses that the vulnerability of different seagrass species to burial and erosion are dependent on plant size, architecture and growth. Allometric relationships between these seagrass characteristics and experimentally-determined burial thresholds were analysed in order to identify which seagrass characteristics better predict the burial effects. These relationships will allow the prediction of the impacts of burial on seagrasses from consideration of architectural characteristics of each species (Chapter 6).

A general discussion (Chapter 7) summarizes the main findings obtained in previous chapters regarding the impacts of the anthropogenic activities on *Z. noltii*.

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CHAPTER 2

Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*)

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Estuarine, Coastal and Shelf Science

2. Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*)

ABSTRACT: The abiotic disturbance of urban wastewater discharge and its effects in the population structure, plant morphology, leaf nutrient content, epiphyte load and macroalgae abundance of *Zostera noltii* meadows were investigated in Ria Formosa coastal lagoon, southern Portugal using both univariate and multivariate analysis. Four sites were assessed, on a seasonal basis, along a gradient from a major Waste Water Treatment Works (WWTW) discharge to a main navigation channel. The wastewater discharge caused an evident environmental disturbance through the nutrient enrichment of the water and sediment, particularly of ammonium. *Z. noltii* of the sites closest to the nutrient source showed higher leaf N content, clearly reflecting the nitrogen load. The anthropogenic nutrient enrichment resulted in higher biomass, and higher leaf and internode length, except for the meadow closest to the wastewater discharge (270 m). The high ammonium concentration (158-663 μM) in the water at this site resulted in the decrease of biomass, and both the leaf and internode length, suggesting a toxic effect on *Z. noltii*. The higher abundance of macroalgae and epiphytes found in the meadow closest to the nutrient source may also affect the species negatively. Shoot density was higher at the nutrient-undisturbed site. Principal component analyses were performed in both the abiotic and biotic data sets, and the significant components and variable loadings were selected using Monte Carlo permutation tests. Three abiotic processes and three morphometric/population structure relationships of *Z. noltii* meadows were revealed and their spatial and seasonal variation was analysed. Two abiotic processes were clearly related to the WWTW discharge, a contrast between water column salinity and nutrient concentration and a sediment contrast between both porewater nutrients and temperature and redox potential, whereas another one was independent of it, the contrast between sediment organic matter and temperature. A multiple regression analysis showed that the first two abiotic processes had a significant effect on the biomass-density dynamics of meadows and on the overall size of *Z. noltii* plants, respectively. Results show that the wastewater discharge is an important source of environmental disturbance and nutrients availability in Ria Formosa lagoon affecting the population structure, morphology and N content of *Z. noltii*. This impact is spatially restricted to areas up to 600 m distant from the WWTW discharge, probably due to the high water renewal of the lagoon.

KEY WORDS: Wastewater, Urban effluents, Nutrient enrichment, Disturbance, Seagrass, *Zostera noltii*, Ria Formosa

2.1. Introduction

Seagrass declines have been reported worldwide, whether natural or human-induced. Most of these declines are attributed to anthropogenic disturbances, such as eutrophication, toxic pollutants and mechanical damages (Short & Wyllie-Echeverria 1996). Urban and industrial development, as well as intensive agriculture, in proximity of coastal areas have resulted in the increase of nutrient inputs to the near shore and estuarine ecosystems all over the world. Eutrophication of coastal waters may follow the increased nutrient loads, promoting the deterioration of water quality (Short & Wyllie-Echeverria 1996). The reduction of water transparency limits the light penetration into the water column generating an important threat to seagrasses by reducing the light availability for photosynthesis. Although direct toxic effects of nutrients have been demonstrated (Burkholder et al. 1992, Van Katwijk et al. 1997, Brun et al. 2002), seagrass declines seem to be primarily related to the indirect effects associated with eutrophication, namely the responses of other primary producers to the nutrient loads (Hemminga & Duarte 2000). Macroalgae, phytoplankton and epiphytes proliferate as nutrient inputs increase (Borum 1985, Tomasko & Lapointe 1991, Frankovich & Fourqurean 1997, Wear et al. 1999, Hauxwell et al. 2003), because they can rapidly assimilate the nutrients from the water column (Duarte 1995). The overgrowth of macroalgae and epiphytes leads to the shading and suffocation of seagrass meadows, contributing to seagrass declines (Duarte 1995, Hughes et al. 2004, Lapointe et al. 2004). Moreover, other indirect effects associated with eutrophication, such as sediment anoxia (Hemminga & Duarte 2000) may also be detrimental for seagrasses (Terrados et al. 1999).

As the other primary producers, seagrasses also respond to the increase of nutrients. However, the lower rates of nutrients uptake under abundant nutrient supply makes seagrasses inferior competitors compared with macroalgae (Duarte 1995). In a meta-analysis approaching the nutrient effects on seagrasses, Hughes et al. (2004) identified the biomass as one of the seagrass parameters that respond positively to the *in situ* nutrient enrichment of the sediment (particularly the aboveground biomass). The water column nutrient additions had strong negative effects on seagrass biomass, because it also results in increased epiphyte biomass (Hughes et al. 2004). On the other hand, the seagrass nutrient content reflects to a certain extent the nutrient availability in marine ecosystems. Higher nutrient concentrations have been related with higher nutrient contents in plant tissues (Borum et al. 1989, Duarte 1990, Fourqurean et al. 1992, Udy & Dennison 1997b, Lee et al. 2004). Even though the effects of increased nutrients on seagrasses have been extensively documented by experimental and field studies, the direct impact of an urban wastewater effluent on seagrass meadows was never assessed.

Zostera noltii is a small seagrass species distributed along the intertidal and subtidal areas of the Northern and Western Europe, Mediterranean Sea and North-West Africa (Den Hartog 1970). The decline of *Z. noltii* meadows has been reported in the Wadden Sea (Philippart & Dijkema 1995), the southern coasts of Spain (e.g. Palmones River estuary; Niell et al. 1996, Hernández et al. 1997) and in the Portuguese coast (e.g. Mondego estuary; Oliveira & Cabeçadas 1996, Cardoso et al. 2004), mostly as a consequence of eutrophication. In Ria Formosa lagoon, southern Portugal, *Z. noltii* is the most abundant seagrass, covering large areas of the lower intertidal. This species plays an important role in the lagoon productivity (Santos et al. 2004). Even though this system constitutes a natural park, the conservation status of this species in the lagoon is unknown, due to the lack of long-term monitoring studies. Thus, the assessment of the effects caused by the increasing anthropogenic nutrient load assumes a major importance in order to prevent potential seagrass declines.

The main objective of this study was to determine the relationships among the urban wastewater discharge and the population structure, plant morphology, nutrient content, epiphyte load and macroalgae abundance of *Z. noltii* meadows of Ria Formosa lagoon, along a gradient from a major urban wastewater discharge to the main navigation channel of the lagoon.

2.2. Methods

2.2.1. Study sites

Ria Formosa lagoon is a mesotidal system located in the southern coast of Portugal (Fig. 2.1). The lagoon has a high spring tide surface area of 84 Km^2, with an exposed intertidal area of approximately 80% (Andrade 1990). The lagoon is separated from the Atlantic Ocean by a system of five sand barrier islands and six inlets. In each tidal cycle about 50-75% of the water in the lagoon is renovated. The tidal amplitude ranges from 3.50 m on spring tides to 1.30 m on neap tides (Andrade 1990), and salinity ranges from 35.5 to 36 PSU along the year (Falcão 1996). Four continuous, intertidal *Z. noltii* meadows were selected along a tidal creek from a Waste Water Treatment Works (WWTW) effluent to a main navigation channel of Ria Formosa (Fig. 2.1). Site 1 was the *Z. noltii* meadow closest to the WWTW discharge (270 m), while site 4 was located in the main channel where there was no influence of the wastewater effluent (1500 m). Sites 2 and 3 were 520 m and 610 m distant from the WWTW discharge, respectively.

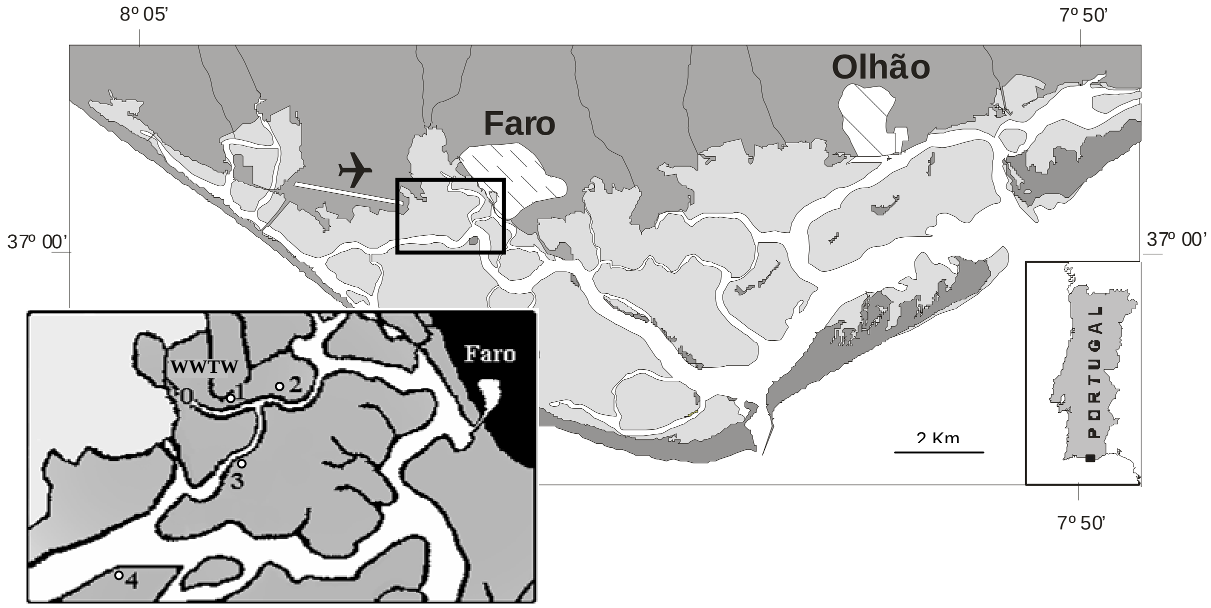

Fig. 2.1. Map of Ria Formosa, southern Portugal, with the location of the sampling sites from the wastewater effluent (site 0) to the main navigation channel (site 4)

2.2.2. Environmental variables

Environmental parameters of water and sediment were determined seasonally, in July 2001 (summer), November 2001 (autumn), February 2002 (winter) and May 2002 (spring), at the *Z. noltii* sampling sites (sites 1 to 4, Fig. 2.1). The effluent water of the WWTW was also characterised (site 0, Fig. 2.1). Water salinity, temperature and pH were recorded *in situ* in each site. Three water samples (100 ml each) were collected in each site, at low tide, filtered (Whatman cellulose acetate filters, 0.45 μm pore size) and frozen until nutrient analysis. Ammonium was determined manually using a spectrophotometric method (Solorzano 1969), while nitrates+nitrites and phosphates were determined with an Autoanalyser system (Skalar, Sans Plus).

The sediment measurements were done in the first 5 cm of the sediment surface, within the *Z. noltii* rhizosphere. The redox potential (Eh) was measured *in situ* with a metal electrode (Russell RL100). Sediment cores were collected during low tide, and sliced *in situ*. A total of six minicores (3.5 cm of diameter) were pooled for each sample (three samples per site), in order to reduce the effect of the nutrient patchiness in the sediment. The sediment samples were centrifuged (3000 rpm, 20 min. at *in situ* sediment temperature) and the supernatant water was filtered (Whatman acetate cellulose filters, 0.45 μm pore size) and frozen until nutrient analysis. Sediment samples were then homogenised and a subsample was dried (48 h at 60 ºC) and combusted (4 h at 450 ºC) to determine the organic matter content. In summer and winter, another sediment subsample was dried and analysed to determine the C, N and P content of the sediment. The C and N content was analysed using a Carlo-Erba elemental analyser (EA1108). To measure the P content, the dried sediment was burned for one hour at 550°C, 0.2 M HCl was added and then incubated for one hour at 100 °C. The extracted orthophosphate was analysed using spectrophotometric analysis (Koroleff 1983). The remaining sediment was analysed only once, in summer, to determine the grain size (% of sand and % of silt plus clay) following Erftemeijer & Koch (2001).

2.2.3. Seagrass characteristics

Five randomly distributed samples of *Z. noltii* were collected seasonally from each site with a 12 cm diameter core. The number of shoots was counted in each sample to estimate shoot density. The leaf length, the leaf width, the leaf number per shoot and the sheath length was measured from intact shoots, the internode length and diameter was measured from intact rhizome internodes and the root length was recorded from intact roots (accuracy of 1.0 mm). The above and belowground biomass of *Z. noltii* was determined by drying the samples at 60 ºC for 48 h. *Z. noltii* leaves were analysed to determine the C, N and P content, both in summer and in winter, using the same methods as described above for the sediment. The elemental ratios of C:N and C:P were calculated on a mol:mol basis.

2.2.4. Macroalgae and epiphytes

The macroalgae present in each *Z. noltii* core was separated and dried at 60 ºC during 48 h. In summer, 15 shoots were randomly taken from each sample to assess the abundance of epiphytes on *Z. noltii* leaves. All epiphytes were smoothly scrapped from the leaves and collected on Whatman GF/F filters. Epiphytes and shoots were separately dried at 60 ºC for 48 h, to estimate the percentage of epiphytes on a dry weight basis.

2.2.5. Statistical analysis

2.2.5.1. Univariate analysis

Significant differences in data sets were investigated using one- or two-way ANOVA, after testing for homogeneity of variances and normality of distributions. When necessary, variables were log transformed to fit ANOVA assumptions. When ANOVA indicate significant differences among sites and/or seasons, Tukey’s multiple comparison test was applied to determine which site(s) and/or season(s) were significantly different from each other. When ANOVA assumptions were not verified, comparison of data sets was performed using the non-parametric test of Kruskal-Wallis. Significance levels were tested at $p < 0.05$ (Sokal & Rohlf 1995).

2.2.5.2. Multivariate analysis

Principal Component Analysis (PCA) was used to analyse both abiotic (12 variables) and biotic (11 variables) data sets. The significance of the statistics performed to decide which eigenvectors to use as principal components (PCs) and which variables were correlated to each PC were estimated using the permutation tests of Monte Carlo methods (Manly 2006). To select which PCs to analyse, both a test for the equality of roots (Jackson 1991) and a test for the significance of the eigenvalues were performed in the original data set and in 10000 randomly permutated data sets. The null hypothesis of each PC describing only random variation was tested by comparing the magnitude of the statistic of the original data set with those obtained from the permutated data sets. Both the Scree plot and the Broken Stick techniques were used to confirm the results obtained. To assess the significance of the loadings of each variable in each selected PC two statistics were used, the correlation of each variable with each PC and a modified version of this statistic. As the first statistic overestimates the loadings of non-significant PCs relative to their eigenvalues:

$$\frac{u_{ij}\sqrt{\lambda_i}}{s_j}$$

where u_{ij} is the loading of the variable *j* in principal component *i*, λ_i is the eigenvalue of the principal component *i* and S_j is the standard deviation of variable *j*; the loadings and eigenvalues were squared in order to make the statistic less sensitive to high loadings generated by random error:

$$\frac{u_{ij}^2\lambda_i^2}{s_j}$$

In this way, only high loadings of PCs with high eigenvalues are likely to be considered significant. The z-scores of each sample in each selected PC were calculated to analyse spatial and temporal patterns of the processes identified by each PC. Multiple regressions of each selected biotic PC (dependent variable) on the selected abiotic PCs (independent variables) were performed to determine which environmental processes are significantly related to the biotic processes determined by the PCA analysis.

2.3. Results

2.3.1. Univariate analysis

2.3.1.1. Environmental variables

The concentration of nutrients in the water (Fig. 2.2A) showed an evident and significant gradient from the wastewater effluent (site 0) to the main channel (site 4, Table 2.1). The wastewater effluent (site 0) provided an ammonium input that ranged between 571 ± 56 µM (summer) and 1801 ± 16 µM (spring). At the seagrass meadows along the effluent gradient, ammonium was the most available nutrient in the water column, varying from 158-663 µM at site 1 to 0.6-4.2 µM at site 4. The nutrients in the sediment porewater also followed the gradient originated by the wastewater discharge (Fig. 2.2B, Table 2.1), except for site 1 that showed intermediate values. There were higher nutrient concentrations at site 2 (80-217 µM for ammonium, 0.25-7.0 µM for nitrates+nitrites, 22-52 µM for phosphates) and lower concentrations at site 4 (12-38 µM for ammonium, 0.2-0.9 µM for nitrates+nitrites, 2.4-13.9 µM for phosphates).

Table 2.1. Statistical results and significance of ANOVA (F) examining the effects of site and season on the nutrient concentrations of the water and sediment porewater

	Site	Season	Site × Season
Water			
Ammonium	5027.3***	448.2***	232.7***
Nitrates+Nitrites	144.9***	17.3***	13.3***
Phosphates[a]	6424.5***	319.6***	78.6***
Sediment porewater			
Ammonium[a]	113.1***	15.8***	6.9***
Nitrates+Nitrites	7.4***	6.3**	8.4***
Phosphates	51.4***	44.1***	7.7***

(*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, ([a]) Log transformed data prior ANOVA

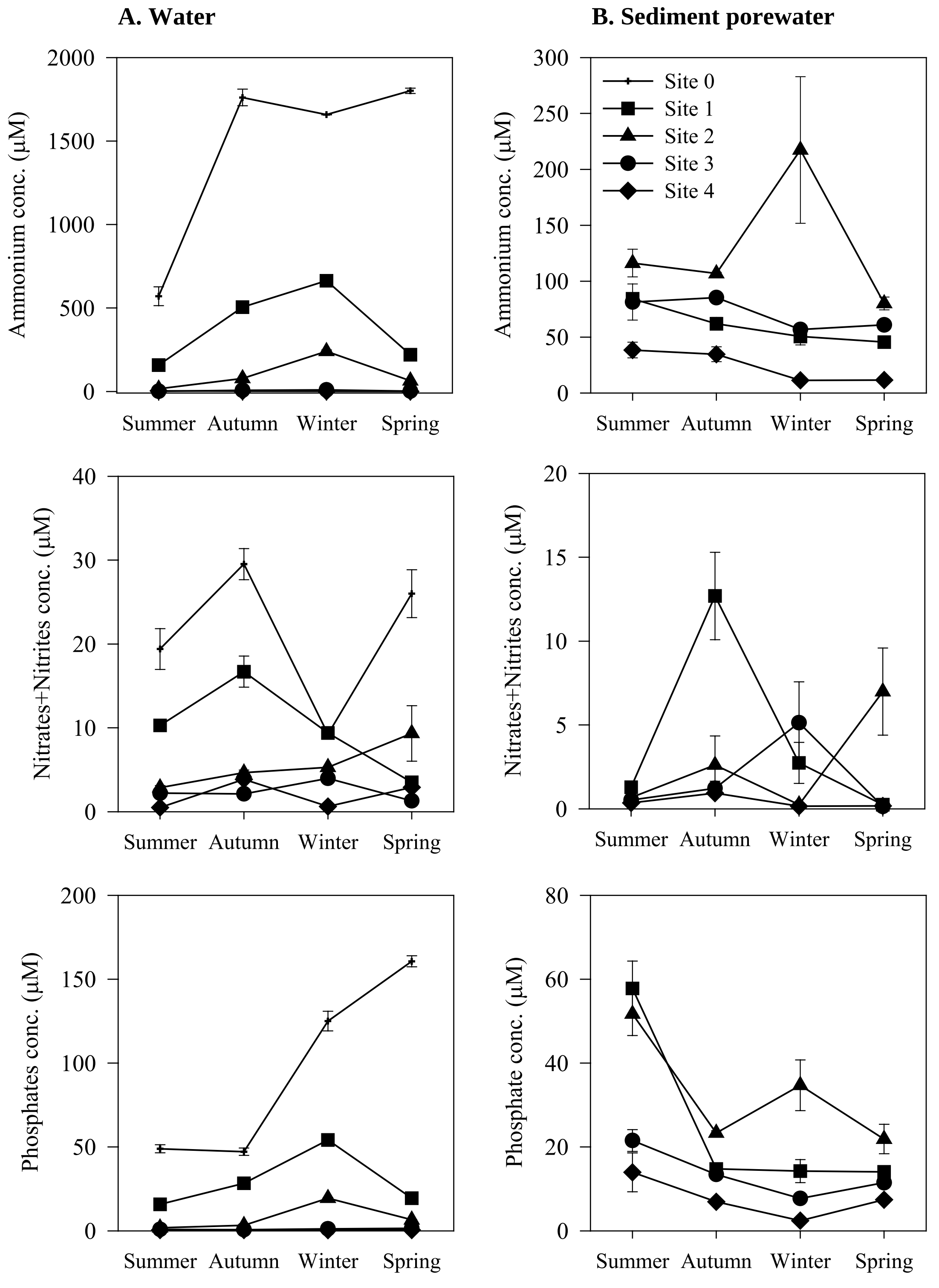

Fig. 2.2. Seasonal and spatial variation of nutrients (ammonium, nitrates+nitrites and phosphates) in the (A) water (site 0 to 4) and in the (B) sediment porewater (site 1 to 4). Error bars represent standard errors

The organic matter content of the sediment (Table 2.2) at sites 2 and 3 was significantly higher than at sites 1 and 4. The redox potential showed more negative values at site 2, although not significantly different from the others sites (Table 2.2). Site 4 showed a significantly different grain size of the sediment (Table 2.2), with more sand (33%) and less fine sediments (66%), than the other sites. The C, N and P content of the sediment was not significantly different between summer and winter. The C content of the sediment of site 2 (3 % DW) was significantly higher than in the other sites, while the N content of site 4 sediment (0.16 % DW) was significantly lower (Table 2.2). No significant differences were detected in the sediment P content among sites.

Table 2.2. Sediment characteristics of *Zostera noltii* sampling sites. Values represent annual means ± standard errors of organic matter (OM, n = 12), redox potential (Eh, n = 4) and elemental contents (CNP, n = 6), except for grain size (n = 3). Different letters denote significant differences among sites in one-way ANOVA (F values)

	Site 1	Site 2	Site 3	Site 4	F
OM (% DW)	6.3 ± 0.3^a	8.6 ± 0.3^b	8.1 ± 0.3^b	5.2 ± 0.2^a	24.67***
Eh (mV)	-227 ± 18	-309 ± 21	-234 ± 38	-192 ± 30	3.14^{ns}
Grain size					
Sand (% DW)	13.5 ± 2.3^a	18.2 ± 0.3^a	12.3 ± 2.1^a	33.4 ± 3.1^b	19.11***
Silt + Clay (% DW)	86.5 ± 2.3^a	81.6 ± 0.5^a	87.6 ± 2.2^a	65.8 ± 3.0^b	21.23***
Nutrient content					
C (% DW)	2.29 ± 0.14^a	3.01 ± 0.18^b	2.22 ± 0.19^a	2.14 ± 0.08^a	6.88*
N (% DW)	0.24 ± 0.01^a	0.26 ± 0.00^a	0.25 ± 0.01^a	0.16 ± 0.01^b	25.16***
P (% DW)	0.23 ± 0.01	0.22 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	2.95^{ns}

(*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, (^{ns}) not significant

2.3.1.2. Seagrass characteristics

The C, N and P content of *Z. noltii* leaves (Fig. 2.3) was significantly higher in winter than in summer. The N content of *Z. noltii* leaves from site 4 was significantly lower than from sites 1 to 3. However, no significant differences were found in C and P leaf content among the study sites (Table 2.3). The C:N ratio of *Z. noltii* leaves from site 4 was significantly higher than from sites 1 and 3 (Fig. 2.3D), while the C:P ratio did not vary significantly among the study sites (Fig. 2.3E). Both the C:N and C:P ratios were significantly higher in summer than in winter.

Table 2.3. Statistical results and significance of ANOVA (F) or Kruskal-Wallis (H) tests examining the effects of site and season on plant variables

	Site	Season	Site × Season
Total biomass[a]	F = 29.04***	F = 19.78***	F = 5.81***
Density	F = 23.11***	F = 40.43***	F = 11.16***
Leaf length	H = 178.14***	H = 291.00***	-
Internode length	H = 109.37***	H = 179.90***	-
Leaf C content	F = 1.48[ns]	F = 52.83***	F = 0.24[ns]
Leaf N content	F = 6.06**	F = 67.54***	F = 1.47[ns]
Leaf P content	F = 3.30*	F = 46.71***	F = 0.24[ns]
Leaf C:N ratio	F = 7.34**	F = 44.02***	F = 2.08[ns]
Leaf C:P ratio	F = 2.34[ns]	F = 28.38***	F = 0.22[ns]

(*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, ([ns]) not significant, ([a]) Log transformed data prior ANOVA

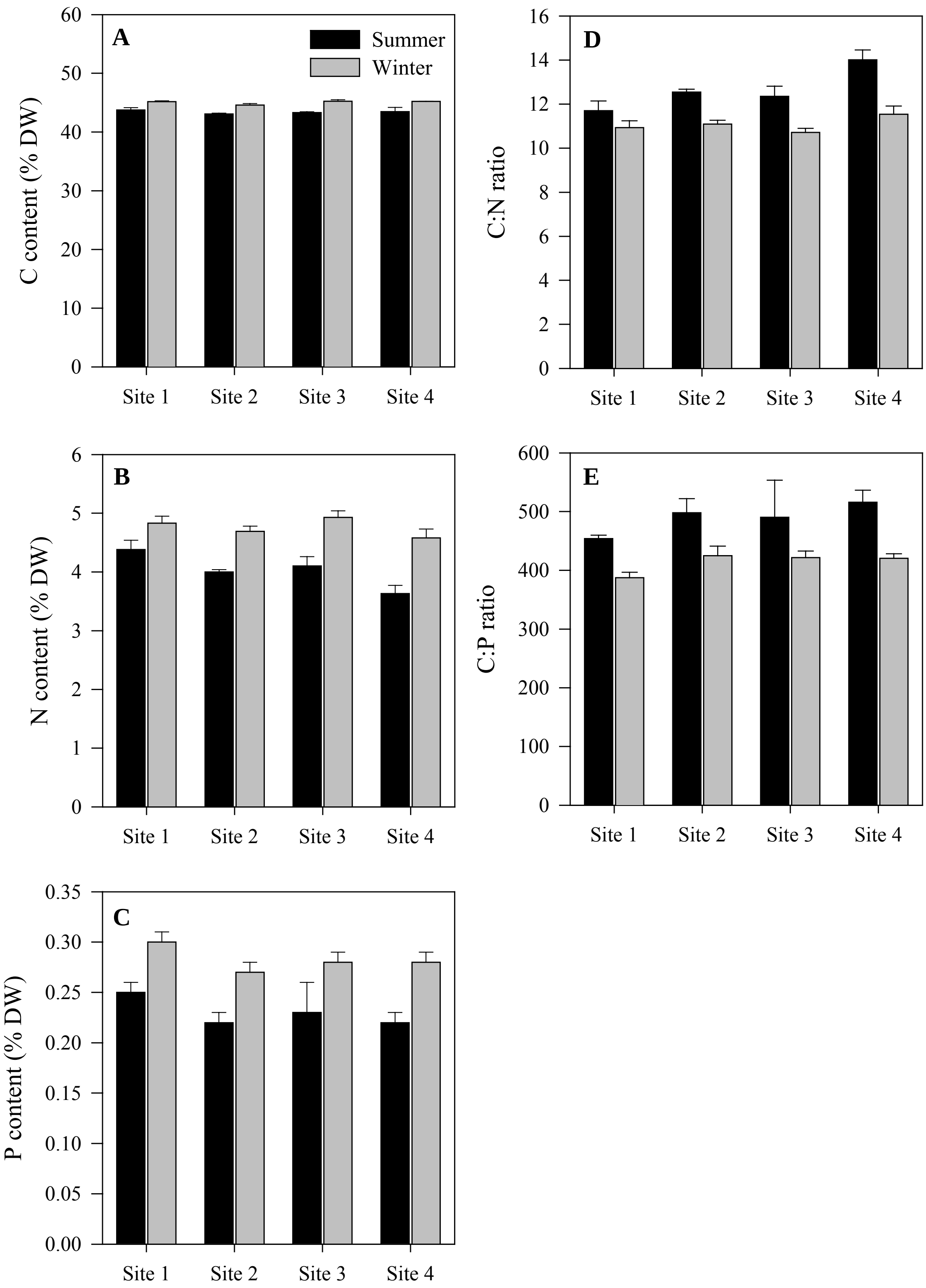

Fig. 2.3. Seasonal and spatial variation of (A) carbon content, (B) nitrogen content, (C) phosphorous content, (D) carbon:nitrogen ratio and (E) carbon:phosphorus ratio of *Zostera noltii* leaves. Error bars represent standard errors

The total biomass of *Z. noltii*, as well as the above and belowground biomass, showed significant effects of site and season (Table 2.3). The total biomass at site 1 was significantly lower than at the other sites (Fig. 2.4A). This site showed the lowest total biomass along the sampling period (always less than 210 g DW m^{-2}). The seasonal variation of biomass was much higher in site 2 (127-429 g DW m^{-2}) than in other sites (119-210 g DW m^{-2} for site 1, 172-372 g DW m^{-2} for site 3, and 229-310 g DW m^{-2} for site 4). The seasonal variation of the aboveground biomass followed the trend of total biomass, increasing in summer and approaching belowground biomass in winter. In general, belowground biomass was lower than aboveground biomass.

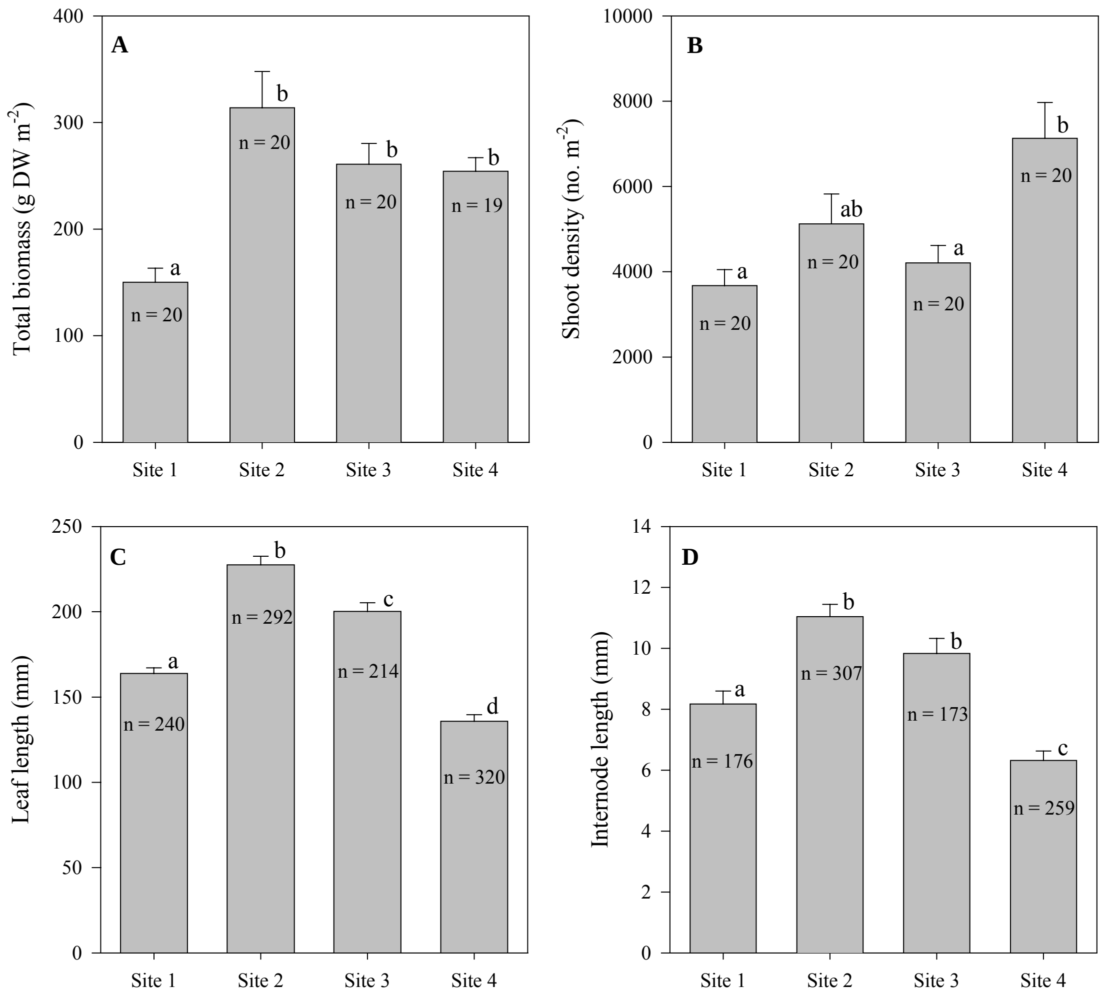

Fig. 2.4. Spatial variation of *Zostera noltii* (A) total biomass, (B) shoot density, (C) leaf length and (D) internode length (annual means). Error bars represent standard errors. Different letters denote significant differences

Shoot density was significantly higher at site 4 relative to sites 1 and 3 (Fig. 2.4B). In general, site 4 showed significantly higher density throughout the sampling period, particularly in summer, when density reached more than 13000 shoots m^{-2}. A significant effect of site and season on the leaf and internode length was found (Table 2.3). Both measures were significantly higher at site 2 and lower at site 4 (Figs. 2.4C & 2.4D). All sites showed the longest leaves in autumn (194 mm for site 1, 328 mm for site 2, 285 mm for site 3 and 235 mm for site 4), whereas longer internodes were observed in spring (12.9 mm for site 1, 17.3 for site 2, 17.6 mm for site 3 and 9.2 mm for site 4).

2.3.1.3. Macroalgae and epiphytes

The biomass of macroalgae within *Z. noltii* meadows, mainly Ulvales, was significantly higher at site 1 (Fig. 2.5A), particularly in spring, when these opportunistic algae peaked. Epiphytes on *Z. noltii* leaves, mostly diatoms, showed significantly higher percentage at site 1 (6.8%) relative to sites 2 and 4 (0.8 and 0.5%, respectively; Fig. 2.5B).

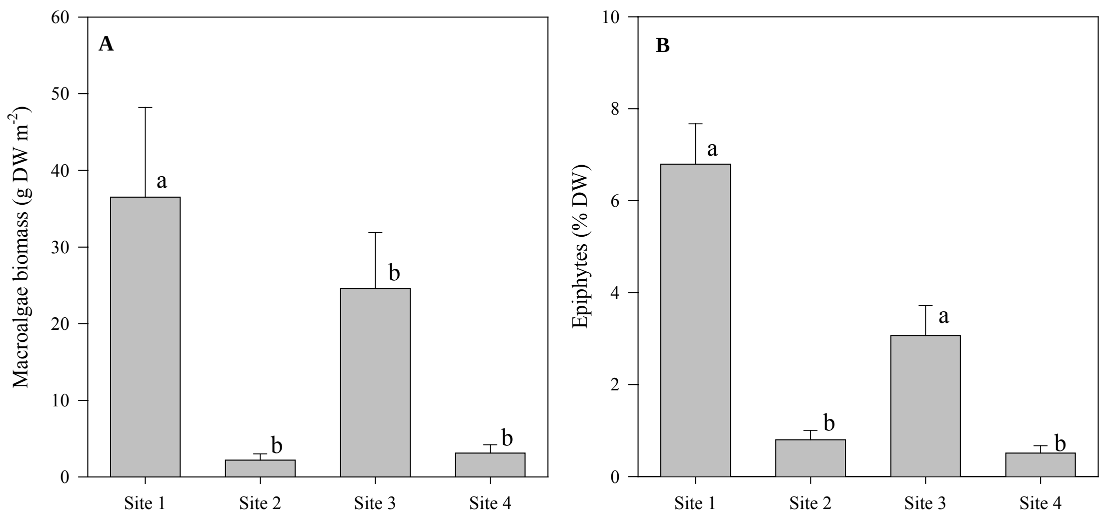

Fig. 2.5. Algae abundance in *Zostera noltii* meadows. (A) Macroalgae biomass (annual mean) and (B) epiphyte load on seagrass leaves in summer. Error bars represent standard errors. Different letters denote significant differences

2.3.2. Multivariate analysis

Three meaningful PCs were selected both for the abiotic and biotic data sets (Tables 2.4 & 2.5). The three abiotic PCs account for 76.8% of total variation (Table 2.4). The first one revealed a process where nutrients (ammonium, nitrates+nitrites and phosphates in the water column and nitrates+nitrites in the sediment porewater) are negatively related to the water column salinity (Table 2.4).

Table 2.4. Eigenvalues, % of explained variance (EV) and variables loadings to the eigenvectors of principal components (PC) extracted from the abiotic data correlation matrix. Significant PCs and their significant loadings are in bold

	Eigen value	EV (%)	NH4 sed.	NO3 sed.	PO4 sed.	OM sed.	Eh sed.	pH sed.	Temp. sed.	NH4 water	NO3 water	PO4 water	Sal. water	Temp. water
PC 1	4.21	35.1	-0.09	**-0.34**	-0.01	-0.11	-0.02	0.09	0.25	**-0.45**	**-0.42**	**-0.42**	**0.45**	0.21
PC 2	2.99	24.9	**0.39**	-0.05	**0.50**	0.23	**-0.50**	-0.30	0.27	0.04	0.12	0.07	0.04	**0.35**
PC 3	2.01	16.8	-0.38	0.06	0.10	**-0.54**	0.19	-0.02	**0.46**	0.16	0.18	0.17	-0.14	**0.45**

This PC exhibits a clear seasonality (Fig. 2.6A), indicating high inputs of nutrient enriched freshwater in autumn and winter, when salinity is lower, and low inputs in spring and summer. The seasonal pattern is very evident at site 1 and fades away to site 4, where no meaningful seasonal variation was found. This indicates that the freshwater input with high nutrients originates from the wastewater discharge. The increase of the z-scores from site 1 to site 4 also supports this hypothesis as it reflects the increased influence of the lagoon water with higher salinity and lower nutrient concentrations. The second PC reveals a sediment biogeochemical process where both the sediment porewater nutrients (ammonium and phosphate) and temperature (water column) are negatively related to the sediment redox potential (Table 2.4). When the redox potential is lower (a strong reducing environment) the temperature and the porewater ammonium and phosphate are higher. This process also exhibited a clear seasonal pattern (Fig. 2.6B) characterized by a summer high of ammonium, phosphate, reducing power and temperature and the opposite in autumn, winter and spring. Both the z-scores and the seasonal pattern are high up to site 3, decreasing to site 4, where no meaningful seasonal variation was found. This suggests that the influence of the urban effluent is evident up to site 3 and not at site 4. The third PC reveals a contrast between the organic matter in the sediment and temperature (sediment and water column, Table 2.4). In summer and spring, when temperatures are higher, the sediment organic matter was lower, probably due to higher remineralization (Fig. 2.6C). Contrary to the other PCs, there is no evident decrease of the z-scores and the seasonal pattern from site 1 to site 4, indicating that this process is not related to the urban effluent discharge.

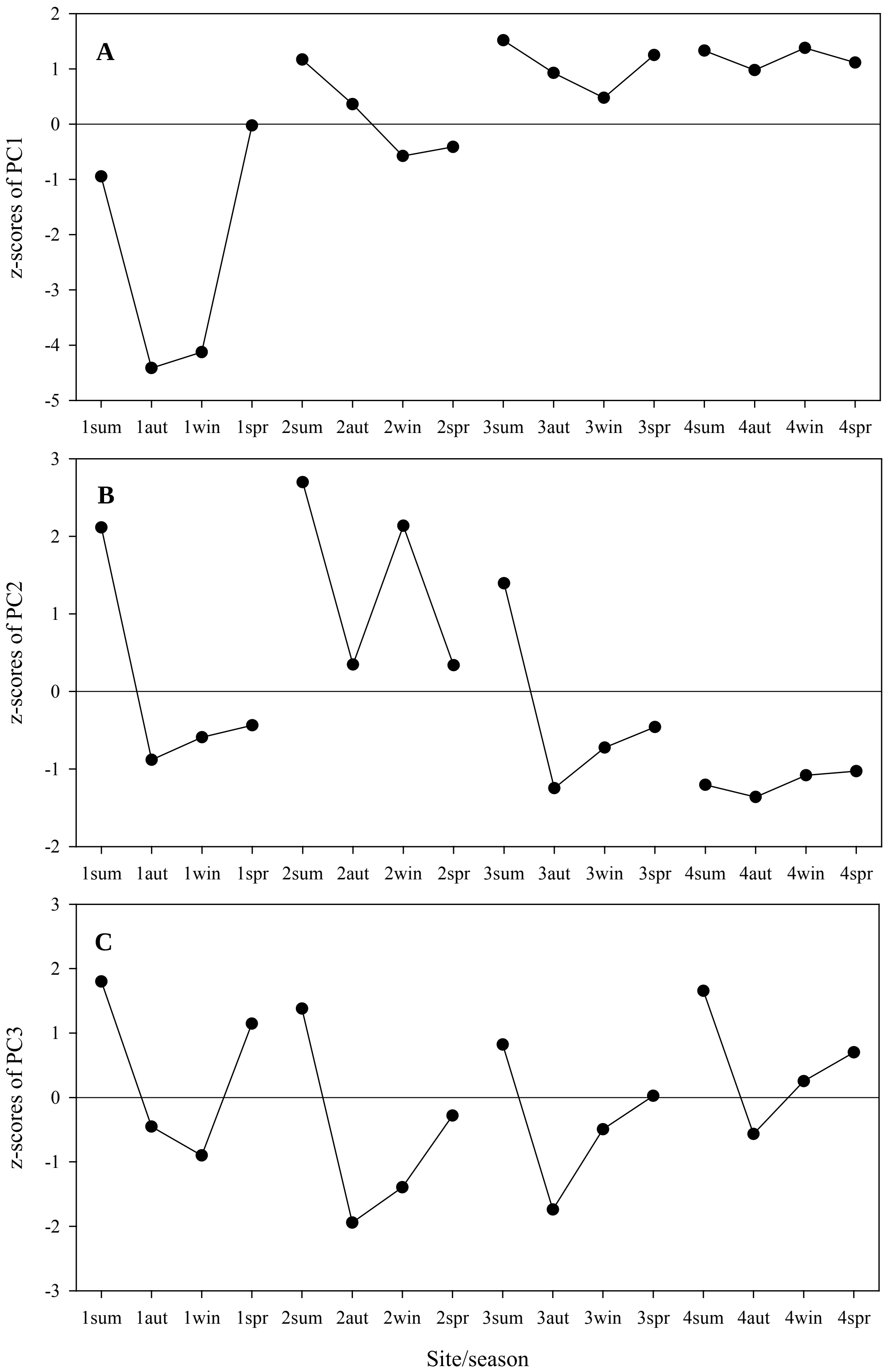

Fig. 2.6. Spatial and temporal variation of the z-scores of the (A) first PC, (B) second PC and (C) third PC for the abiotic data

Three PCs were also selected for analysis for the biotic data set (Table 2.5). The three PCs together accounted for 79.7% of total variation. The first PC represents the diameter of *Z. noltii* rhizomes and the length of roots, which are negatively related to the length of leaves (Table 2.5). The z-score analysis showed that the relative magnitude of the belowground components was higher in spring whereas the leaf length was more important in autumn. This seasonal pattern was consistent in all sites, even though it was less pronounced in site 1 where the relative magnitude of the rhizome diameter and root length was higher (Fig. 2.7A). The second PC represents the overall size of *Z. noltii* plants, where a positive relationship among the leaf metrics (leaf width, sheath length and leaf length, this one with $p = 0.07$) and the rhizome internode length was revealed (Table 2.5). The overall size of the plants did not vary much spatially or seasonally (Fig. 2.7B). The third PC reveals a positive relationship between *Z. noltii* total biomass (above and belowground) and density (Table 2.5). The analysis of the z-scores shows that, in general, the total biomass and density increased along the sites 1 to 4 and that these variables have a high in summer and a low in winter (Fig. 2.7C). Exceptions to this pattern are the autumn low in site 1 and the spring high in site 2.

Table 2.5. Eigenvalues, % of explained variance (EV) and variables loadings to the eigenvectors of the principal components (PC) extracted from the biotic data correlation matrix. Significant PCs and their significant loadings are in bold

	Eigen value	EV (%)	Above biom.	Below biom.	Algae biom.	Shoot density	Leaf length	Leaf width	Leaf no.	Sheath length	Intern. length	Intern. diam.	Root length
PC 1	3.46	31.4	0.00	-0.01	0.29	0.18	**-0.40**	0.08	0.35	-0.36	0.33	**0.40**	**0.44**
PC 2	3.22	29.3	-0.30	0.15	-0.29	0.18	-0.32	**-0.51**	-0.07	**-0.38**	**-0.35**	-0.31	0.21
PC 3	2.10	19.1	**0.55**	**0.51**	-0.23	**0.52**	-0.09	0.20	-0.24	-0.04	0.04	-0.03	0.04

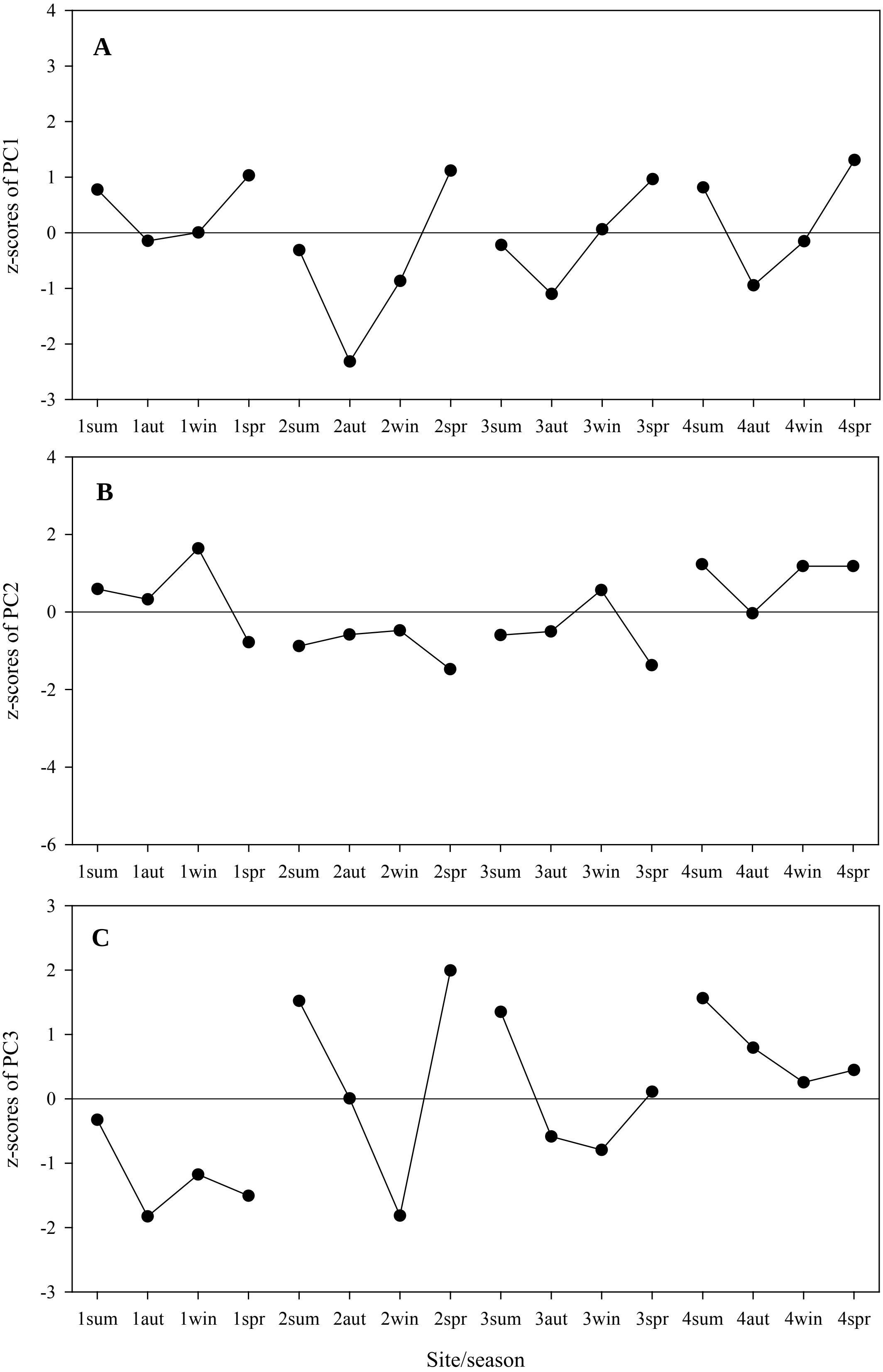

Fig. 2.7. Spatial and temporal variation of the z-scores of the (A) first PC, (B) second PC and (C) third PC for the biotic data

The three main biological patterns described for *Z. noltii* populations by the three significant biotic PCs were significantly related to the three meaningful abiotic processes as revealed by the multiple regression analysis (Table 2.6). The relative sizes of rhizomes/roots versus leaves, described by the first PC is highly correlated ($p = 0.001$) to the third abiotic PC, which represents the temperature contrast with sediment organic matter. When temperatures are higher and the organic matter content of the sediment is lower, the rhizome diameter and the root length of *Z. noltii* are higher and their leaves are shorter. The process described by the second biotic PC, which represents the overall size of the plants, is correlated (with $p = 0.08$) to the second abiotic PC, which represents the contrast between both the sediment porewater nutrients (ammonium and phosphate) and temperature (water column) with the sediment redox potential. This suggests that the size of *Z. noltii* plants is more influenced by within sediment ammonium and phosphate, which are higher in the summer (Fig. 2.2B), rather than other variables such as water column nutrients. Finally, the biological process described by the third biotic PC, which represents the biomass-density dynamics of *Z. noltii* populations, was significantly correlated ($p = 0.04$) to the process described by the first abiotic PC, which is clearly related to the urban wastewater discharge, as it describes a pattern of high nutrients and low salinity in the water.

Table 2.6. Results of multiple regression analysis between the z-scores of the biotic PCs (dependent variable) and the abiotic PCs (independent variables)

Dependent	Regression			Independent					ANOVA	
variable	r^2	SE	n	variable	Coefficient	SE	t	p	F	p
Biotic PC1	0.59	0.71	16	Abiotic PC1	-0.11	0.10	-1.02	0.33	5.79	0.01
				Abiotic PC2	-0.21	0.14	-1.53	0.15		
				Abiotic PC3	0.68	0.16	4.13	0.001		
Biotic PC2	0.33	0.90	16	Abiotic PC1	-0.19	0.13	-1.45	0.17	1.99	0.17
				Abiotic PC2	-0.33	0.18	-1.90	0.08		
				Abiotic PC3	0.30	0.21	1.43	0.18		
Biotic PC3	0.43	1.05	16	Abiotic PC1	0.36	0.15	2.36	0.04	2.95	0.08
				Abiotic PC2	0.03	0.21	0.15	0.89		
				Abiotic PC3	0.27	0.24	1.10	0.29		

2.4. Discussion

The urban wastewater discharge into the Ria Formosa lagoon establishes an evident environmental disturbance, which was related to changes in the *Z. noltii* population structure, plant morphology and tissue nutrient contents. The coupling between the environmental disturbance and the biological processes operating within *Z. noltii* populations was clearly showed by the significant correlations found between the main abiotic and biotic processes revealed by the multivariate analysis. The high availability of nutrients throughout the year, both in the water and in the sediment porewater, reflected the effects of the wastewater effluent. The abiotic process explaining more variation (35%) was clearly related to the wastewater discharge (Table 2.4), as it described the negative relationship between nutrients and salinity in the water and a spatial pattern that faded away with the distance to the wastewater effluent. This supports the fact that the wastewater discharge is the major source of water column nutrients. The availability of nutrients decreased with the distance to the WWTW in a way that the *Z. noltii* meadow near the discharge was exposed to ammonium concentrations in the water of up to three orders of magnitude higher than the distant meadow. As well, the sediment of the sites along the wastewater creek was much richer in nutrients, in organic matter and in silt plus clay than the undisturbed site (Table 2.2). This reflects both the effluent inputs and the lower hydrodynamic forces of the inner sites compared to the site located on the main navigation channel. During the period of this study, an unexpected artificial input of sediments originated from works related to the airport expansion towards the lagoon may have altered the sediment condition of site 1. This probably masked the influence of the wastewater discharge on the sediments of this site, which showed lower nutrient concentrations and organic matter contents than contiguous sites (Fig. 2.2B, Table 2.2). Nevertheless, the sediment biogeochemical process described by the second abiotic PC, which describes the positive relationship between the nutrients and anoxia condition of the sediment, was clearly related to the wastewater discharge. This effect was evident up to site 3, about 600 m distant from the WWTW, but not in site 4 (Fig. 2.6B), about 1500 m distant from the WWTW, revealing the extent of the WWTW disturbance into the Ria Formosa lagoon.

As a consequence of the environmental nutrient enrichment caused by the wastewater discharge, both the *Z. noltii* leaves and the sediment organic matter of the nearby sites showed significantly higher N content, revealing a physiological response of the biota to the N input. The seasonal variation of the nitrogen and phosphorous inputs by the wastewater discharge was also reflected in the N and P content of *Z. noltii* leaves (Fig. 2.3), which were higher in winter when there were higher inputs of nutrients (Fig. 2.2A). The tissue nutrient content of seagrasses is a good indicator of the environmental nutrient enrichment (Udy & Dennison 1997b), since it reflects the local nutrient availability, as observed in this study. In particular, *Z. noltii* may be a good biological indicator of nutrient loading to coastal ecosystems, as this is a fast growing species (Hemminga & Duarte 2000) and thus its internal nutrient contents rapidly reflect the environmental conditions.

The high nutrient concentrations observed at site 1 (300 m distant from the WWTW discharge), both in the case of ammonium (158.3-663.4 μM) and of phosphate (15.8-54.2 μM), may have toxic effects for *Z. noltii* as suggested by the lowest biomass of the species throughout the sampling period. Brun et al. (2002) findings corroborate our observations as ammonium concentrations of 200 μM were shown to have inhibitory toxic effects on *Z. noltii* growth and survival. Ammonium toxicity was also demonstrated for *Zostera marina* at concentrations of 125 μM, with plants becoming necrotic within 2 weeks (Van Katwijk et al. 1997). The evidence of toxicity at site 1 was also reflected in the lower length of leaves and internodes. Similarly, *Z. marina* plants exposed to high ammonium concentrations (75 μM and 125 μM) were also generally smaller, with shorter leaves (Van Katwijk et al. 1997). The lowest *Z. noltii* biomass observed in winter at site 2, when nutrient concentrations were higher (240 μM of ammonium and 19 μM of phosphate), also suggests the effects of toxicity.

The *Z. noltii* population structure clearly reflected the effects of the urban wastewater discharge, as revealed by the significant correlation between the processes describing the biomass-density dynamics of *Z. noltii* populations (third biotic PC) and the wastewater discharge (first abiotic PC). The process describing the variation of biomass and density revealed a spatial pattern with *Z. noltii* biomass and density increasing with the distance to the wastewater effluent, but also a seasonal pattern, typical of temperate seagrasses, which seems to be amplified by the effect of the wastewater discharge (Fig. 2.7C).

On the whole, the shoot density of *Z. noltii* meadows was higher at the nutrient-undisturbed site 4 (Fig. 2.4). The nutrient enrichment may promote the shoot mortality and/or reduce the shoot recruitment (Hauxwell et al. 2003), resulting in shoot density decreases. Van Katwijk et al. (1997) also found that high ammonium concentrations (125µM) decreased the number of shoots for *Z. marina* plants, as a consequence of increased necrosis and plant death. Despite shoot density declines have been related with increasing nutrient loads (Tomasko & Lapointe 1991, Perez et al. 1994, Hauxwell et al. 2003), the opposite response was also reported (Short 1987, Van Lent et al. 1995, Lee & Dunton 2000, Ibarra-Obando et al. 2006).

In general, plant morphometry, such as leaf length and internode length were higher at the nutrient-enriched sites (Fig. 2.4), except at site 1, where the toxic effects of nutrients resulted in shorter leaves and internodes. While some studies have demonstrated that seagrass morphometry, such as canopy height or leaf length, increases with increasing nutrients availability (Perez et al. 1994, Udy & Dennison 1997a,b, Udy et al. 1999, Lee & Dunton 2000), others found no clear pattern of shoot height along nitrogen availability gradients (Lee et al. 2004). The morphological responses to nutrient enrichment may not show a clear trend, since seagrass growth is also influenced by other environmental variables, such as light, temperature, salinity, sediment sulphite (Hemminga & Duarte 2000) or the concentration threshold for nutrient toxicity, which can distort the nutrients effect. This interaction was also observed in this study, as the morphometry of the plants, which was described by the two main processes occurring within *Z. noltii* populations, was significantly related to different abiotic processes. The process describing the relative sizes of rhizomes/roots versus leaves (first PC) was correlated to the abiotic process describing the contrast between the temperature and the sediment organic matter (third abiotic PC), which is related to seasonal effects. On the other hand, the process describing the overall size of the plants (second PC) was related to the contrast between the sediment porewater nutrients and temperature with the sediment redox potential (second abiotic PC), which reflected the influence of the urban effluent.

The higher macroalgae biomass and epiphyte load on *Z. noltii* leaves found in the meadow closest to the nutrient source (site 1, Fig. 2.5) reflects the higher availability of nutrients. At high nutrient levels, opportunistic macroalgae and epiphytes are better competitors than seagrasses, since they have higher nutrient uptake and faster growth rates (Duarte 1995). Thus, they will proliferate and a shift from a seagrass-dominated to a macroalgae-dominated community may occur with increased eutrophication (Sand-Jensen & Borum 1991, Short et al. 1993, Duarte 1995, Hauxwell et al. 2003, Cardoso et al. 2004).

Since the loss of seagrass habitats has enormous ecological implications for coastal systems (e.g. decreased productivity and biodiversity, increased sediment resuspension and erosion), and its recovery is likely to be slow or never occur (Duarte 1995, Hemminga & Duarte 2000), the conservation of existent seagrass meadows should be imperative to prevent irretrievable losses and, in particular, the effects of the urban wastewater on these communities must be understood and monitored.

We showed here how the nutrient enrichment originated by the urban wastewater discharge affected the population structure, morphology and N content of *Z. noltii*. The multivariate analysis clearly identified the wastewater discharge as an important source of environmental disturbance and nutrients availability in Ria Formosa lagoon. The high ammonium concentrations (158.3-663.4 μM) at the site closest to the nutrient source showed toxic effects on this species by reducing total biomass and both leaf and internode size. In addition, the higher abundance of macroalgae and epiphytes at this site may also affect the species negatively. The multiple regression analysis showed that two of the main biotic processes operating within *Z. noltii* populations, i.e. the overall size of the plants and the biomass-density dynamics, were significantly correlated to the abiotic processes clearly related to the effects of the urban wastewater, i.e. to the nutrients and anoxia conditions of the sediment, and to the urban wastewater discharge, respectively. The adverse effects of the urban wastewater in *Z. noltii* meadows of Ria Formosa seem to be spatially restricted to areas up to 600 m distant to the WWTW discharge, which is a relatively small spatial impact. The water quality of this coastal system is maintained by a high water renewal (Andrade 1990) as the tidal amplitude is high, up to 3.5 m, and the average depth of the lagoon is low, about 2.5 m.

Acknowledgements. This study was funded by the EC project "Monitoring and Managing of European Seagrass Beds" (EVK3-CT-2000-00044). We are grateful to A. Alexandre, A. Quaresma, C. Alves, C. Godinho, J. Silva, J.A. Bentes and P. Teixeira for fieldwork assistance. S.C., R.M. and V.V. were supported by PhD grants from Fundação para a Ciência e a Tecnologia (FCT).

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CHAPTER 3

Biomass-density relationships of the seagrass *Zostera noltii*: a tool for monitoring anthropogenic nutrient disturbance

Cabaço S, Machás R, Santos R (2007)

Estuarine, Coastal and Shelf Science 74: 557-564

3. Biomass-density relationships of the seagrass *Zostera noltii*: a tool for monitoring anthropogenic nutrient disturbance

ABSTRACT: In order to assess how anthropogenic nutrient disturbance affects the populations of the seagrass *Zostera noltii*, the temporal variation of the stand biomass and shoot density expressed by the population-specific biomass-density relationships were studied along nutrient gradients associated with discharge from Waste Water Treatment Works, in two coastal systems of southern Portugal. Two sites were studied in Ria Formosa lagoon (Faro NW and Tavira) and one in the Arade estuary. Four stations were sampled along the nutrient gradient in each of two sites (Faro NW along 2 years, and Arade along 1 year) and two stations were sampled in Tavira. The *Z. noltii* population structure reflected the anthropogenic nutrient disturbance. The nutrient-disturbed stations showed significant correlations between biomass and density, whereas at nutrient-undisturbed stations the biomass-density data were uncorrelated. The *Z. noltii* population parameters derived from the temporal variation of the population structure, i.e. the slope of the biomass-density relationships, the coefficient of variation of biomass and the ratio of the maximum/minimum biomass increased with nutrient availability, whereas the intercept of the biomass-density relationship decreased with nutrient availability. These patterns resulted mostly from the higher variability of biomass in nutrient disturbed stations as shoot density varied little among stations. The higher biomass variability may reflect both the beneficial effects (availability) and the detrimental effects (toxicity) of nutrients along the year. Very high concentrations of DIN (> 400 μM) caused toxic effects on plants limiting the biomass development. The described relationships and patterns of variation may be used to assess the global nutrient-disturbance level of areas within the coastal systems in southern Portugal. Validation of these patterns for other geographical areas and other seagrass species may provide a general, valuable tool, to assess the anthropogenic nutrient disturbance in coastal areas.

KEY WORDS: Biomass-density relationship, Population structure, Nutrients, Wastewater, Disturbance, Seagrass, *Zostera noltii*

3.1. Introduction

Nutrients are basic requirements for plant growth and development. Environmental variation of nutrient availability is expected to result in changes to plant physiology and morphology, and consequently in changes of the population structure. The biomass-density relationships can respond to different environmental conditions (e.g. light, nutrients), such that differences among slopes may constitute a valuable measure of intraspecific competition (self-thinning lines) or reflect differing ecology among meadows and, therefore, may be a useful tool in assessing plant population dynamics (Weller 1987a,b).

Nutrient variation is known to affect the thinning lines of non-clonal terrestrial plants (Morris 2003). Populations may structure a) along the same biomass-density line, with the rate of propagation along this line increasing with the increase of nutrients (Yoda et al. 1963, White & Harper 1970, Bazzaz & Harper 1974, Morris 1995), or b) along different biomass-density lines, with the slope of this line decreasing (White 1981, Westoby 1984) or increasing (Furnas 1981, Morris & Myerscough 1985, Gibson & Good 1986, Morris & Myerscough 1991, Morris 1999, Morris 2002) as nutrients increased. Such patterns have been associated to different intraspecific competition processes operating within the meadows (Morris 2003). However, clonal plants have different mechanisms involved in growth, competition and mortality (De Kroon 1993, Hara & Srutek 1995). Competition may be alleviated through the physiological integration among modules (De Kroon 1993), and the overproduction of shoots may be prevented by controlling ramet formation (De Kroon & Kwant 1991, De Kroon 1993, De Kroon & Kalliola 1995).

Seagrasses are similarly clonal in nature and seem to regulate the population level processes in much the same way as terrestrial plants. Marbà et al. (2002) demonstrated that seagrass species can share resources between neighbour modules through physiological integration mechanisms. The inhibition of foliar development of shoots was described by van Tussenbroek et al. (2000) for the seagrass *Thalassia testudinum* under crowded conditions, resembling the density-dependant regulatory mechanisms described for terrestrial clonal plants (De Kroon 1993). Such mechanisms allow seagrasses to regulate shoot density within meadows through a basic growth programme, imprinted on the spacing between shoots along their rhizomes (Marbà & Duarte 2003). This allows seagrass species to maintain crowded conditions without undergoing shoot mortality. Hence, biomass and density of seagrass meadows are population components that ultimately express the physiological- and plant-level mechanisms (e.g. Olesen & Sand-Jensen 1994). To our knowledge there are no studies relating the biomass-density relationships of seagrass species to different levels of nutrient availability. However, van Tussenbroek et al. (2000) reported the re-activation of the undeveloped shoots (due to density-regulation or dormancy mechanisms) after nutrient addition, whereas Hughes et al. (2004) showed that seagrass biomass responded positively to the sediment nutrient enrichment. From the available plant- and population-level evidence, it can be hypothesized that the biomass-density relationships within the seagrass meadows are affected by different nutrients levels. The present study aims to assess how the biomass-density relationships of the seagrass *Zostera noltii* vary along anthropogenic nutrient gradients resulting from the discharge of Waste Water Treatment Works. This information could be interpreted as a useful biological indicator to monitor the nutrient loading of coastal systems.

3.2. Methods

3.2.1. Study area

This study was conducted in estuarine and lagoonal systems located in the south coast of Portugal (Fig. 3.1). One study site was located in the Arade estuary, which is a mesotidal torrential system and the second largest estuary in the Algarve (Instituto Portuário do Sul 2002). Two other study sites, Faro NW and Tavira, were located in the Ria Formosa lagoon, which is a mesotidal coastal system with a surface area of 84 Km^2 and an exposed intertidal area of about 80% (Andrade 1990). *Z. noltii* populations distributed along the nutrient gradients-created by the local urban wastewater discharge were assessed in each site.

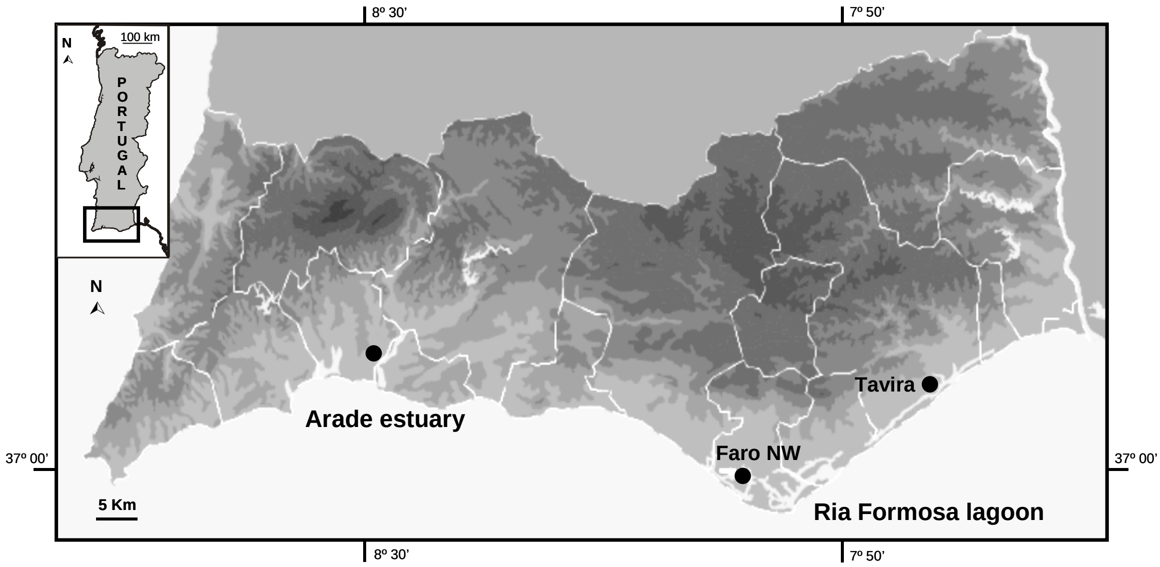

Fig. 3.1. Map of Portugal with the location of the study sites in the south coast of Portugal: Faro NW and Tavira at Ria Formosa lagoon and Arade estuary

3.2.2. Sampling

The first part of this study was conducted during 2001-2002 in Ria Formosa lagoon, at the study site of Faro NW. Four sampling stations were established along the nutrient gradient in homogeneous *Z. noltii* meadows, located at similar tidal elevation, with station 1 closest to the wastewater effluent and thus the most nutrient enriched, while station 4 was located in the main channel where there was no influence of the wastewater discharge. In each station, five randomly distributed samples were collected with a 12 cm diameter corer, to a depth of 15 cm, from July 2001 to July 2002, with a two months interval. In each sample, the number of shoots was counted to estimate shoot density. Plants were dried at 60 ºC during 48 h to determine stand biomass (above plus belowground biomass).

Nutrient concentrations of the water were determined seasonally, in July 2001 (summer), November 2001 (autumn), February 2002 (winter) and May 2002 (spring), at the *Z. noltii* sampling stations. Three water samples (100 ml each) were collected in each station, at low tide, filtered (Whatman cellulose acetate filters, 0.45 μm pore size) and frozen for later nutrient analysis. Ammonium was determined using a spectrophotometric method (Solorzano 1969), while nitrates+nitrites and phosphates were determined with an Autoanalyser system (Skalar, Sans Plus).

The second part of this study was conducted during 2005 at Faro NW (4 sampling stations), Tavira (2 sampling stations), and Arade estuary (4 sampling stations). In each station, four randomly distributed samples were collected with a 12 cm diameter corer, on a seasonal basis, in February 2005 (winter), April 2005 (spring), August 2005 (summer) and November 2005 (autumn). The winter sampling at the Arade estuary was not conducted due to weather and logistical problems. The samples were analysed for shoot density estimates and stand biomass as describe above. Data obtained for Faro NW during 2001-2002 and 2005 were analysed together. Nutrient concentrations were determined seasonally at all sampling stations and analysed as previously described.

3.2.3. Biomass-density relationships

The temporal variation of the relationship between the log of stand biomass and the log of shoot density were analysed in each station of the three study sites. Stand biomass was preferred over mean plant biomass as the variable to be plotted against shoot density to avoid problems of interpretation of results (Weller 1987a, Scrosati 1997). The linear relationship between log stand biomass and log shoot density was determined for each site through principal component analysis (PCA), because neither variable can be considered as a fixed variable (Weller 1987a). The statistical differences among the slopes obtained for each site were determined by comparing 95% confidence intervals of the slopes; the non-overlap of the 95% confidence intervals indicated significant differences among slopes (Weller 1987a, Sokal & Rohlf 1995). The strength of the linear relationship in each data set was measured by the Pearson correlation coefficients. Statistical significance of each correlation was examined by testing the null hypothesis that log stand biomass and log shoot density were uncorrelated (Sokal & Rohlf 1995).

3.3. Results

The relationships between the stand biomass and the shoot density of *Z. noltii* meadows yielded a positive relationship for all stations along the three nutrient gradients studied (Fig. 3.2 to 3.4). The correlation between stand biomass and shoot density was significant ($p < 0.05$, Table 3.1) in stations 1, 2 and 3, i.e. the nutrient-disturbed stations, of the study sites of Faro NW and Arade (modest to very strong correlations, $0.45 < r < 0.94$; Table 3.1). At the nutrient-disturbed station of Tavira the significance level of the correlation was $p = 0.08$ (Table 3.1). On the contrary, the nutrient-undisturbed stations showed no significant correlation between biomass and density (very weak to weak correlations, $0.04 < r < 0.38$; Table 3.1). The slopes of the biomass-density relationships decreased along the studied gradients for all the study sites (Table 3.1), as nutrients availability decreased, except for station 1 of Arade. Slopes vary from 1.13 at station 1 to 0.09 at station 4 for the study site of Faro NW, from 0.74 at station 2 to 0.42 at station 4 for Arade, and from 0.91 at station 1 to 0.23 at station 2 for Tavira (Table 3.1). Significant differences among the slopes were found for the study sites of Faro NW (station 1 was significantly different from station 4) and

Arade (station 1 was significantly different from stations 2 and 3), as indicated by non-overlap of the 95% confidence intervals (Table 3.1). The overlapping of the 95% confidence intervals found for Tavira indicated that slopes were not significantly different (Table 3.1). This was caused by the large confidence intervals found in this site. Opposite to the slopes, the intercept of the biomass-density relationships increased along the nutrient gradient for all the study sites, except in station 1 of Arade (Table 3.1).

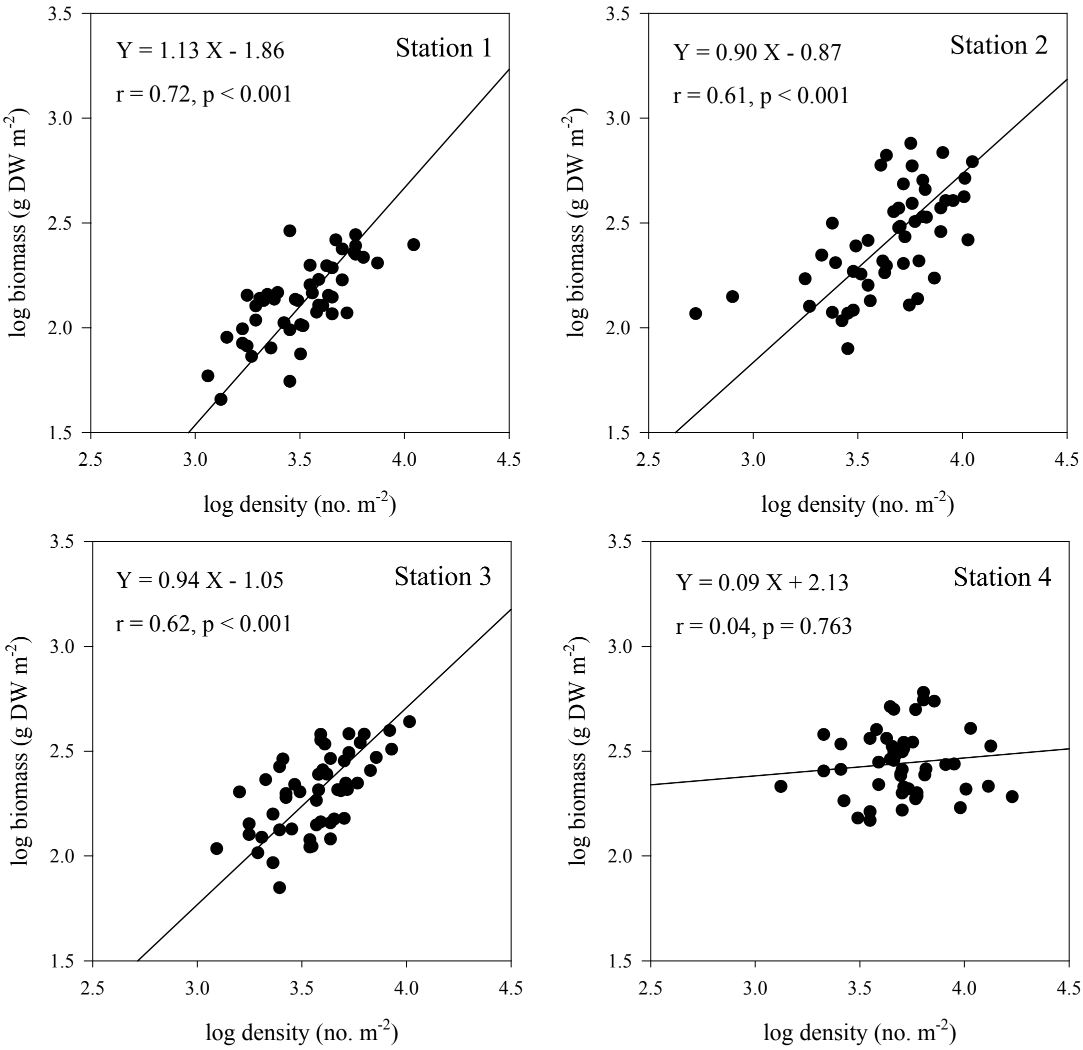

Fig. 3.2. Dynamic relationship between stand biomass and shoot density of *Zostera noltii* along the nutrient gradient originated by the urban wastewater discharge of Faro NW

Table 3.1. Biomass-density linear relationships of *Zostera noltii*, along the nutrient gradient originated by the urban wastewater discharges of Faro NW, Arade and Tavira

Sites		Slope	95% C.I.	Intercept	r	p	n
Faro NW	Station 1	1.13	0.86 to 1.51	-1.86	0.72	< 0.001	51
	Station 2	0.90	0.60 to 1.32	-0.87	0.61	< 0.001	51
	Station 3	0.94	0.63 to 1.38	-1.05	0.62	< 0.001	51
	Station 4	0.09	-0.43 to 0.65	2.13	0.04	0.763	50
Arade	Station 1	0.17	0.05 to 0.28	1.39	0.73	0.008	12
	Station 2	0.74	0.45 to 1.16	-0.38	0.84	0.001	12
	Station 3	0.50	0.37 to 0.64	0.61	0.94	< 0.001	12
	Station 4	0.42	-0.24 to 1.67	0.86	0.38	0.225	12
Tavira	Station 1	0.91	0.12 to 4.65	-1.03	0.45	0.082	16
	Station 2	0.23	-0.35 to 1.01	1.67	0.21	0.447	16

The range of both biomass and density data was wider in stations 1, 2 and 3 in Faro NW (Fig. 3.2) and Arade (Fig. 3.3), and in station 1 in Tavira (Fig. 3.4) than at stations 4 of Faro NW and Arade, and at station 2 of Tavira, respectively. In particular, the lowest biomass values of nutrient-disturbed stations were much lower than undisturbed stations. The coefficient of variation of biomass and the ratio of maximum/minimum biomass showed a clear pattern, consistently decreasing along the nutrient gradients (Table 3.2), except for the station 1 of Arade. On the other hand, no pattern was found for the coefficient of variation of density and for the ratio of maximum/minimum density (Table 3.2). The coefficients of variation and the ratios between the maximum and minimum values were higher for biomass than for density (Table 3.2).

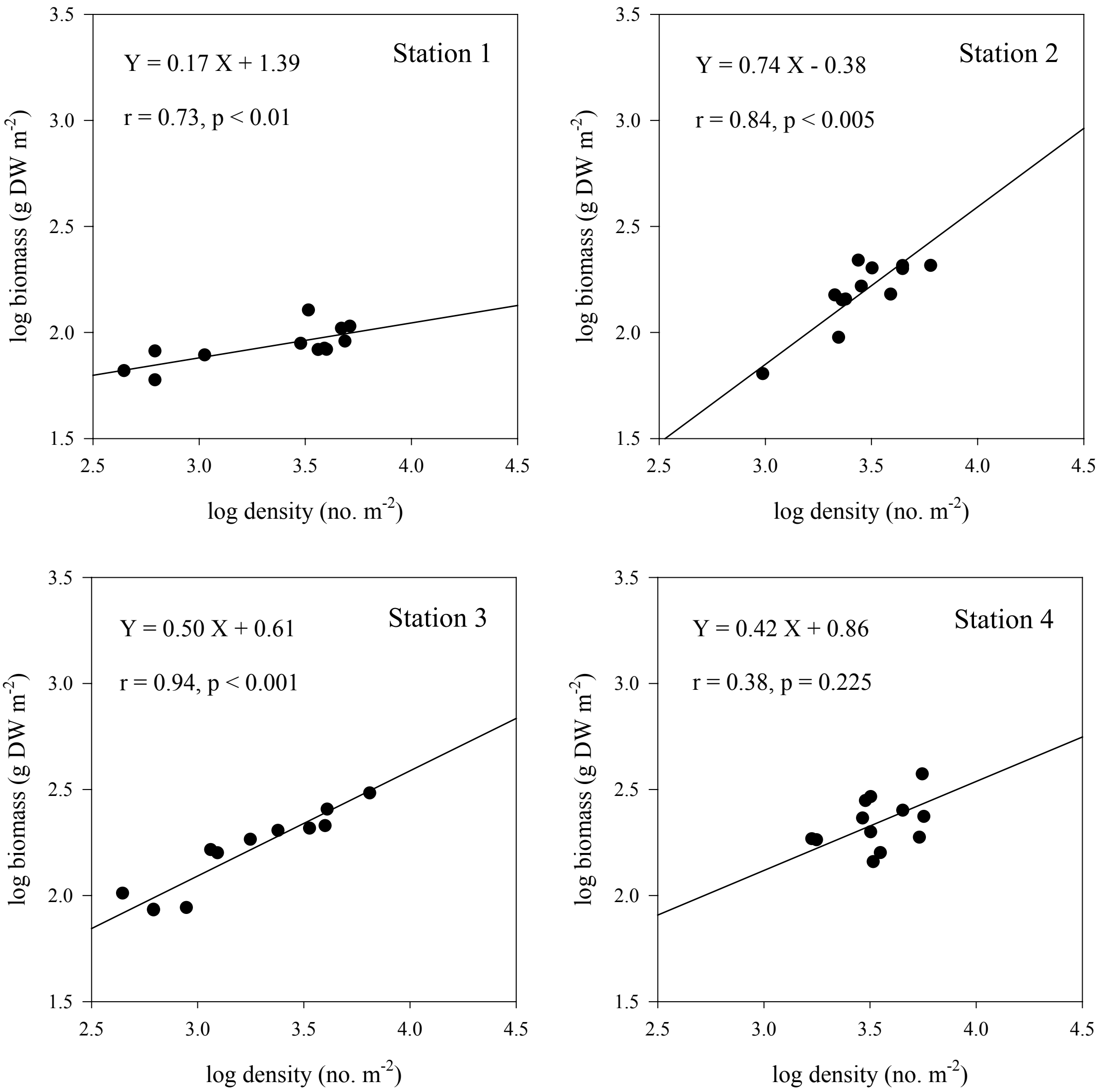

Fig. 3.3. Dynamic relationship between stand biomass and shoot density of *Zostera noltii* along the nutrient gradient originated by the urban wastewater discharge of Arade

The slope (Fig. 3.5A), the coefficient of variation of biomass (Fig. 3.6A) and the ratio of maximum/minimum biomass (Fig. 3.6B) increased along the disturbance gradient as DIN concentration increased, while the intercept (Fig. 3.5B) showed the opposite trend. This general pattern was disrupted at DIN concentrations higher than 400 μM, at station 1 of Arade, when the slope (Fig. 3.5A), the coefficient of variation of biomass (Fig. 3.6A) and the ratio of maximum/minimum biomass (Fig. 3.6B) dropped to low values and the intercept (Fig. 3.5B) increased.

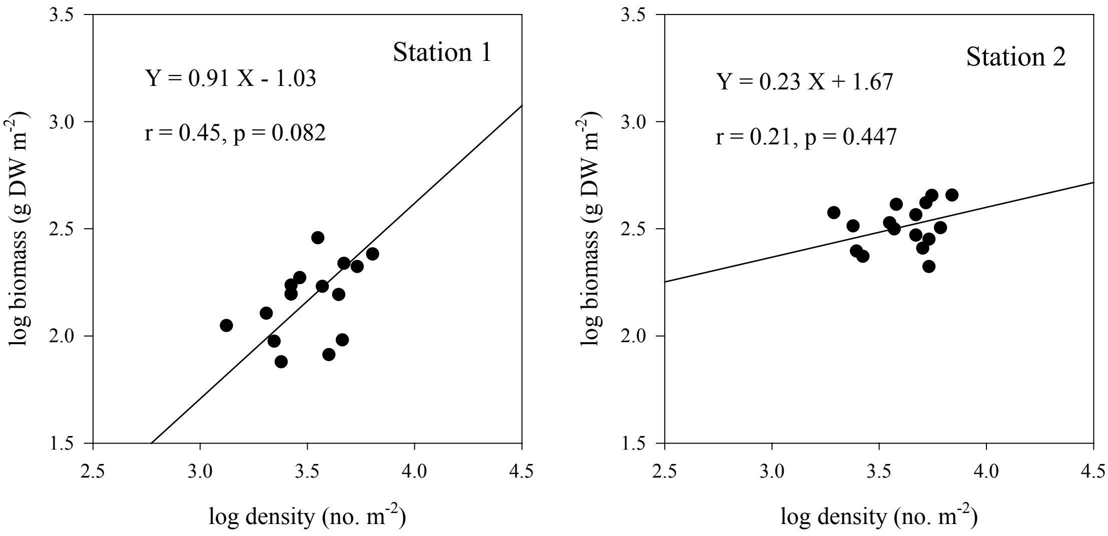

Fig. 3.4. Dynamic relationship between stand biomass and shoot density of *Zostera noltii* along the nutrient gradient originated by the urban wastewater discharge of Tavira

Table 3.2. Coefficient of variation (CV) of log biomass and density, and ratio between the maximum and minimum log biomass and density of *Zostera noltii* populations along the nutrient gradients originated by the urban wastewater discharges of Faro NW, Arade and Tavira

Sites		CV biomass	CV density	Biomass max/min	Density max/min
Faro NW	Station 1	11.60	6.38	1.74	1.35
	Station 2	10.27	7.25	1.52	1.49
	Station 3	8.33	5.56	1.43	1.30
	Station 4	6.37	5.65	1.28	1.35
Arade	Station 1	4.60	12.04	1.19	1.40
	Station 2	7.25	5.92	1.30	1.26
	Station 3	8.82	11.79	1.29	1.44
	Station 4	5.09	4.93	1.19	1.16
Tavira	Station 1	7.93	5.08	1.31	1.22
	Station 2	4.07	4.54	1.14	1.17

3.4. Discussion

The nutrient enrichment originated by the urban wastewater discharges clearly affects the population structure of *Z. noltii* meadows, as indicated by the variation of the biomass-density relationships along the disturbance gradients (Fig. 3.2 to 3.4). Nutrient-disturbed stations showed significant correlations between biomass and density, whereas at nutrient-undisturbed stations, the biomass-density data were uncorrelated (Table 3.1). This pattern results from the higher variability of the biomass values found in nutrient-disturbed stations than in undisturbed stations. Differences among the biomass-density relationships were mainly attributable to changes in biomass, as shoot density varied little among stations, suggesting that *Z. noltii* biomass is more sensitive than density to nutrient availability. This observation is supported by Hughes et al. (2004) meta-analysis of the effects of nutrients on seagrasses, which showed that nutrient enrichment has a positive effect on biomass, whereas the effect on shoot density was negligible.

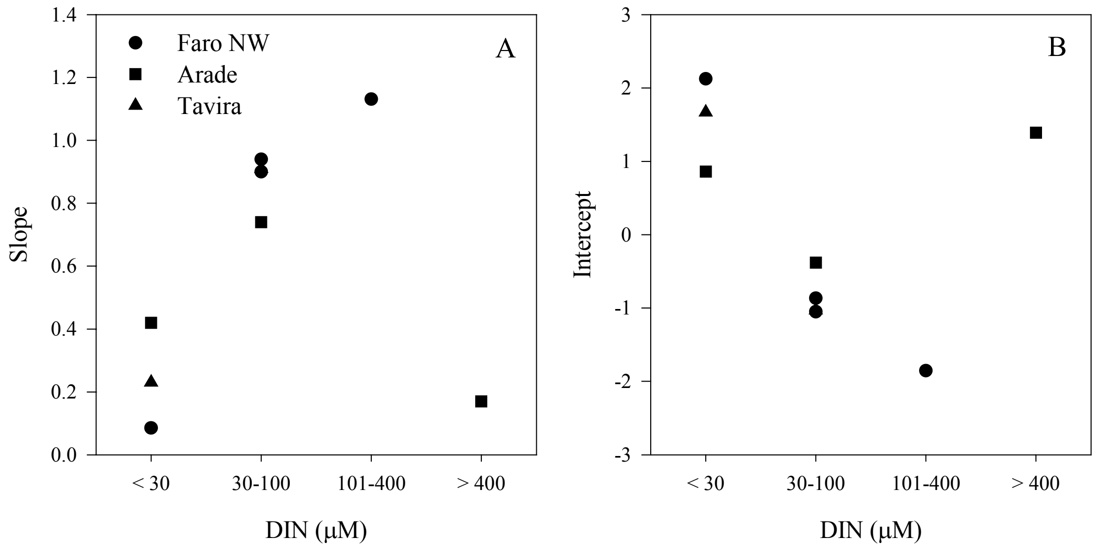

Fig. 3.5. (A) Slope and (B) intercept of the biomass-density relationships of *Zostera noltii* populations of Faro NW, Arade and Tavira along the dissolved inorganic nitrogen (DIN) gradient

The patterns of variation of the *Z. noltii* population parameters described here, namely the slope of the biomass-density relationships, the coefficient of variation of biomass and the maximum/minimum ratio of biomass, which increase with nutrient availability, and the intercept of the biomass-density relationship, which decreases with nutrient availability (Figs. 3.5 & 3.6) reflect the higher variability of *Z. noltii* biomass in stations closer to the urban effluent source. In contrast, the nutrient-undisturbed stations showed a more conservative range of biomass-density data, varying more in density but very little in biomass, with no clear seasonal trend. Supporting our results, Asmus et al. (2000) reported little variation of biomass during the year in natural, undisturbed *Z. noltii* meadows of Ria Formosa. As well, no clear seasonal pattern was found in *Z. noltii* aboveground and belowground biomass in the Atlantic coast (Bassin d'Arcachon, France; Castel et al. 1996) and in the Mediterranean (Plus et al. 2001, Pergent-Martini et al. 2005). Pergent-Martini et al. (2005) reported significant differences in density depending on the sampling period. The higher biomass variability found in nutrient-disturbed stations probably reflects both the beneficial effects (availability) and the detrimental effects (toxicity) of nutrients along the year. Brun et al. (2002) showed that ammonium might have a toxic effect on *Z. noltii* survival and growth or might result in increased plant growth, depending on the season and on the interactions with other nutrients (e.g. phosphates). Sucrose seemed to play an important role in these differences, as growth inhibition in response to nutrients addition was always accompanied by sucrose mobilization (Brun et al. 2002). In spring, with higher light and temperature, the higher photosynthetic C flux seemed to be sufficient to meet C demands arising from the N assimilation, thus alleviating the toxic effect of nutrients. On the other hand, a drop in sucrose content to very low levels was observed in response to ammonium enrichment in winter, when sucrose was not mobilized (Brun et al. 2002). These opposing effects may account for the high plasticity of *Z. noltii* biomass found in nutrient-disturbed stations. High plasticity was reported for *Z. noltii* as an acclimation mechanism to different environmental conditions (Peralta et al. 2000, 2005, 2006). Intraspecific plasticity is a mechanism that allows clonal plants to adapt to changes in resources (Slade & Hutchings 1987, Dong et al. 1996).

Other environmental factors than nutrient load from urban wastewater may play an important role in controlling the biomass/density dynamics of *Z. noltii*, such as the light attenuation from phytoplankton blooms under eutrophic conditions or the sediment nutrients. However, it is not likely so as both coastal systems studied are not eutrophic due to the high water renewal that maintains water quality. As well, a multivariate analysis of the relationships among environmental variables of both water column and sediment, and *Z. noltii* population descriptors (Cabaço et al. submitted, Chapter 2) shows that the biomass-density dynamics of *Z. noltii* meadows is significantly related to the nutrient load of urban effluents and not to the sediment condition.

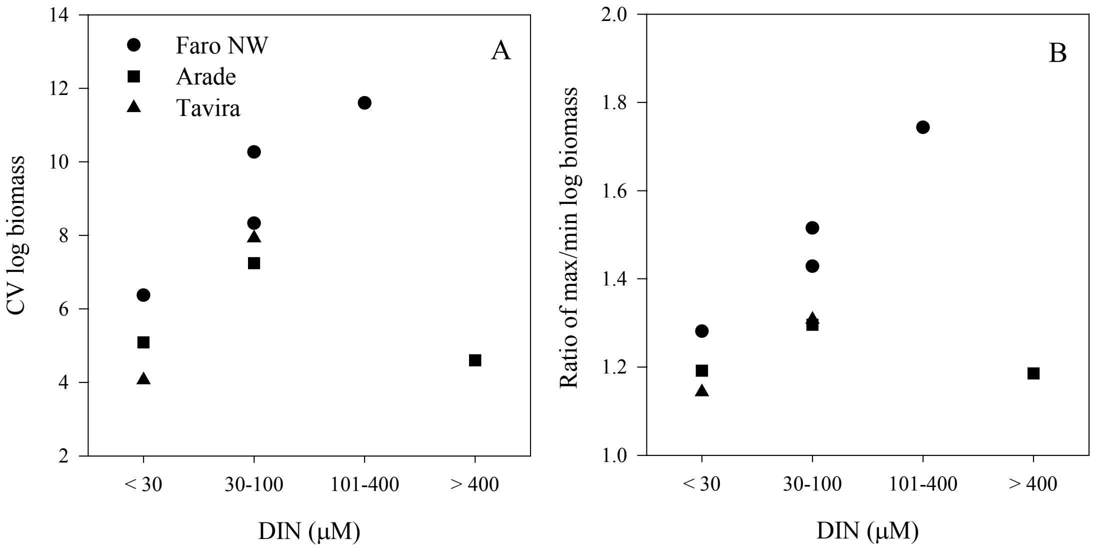

Fig. 3.6. (A) Coefficient of variation (CV) of log biomass and (B) ratio of maximum/minimum log biomass of the biomass-density relationships of *Zostera noltii* populations of Faro NW, Arade and Tavira along the dissolved inorganic nitrogen (DIN) gradient

The *Z. noltii* population of station 1 of Arade was an exception to the general pattern described for all other populations, illustrating a situation of severe stress, where only the detrimental effects of nutrients prevail. The extremely high nutrient concentrations characterizing this station must have caused strong toxic effects on plants, limiting the biomass development (Fig. 3.3). Brun et al. (2002) showed that continuous exposure of high concentrations of ammonium (> 200 μM) had a toxic effect on *Z. noltii* survival and growth. In consequence, the slope and the intercept of the biomass-density relationship of *Z. noltii*, as well as the coefficient of variation of biomass and the ratio of maximum/minimum biomass, shifted to values similar to the undisturbed station (Figs. 3.5 & 3.6). Nonetheless, the significant correlation of the biomass-density data discriminate this highly disturbed station from the nutrient-undisturbed one, where biomass and density were uncorrelated (Table 3.1).

The slopes of the biomass-density relationships were not significantly different from each other for all the stations, due to the high variability of biomass-density data, particularly in nutrient-disturbed stations. Yet, significant differences were detected between the more nutrient-disturbed and undisturbed stations in Faro NW and Arade. A reduction in slopes at lower nutrient levels was also described for non-clonal terrestrial plants (Furnas 1981, Morris & Myerscough 1985, Gibson & Good 1986, Morris & Myerscough 1991, Morris 1999, Morris 2002). This pattern was related to an increase of the intraspecific competition as nutrients availability decrease (Morris 2003). For clonal plants, physiological integration moderates the competition between shoots (De Kroon 1993), but little is known about the dynamics of genets in clonal plants and in seagrasses in particular. The flatter slopes found for *Z. noltii* may reflect a higher competition between genets as nutrient availability decreases and within population size inequality in plants decreases. Schwinning & Weiner (1998) suggested that, despite different life-strategies, the factors determining the competition among clonal plants are ultimately the same as in competition among non-clonal plants, and may be deducible from differences in plant allometry and plasticity.

The significant correlations observed between the temporal data of biomass and density of *Z. noltii* populations disturbed by nutrients as opposed to the non-significant relationships observed in undisturbed populations, together with the population parameters of biomass-density slope and intercept, coefficient of variation of biomass and ratio of maximum/minimum biomass, may be used to assess the global nutrient-disturbance level of areas within the Ria Formosa lagoon and the Arade estuary in southern Portugal. Validation of these patterns for *Z. noltii* populations elsewhere and for other seagrass species may provide a general, valuable tool to assess the anthropogenic nutrient disturbance in coastal areas.

Acknowledgements. This study was funded both by the EC project "Monitoring and Managing of European Seagrass Beds" (EVK3-CT-2000-00044) under the 5th Framework Programme and by a contract with Hidrotec/Águas do Algarve to monitor the macrophyte communities of Algarve coastal systems. We are grateful to A. Alexandre, A.H. Cunha, A. Fernandes, A. Quaresma, C. Alves, C. Godinho, J. Silva, J.A. Bentes, P. Morais, P. Neves and P. Teixeira for fieldwork assistance. We thank R. Scrosati for clarifying the statistical analysis on the biomass-density relationship, and C.M. Duarte for comments on data analysis. We thank to two anonymous reviewers that greatly improved the manuscript. S.C. and R.M. were supported by a PhD grant from Fundação para a Ciência e a Tecnologia (SFRH/BD/2880/2000 and Praxis XXI/BD/19579/99).

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CHAPTER 4

Population-level effects of clam harvesting on the seagrass *Zostera noltii*

Cabaço S, Alexandre A, Santos R (2005)

Marine Ecology Progress Series 298: 123-129

4. Population-level effects of clam harvesting on the seagrass *Zostera noltii*

ABSTRACT: Seagrass declines have been reported worldwide, mostly as a consequence of anthropogenic disturbance. In Ria Formosa lagoon, southern Portugal, the intertidal meadows of *Zostera noltii* are highly disturbed by clam harvesters. The most common technique used to collect the clams consists of digging and tilling the sediment with a modified knife with a large blade. Here we present both descriptive and experimental evidence of the negative effects of clam harvest on the *Z. noltii* populations of Ria Formosa. A comparison between disturbed and undisturbed meadows suggests that clam harvesting activities change the species population structure by significantly reducing shoot density and total biomass, particularly during August, when the harvest effort is higher. Experimental harvest revealed a short-term impact on shoot density, which rapidly recovered to control levels during the following month. An experimental manipulation of rhizome fragmentation revealed that plant survival is reduced only when fragmented rhizomes are left with 1 intact internode. Shoot production and rhizome elongation and production of fragmented rhizomes having 2 to 5 internodes were not significantly affected, even though growth and production were lower when only 2 internodes were left. Experimental shoot damage at different positions along the rhizome had a significant effect on plant survival, rhizome elongation, and production only when the apical shoot was removed. Our results show that clam harvest can adversely affect *Z. noltii* meadows of Ria Formosa while revealing a low modular integration that allows the species to rapidly recover from physical damage.

KEY WORDS: Clam harvest, Physical damage, *Zostera noltii*, Seagrass, Disturbance, Population recovery

4.1. Introduction

Seagrass decline is a worldwide phenomenon. Although natural disturbances are recognized, most declines are attributed to anthropogenic disturbances (Short & Wyllie-Echeverria 1996). Direct mechanical damage reported to disturb seagrasses include dredging (Zieman 1982, Phillips 1984, Thayer et al. 1984, Coles et al. 1989), propeller scarring (Zieman 1976, Walker et al. 1989, Dawes et al. 1997), boat mooring and anchoring (Williams 1988, Walker et al. 1989, Creed & Amado Filho 1999), and docks (Burdick & Short 1999). Fishing gear practices (Ardizzone et al. 2002, Orth et al. 2002, Uhrin et al. 2005) and fishing techniques associated with clam harvest and clam culture (Peterson et al. 1983, Fonseca et al. 1984, Peterson et al. 1987, Everett et al. 1995, Boese 2002, Neckles et al. 2005) have also been shown to negatively impact seagrasses, including declines in seagrass cover and failure of seagrass restoration in the Dutch Wadden Sea (De Jonge & De Jong 1992).

Sporadic and continuous mechanical damage results in partial or complete removal of plants from the substratum (Short & Wyllie-Echeverria 1996). As a result of plant removal, secondary effects like decreased seagrass cover, productivity, and biodiversity and increased habitat fragmentation, sediment resuspension, erosion, and alteration of physical processes (e.g. water currents) may result in long-term effects such as community restructuring (Hemminga & Duarte 2000).

The Ria Formosa lagoon, southern Portugal, is a highly productive ecosystem dominated by the intertidal seagrass *Zostera noltii*. *Z. noltii* is a small species that develops extensive meadows sustaining high gross primary production (Santos et al. 2004). These meadows play an important role in the bivalve recruitment (A.H. Cunha & R. Santos unpubl. data) and biodiversity of Ria Formosa lagoon, including economically important species such as cephalopods, crustaceans, and fish. Clam harvest and clam culture are the main commercial activities of the lagoon, representing more than 90% of national clam production (Direcção Regional das Pescas e Aquicultura do Sul pers. comm.). These activities take place along the intertidal areas, where *Z. noltii* meadows develop. The most common technique used by local clam harvesters consists of manually digging and tilling the sediment using a modified knife with a large blade. This technique severs shoots and rhizomes and causes plant burial.

The main objectives of this study were to (1) analyse the effects of clam harvesting, as it is performed by local fishermen, on *Z. noltii* population density and biomass through the comparison of disturbed and undisturbed meadows; (2) test the effects of clam harvesting on *Z. noltii* density and its recovery through *in situ* experimental manipulation; and (3) determine the effects of physical damage caused by clam harvesting technique in plant survival, growth, and production, through the experimental manipulation of both rhizome and shoot fragmentation at different modular levels, i.e. altering the intact number of modular units.

4.2. Methods

This study was conducted from June to November 2001 in the Ria Formosa lagoon, southern Portugal (Fig. 4.1). The lagoon is a mesotidal system with a high spring tide surface area of 84 km^2 and an exposed intertidal area of about 80% (Andrade 1990). The lagoon is separated from the Atlantic Ocean by a system of 5 sand barrier islands and 6 inlets. The tidal amplitude ranges from 3.50 m on spring tides to 1.30 m on neap tides. Sampling was performed in a *Z. noltii* meadow under clam harvesting disturbance and in an adjacent undisturbed meadow. The disturbed meadow is a free access area frequently used for commercial clam harvest. The undisturbed meadow is part of a private clam culture concession where trespassing is not allowed. Clam harvest did not occur in this area for several years. Five randomly distributed samples were collected biweekly from each meadow, with a 12 cm diameter core. In each sample, the number of shoots was counted to estimate shoot density. The total biomass (above plus belowground material) of *Z. noltii* was determined by drying the sample at 60 °C for 48 h.

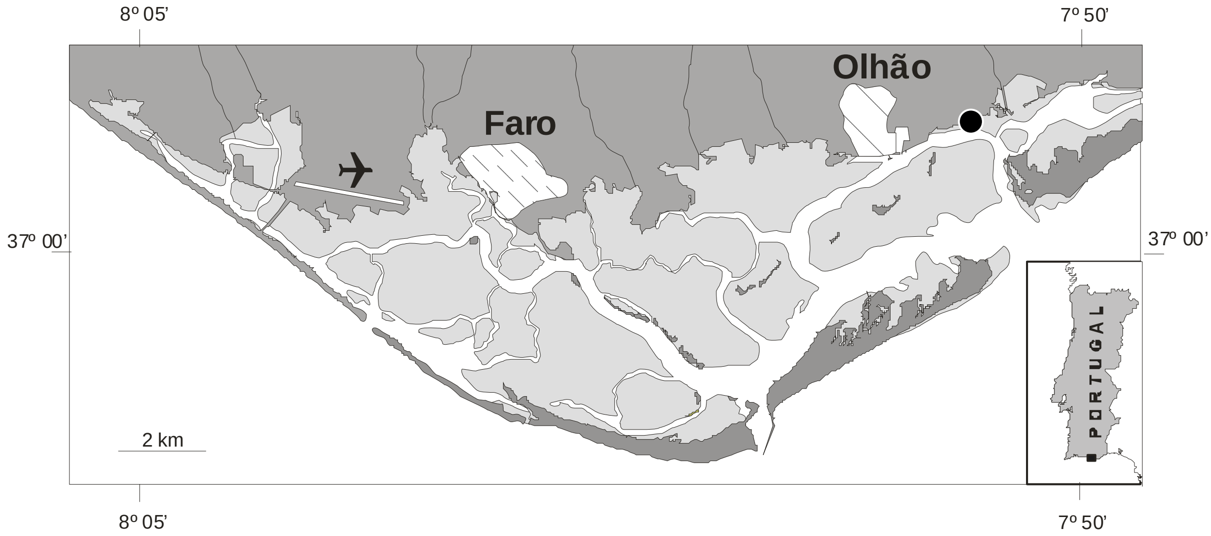

Fig. 4.1. Map of Ria Formosa, southern Portugal with the location of *Zostera noltii* study meadows (•)

The effects of clam harvest on *Z. noltii* population density and its recovery were assessed by *in situ* experimental manipulation. Fourteen permanent plots of 10 × 10 cm were randomly placed in a homogeneous, undisturbed *Z. noltii* meadow. Half of the plots were disturbed using the same technique employed by the local clam harvesters, while the other half remained as control (undisturbed). After the plots were disturbed, the area was allowed to settle for a day, so that the tidal currents would flatten the sediment and remove the loose plants. Plots were monitored the following day and every 2 weeks thereafter for 5 months, by counting all the shoots within each plot.

The physical impact of the clam digging on plant survival, growth, and production was assessed by experimental manipulation to varying degrees of rhizome and shoot fragmentation. In the first experiment, rhizomes were severed at increasing distances from the apical meristem, creating 5 levels of modular units (ramets) composed of 1, 2, 3, 4, or 5 rhizome internodes and including the respective aerial shoots (Fig. 4.2A). Each treatment was independently applied to 10 replicate plants. Plants were carefully collected, severed, and immediately placed in perforated plastic containers filled with local sediment. Apex shoots were tagged to distinguish new modular sets produced during the experimental period. The containers were randomly placed in the same meadow maintaining the local sediment level. The experiment was initiated in August 2003 and concluded after 30 days. Plant survival was determined and plants were examined for the number of new shoots and internodes produced to estimate the shoot and internode production rates (no. d^{-1}) and for the length of newly developed rhizome to calculate rhizome elongation rate (mm d^{-1}). The new internodes were dried at 60 °C for 48 h, to estimate rhizome production rate (g DW d^{-1}).

In a second experiment, shoots were cut at their base, removing the basal meristem, to simulate damage caused by clam digging. Four levels of shoot damage, relative to shoot position on the rhizome, were generated: no damage (control), 1 shoot cut off (the closest to the apex shoot), 2 shoots cut off (leaving the apex shoot only), and only the apex shoot cut off (Fig. 4.2B). Each treatment was independently applied to 10 replicate plants consisting of 3 rhizome nodes and associated shoots, including the apical shoot. Plants with 3 modules were selected, as there were no significant differences in growth and production of plants with 3, 4, or 5 modules and it is very difficult to find intact plants with 5 modules. Plants were harvested, severed, and immediately placed in perforated plastic containers as described above. The experiment was initiated in September 2003 and concluded after 30 days. The plant parameters were analysed as described above.

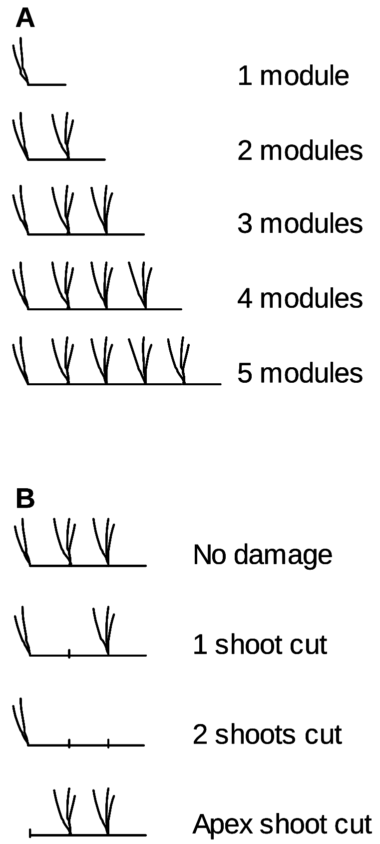

Fig. 4.2. Schematic representation of the experimental manipulation of (A) rhizome fragmentation and (B) shoot damage of *Zostera noltii* plants

Prior to statistical analyses, data were tested for homogeneity of variance and normality of distribution. When necessary, data were log-transformed to fit assumptions. Differences in shoot density and biomass between disturbed and undisturbed meadows were investigated using two-way ANOVA with disturbance and date as main effects. The recovery of shoot density after experimental disturbance was compared with controls using a Student's t-test for each sampling moment after data log-transformation. One-way ANOVA was used to test the effects of experimental damage of rhizomes and shoots on shoot and internode production, and in rhizome growth and production. When ANOVA indicated a significant difference, Tukey's multiple comparison test was applied to determine where significant differences occurred. Significant differences were considered at a probability value of $p < 0.05$ (Sokal & Rohlf 1995).

4.3. Results

The shoot density of the *Z. noltii* meadow under clam harvest disturbance was significantly lower than the undisturbed meadow (Fig. 4.3A), except on 1 June, 1 July, 15 July, and 1 October. The biomass of the disturbed meadow was 2 to 8 times lower during the whole sampling period (Fig. 4.3B). Shoot density and biomass showed no significant differences among sampling dates.

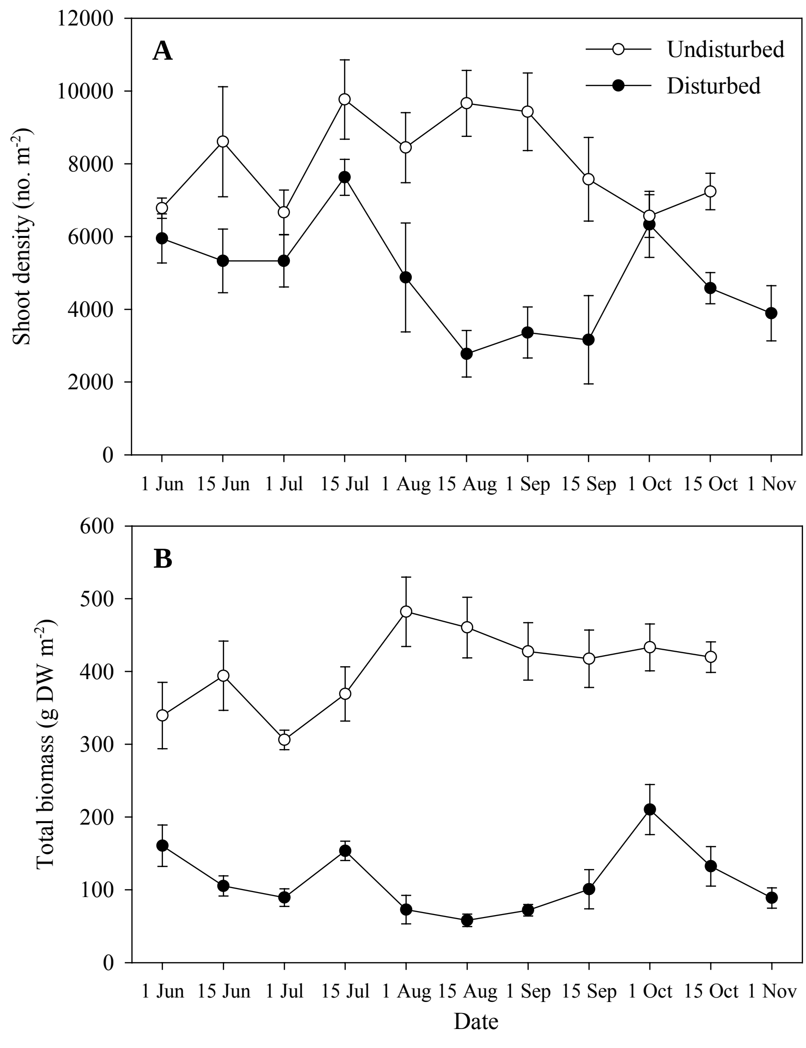

Fig. 4.3. (A) Shoot density and (B) total biomass of *Zostera noltii* meadows under varying levels of clam harvest disturbance (mean ± SE)

Experimental clam harvest significantly reduced the density of *Z. noltii* shoots until 15 days after the digging event (Fig. 4.4). Immediately following disturbance, 43% of shoots were lost and 19% of the remaining shoots had damaged leaves. Thirty days post-disturbance, densities had recovered to non-disturbed levels. From then on, no significant differences were found between treatment and control plots (Fig. 4.4).

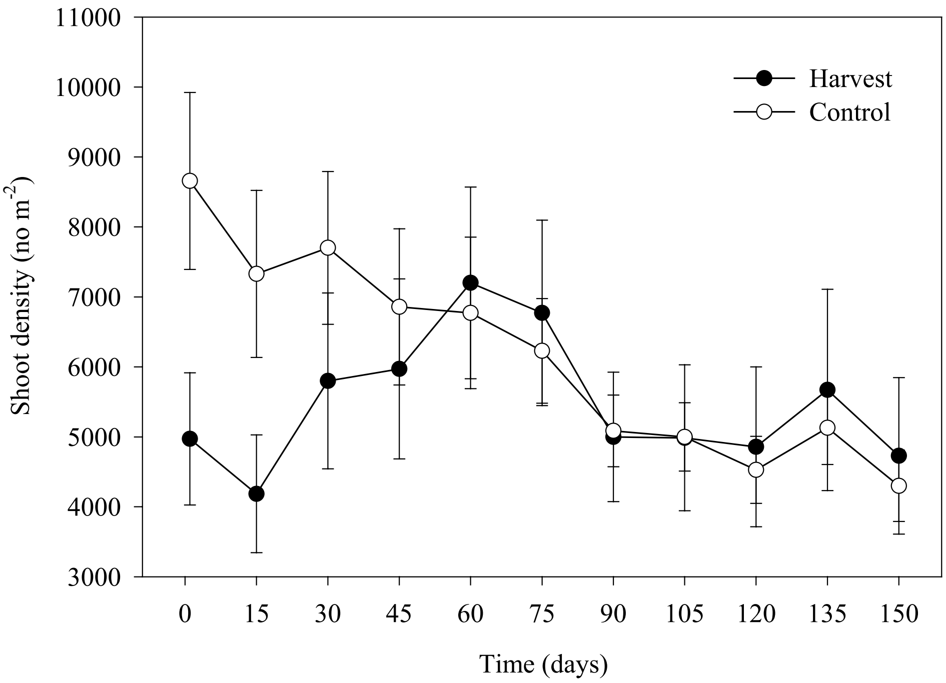

Fig. 4.4. Effects of clam harvest on *Zostera noltii* shoot density (mean ± SE)

Survival of experimentally damaged plants having 1 modular unit was much lower (10%) than plants with 2 to 5 modules (80 to 100%, Fig. 4.5A). This treatment level was not considered in further statistical analysis as only one plant had survived. Rhizome elongation and rhizome production rates were lower in plants with 1 and 2 internodes, compared to plants with 3 to 5 internodes, but no significant effects of rhizome fragmentation (2 to 5 modules) were found in shoot production, internode production, rhizome elongation, or rhizome production rates (Fig. 4.5).

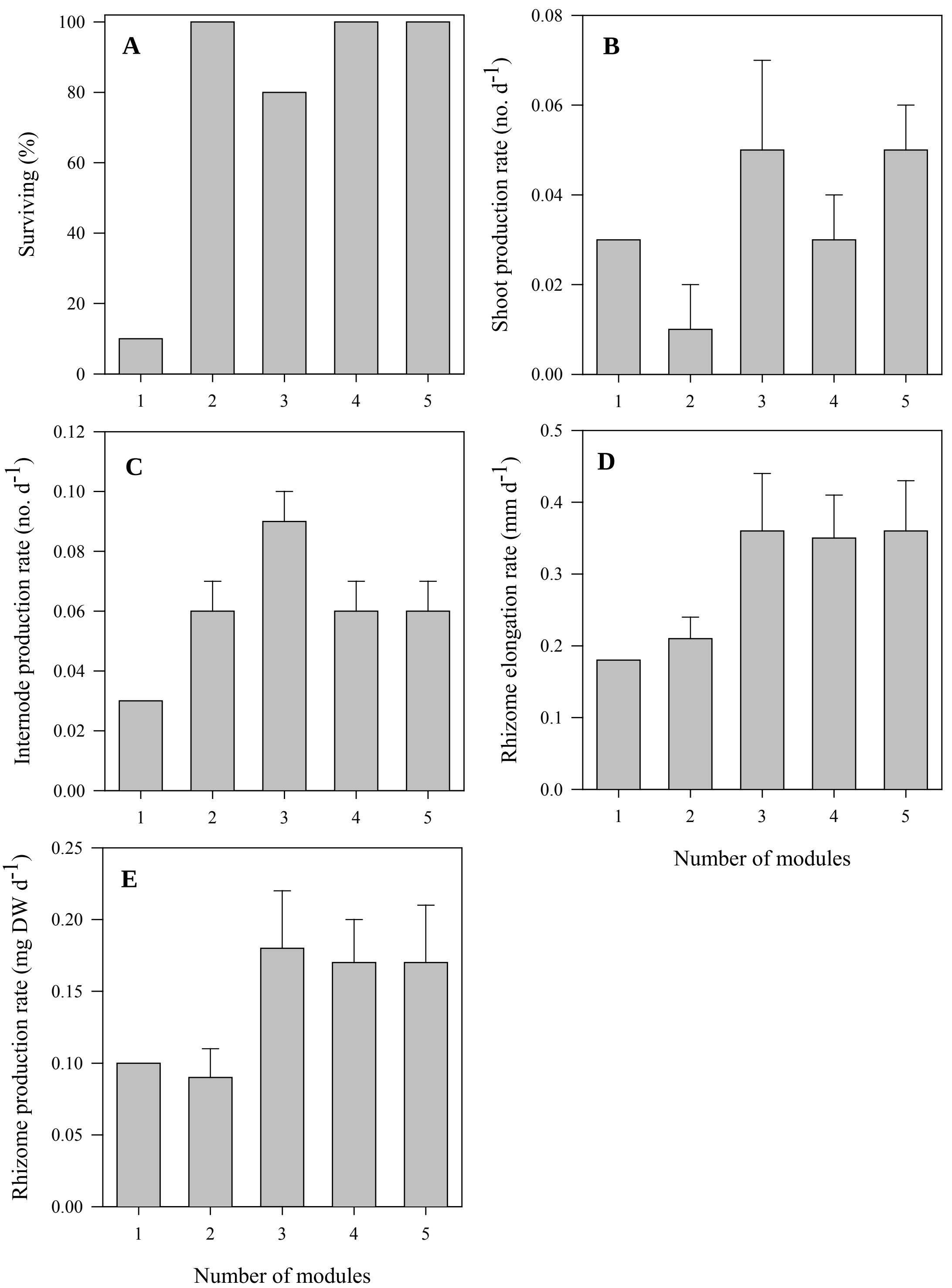

Fig. 4.5. Effects of rhizome fragmentation level (increasing number of connected modules) on (A) survival percentage, (B) shoot production rate, (C) internode production rate, (D) rhizome elongation rate and (E) rhizome production rate of *Zostera noltii* plants (mean ± SE)

The shoot damage experiment showed a negative effect of manipulation on plant survival, as 20% of all plants did not survive the initial cutting (Fig. 4.6A). No differences were found in the survival of plants with 1 or 2 shoots severed. Plant survival was lowest when the apex shoot was cut off (20%). The effects of cutting the apex shoot on shoot production, internode production, rhizome elongation, and rhizome production rates were extreme as practically no growth and production were observed with this treatment (Fig. 4.6). On the other hand, no significant effects were found when shoots other than the apical were severed (Fig. 4.6). No rhizome branching occurred during the experiment.

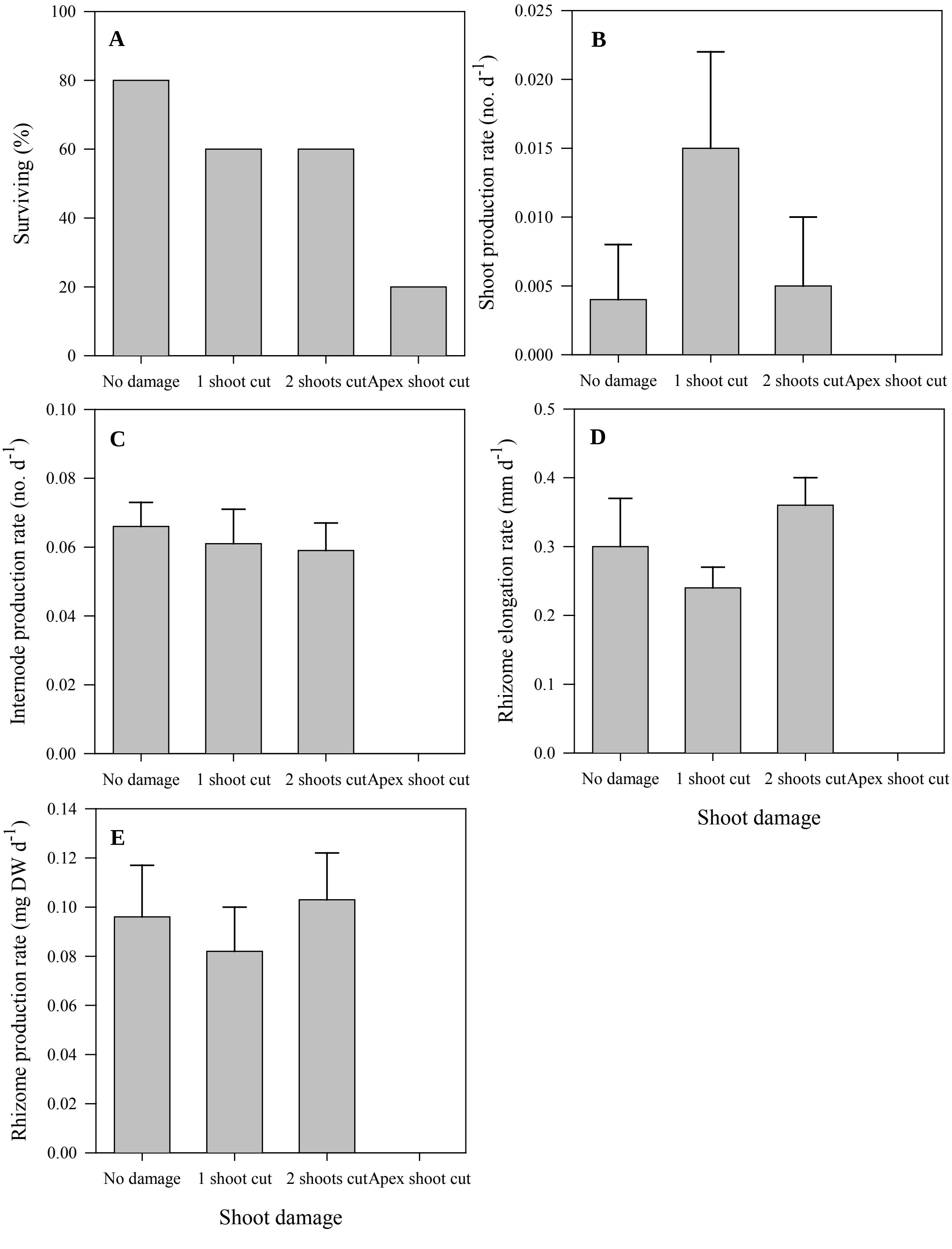

Fig. 4.6. Effects of shoot damage on (A) survival percentage, (B) shoot production rate, (C) internode production rate, (D) rhizome elongation rate and (E) rhizome production rate of *Zostera noltii* plants (mean ± SE)

4.4. Discussion

The *Z. noltii* meadows of Ria Formosa, southern Portugal, are heavily utilized by clam harvesters and have a visually fragmented aspect and a lower seagrass cover. The results of this study provide both descriptive and experimental evidence of the negative effects of clam harvest activity on *Z. noltii* populations. Both shoot density and total plant biomass were lower in meadows described as disturbed, and experimental harvest significantly reduced shoot density up to 15 days post-harvest. Our results indicate that recovery of isolated disturbances in *Z. noltii* meadows will occur for approximately 1 month, as suggested by experimental harvest (Fig. 4.4). The high growth rates and production of *Z. noltii* (Vermaat & Verhagen 1996, Marbà & Duarte 1998, Laugier et al. 1999) seem to buffer the long-term effects of isolated disturbances. Besides the initial reduction in shoot density (43%), shoot damage was also found the day after the experimental disturbance. However, no significant evidence of shoot damage was found 15 days after disturbance and beyond, which illustrates the fast leaf growth of the species (Vermaat et al. 1987, 1993). Boese (2002) found slower recovery for *Z. marina* subjected to experimental clam digging. Significant declines in above- and belowground biomass were observed for 1 month post-digging, and persisted for 10 months, although not significant. Recovery of disturbed *Z. noltii* meadows may occur through vegetative development, as long as modular units with at least 2 rhizome internodes with the respective connected shoots remain on the sediment (Figs. 4.5 & 4.6). This result can be directly applied to the management of *Z. noltii* meadows in Ria Formosa, allowing them to sustain the impacts of local clam harvesting. A secondary effect of exploiting clams or other resources such as molluscs within the *Z. noltii* meadows of Ria Formosa is the disturbance caused by trampling. Negative impacts on seagrass shoots and rhizomes as a result of repeated trampling have been demonstrated elsewhere (Eckrich & Holmquist 2000).

The recovery of commonly disturbed seagrass meadows depends not only on the level of disturbance but also on its frequency (Short & Wyllie-Echeverria 1996). The experimental manipulation of clam harvest in this study consisted of isolated disturbances. Extrapolation to the intertidal areas of Ria Formosa under frequent and intense clam harvest activity must be done with caution. A slower recovery of *Z. noltii* shoot density than that found here would be expected.

Sexual reproduction of *Z. noltii* may also contribute to the recovery of disturbed meadows as indicated by the higher reproductive effort of this species under clam harvesting disturbance (Alexandre et al. 2005). The relevance of sexual reproduction to the species recruitment was demonstrated by Diekmann et al. (2005), who found high genetic variability of *Z. noltii* meadows in Ria Formosa.

Rhizome fragmentation drastically reduced plant survival when only 1 module remained connected to the apical meristem (Fig. 4.5A). The damaged plants were not observed to decay but instead disappeared from the meadow, probably as a result of the buoyancy of the leaves, which caused the limited root system of the modules to disengage. Shoot production, internode production, rhizome elongation, and rhizome production rates were not significantly affected by rhizome fragmentation (Fig. 4.5), even though growth and production were lower when only 2 modules were left. This indicates a low modular integration for *Z. noltii* compared with other seagrasses. Terrados et al. (1997b) found negative effects on both rhizome and leaf growth of the seagrass *Cymodocea nodosa* when the horizontal rhizome was severed up to 11 internodes away from the apical meristem. Marbà et al. (2002) observed that the maximum translocation of carbon and nitrogen along *Z. noltii* rhizomes was lowest among seagrasses, about 9 cm, which is equivalent to a maximum of 3 internodes. The low modular integration observed in *Z. noltii* suggests that the high rhizome elongation and clonal growth rate for this species do not depend much on accumulated reserves in the rhizome. Rather, a direct and immediate investment of photosynthates (soluble carbohydrates) in growth and a low accumulation of insoluble carbohydrate reserves (starch) are expected. In fact, this was observed in a current investigation of the circadian and seasonal variation of *Z. noltii* carbohydrates (J. Silva & R. Santos unpubl. data). This strategy may constitute a valuable feature of *Z. noltii* when withstanding physical disturbances such as those caused by clam harvest.

When shoots were severed at different positions along the rhizome, a strong effect was found on shoot production, internode production, rhizome elongation, and rhizome production rates only when the apical shoot was removed (Fig. 4.6). This supports the hypothesis that apical growth in *Z. noltii* is mostly dependent on apical shoot photoassimilates, contrary to what was observed in other seagrasses that rely on internal translocation of resources along the rhizome (Marbà et al. 2002). Physiological integration between shoots has been interpreted as an adaptive advantage for seagrasses, such that different modules can share resources produced by neighbouring modules and contribute to vegetative spread by apical meristem growth (Marbà et al. 2002). The *Z. noltii* strategy must differ from most seagrasses as it depends less on module integration yet is more able to react to heavy physical disturbance that fragment its clonal structure. In addition, no rhizome branching occurred in *Z. noltii* within the time of the experiment, indicating that apical dominance does not occur in *Z. noltii*, at least within a 30 days response time. Removal of the apical meristem in *Cymodocea nodosa* not only promoted branching but also elongation of the rhizome branches (Terrados et al. 1997b). A change in the growth form of the closest vertical rhizome into horizontal growth was also observed in *C. nodosa* as a result of apical dominance (Terrados et al. 1997a).

In conclusion, clam harvesting activity adversely affects *Z. noltii* populations, despite the great recovery capacity of the species. Meadow recovery may occur even if plants with only 1 or 2 modules, including the apical shoot, remain on the sediment. Clam harvesting in Ria Formosa may not allow the full recovery of *Z. noltii* meadows due to high frequency and intensity of disturbance, particularly during summer. Our results suggest that *Z. noltii* meadows may sustain clam harvest disturbance provided that the meadows are allowed to recover from isolated disturbance for about 1 month.

Acknowledgements. This study was funded by the EC project Monitoring and Managing of European Seagrass Beds (EVK3-CT-2000-00044) under the 5th Framework Programme. We are grateful to A. Quaresma and P. Teixeira for fieldwork assistance, as well as to Mr. Sousa from the clam culture concession. S.C. was supported by a PhD grant from Fundação para a Ciência e a Tecnologia (FCT).

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CHAPTER 5

Effects of burial and erosion on the seagrass *Zostera noltii*

Cabaço S, Santos R (2007)

Journal of Experimental Marine Biology and Ecology 340: 204-212

5. Effects of burial and erosion on the seagrass *Zostera noltii*

ABSTRACT: The effects of experimental burial and erosion on the seagrass *Zostera noltii* were assessed through *in situ* manipulation of the sediment level (-2 cm, 0 cm, +2 cm, +4 cm, +8 cm and +16 cm). Shoot density, leaf and sheath length, internode length, C and N content and carbohydrates of leaves and rhizomes were examined 1, 2, 4 and 8 weeks after disturbance. Both burial and erosion resulted in the decrease of shoot density for all the sediment levels. The threshold for total shoot loss was between 4 cm and 8 cm of burial, particularly during the 2nd week. A laboratory experiment confirmed that shoots did not survive more than 2 weeks under complete burial. There was no evidence of induced flowering by burial or erosion. As well, no clear evidence was found of sediment level effects on leaf and sheath length. Longer rhizome internodes were observed as a response to both burial and erosion, suggesting a plant attempt to relocate the leaf-producing meristems closer to sediment surface or in search of new sediment avoiding the eroded area. The C content of leaves and rhizomes, as well as the non-structural carbohydrates (mainly the starch in rhizomes), decreased significantly along the experimental period, indicating the internal mobilization of carbon to meet the plant demands as a consequence of light deprivation. The significant decrease of N content in leaves, and its simultaneous increase in rhizomes, suggests the internal translocation of nitrogen from leaves to rhizomes. About 50% of the N lost by the leaves was recovered by the rhizomes. Our results indicated that *Z. noltii* has a high sensitivity to burial and erosion disturbance, which should be considered in the management of coastal activities.

KEY WORDS: Burial, Disturbance, Erosion, Seagrass, Sedimentation, *Zostera noltii*

5.1. Introduction

Natural and human-induced activities can change the sedimentary dynamics of coastal areas and impact the aquatic vegetation. Although natural events such as the migration of sand barrier-island inlets (Cunha et al. 2005), earthquakes, volcanic eruptions or hurricanes (Short & Wyllie-Echeverria 1996) may contribute to local and large-scales losses, most of the seagrass declines are attributed to anthropogenic disturbances (Short & Wyllie-Echeverria 1996). Coastal activities that change sedimentary dynamics constitute a threat to seagrass survival, resulting in plant removal from the substratum and in burial or erosion of the seagrass meadows (Short & Wyllie-Echeverria 1996, Hemminga & Duarte 2000). Dredging, coastal engineering, beach stabilization, siltation, among others, are examples of human activities related to sediment redistribution in coastal areas with potential impacts for seagrasses (Onuf 1994, Burdick & Short 1999, Halun et al. 2002, Daby 2003, Ruiz & Romero 2003).

Burial and erosion have been reported to negatively affect seagrasses. Shoot mortality and changes in plant morphology were the main responses to experimental changes in sediment level of *Cymodocea nodosa* (Marbà & Duarte 1994), of *Posidonia oceanica* (Manzanera et al. 1998), of *Zostera marina* (Mills & Fonseca 2003) and of a mixed seagrass meadow (Duarte et al. 1997). Nevertheless, seagrasses may inhabit highly dynamic substrates. Marbà & Duarte (1995) demonstrated the coupling between the patch dynamics of *C. nodosa* and the migration of subaqueous dunes, whereas Cunha et al. (2005) reported on the landscape-scale changes of *Zostera noltii* patches associated with a highly dynamic sand barrier-island inlet.

Z. noltii is a small seagrass species occurring in the intertidal and subtidal areas of the Northern and Western Europe, Mediterranean Sea and North-West Africa (Den Hartog 1970). In the Ria Formosa lagoon (southern Portugal), *Z. noltii* is the most abundant seagrass species, playing an important role in the lagoon productivity (Santos et al. 2004). Changes in the sedimentary dynamics of Ria Formosa occur due to natural causes (e.g. inlet migration, storms) or as a result of human activities such as dredging of navigation channels, opening of artificial inlets, addition of allochthonous sediments in clam culture beds. The present study aimed to assess: (1) the effects of burial and erosion on the shoot density, plant morphology, carbon and nitrogen contents, and non-structural carbohydrates reserves of the seagrass *Z. noltii*, through the *in situ* experimental manipulation of the sediment level; and (2) the survivorship, longevity, production and growth of *Z. noltii* plants submitted to experimental burial in laboratory conditions.

5.2. Methods

5.2.1. *In situ* manipulation of the sediment level

The experiment was conducted in a homogenous and undisturbed *Z. noltii* meadow of the Ria Formosa lagoon (southern Portugal) in June and July 2004. This time of year was chosen to conduct the experiment because this period corresponds to the species higher growth rate (Peralta et al. 2005). The experimental design included 6 different imposed sediment levels: one erosion level (-2 cm), one level with the sediment maintained at the original position (0 cm), and four burial levels (+2, +4, +8 and +16 cm). Experimental treatments were performed using PVC cylinders (12 cm i.d.), placed at a 5 cm depth into the sediment. The cylinders were introduced in the sediment at referred depths to avoid them to be dragged by the strong tidal currents that occur in intertidal areas of the Ria Formosa lagoon. Cylinder height above the sediment was equivalent to the corresponding burial treatment, except in control and erosion where cylinders were placed at the sediment level. Erosion was imposed with a Venturi-effect suction device operated with a diving tank, which gently removed the sediment down to a depth of -2 cm without damaging the plants. The different burial levels were obtained by adding sandy sediments from the adjacent unvegetated area. Each treatment consisted of 20 replicates, randomly installed at about 1 m apart from each other. Five cylinders of each treatment were randomly collected 1, 2, 4 and 8 weeks after the onset of the disturbance. Additionally, five samples were also collected from the surrounding meadow, with a 12 cm diameter core, to control for the cylinder effect. In each sample, the number of shoots was counted to estimate shoot density. The leaf length and the sheath length were measured from intact shoots and the internode length was measured from intact rhizomes. The above and belowground biomass of *Z. noltii* was dried at 60 ºC for 48 h. Subsamples of dried leaves and rhizomes were ground on a ball mill for tissue content analysis (n = 3). C and N contents were determined using a CHN Perkin Elmer elemental analyser. The non-structural, soluble, carbohydrates (sugars) from the ground tissues (5 mg) were extracted in hot ethanol (80 °C) and measured by the phenol sulphuric acid method using glucose as standard (Dubois et al. 1956). Starch was extracted from the ethanol-insoluble fraction. Samples were washed in deionised water, autoclaved for 15 min, hydrolysed to glucose with an enzymatic suspension (α-amilase and amiloglucosidae), and determined spectrophotometrically as described before.

5.2.2. Laboratory burial experiment

In order to estimate the *Z. noltii* survivorship and longevity under complete burial, as well as its growth and production, 60 plants composed of 3 modules (i.e. 1 apical shoot plus 2 shoots and respective internodes) were collected in the field and immediately transported to the laboratory in seawater. Plants with 3 modules were selected as those with fewer modules may have higher survivorship due to clonal integration effects (Cabaço et al. 2005). Each plant was placed in an acrylic container (8.5 cm of diameter) filled with sandy sediments obtained from the same location where plants were collected. Half of the plants were completely buried, while the other half remained as controls, i.e. with sediment maintained at meristem level. Half of the buried (n = 15) and half of the control (n = 15) treatments were monitored once a week for plant survival and growth, while the other half were only checked in the end of the experiment to control the weekly manipulation effect. Fifteen containers of each treatment were randomly assigned into three replicate aquaria, kept with aerated seawater, at field temperature (18 °C) and salinity (35 PSU) conditions, in a plant growth chamber, with light intensity of 150 μmol quanta m^{-2} s^{-1} and a 12 h day:12 h night photoperiod. Apex shoots were tagged to distinguish new modular sets produced during the experimental period. The experiment was initiated in January 2005 and concluded after 30 days, when all the buried plants showed signs of necrosis. Then, the number of new shoots and internodes produced was counted to estimate the shoot and internode production rates (no. d^{-1}). The length of newly developed rhizome was measured to calculate rhizome elongation rate (mm d^{-1}). At the end of the experiment, some of the buried plants ($n = 15$) whose rhizomes showed black spots of necrosis, but not in the meristematic apex, were placed in seawater under normal light conditions to assess its recovery during the following weeks.

5.2.3. Statistical analysis

The effects of *in situ* manipulation of the sediment level were investigated using two-way ANOVA, with burial/erosion treatment and time as main factors. Differences in data sets testing the effects of laboratory burial on plant survival, production and growth were analysed using one-way ANOVA. Prior to statistical analyses, data were tested for homogeneity of variances and normality of distributions. When necessary, variables were log transformed to meet ANOVA assumptions. When ANOVA was significant ($p < 0.05$), the Tukey's multiple comparison test was applied to determine which treatment levels were significantly different. When ANOVA assumptions were not verified, the non-parametric test of Kruskal-Wallis was used (Sokal & Rohlf 1995).

5.3. Results

5.3.1. *In situ* manipulation of the sediment level

The shoot density of *Z. noltii* was significantly lower under the burial and erosion treatments than under both the control and the 0 cm treatment, revealing not only the negative effect of the introduction of the cylinder into the sediment but also a significant effect of the sediment level on density (Fig. 5.1A, Table 5.1). Besides, the shoot density was higher for the controls than for the 0 cm treatment. The shoot density decreased significantly during the second week of the experiment, when total shoot loss was observed under the more intense burial levels of +8 cm and +16 cm. The density of flowering shoots decreased significantly in all treatments along the experiment, but this pattern was also observed in the control meadow (Fig. 5.1B, Table 5.1).

Table 5.1. Statistical results and significance of ANOVA (F) or Kruskal-Wallis (H) tests for the effects of sediment level and time on *Zostera noltii*

Plant variable	Treatment	Time	Treatment × Time
Shoot density	F = 72.98 ***	F = 7.44 ***	F = 1.20 ^{ns}
Flowering shoot density	F = 6.64 ***	F = 6.46 ***	F = 1.22 ^{ns}
Leaf length	H = 116.30 ***	H = 29.12 ***	-
Sheath length	F = 15.76 ***	F = 24.94 ***	-
Internode length	H = 273.64 ***	H = 170.75 ***	-
Leaf C content	F = 10.79 ***	F = 64.33 ***	F = 1.98 *
Leaf N content	F = 19.53 ***	F = 81.57 ***	F = 2.68 **
Rhizome C content	F = 3.78 **	F = 8.67 ***	F = 1.16 ^{ns}
Rhizome N content	F = 4.16 **	F = 78.36 ***	F = 3.30 ***
Leaf sugar content	F = 30.71 ***	F = 1.10 ^{ns}	F = 4.99 ***
Leaf starch content	F = 0.51 ^{ns}	F = 1.37 ^{ns}	F = 1.09 ^{ns}
Rhizome sugar content	F = 2.04 ^{ns}	F = 21.18 ***	F = 1.90 *
Rhizome starch content	F = 3.24 **	F = 18.49 ***	F = 0.82 ^{ns}

(*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, (^{ns}) not significant

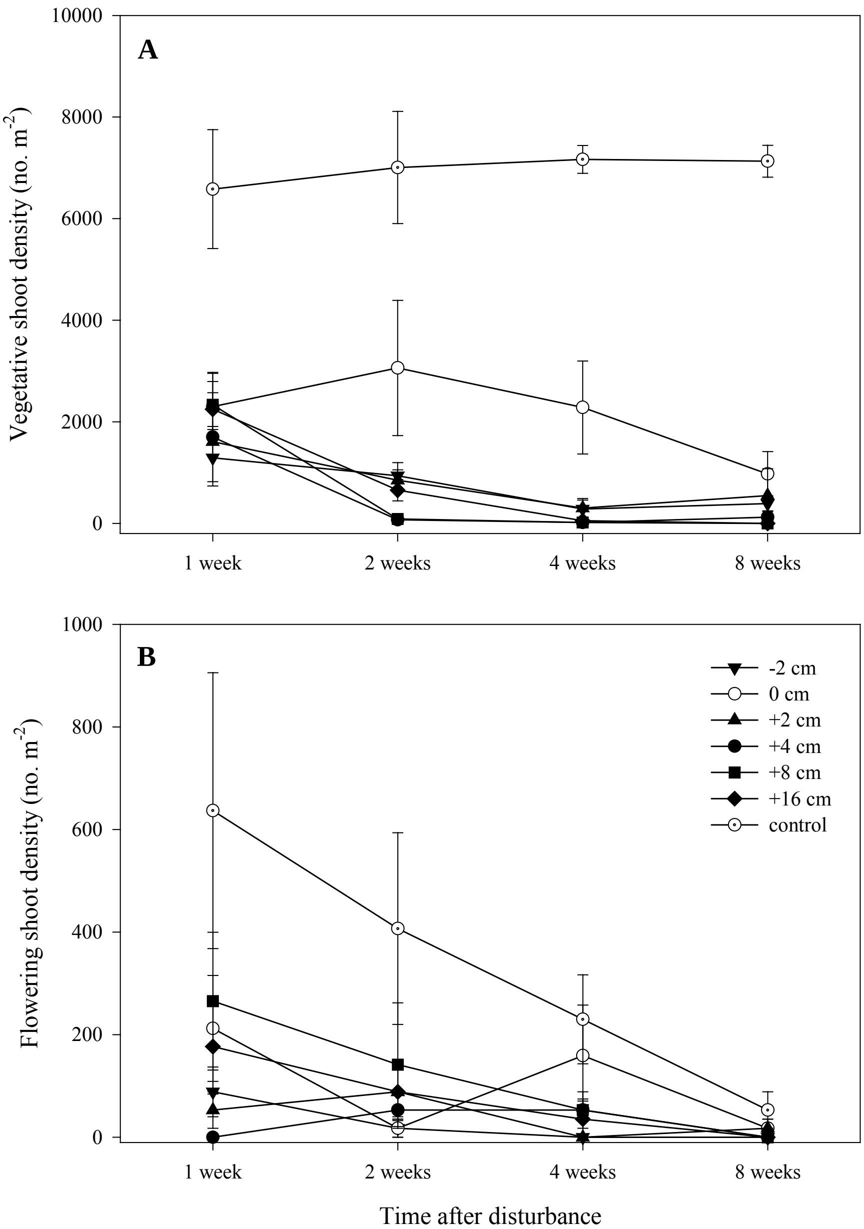

Fig. 5.1. Effects of *in situ* experimental manipulation of the sediment level on (A) vegetative shoot density and (B) flowering shoot density of *Zostera noltii* after 1, 2, 4 and 8 weeks (mean ± SE)

In spite of the observed decline of shoot density, the corresponding shoot biomass did not decrease (data not shown). The detached leaves remained under the sediment except in the erosion treatment where aerial biomass was carried away. The loss of aboveground biomass (detached leaves) occurred during the first week of the erosion treatment, remaining fairly constant in the period between the 1st and 8th week after disturbance. A portion of the leaves detached throughout the burial experiment remained green and did not show signs of necrosis. This was also observed in the laboratory burial experiment, in which detached green leaves were observed from the first week onwards.

At the end of the first week of experiment, the leaf length of the *Z. noltii* plants under the burial level of +16 cm and of control were significantly higher than of plants under -2 cm, 0 cm and +2 cm treatments (Fig. 5.2A). Then, the leaf length of *Z. noltii* decreased continuously along the experiment in all treatments (Fig. 5.2A) but not in the control. As well, the sheath length (Fig. 5.2B) decreased significantly along the experiment except in the -2 cm treatment where it remained high. The sheath length of the +2 cm treatment was as high as the -2 cm treatment after 4 weeks.

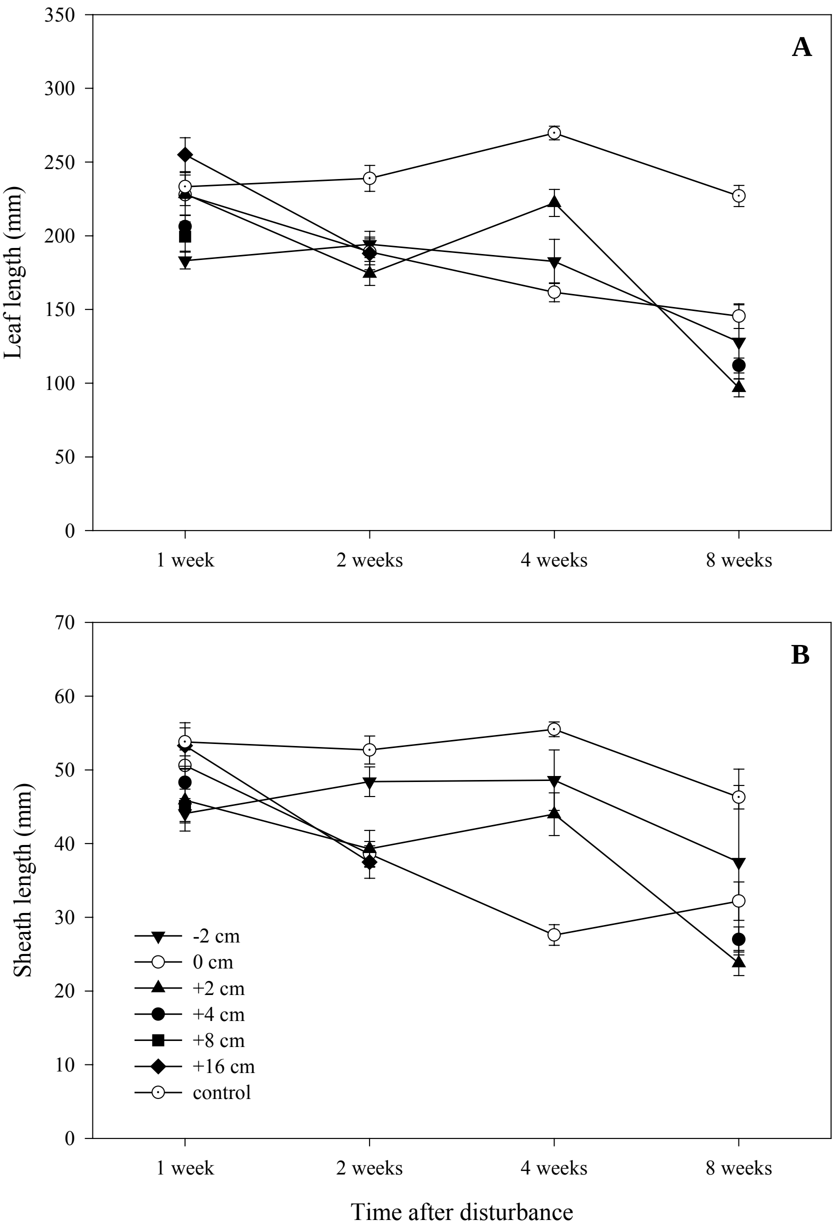

Fig. 5.2. Effects of *in situ* experimental manipulation of the sediment level on (A) leaf length and (B) sheath length of *Zostera noltii* plants after 1, 2, 4 and 8 weeks (mean ± SE). Missing data were due to lack of intact leaves

The internode length of the rhizomes decreased significantly along the experiment in all treatments (Table 5.1), but it was significantly higher than the control until the 4th week (Fig. 5.3). The internode length of the rhizomes of the 0 cm treatment was significantly lower than the other treatments indicating a significant effect of burial and erosion on internode length on top of the effect of the introduction of the cylinder into the sediment.

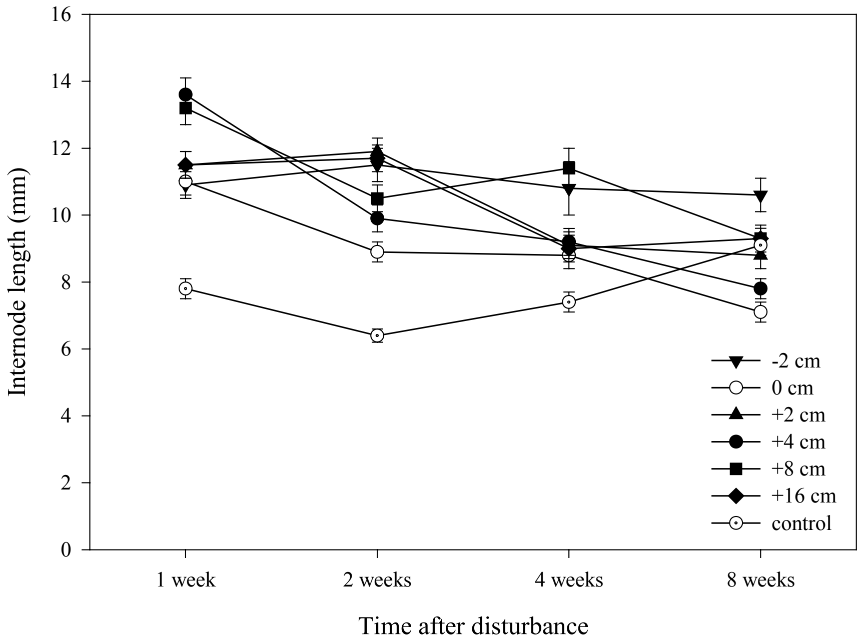

Fig. 5.3. Effects of *in situ* experimental manipulation of the sediment level on internode length of *Zostera noltii* rhizomes after 1, 2, 4 and 8 weeks (mean ± SE)

The C and N content of both leaves and rhizomes of *Z. noltii* varied significantly among treatments and sampling time (Table 5.1). In general, the C content of leaves and rhizomes and the N content of leaves of all treatments decreased along the experiment while the N content of the rhizomes increased (Fig. 5.4). These patterns were mostly caused by the experimental disturbance than by burial level as all treatments, including the 0 cm treatment, showed the same variation pattern, which was different from the control. On the other hand, the C content of leaves from more intense burial treatments (+4 cm, +8 cm, +16 cm) was significantly lower than other treatments indicating a burial level effect (Fig. 5.4A). The significant relationship between the leaf and rhizome

N contents along the 8 weeks of the experiment (Fig. 5.5) revealed that there was an active transfer of nitrogen, of about 50%, from the leaves to the rhizomes.

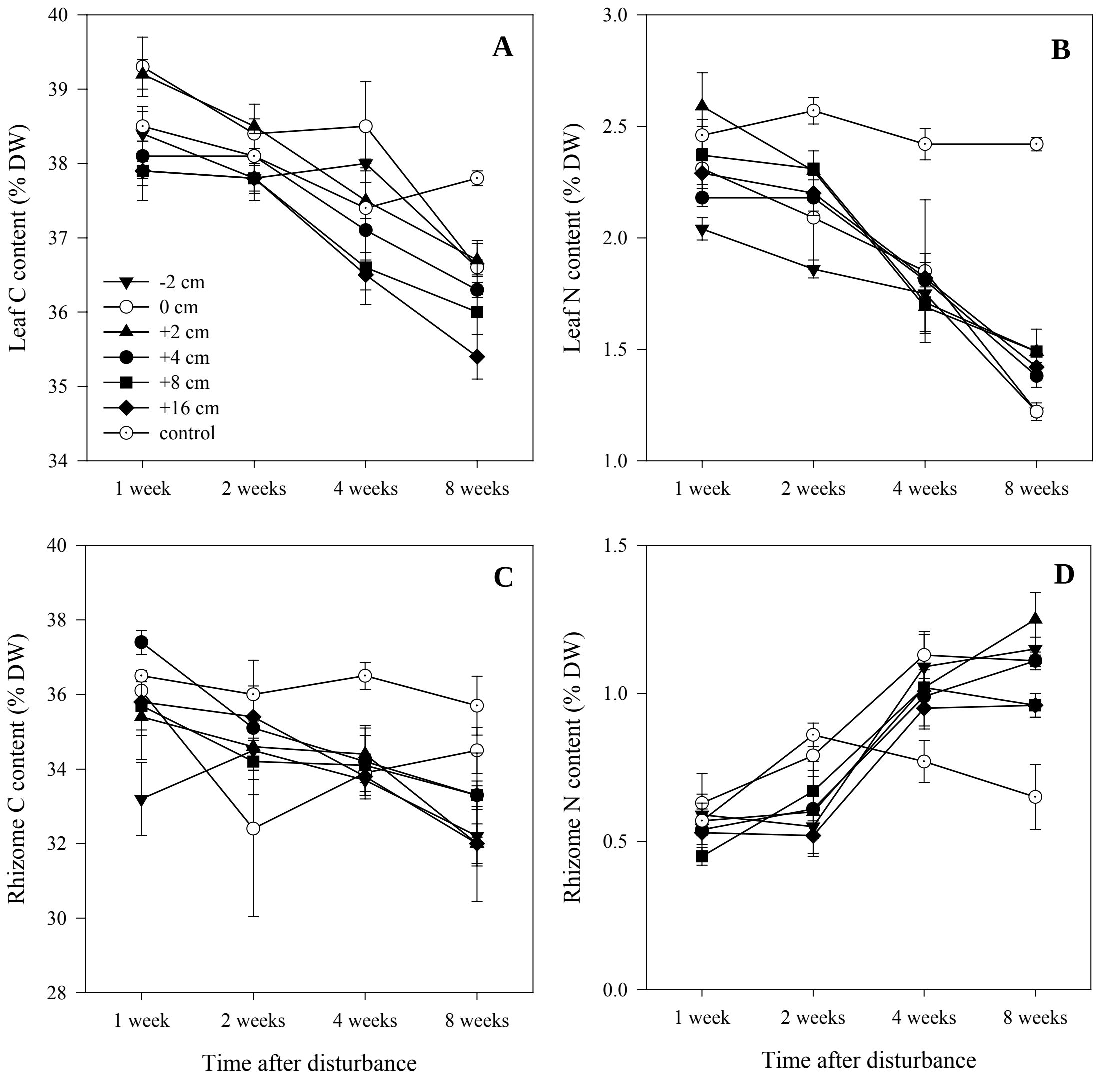

Fig. 5.4. Effects of *in situ* experimental manipulation of the sediment level on (A) leaf carbon, (B) leaf nitrogen, (C) rhizome carbon and (D) rhizome nitrogen contents of *Zostera noltii* after 1, 2, 4 and 8 weeks (mean ± SE)

The soluble sugar of *Z. noltii* leaves increased significantly with the erosion treatment (-2 cm) and with the +4 cm burial treatment (Fig. 5.6A). No significant differences were found along the sampling time (Table 5.1). On the contrary, the soluble sugar content in the rhizomes (Fig. 5.6C) did not vary significantly among treatments, but varied in time (Table 5.1), decreasing significantly after 4 and 8 weeks in all treatments. No significant differences were found for the starch content of leaves among treatments or time (Fig. 5.6B, Table 5.1). On the other hand, the starch content of rhizomes varied significantly among treatments and time (Table 5.1). This was due to the significantly lower starch content of the rhizomes from the -2 cm treatment relative to the other treatments and control in the first 2 weeks of the experiment (Fig. 5.6D). After 4 weeks, no significant differences were found.

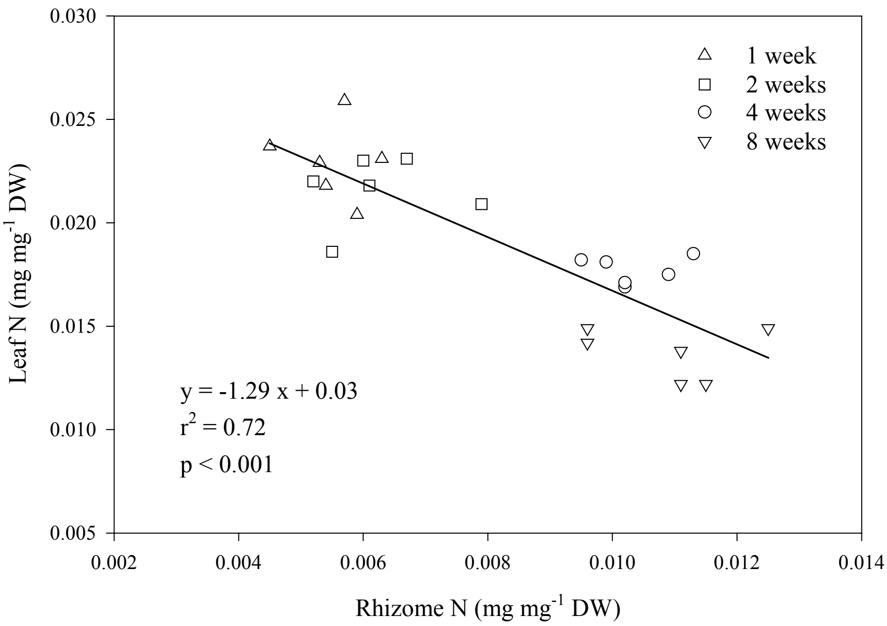

Fig. 5.5. Relationship between the mean N content of leaves and the mean N content of rhizomes of *Zostera noltii* along the 8 weeks of the experiment

The same significance levels reported in Table 5.1 were found for plant variables when only the treatments, excluding the control, were considered in statistical analysis, expect for the flowering shoot density and the rhizome C content, which became not significant.

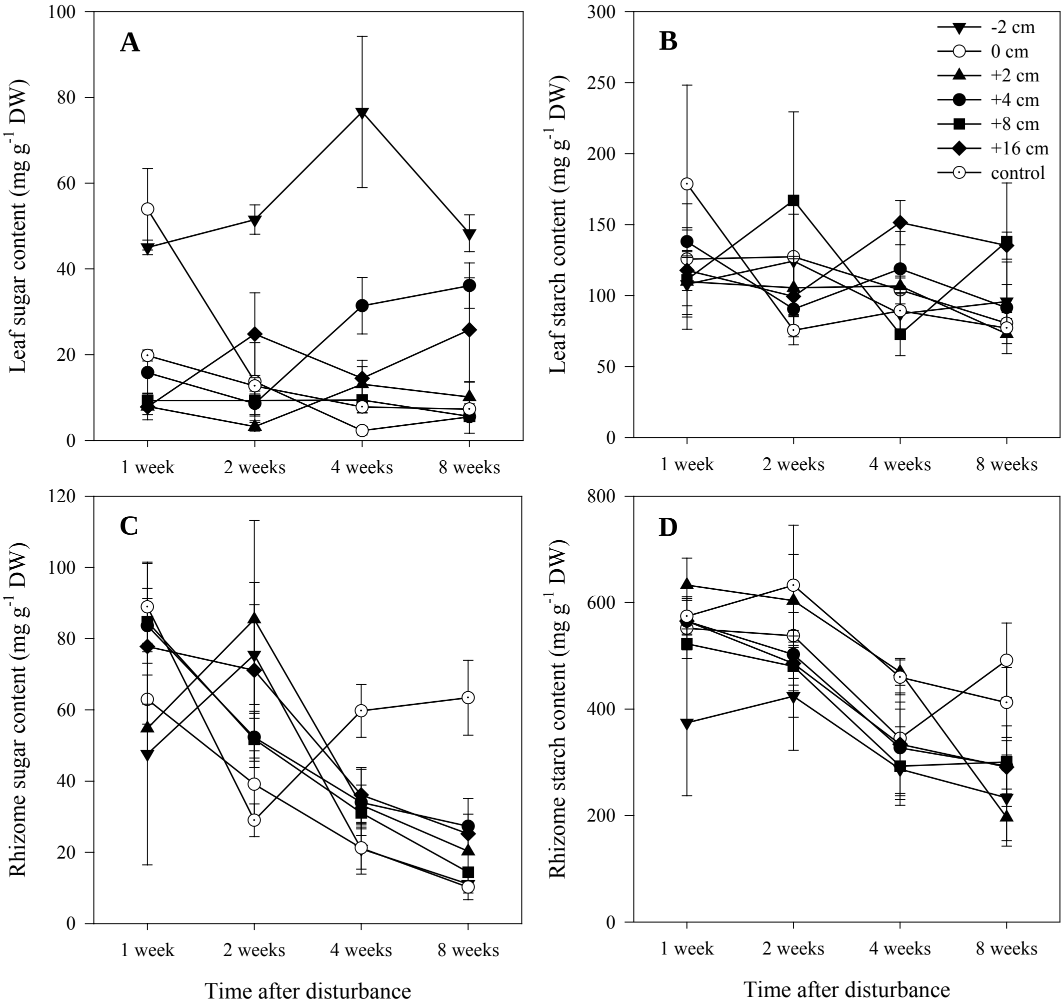

Fig. 5.6. Effects of *in situ* experimental manipulation of the sediment level on (A) leaf soluble sugar, (B) leaf starch, (C) rhizome soluble sugar and (D) rhizome starch contents of *Zostera noltii* after 1, 2, 4 and 8 weeks (mean ± SE)

5.3.2. Laboratory burial experiment

The survival of *Z. noltii* plants buried in the laboratory experiment decreased significantly after 1 and 2 weeks, reaching 0% after 3 weeks (Table 5.2). On the contrary, the survival of control plants was 100% at the end of the experiment. No growth and production occurred on buried plants as opposed to the control plants, where rhizome growth and production of shoots and internodes was observed (Table 5.2). No significant differences were found between plants manipulated every week along the experiment and plants manipulated only at the end of the experiment (Table 5.2). The rhizomes buried in this experiment did not recover within 1 and 2 weeks of recovery time. Apparently they were already necrotic (black) after 1 week of recovery.

Table 5.2. Effects of laboratory burial on survival, production and growth of *Zostera noltii* plants (mean ± SE), and significance of one-way ANOVA

	Control	Burial	p
Plant survival (%)			
1 week	100 ± 0.0	93.3 ± 6.7	< 0.001
2 weeks	100 ± 0.0	33.3 ± 17.6	< 0.001
3 weeks	100 ± 0.0	0.0 ± 0.0	< 0.001
4 weeks	100 ± 0.0	0.0 ± 0.0	< 0.001
	(100 ± 0.0)	(0.0 ± 0.0)	
Shoot production (no. d^{-1})	0.06 ± 0.01	0.00 ± 0.00	< 0.001
	(0.05 ± 0.01)	(0.00 ± 0.00)	
Internode production (no. d^{-1})	0.06 ± 0.01	0.00 ± 0.00	< 0.001
	(0.05 ± 0.01)	(0.00 ± 0.00)	
Rhizome elongation (mm d^{-1})	0.38 ± 0.06	0.00 ± 0.00	< 0.001
	(0.37 ± 0.05)	(0.00 ± 0.00)	

Values in brackets were obtained in plants that were manipulated only at the end of the experiment rather than every week

5.4. Discussion

The results of this study provide experimental evidence of the negative effects of burial and erosion events on the seagrass *Z. noltii*. Experimental burial and erosion resulted in the decrease of shoot density for all the sediment levels, suggesting that the relative importance of mortality versus recruitment increased. Laboratory data showed that shoots did not survive more than 2 weeks under complete burial (Table 5.2).

Positive effects of burial on shoot mortality have been previously reported for other seagrass species (Marbà & Duarte 1994, Manzanera et al. 1998, Mills & Fonseca 2003), but contrary to our observations, mortality was related to sediment depth. These studies were conducted in larger seagrasses (*C. nodosa*, *P. oceanica* and *Z. marina*) that could easily support low levels of sedimentation, being only strongly affected at considerable burial depths. In a mixed seagrass meadow, Duarte et al. (1997) described a pattern of species loss in which the effects of burial on mortality were dependent of seagrass size. Our results were consistent with this pattern, as *Z. noltii* is one of the smallest seagrass species and all burial treatments resulted in a high loss of shoots. The threshold for total shoot loss was between 4 cm and 8 cm of burial (Fig. 5.1A).

Despite both natural and human-induced disturbances have been reported to increase seagrass flowering intensity (Gallegos et al. 1992, Marbà & Duarte 1995, Alexandre et al. 2005), there was no evidence of induced flowering in *Z. noltii*. The flowering shoot density decreased in all treatments (Fig. 5.1B), following the decrease of vegetative shoots along the experiment and the natural flowering decrease observed in the control surrounding area.

The leaf length response of seagrasses to burial seems to be species-specific. Marbà & Duarte (1994) showed that *C. nodosa* responded to burial by increasing the length of leaves whereas Mills & Fonseca (2003) showed that *Z. marina* had the opposite response. The experiment developed here showed a reduction of the leaf length of *Z. noltii* in all treatments including the control for the effect of the experimental manipulation. As there were no clear differences between treatments we conclude that the observed pattern was an effect of the cylinder introduction into the sediment, which probably damaged the rhizomes.

On the other hand, it has been reported that seagrasses have the ability to increase their vertical internodal length as a response to burial (Marbà & Duarte 1994, Marbà et al. 1994a,b, Marbà & Duarte 1995, Duarte et al. 1997). Even though *Z. noltii* lacks vertical rhizomes, significantly longer internodes of the horizontal rhizome were observed both as a response to burial and erosion, and to the experimental manipulation (Fig. 5.3). However, contrary to the leaf length response, there was a significant effect of burial and erosion compared to the experimental manipulation control. The response to burial may reflect the plant attempt to relocate the leaf producing meristems closer to sediment surface, through the bending of the horizontal rhizome towards the sediment surface. This bending was found in our samples but it was not possible to quantify this effect. The response observed for the plants subject to erosion may reflect the plant response in search of sediment, avoiding the eroded area. The experimental manipulation induced the same effect, probably as a consequence of the damage of the apical portions of rhizomes by the cylinders as Terrados et al. (1997) reported for *C. nodosa*.

The overall decay of *Z. noltii* C content of leaves and rhizomes along the experimental period (Fig. 5.4), particularly in severe burial treatments, indicates the internal mobilization of carbon to meet the plant demands as a consequence of the light deprivation. This is also reflected by the observed decrease of the non-structural carbohydrates, mainly the starch content in rhizomes. Brun et al. (2003a,b) also found that *Z. noltii* under low-light conditions quickly mobilizes non-structural carbohydrates to meet carbon demands. However, this species seems to have a poor ability to survive under low-light conditions due to the small starch reserves and the lack of sucrose mobilization within the plant in comparison with other seagrass species (Brun et al. 2003a). The fact that most of the *Z. noltii* plants under the complete burial experiment died between the 1st (7% mortality) and the 2nd week (67% mortality) may be a consequence of the low storage capacity of this species. Contrasting with the burial treatments, erosion resulted in an interesting and significant increase of the leaf sugar content (Fig. 5.6). This production of sugars in the leaves may represent a physiological response of the plants previous to the biomass investment for the space occupation process in the search for non-eroded sediments. Curiously, the internode length of the eroded *Z. noltii* plants was significantly higher after 8 weeks than of plants of burial treatments.

The decrease of the N content in *Z. noltii* leaves, and its simultaneous increase in rhizomes along the experimental period (Fig. 5.5), suggests the internal translocation of nitrogen from the senescent photosynthetic tissues (leaves) into the storage organs (rhizomes). About 50% of the N lost by the leaves was recovered by the plant to the rhizomes. The burial laboratory experiment revealed that shoots died before rhizomes showed signs of necrosis. This internal translocation may represent a survival strategy that allows *Z. noltii* to recover if the burial event reverts in a short-term period.

Z. noltii did not show signs of recovery within the time range of the experiment, 2 months, independent of the sediment level. However, the species may survive and recover from short-term events of burial of about 1 week. The complete burial laboratory experiment revealed a mortality threshold of the plants between 1 and 2 weeks, when mortality increased one order of magnitude from about 7% to 70%. Additionally, the field experiment showed that the shoot density after one week was not significantly different from the experimental manipulation control but it decreased sharply during the second week. Duarte et al. (1997) found that other small seagrasses, such as *Halophila ovalis* and *Halodule uninervis* were able to recover within 4 months after burial disturbance, with some species reaching much higher densities in high-burial treatments than in controls.

In the Ria Formosa lagoon, the natural inlet migration and inlet relocation result in deep sedimentary changes with burial and erosion rates happening in a scale of meters (Vila-Concejo et al. 2003), which is not compatible with the low burial threshold for

Z. noltii, of the order of centimeters. Such events induce dramatic changes in the seagrass landscapes that take many years to recover, as those reported by Cunha et al. (2005). However, these are extreme disturbances that occur only sporadically and near the inlets. Most changes in the sedimentary dynamics of coastal systems result from disturbances that are not so drastic, involving sediment redistribution at lower levels.

In conclusion, the critical level of burial or erosion tolerated by the seagrass *Z. noltii* is extremely low (between +4 and +8 cm), due to the small size of this species and the lack of vertical rhizomes. Despite *Z. noltii* being a fast-growing species, recovery did not occur within the experimental period (2 months), even though laboratory experiment revealed that plants survived for 1-2 weeks under complete burial. Since slight burial and erosion events negatively affect *Z. noltii*, the coastal activities in the Ria Formosa lagoon or elsewhere that result in sediment level changes should take into account the high sensitivity of this species.

Acknowledgements. This study was funded by the EC project "Monitoring and Managing of European Seagrass Beds" (EVK3- CT-2000-00044). We are grateful to A. Alexandre, A.H. Cunha, C. Godinho, J. Silva, P. Morais and P. Teixeira for precious fieldwork assistance. Additional thanks to J. Silva for valuable comments on the manuscript. We thank C.M. Duarte for helpful contribution on the experimental design and comments on the manuscript. S.C. was supported by a PhD grant from Fundação para a Ciência e a Tecnologia (SFRH/BD/2880/2000).

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CHAPTER 6

The impacts of sediment burial and erosion on seagrasses: a review

Cabaço S, Santos R, Duarte CM (submitted)

Marine Ecology Progress Series

6. The impacts of sediment burial and erosion on seagrasses: a review

ABSTRACT: The available information from both experimental and descriptive studies on the effects of sediment burial and erosion on seagrasses was compiled in order to synthesize the information regarding the species-specific impacts and to relate them to plant size, architecture and growth. Burial thresholds, i.e. the burial levels causing 50% and 100% shoot mortality, and mortality-burial curves were estimated for the 15 seagrass species subject to experimental burial. All the species investigated reached 50% mortality at burial levels ranging from 2 cm (*Halophila ovalis*) to 19.5 cm (*Posidonia australis*). *Posidonia australis* was the most tolerant seagrass species to burial, while *Thalassia testudinum* was the most tolerant species to erosion. The implications of plant size in the burial levels sustained by seagrass species were examined through allometric relationships. The analysis revealed strong relationships between the burial thresholds and the shoot mass, the rhizome diameter, the aboveground biomass, the horizontal rhizome elongation and the leaf length of seagrass species. The leaf size and the rhizome diameter are the best predictors of the capacity of seagrasses to resist burial. The burial thresholds estimated for seagrass species were in many cases in agreement with the burial impacts described by field observations (bioturbation), while in some cases it was related to the species long-term colonization capacity (dune migration). Most human-induced impacts result in deep changes of the sedimentary environment, with permanent negative effects on seagrass meadows (regression and complete destruction), whereas natural events, whether catastrophic (hurricane) or regular (dune migration), allow the recovery and/or adaptation of seagrasses to the burial/erosion sediment dynamics. The available information on the effects of burial and erosion on seagrasses indicate that seagrasses are highly vulnerable to changes of the sediment level. The extent of the effects is species-specific and depends on the magnitude and on the frequency of disturbance.

KEY WORDS: Seagrass, Burial, Erosion, Sediment, Impacts, Disturbance, Sediment redistribution, Plant size

6.1. Introduction

Concerns about widespread seagrass loss have lead to examination of the contribution of loss sources (e.g. Duarte 2002, Orth et al. 2006). Whereas the negative consequences of eutrophication are believed to be a major driver of losses, intense sediment dynamics involving excessive burial or erosion have also been identified as widespread causes of loss. Events such as hurricanes, typhoons and storms induce intense sedimentary dynamics that may result in large-scale destruction of seagrass habitat (Preen et al. 1995, Fourqurean & Rutten 2004). The motion of sand waves (Marbà et al. 1994a) and migration of barrier-islands (Cunha et al. 2005) by currents can lead to intense burial and erosion. Sediment redistribution may also result from small-scale disturbances related to the activities of burrowing animals (Suchanek 1983, Philippart 1994, Duarte et al. 1997, Dumbauld & Wyllie-Echeverria 2003), which affects seagrasses locally through the mobilization of sediments at small scales within meadows (Suchanek 1983). Lastly, global warming is leading to increased sea-level rise, with a parallel tendency for coastal erosion, causing seagrass loss (Duarte 2002).

Human activities in coastal areas may also affect sedimentary processes, severely affecting seagrass meadows. Coastal works (e.g. harbors, docks, breakwaters), beach stabilization, dredging and excess siltation from changes in land catchments, are examples of anthropogenic activities that result in changes of the sedimentary dynamics and consequent seagrass loss (Onuf 1994, Terrados et al. 1998, Burdick & Short 1999, Halun et al. 2002, Ruiz & Romero 2003, Badalamenti et al. 2006). Frequently, such human-induced activities result in complete, perhaps irreversible, disappearance of seagrass meadows from coastal areas. The construction of permanent structures (e.g. harbors, Ruiz & Romero 2003) and the local modifications of hydrodynamics and sedimentary dynamics (e.g. land reclamation, Meinesz et al. 1991) may exclude seagrasses from such unfavorable environments (Meinesz et al. 1991, Duarte 2002).

The responses of seagrasses to sediment burial and erosion have been assessed through both experimental and descriptive studies, but a comparative analysis of the available information to provide a synthesis of the response of seagrass species to burial and erosion is still lacking. This study aims to compile the available information on species-specific impacts of burial and erosion disturbance to test the hypotheses that the vulnerability of different seagrass species to burial and erosion are dependent on plant size, architecture and growth. Allometric relationships between these seagrass characteristics and experimentally-determined burial thresholds were analysed in order to identify which seagrass characteristics better predict the burial effects. These relationships will allow the prediction of the impacts of burial on seagrasses from consideration of architectural characteristics of each species.

6.2. Methods

6.2.1. Experimental effects of burial and erosion on seagrass species

Available data on the experimental effects of burial and erosion on seagrass species were compiled from both published literature and reports (Table 6.1). The experiments typically involved different treatments of burial pulses followed by the observation of the response of the seagrasses. None of the experiments involved a sustained sediment supply. Most experiments were conducted in the field, and involved several (3 to 6) burial treatments and, in some cases (4 species tested) an erosion treatment, as well as controls. Treatments were imposed using PVC cylinders or plots placed in the sediment to a height above the sediment equivalent to the corresponding burial treatment or at the maximum burial level tested.

Table 6.1. Seagrass species for which resistance to burial and erosion have been examined, and the corresponding sources

Species	Location	Reference
Cymodocea nodosa	Alfacs Bay, Spain (Lab)	Marbà & Duarte 1994
Cymodocea rotundata	The Philippines*	Duarte et al. 1997
Cymodocea serrulata	The Philippines*	Duarte et al. 1997
Enhalus acoroides	The Philippines*	Duarte et al. 1997
Halodule uninervis	The Philippines*	Duarte et al. 1997
Halophila ovalis	The Philippines*	Duarte et al. 1997
Posidonia australis	West Australia	Ruiz 2000
Posidonia oceanica	NE Spain	Manzanera et al. 1998
Posidonia oceanica	SE Spain	Ruiz 2000
Posidonia sinuosa	West Australia	Ruiz 2000
Syringodium filiforme	Caribbean, Mexico	Cruz-Palacios & van Tussenbroek 2005
Syringodium isoetifolium	The Philippines*	Duarte et al. 1997
Thalassia hemprichii	The Philippines*	Duarte et al. 1997
Thalassia testudinum	Caribbean, Mexico	Cruz-Palacios & van Tussenbroek 2005
Zostera marina	Beaufort, USA	Mills & Fonseca 2003
Zostera noltii	South Portugal	Cabaço & Santos 2007

(*) Mixed seagrass meadow

Shoot mortality (M), when not provided by the authors, was calculated as:

$$M\ (\%) = (d_i - d_f)/d_i \times 100$$

where d_i is initial shoot density and d_f is final shoot density. Mortality of seagrass species was determined for each burial level tested and averaged for the overall experimental period, when the authors considered more than one sampling moment (e.g. Duarte et al. 1997). The burial thresholds of each seagrass species was defined as the burial levels causing 50% and 100% of mortality, and derived from information on changes in M with increasing burial, or interpolated from the data provided if necessary.

6.2.2. Scaling between burial thresholds and plant size, architecture and growth

The plant size:burial ratio (SBR) was estimated as the ratio between the leaf length of each seagrass species and the maximum burial level tested. The plant size:erosion ratio (SER) was estimated as the ratio between the anchoring depth of each species and the maximum erosion level tested. The anchoring depth was calculated as the sum of the leaf sheath length (which remain within the sediment), the length of the vertical rhizome (if present) and the diameter of the horizontal rhizome, as defined by Cruz-Palacios & van Tussenbroek (2005). The anchoring depth of *Zostera noltii* was calculated based on our own observations that only one-third of the sheath length is actually buried.

Seagrass characteristics (plant size, architecture and growth; Table 6.2) were derived from the information provided in the literature, compiled by Duarte et al. (1998), Marbà & Duarte (1998) and Marbà & Duarte (2003), and amended, when necessary, with recently published information. Data on seagrass leaf length (minimum and maximum leaf length for each species) was obtained from Phillips & Meñez (1988) and Kuo & Den Hartog (2001). The relationships between the burial thresholds (Y) and seagrass characteristics (X) were tested by fitting allometric equations of the form $Y = a.X^b$, using least squares linear regression analyses on log-transformed variables (Niklas 1994, Sokal & Rohlf 1995).

6.2.3. Descriptive impacts of burial and erosion on seagrass meadows

The information from descriptive-field studies reporting the effects of natural- and human-induced changes of the sedimentary dynamics on seagrass meadows were compiled from the published literature to summarise the most important effects of burial and erosion for each species.

Table 6.2. Size, growth and biomass of the seagrass species examined. Mean aboveground biomass (AB, g DW m^{-2}), shoot mass (SM, g DW), shoot density (SD, shoots m^{-2}), leaf length (LL, cm), rhizome diameter (RD, mm), horizontal internodal length (HIL, mm), vertical internode length (VIL, mm), horizontal rhizome elongation rate (HE, cm yr^{-1}), vertical rhizome elongation rate (VE, cm yr^{-1}), internodes between shoots (IS, no. of internodes) and length between shoots (LS, cm). Data compiled from Marbà & Duarte (1998), Duarte et al. (1998) and Marbà & Duarte (2003). Leaf length range compiled from Phillips & Meñez (1988) and Kuo & Den Hartog (2001). *Zostera noltii* data was updated with data from Cabaço & Santos (2007)

Species	AB	SM	SD	LL	RD	HIL	VIL	HE	VE	IS	LS
C. nodosa	245.7	0.090	2028.5	10-30	2.73	25.34	1.36	40.2	1.43	2.44	2.77
C. rotundata	27.1	0.065	215.5	7-15	2.44	28.75	1.97	209.9	1.53	7.61	4.80
C. serrulata	76.1	0.121	313.3	6-15	2.78	37.97	5.22	153.0	13.10	1.61	5.30
E. acoroides[a]	300.2	1.465	21.0	30-150	14.13	4.70		3.1		14.22	6.68
H. uninervis	297.9	0.027	71.5	6-15	1.37	20.97	4.95	101.2	4.10	5.17	270
H. ovalis[a]	39.0	0.016	1737.1	1-4	1.30	17.00		357.5		1.00	1.70
P. australis	241.3	0.875	605.0	30-60	7.21	14.90	-	9.3	1.42	7.36	6.00
P. oceanica	646.7	0.657	503.5	40-50	10.75	2.71	0.95	2.8	2.70	-	-
P. sinuosa	208.9	0.265	743.0	30-70	5.53	10.58	2.68	3.6	12.62	3.80	4.47
S. filiforme	185.4	0.037	4080.0	10-30	2.77	23.30	5.60	122.5	3.36	1.00	3.06
S. isoetifolium	52.0	0.038	1514.5	7-30	1.74	26.65	11.09	109.1	8.55	1.50	3.70
T. hemprichii	201.3	0.156	844.4	10-40	3.63	4.25	1.03	54.1	3.25	20.18	6.34
T. testudinum	907.1	0.238	1315.0	10-60	5.96	14.80	2.03	69.3	3.89	13.37	6.63
Z. marina[a]	418.9	0.323	852.6	-	3.50	10.58		26.1		5.59	6.08
Z. noltii[a]	122.1	0.011	7926.5	6-22	1.54	10.87		68.4		2.66	2.07

([a]) Species without vertical rhizomes. (-) Data not available

6.3. Results

6.3.1. Experimental effects of burial and erosion on seagrass species

6.3.1.1. Experimental burial

The effects of experimental burial have been reported for 15 species, corresponding to about one-third of the seagrass flora (Table 6.1) and encompassing a broad size range. From all the seagrass species analysed, including a wide range of plants size, some did not experience 100% mortality (*Cymodocea serrulata*, *Enhalus acoroides*, *Halodule uninervis*, *Posidonia australis*, *Posidonia sinuosa*, *Syringodium isoetifolium*, *Thalassia hemprichii*, *Thalassia testudinum*) even at the highest burial levels applied (16-30 cm, Table 6.3). Among those species, only *Enhalus acoroides* does not have vertical rhizomes. The burial level causing total shoot loss varied between 2 cm for *Halophila ovalis*, and 15 cm for *Posidonia oceanica*. All species investigated showed 50% mortality within the time span of the experiment (Table 6.3). The corresponding burial threshold for 50% mortality ranged from 2 cm for *Cymodocea rotundata*, *Cymodocea serrulata*, *Halophila ovalis* and *Zostera noltii*, and 19.5 cm for *Posidonia australis*, but most species experienced 50% mortality within the 2-4 cm range. *Cymodocea serrulata* and *Enhalus acoroides* showed relatively low shoot mortality (ca. 40% and 20%, respectively) after 10 months under 16 cm of sediment, indicating a capacity to survive long-term burial (Figs. 6.1A & 6.1C).

Species lacking vertical rhizomes, such as *Zostera marina* and *Zostera noltii*, experienced high mortality (70-90%) under low burial levels (2-4 cm, Fig. 6.1D), while species with vertical rhizomes, such as *Posidonia* spp. showed high mortality only under high burial levels (> 10 cm, Fig. 6.1E). However, some seagrass species with vertical rhizomes also showed high mortality under low burial levels of 4-5 cm (80% mortality, *Syringodium filiforme* and 60% mortality, *Halodule uninervis*, Fig. 6.1B).

Table 6.3. Details of the experimental design to test the effects of burial on seagrasses (burial levels tested, the duration of the experiments, the size:burial ratio (SBR)) and the resulting effect on seagrass survival summarised in the experimental burial levels causing 50% and 100% mortality

Species	Burial levels (cm)	Experimental period (days)	SBR	Burial level (cm)	
				50% mort.	100% mort.
C. nodosa	1, 2, 4, 7, 13, 16	35	0.6	4	13
C. rotundata	2, 4, 8, 16	60, 120, 300		2	8
C. serrulata	2, 4, 8, 16	60, 120, 300		2	-
E. acoroides	2, 4, 8, 16	60, 120, 300		4	-
H. uninervis	2, 4, 8, 16	60, 120, 300		4	-
H. ovalis	2, 4, 8, 16	60, 120, 300		2	2
P. australis	10, 15, 20, 30	50	1.3 [c]	19.5	-
P. oceanica [a]	5/7, 9/10, 13/14	250	1.4	14	14
P. oceanica [b]	3, 6, 9, 12, 15	45	1.3 [d]	10.2	15
P. sinuosa	10, 15, 20, 30	50	1.3 [c]	15.4	-
S. filiforme	3.5/4.5, 4/5, 6.5/7.5, 9/10	60	0.8	4.5	10
S. isoetifolium	2, 4, 8, 16	60, 120, 300		8	-
T. hemprichii	2, 4, 8, 16	60, 120, 300		4	-
T. testudinum	3.5/4.5, 4/5, 6.5/7.5, 9/10	60	1.2	5	-
Z. marina	4, 8, 12, 16	12, 24	1.0	4	12
Z. noltii	2, 4, 8, 16	7, 14, 28, 56	< 1*	2	8

(-) Total shoot loss did not occur for the tested burial levels; ([a]) Manzanera et al. 1998; ([b]) Ruiz 2000; ([c]) Leaf length from Smith & Walker (2002) for the same area; ([d]) Leaf length from Manzanera et al. (1998); (*) intertidal species, leaves are buried even at low burial levels

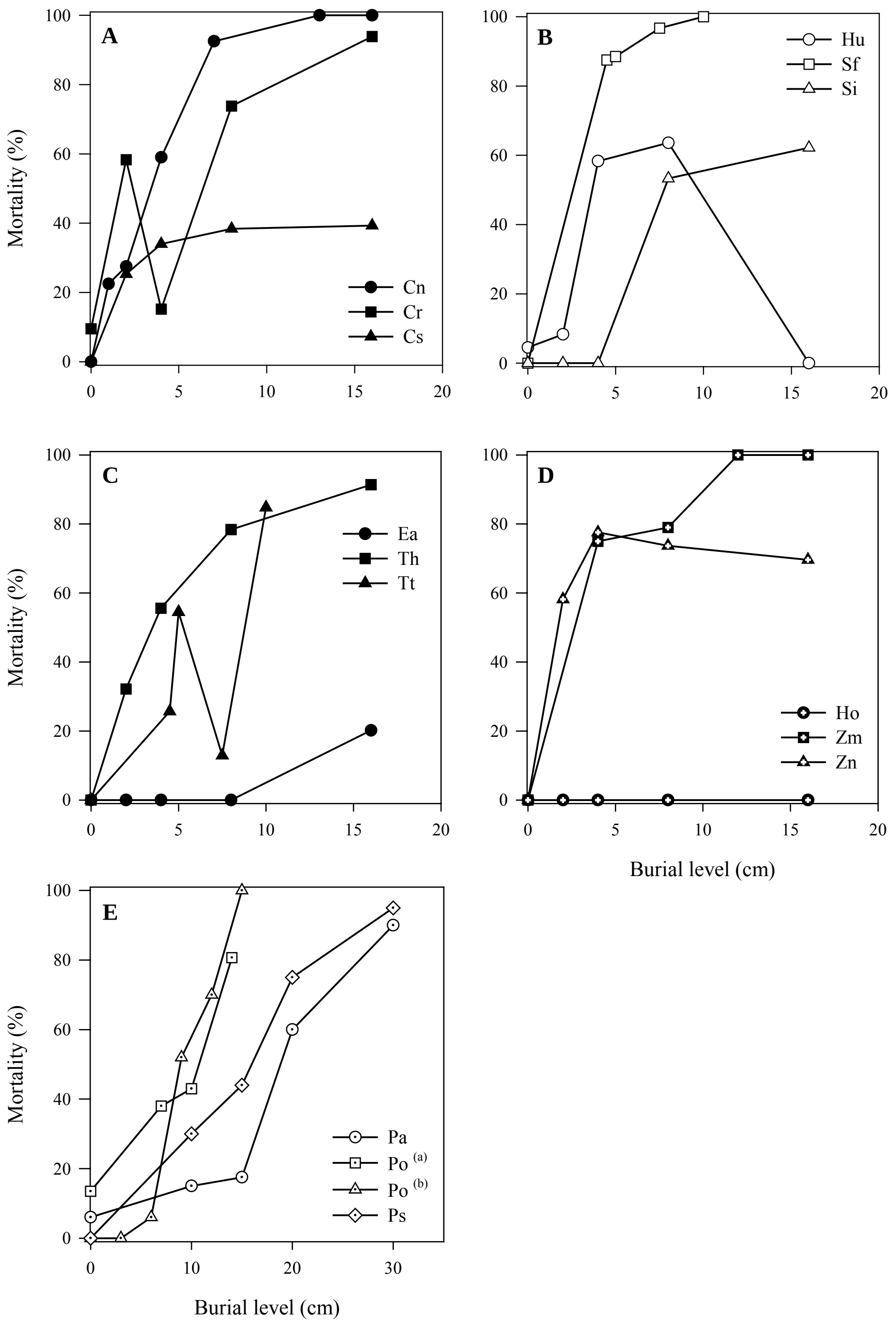

Fig. 6.1. The relationship between shoot mortality and burial levels in seagrasses subject to experimental burial. Cn: *Cymodocea nodosa*, Cr: *Cymodocea rotundata*, Cs: *Cymodocea serrulata*, Ea: *Enhalus acoroides*, Hu: *Halodule uninervis*, Ho: *Halophila ovalis*, Pa: *Posidonia australis*, Po: *Posidonia oceanica*, Ps: *Posidonia sinuosa*, Sf: *Syringodium filiforme*, Si: *Syringodium isoetifolium*, Th: *Thalassia hemprichii*, Tt: *Thalassia testudinum*, Zm: *Zostera marina*, Zn: *Zostera noltii*.

([a]) Manzanera et al. 1998, ([b]) Ruiz 2000

The mortality-burial curve for *Halophila ovalis* (Fig. 6.1D), which showed 0% of mortality for all the burial levels tested on the overall experimental period (300 days), reflect the great recovery capacity of this species rather than its high tolerance to burial. This species must have experienced mortality immediately after burial, at least for burial levels > 4 cm but rapidly recovered to densities of 4- to 5-fold higher than controls (Duarte et al. 1997). This resulted in no detrimental effects of burial, even when a 2 months time-scale was considered. Similarly, the 0% mortality found for *Halodule uninervis* at the 16 cm burial level (Fig. 6.1B) illustrates the species recovery especially after 10 months, when density increased substantially (Duarte et al. 1997).

The main responses of each seagrass species to experimental burial are summarized in Table 6.4. An increase of shoot mortality was the main response found for seagrass species under experimental burial, whether it was an immediate reaction, as was the case for *Cymodocea serrulata*, *Halodule uninervis* and *Syringodium isoetifolium*, or by the end of the experiment as found for *Enhalus acoroides*. An increase in the vertical internode length was also a common response among those species with vertical rhizomes, such as *Cymodocea nodosa*, *Cymodocea rotundata*, *Halodule uninervis*, *Syringodium isoetifolium* and *Thalassia hemprichii*. However, no significant relationship was found between the vertical elongation rate of species and the experimental burial thresholds. This suggests that the response of increasing internode length occurs at levels below the 50% mortality levels.

Table 6.4. Summary of the main responses of seagrass species to experimental burial

Species	Main responses to burial
C. nodosa	- Increased shoot mortality
	- Increased length of the youngest vertical internode (up to 4 cm of burial)
	- Increased leaf turnover rate (up to 2 cm of burial)
	- Increased vertical growth rate (up to 4 cm of burial)
	- Increased leaf sheath length (up to 4 cm of burial)
C. rotundata	- Shoot density decline
	- Increased vertical internode length (up to 4-8 cm of burial)
	- No changes in age distribution
	- No response of shoot size, sheath length and leaf specific weight
C. serrulata	- Initial shoot density decline in high burial levels (8 and 16 cm) followed by shoot density recovery
	- No response of vertical internode length
	- No changes in age distribution
	- No response of shoot size, sheath length and leaf specific weight
E. acoroides	- Shoot density decline only by the end of the experiment (300 days)
	- No response of shoot size, sheath length and leaf specific weight
H. uninervis	- Initial shoot density decline in high burial levels (8 and 16 cm) followed by shoot density recovery
	- Increased vertical internode length (up to 2 cm of burial)
	- Changes in age distribution
	- No response of shoot size, sheath length and leaf specific weight
	- Increased branching frequency (up to 8 cm of burial)
H. ovalis	- Early increase of shoot density at intermediate burial levels (4 and 8 cm of burial)
	- No response of shoot size, sheath length and leaf specific weight
P. australis	- Increased shoot mortality
	- Increased sheath length in 20 cm burial level
	- Decreased shoot biomass and leaf growth
	- Decreased leaf surface area

P. oceanica	- Increased shoot mortality
	- Decreased leaf growth and leaf length under moderate burial (6 cm)
	- Decreased shoot biomass and leaf no. per shoot in high burial levels (9 cm)
	- No response of sheath length
	- Decreased rhizome starch content in 3 cm burial level
	- Decreased leaf surface area
P. sinuosa	- Increased shoot mortality
	- Decreased leaf growth
	- Decreased sheath length and internode length under extreme burial (> 30 cm)
	- No response of shoot biomass and leaf number per shoot
	- Decreased leaf surface area
S. filiforme	- Decreased shoot density
	- Decreased horizontal rhizome length
	- No response in the number of shoots per rhizome
S. isoetifolium	- Initial shoot density decline in high burial levels (8 and 16 cm) followed by shoot density recovery
	- Increased vertical internode length (up to 4 and 8 cm of burial)
	- Changes in age distribution (increase in recruitment of young shoots (< 1 yr))
	- No response of shoot size, sheath length and leaf specific weight
	- Increased branching frequency (up to 8 cm of burial)
T. hemprichii	- Shoot density decline
	- Increased vertical internode length (up to 8 cm of burial)
	- Changes in age distribution (selective loss of young shoots (< 1 yr) and reduced recruitment)
	- No response of shoot size, sheath length and leaf specific weight
	- Increased branching frequency (up to 4 cm of burial)
T. testudinum	- Decrease or no response of shoot density
	- No response in horizontal rhizome length
	- No response in the number of shoots per rhizome

Z. marina	- Increased mortality
	- Decreased productivity
	- No changes in sheath length
	- Decreased leaf length and leaf surface area
Z. noltii	- Decreased shoot density
	- No response of flowering shoot density
	- No response of leaf length and sheath length
	- Increased horizontal internode length
	- Decreased leaf and rhizome C content in high burial levels (4 cm, 8 cm and 16 cm)
	- Decreased leaf N content and simultaneous increase in rhizomes
	- Increased leaf sugar content in intermediate burial level (4 cm)
	- No response of leaf and rhizome starch content

The length of the leaf sheath increased in response to burial in some species (*Cymodocea nodosa* and *Posidonia australis*) but decreased in others (*Posidonia oceanica*, *Posidonia sinuosa*, *Zostera marina* and *Zostera noltii*). Most of the species showed no response in leaf morphometry to changes in the sediment level (*Cymodocea rotundata*, *Cymodocea serrulata*, *Enhalus acoroides*, *Halodule uninervis*, *Halophila ovalis*, *Syringodium isoetifolium*, *Thalassia hemprichii*, *Zostera marina* and *Zostera noltii*; Table 6.4). On the other hand, a significant and positive relationship between the sheath length of plants at the 50% mortality burial level and the 50% burial threshold was found for species subject to experimental burial (Fig. 6.2).

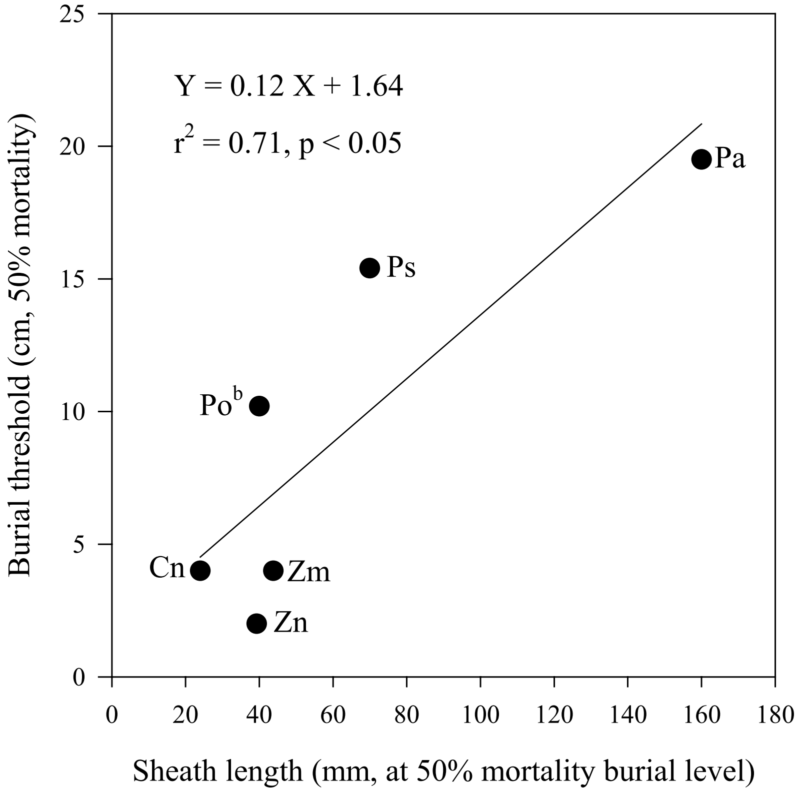

Fig. 6.2. The relationship between the burial threshold causing 50% mortality and the length of the leaf sheath at the 50% mortality burial level in seagrasses subject to experimental burial. Legends as in Fig. 6.1

6.3.1.2. Experimental erosion

The effects of experimental erosion were only tested for 4 seagrass species (Table 6.5). None of the tested species experienced 100% mortality in response to erosion, even when deep erosion was applied (e.g. -10 cm for *Syringodium filiforme* and *Thalassia testudinum*). The maximum erosion levels tested exceeded the anchoring depth of *Syringodium filiforme*, *Thalassia testudinum* and *Zostera noltii* (SER < 1, Table 6.5). *Syringodium filiforme* and *Zostera noltii* experienced 50% mortality at erosion depths of -4.5 cm and -2 cm respectively, whereas *Cymodocea nodosa* and *Thalassia testudinum* showed relatively low mortality (< 30%) at -2 cm and -10 cm, respectively (Fig. 6.3).

Table 6.5. Details of the experimental design to test the effects of erosion on seagrasses (erosion levels tested, the duration of the experiments, the size:erosion ratio (SER)) and the resulting effect on seagrass survival summarised in the experimental erosion levels causing 50% and 100 % mortality

Species	Erosion levels (cm)	Experimental period (days)	SER	Erosion level (cm) causing	
				50% mort.	100% mort.
C. nodosa	-2	35	1.1	-	-
S. filiforme	-3.5/-4.5, -4/-5, -6.5/-7.5, -9/-10	60	0.4	-4.5	-
T. testudinum	-3.5/-4.5, -4/-5, -6.5/-7.5, -9/-10	60	0.9	-	-
Z. noltii	-2	7, 14, 28, 56	0.9	-2	-

(-) Shoot loss did not occur for the erosion levels tested

Thalassia testudinum seemed to be the most tolerant species to erosion, as no responses in shoot density and in the length of the horizontal rhizome were observed (Table 6.6). Moreover, the mortality-erosion relationship for this species showed that no mortality occurred until the erosion depth reached -5 cm. At the -7.5 cm erosion level, this species experienced 30% shoot loss (Fig. 6.3).

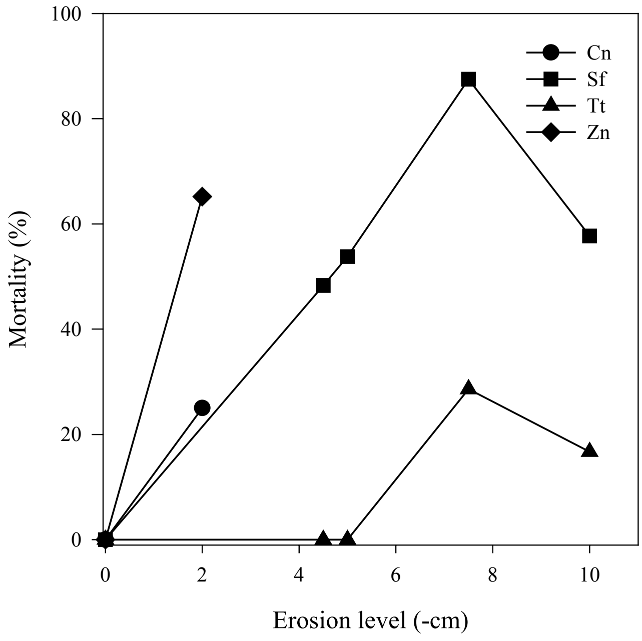

Fig. 6.3. The relationship between shoot mortality and erosion levels in seagrasses subject to experimental erosion. Legends as in Fig. 6.1

The main responses to experimental erosion of the seagrass species tested are summarized in Table 6.6. An increase in shoot mortality was a common response of *Cymodocea nodosa*, *Syringodium filiforme* and *Zostera noltii* to erosion. The plant morphometry showed no general trend in response to erosion, but three out the four species tested experienced reduced rhizome growth, as *Cymodocea nodosa* and *Syringodium filiforme* showed a decrease in the vertical internode length and of the horizontal rhizome length, respectively; whereas *Zostera noltii* showed an increase of the horizontal internode length (Table 6.6).

Table 6.6. Summary of the main responses of seagrass species to experimental erosion

Species	Main responses to erosion
C. nodosa	- Increased shoot mortality
	- Decreased vertical internode length and vertical growth rate
	- Decreased leaf turnover rate and leaf sheath length
S. filiforme	- Decreased shoot density
	- Decreased horizontal rhizome length in high erosion level (-9 cm)
	- No response in the number of shoots per rhizome
T. testudinum	- No response of shoot density
	- No response of horizontal rhizome length and number of shoots per rhizome length
Z. noltii	- Decreased shoot density
	- No response of flowering shoot density
	- No response of leaf length and sheath length
	- Increased internode length
	- No response of leaf and rhizome C and N content
	- Increased leaf sugar content, but no response of rhizome sugar content
	- No response of leaf starch content, but decrease of rhizome starch content

6.3.2. Scaling between burial thresholds and plant size, architecture and growth

The capacity of seagrass species to resist sediment burial was strongly size-dependent (Figs. 6.4 & 6.5). Both burial thresholds, 50% and 100% mortality, were significantly related to the shoot mass, the rhizome diameter, the aboveground biomass, the horizontal elongation rate and the size of leaves, and their scaling allometric slopes were similar across species. The 50% and 100% burial thresholds were scaled to, respectively, the 0.31 and 0.28 power of the shoot mass (Figs. 6.4A & 6.5A), the 0.57 and 0.59 power of the rhizome diameter (Figs. 6.4B & 6.5B), the 0.39 and 0.41 power of the aboveground biomass (Figs. 6.4C & 6.5C), the -0.34 and -0.26 power of the horizontal elongation rate (Figs. 6.4D & 6.5D), the 0.60 and 0.51 power of minimum leaf length (Figs. 6.4E & 6.5E) and the 0.81 and 0.77 power of maximum leaf length (Figs. 6.4F & 6.5F).

This scaling relationships indicate that large species, with high shoot mass, high aboveground biomass, thick rhizomes, low horizontal rhizome elongation rates and long leaves, such as *Posidonia* spp., have a great capacity to resist sediment burial, but that the capacity to resist burial increases more slowly than plant size (allometric slopes < 1). Mortality rates of 50% and 100% were only observed under high burial levels (Table 6.3). On the other hand, small seagrass species, such as *Cymodocea rotundata*, *Cymodocea serrulata*, *Halophila ovalis* and *Zostera noltii*, characterised by low shoot mass, low aboveground biomass, thin rhizomes, high horizontal rhizome elongation and small leaves, are more sensitive to burial disturbance. Low burial levels down to 2 cm resulted in 50% mortality for the first three species, while for *Halophila ovalis* mortality attained 100% (Table 6.3). The burial thresholds for seagrass species were independent ($p > 0.05$) of the vertical and horizontal internode length, to the vertical elongation rate, to the number of internodes and distance between shoots, and to the shoot density.

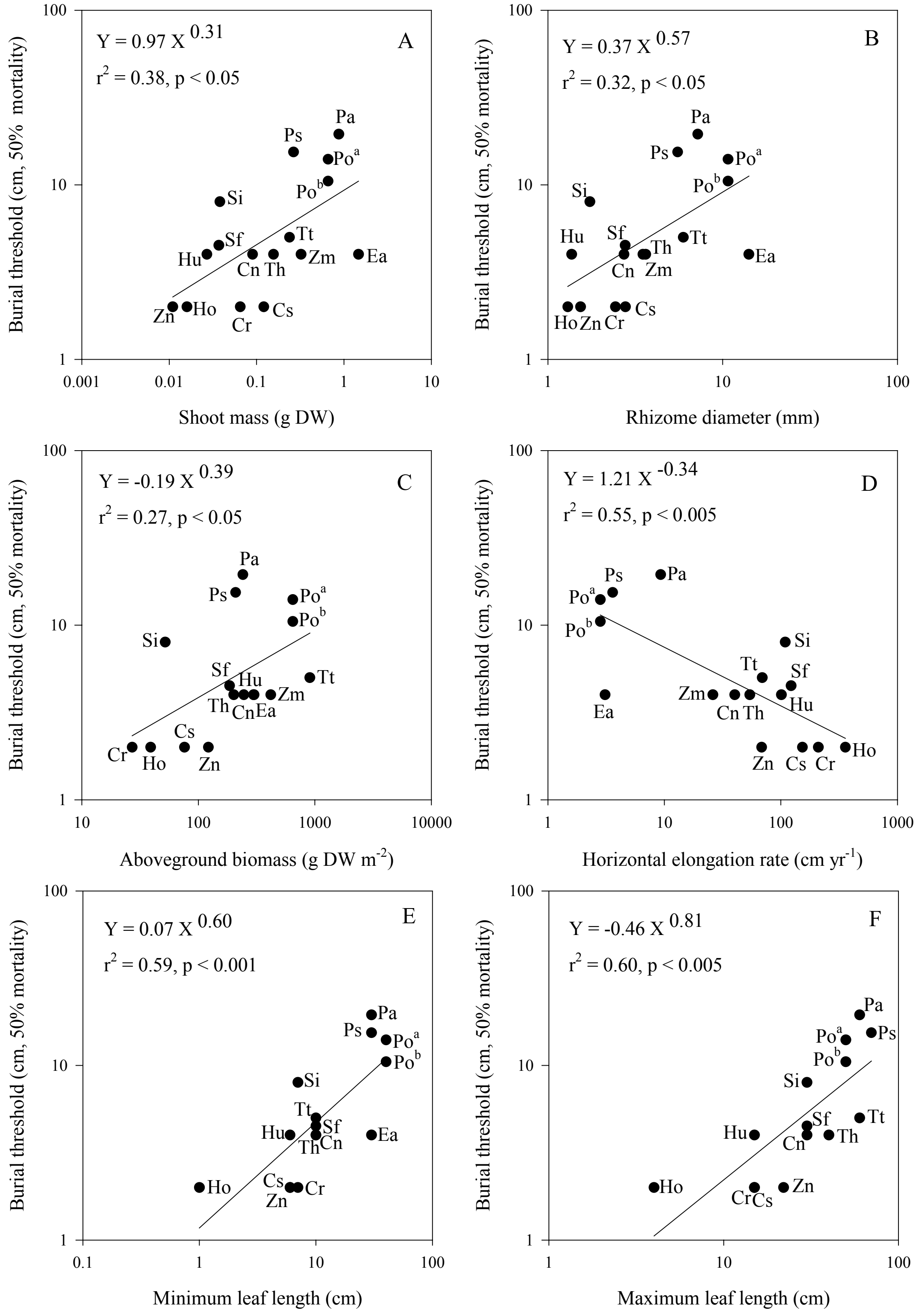

Fig. 6.4. Allometric relationships between the burial threshold causing 50% mortality and (A) the shoot mass, (B) the rhizome diameter, (C) the aboveground biomass, (D) the horizontal rhizome elongation, (E) the minimum leaf length and (F) the maximum leaf length of seagrass species. Legends as in Fig. 6.1

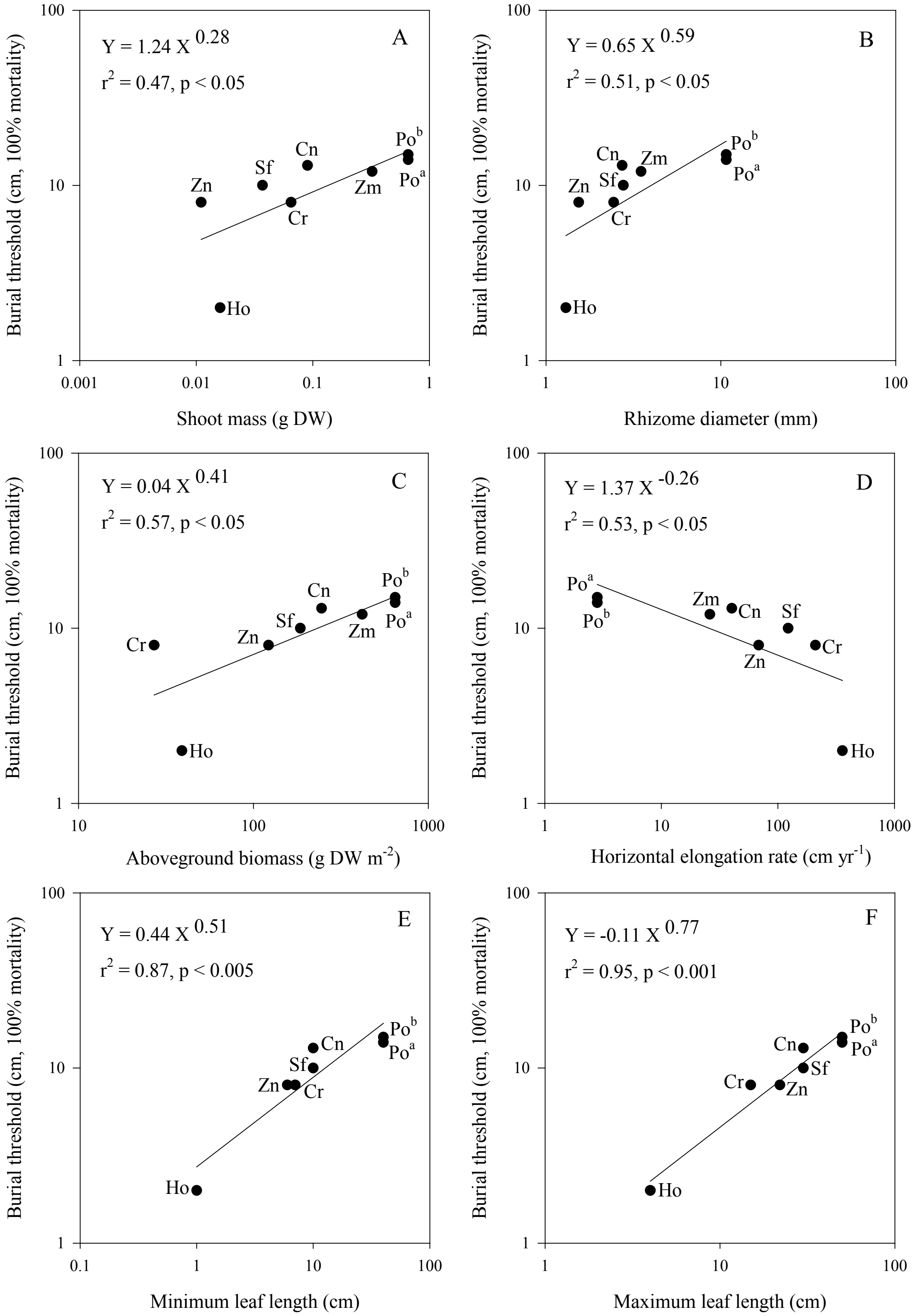

Fig. 6.5. Allometric relationships between the burial threshold causing 100% mortality and (A) the shoot mass, (B) the rhizome diameter, (C) the aboveground biomass, (D) the horizontal rhizome elongation, (E) the minimum leaf length and (F) the maximum leaf length of seagrass species. Legends as in Fig. 6.1

6.3.3. Descriptive impacts of burial and erosion on seagrass meadows

Field observations on the impacts of burial and erosion events, whether natural or human-induced, have been described for several seagrass species worldwide (Table 6.7). The natural disturbances ranged from small-scale events, such as bioturbation, to large-scale changes, such as barrier-island migration and hurricanes (Table 6.7). Such disturbances resulted in different magnitudes of burial, ranging from around 6 cm of burial as in the case of bioturbation (Suchanek 1983) to more than 70 cm of burial as in the case of hurricanes (Marbà et al. 1994b). Disturbance due to barrier-island migration and hurricanes resulted in deep changes of the sediment dynamics and drastic decline of seagrass communities. Even so, seagrass meadows are able to recover, depending on the magnitude of burial. Fourqurean & Rutten (2004) reported that seagrass meadows may recover within few months, from surviving shoots, from a few centimeters of sediment deposition to years, through recolonization, when buried 50 cm after the passage of a category 2 hurricane (Table 6.7). Cunha et al. (2005) reported that *Zostera noltii* meadows recovered, through recolonization, 10 years after the passage of a sand-barrier inland over them. Marbà and co-workers report that recovery from the burial by sand-waves involved recolonization from horizontally-branching rhizomes of a few surviving shoots that managed to grow vertically to reach the sediment surface from the 10-20 cm burial affecting a *Cymodocea nodosa* stand (Marbà & Duarte 1995) and the more than 70 cm burial affecting a *Thalassia testudinum* buried by the migration of mega-ripples displaced by a hurricane (Marbà et al. 1994b). On the other hand, the effects of burrowing animals (bioturbation) on seagrasses, although at a small-scale, resulted in the decrease of seagrass cover, abundance and growth (Table 6.7), and may play an important role in the long-term decline of seagrasses, particularly when compound with other disturbances (e.g. *Zostera noltii* in the Dutch Wadden Sea, Philippart 1994).

These observations suggest that burial disturbances derived from events that have a return time sufficient for seagrass recovery allow the maintenance of patchy seagrass meadows, through the coupling of the plant population dynamics to the sediment disturbance. The patch dynamics of *Cymodocea nodosa* was reported to be coupled with the migration rate of subaqueous dunes that arrested the population in a continuous colonization process yielding a patchy landscape (Marbà et al. 1994a, Marbà & Duarte 1995). A similar process at a decadal time scale was described by Cunha et al. (2005) for *Zostera noltii* experiencing burial loss from a barrier-island migration and subsequent recolonization.

The field observations on the effects of human-induced disturbances that result in burial and erosion events were mainly related to coastal construction and dredging activities (Table 6.7). Most of these impacts were reported for *Posidonia oceanica*, a Mediterranean threatened species, whose meadows have been destroyed and altered (e.g. reduction of shoot density, biomass and productivity), due to the direct effects of the sediment redistribution, but also due to the associated effects of the increased water turbidity (Guidetti 2001). In some cases, seagrass recovery was apparent after 2-3 years of human-induced disturbances such as the deposition of dredged materials (Eldridge et al. 2004, Sheridan 2004), whereas complete destruction of seagrass meadows or continuous meadow regression were identified as a consequence of continuous disturbances related to coastal construction and use (Ruiz 2000, Badalamenti et al. 2006).

Table 6.7. Field observations of the natural and human-induced impacts of burial and erosion events on seagrasses

Species	Disturbance	Factors/Intensity	Effects on seagrasses
Natural disturbances			
Cymodocea nodosa	Dune migration (Marbà et al. 1994a, Marbà & Duarte 1995)	- Sand dunes height of 0.22 m (0.07-0.65 m) - Dune migration of 13 m yr^{-1} (5.4-32.3 m yr^{-1}) - Period between successive dunes of 1.5 yrs	- Patchy landscape with scattered meadows at migrating slope and exposed dead rhizomes at regressing slope - Period of denuded sediment of 3-9 mo
Posidonia oceanica	Dune migration (Boudouresque et al. 1984)	- Regular and small sand dunes	- Rhizome growth related to the sedimentation rate
Thalassia testudinum	Hurricane (Wanless et al. 1988, Gallegos et al. 1992, Marbà et al. 1994b, van Tussenbroek 1994)	- Changes in sediment dynamics: erosion, deposition and sediment redistribution	- High mortality, burial of shoots and exposure of rhizomes - Increased vertical growth in buried shoots and decreased in eroded ones - Decreased shoot density and increased flowering intensity
	Bioturbation (Suchanek 1983, Valentine et al. 1994)	- Extensive burrow construction - Increase of sediment transport (4 g m^{-2} d^{-1}) and siltation over shoots	- Decline of area covered (regression > 1 m in 7 mo) - Decreased shoot density and productivity - Complete burial in high bioturbation areas
Zostera japonica	Bioturbation (Dumbauld & Wyllie-Echeverria 2003)	- Burial of shoots, and dispersal and burial of seeds	- Decreased shoot density, seeds and germination - Decreased sprout survival and growth
Zostera marina	Storm (Cabello-Pasini et al. 2002)	- Sediment resuspension (periods of light limitation)	- Decreased survival and population loss - Increased internode length - Decreased sugar and starch levels

Zostera noltii	Bioturbation (Philippart 1994)	- Cover of seagrass shoots by burrowing materials (sediment layer of 26 cm yr^{-1})	- Decline of seagrass meadows and shoot density - Decreased seagrass growth and survival - Decreased rhizomes, seeds and seedlings survival
	Barrier-islands inlet migration (Cunha et al. 2005)	- Change of sedimentary dynamics and hydrodynamics - Natural inlet migration of 119-187 m yr^{-1}	- Change in landscape coinciding with inlet migration - Increased patch number and area with the sediment stabilization due to the acceleration of the inlet migration
Zostera novazelandica	Bioturbation (Woods & Schiel 1997)	- Burrows within meadows (9.4 burrows m^{-2}), decreasing in abundance towards the centre	- Destabilization and loss of meadows (22.5% in 6 mo) - Increased meadow erosion - Decreased shoot density
Seagrass meadows	Sand movement (Kirkman 1978)	- Rapid increase in sand deposition	- Seagrass decline
SE-Asia seagrass meadows[1]	Siltation (Gacia et al. 2003)	- Sediment deposition (also trapped by seagrasses) - Deterioration of light and sediment conditions	- Detrimental effects for seagrasses - Sediment deposition within meadows is species-specific - Sediment deposition by seagrasses is small compared to the deposition by the water column
Seagrass meadows[2]	Hurricane (Fourqurean & Rutten 2004)	- Sediment deposition (< 1 to 50 cm) and erosion	- Severe loss of seagrasses - Rapid seagrass recovery under few cm of burial, but slow recovery (> 3 yr) of 50 cm buried meadows - Slowest recovery of eroded seagrass meadows

Human-induced disturbances			
Posidonia oceanica	Coastal construction and engineering (Meinesz et al. 1991, Guidetti & Fabiano 2000, Ruiz 2000, Guidetti 2001, Ruiz & Romero 2003, Badalamenti et al. 2006)	- Changes of sedimentary dynamics and hydrodynamics - Increase of sediment deposition - Resuspension of silty sediments and increased water turbidity - Irreversible replacement of the natural environment	- Destruction or severe alteration of seagrass meadows - Significant mortality and meadow regression - Decline in seagrass density, biomass and productivity - Burial-induced vertical growth responses - Changes in photosynthetic activity - Decreased rhizome carbohydrates reserves
Thalassia testudinum	Dredging (Eldridge et al. 2004)	- Sediment deposition in seagrass meadows (dredged material burial of 7-10 cm) - Reduction of water transparency - Addition of organic matter to sediments - Lethal sulphide levels and increased ammonium in sediment	- Continued reduction in biomass - Unsuitable sediment conditions for seagrass recolonization (up to 2.5 yrs)
Zostera noltii	Dredging (inlet relocation) (Cunha et al. 2005)	- Change of sedimentary dynamics and hydrodynamics - Deposition of sand over seagrass meadows	- Dramatic changes in seagrass landscape - Decreased landscape cover and increased patch fragmentation

Seagrass meadows[3]	Dredging (Larkum & West 1990)	- Increase of wave height - Erosion of seagrass meadows	- Long-term deterioration of seagrass meadows - Decreased seagrass cover - Fragmentation of meadows - Decreased leaf biomass after a storm event
Seagrass meadows	Dredging deposits (Sheridan 2004)	- Deposition of dredged materials (0.14-18.7 ha) over seagrass meadows - Increased water turbidity	- Decreased seagrass cover - Seagrass recovery, as increased biomass, noticeable after 2 yrs and widespread after 3 yrs

([1]) Mixed meadows of *Enhalus acoroides*, *Cymodocea rotundata*, *Cymodocea serrulata*, *Halodule uninervis, Thalassia hemprichii*;

([2]) Dominant seagrass: *Thalassia testudinum*, other seagrasses: *Syringodium filiforme*, *Halodule wrightii*, *Halophila decipiens*, *Halophila engelmanni*;

([3]) *Posidonia australis* and *Zostera capricorni*

6.4. Discussion

6.4.1. Experimental burial and erosion

The comprehensive analysis of the available literature on the experimental effects of burial and erosion on seagrasses confirms that seagrasses are highly vulnerable to changes in sediment level. All species studied showed at least 50% mortality when subject to experimental burial. Moreover, in most of these species this level of mortality was induced by low burial levels (2-4 cm, Table 6.3). However, some seagrass species are able to survive relatively high burial levels, and they did not experience 100% mortality under experimental burial. The burial tolerance was more evident in large seagrass species with vertical rhizomes, while the small-size seagrasses lacking vertical rhizomes were most sensitive to burial. An exception to this pattern was *Enhalus acoroides*, a large seagrass that does not have vertical rhizomes. This species is one of the most tolerant species to burial (20% mortality along 10 months under 16 cm of burial, Fig. 6.1), probably due to the large size of its leaves, which can exceed one meter in length. Despite the high sensitivity to burial showed by some species, such as *Cymodocea serrulata* (50% mortality burial threshold of 2 cm), their great recovery capacity (Duarte et al. 1997) results in a long-term ability to sustain burial. *Posidonia australis* was the most tolerant seagrass species to burial, not reaching 100% mortality for the burial levels tested (up to 30 cm), and reaching 50% of mortality only at 19.5 cm of burial. On the other hand, *Zostera noltii*, *Halophila ovalis*, *Cymodocea rotundata* and *Cymodocea serrulata* were the less tolerant species to burial, with a burial threshold of 50% mortality at the 2 cm burial level. Among these, *Halophila ovalis* was the most sensitive species to burial, experiencing 100% mortality under 2 cm of burial (Table 6.3).

The available data does not show any significant relationship between the vertical rhizome elongation and the burial thresholds, even though the stimulation of the vertical rhizome growth by sediment accretion was experimentally demonstrated for several seagrass species. Marbà & Duarte (1998) reported that the seagrass vertical elongation rate is species-specific, but it is independent of seagrass size. This explains why no correlation was found between the vertical elongation rate, which is size-independent, and the burial thresholds, which were found to be size-dependent.

Seagrasses were also vulnerable to sediment erosion. The capacity of seagrasses to sustain erosion disturbance was also dependent on plant size, specifically with the size of the belowground modules, which determine the anchoring depth of species. *Thalassia testudinum* was the species most resistant to erosion disturbance among those tested (Fig. 6.3). This species has the deepest anchorage system (around -8.5 cm), and shows woody rhizomes and sturdy, unbranched roots (Cruz-Palacios & van Tussenbroek 2005), which certainly play an important role in its capacity to survive erosion.

6.4.2. Scaling between burial thresholds and plant size, architecture and growth

The burial thresholds were strongly size-dependent, scaling with the plant shoot mass, rhizome diameter, aboveground biomass, horizontal elongation rate and leaf length (Figs. 6.4 & 6.5). The leaf length is the best predictor of the vulnerability to burial (Figs. 6.4 & 6.5). The rhizome diameter, which is a very conservative trait within species (Marbà & Duarte 1998) closely related to plant size (Figs. 6.4 & 6.5), was also found to be a good predictor of the vulnerability of seagrass species to burial. The rhizome diameter is also related to the resource storage capacity (Duarte & Chiscano 1998, Marbà & Duarte 1998), and therefore to the capacity of seagrasses to resist burial. Sediment burial results in the reduction of the available photosynthetic area of seagrass leaves, forcing the plants to use stored resources to survive (Alcoverro et al. 1999). Cabaço & Santos (2007) reported a decrease in carbon and carbohydrates content of *Zostera noltii* rhizomes as a consequence of the burial-induced light deprivation. Species with high storage capacity, as indicated in thicker rhizomes, are better able to survive burial disturbance. The robust relationships described indicate that the vulnerability of seagrasses to sediment burial decreases with increasing leaf length and rhizome diameter. Whereas the species for which sediment burial thresholds have been experimentally determined comprise only a third of the seagrass flora, the allometric relationships developed here allow predictions on the vulnerability to sediment burial to be formulated from easily determined architectural traits of yet untested seagrass species.

Although fast growing species, such as *Halophila ovalis* and *Zostera noltii*, were extremely sensitive to sediment burial with relatively low burial thresholds, the high horizontal elongation rate of these species allows a fast recovery after burial. The horizontal rhizome elongation rate, which is largely species-specific and is strongly size-dependent (Marbà & Duarte 1998), is the main mechanism involved in space occupation. As the horizontal rhizome elongation is negatively scaled to the burial thresholds, it provides valuable information on the recovery capacity of each species after burial disturbance. Seagrass characteristics that are independent of plant size, such as the vertical elongation rate and the vertical and horizontal internode length (Marbà & Duarte 1998), were not significantly related to the burial thresholds, which proved to be a strongly size-dependent trait.

6.4.3. Descriptive studies

Both natural and human-induced disturbances may lead to drastic changes in the sedimentary dynamics that seriously impact seagrass meadows, but whereas recovery typically follows after natural disturbances, such as inlet migration and hurricanes

(Fourqurean & Rutten 2004, Cunha et al. 2005) that typically involve pulses of sediment redistribution, human-induced disturbances, such as coastal works (Ruiz & Romero 2003) often lead to permanent loss. Human-induced disturbances generally result in long-lasting changes in the sedimentary environment that preclude seagrass recovery.

Seagrass recovery from large pulses of burial and erosion following natural disturbances is relatively independent of their specific burial thresholds, depending strongly on their longer-term colonization capacity and patch dynamics. Two examples are presented in the literature. The first one is related to the regular, periodic pattern of some natural disturbances, such as the migration of subaqueous dunes, which allows *Cymodocea nodosa* to adapt its life strategy to this particular sedimentary environment (Marbà et al. 1994a, Marbà & Duarte 1995). Even though, the burial thresholds estimated for *Cymodocea nodosa* of 4 cm (50% mortality) and 13 cm (100% mortality) are well below the mean height of the sand dunes studied (22 cm), the progressive process of dune migration allows the plants to couple its patch dynamics to the sedimentary dynamics in a continuous colonization process. The coupling between the species flowering frequency and the dynamics of dune progression (Marbà et al. 1994b, Marbà & Duarte 1995) also supported the species adaptation to this natural disturbance.

The second example is the coupling of *Zostera noltii* patch dynamics to the effects of inlet migration and relocation in Ria Formosa lagoon, southern Portugal (Cunha et al. 2005). The low burial thresholds estimated for *Zostera noltii*, of 2 cm (50% mortality) and 8 cm (100% mortality), are in a completely different scale to the dramatic changes caused by the artificial inlet migration and relocation in this landscape. However, the long-term patch dynamics of the species was coupled to the burial caused by the passage of the lagoon inlet over the seagrass meadows (10 years).

Small-scale disturbances, such as the impacts resulting from the burrowing animal activities, should not be neglected. Regression of seagrass meadows and a decrease of plant growth and survival were reported as a consequence of bioturbation (Table 6.7). The negative effects of bioturbation on the *Thalassia testudinum* cover and abundance are clearly explained by the 50% mortality burial threshold of the species (5 cm), which was similar to the mean height (5.46 cm) of the sediment mounds built by ghost shrimps (Suchanek 1983).

The indirect effects originated by the changes of the sedimentary dynamics also play an important role in the adverse effects related to the burial and erosion events. The decline of *Posidonia oceanica* meadows reported in the Mediterranean basin as a consequence of human-induced changes of the sedimentary dynamics (e.g. coastal works), were often accompanied by events of increased turbidity (Table 6.7). Even though the direct burial disturbance involved considerable changes of the sediment level, this species has a relatively high capacity to sustain burial, with burial thresholds of 10.2-14 cm for 50% mortality, and 14-15 cm for 100% mortality. This suggests that the increased water turbidity may have been responsible for the reported decline.

6.4.4. Mechanisms of seagrass mortality upon sediment burial or erosion

Disturbance of seagrass meadows upon sediment burial and erosion has direct and indirect components. Indirect or secondary effects associated with post-disturbance processes, such as increased turbidity, can greatly affect seagrasses. The sediment redistribution by currents, wind and tides, are often involved in such processes, which may last from several months (Guidetti & Fabiano 2000) to years (Eldridge et al. 2004). The resuspension of sediment, particularly of fine sediment particles, result in increased turbidity of coastal waters, which limit the light availability for seagrass photosynthesis

(Ruiz & Romero 2003). The decrease of carbohydrate reserves and the increase of shoot mortality may follow if the light limitation extends for long periods (Alcoverro et al. 1999). The capacity of seagrasses to endure under such circumstances depends on the storage capacity of each species (Duarte & Chiscano 1998).

Sediment redistribution can also involve the modification of the sediment attributes, resulting in unsuitable conditions for seagrasses. The addition of organic matter due to the deposition of dredging materials (Eldridge et al. 2004), or the erosion of the bottom exposing sediment layers depleted of oxygen (Hemminga & Duarte 2000) may result in anoxia conditions. Such anoxic sediments may be detrimental to seagrasses by promoting growth inhibition and mortality (Terrados et al. 1999). Increased levels of toxic compounds, such as sulfides, may also be associated with this process and impact seagrasses, especially when in lethal concentrations. Sulfide has been considered a major contributing factor in seagrass diebacks (Koch & Erskine 2001) and a limiting factor in seagrass recover (Eldridge et al. 2004).

Plant burial results in the reduction of the available photosynthetic biomass, which may negatively affect the growth and survival of plants. Substantial shoot mortality and increase of the vertical growth of the surviving shoots are the main effects/responses described for several species (Boudouresque et al. 1984, Marbà & Duarte 1994, Marbà & Duarte 1995, Duarte et al. 1997, Manzanera et al. 1998, Mills & Fonseca 2003, Cruz-Palacios & van Tussenbroek 2005, Cabaço & Santos 2007). Changes in plant morphometry, such as longer leaves and leaf sheaths (e.g. Marbà & Duarte 1994), and longer internodes (e.g. Duarte et al. 1997), have also been reported as a response to burial. Burial may also increase the probability of exposure of the leaf meristems, which may be buried in the sediments, to anoxic conditions, which has been shown to lead to seagrass mortality (Borum et al. 2005).

Erosion exposes belowground tissues to the colonization by organisms, and drilling organisms can penetrate the rhizomes, disrupting gas flow and affecting seagrass survival. In addition, erosion exposes the belowground components to waves and currents that can, because these elements are not flexible like leaves, be broken and advected out.

The results presented here show that the effects of burial on seagrasses are strongly dependent on plant size. In a mixed seagrass meadow, Duarte et al. (1997) described a pattern of species loss after burial-induced disturbance, in which mortality increased with decreasing seagrass size. The size of seagrass modules, the resource allocation within modules, and the life-strategy of each species result in a differential capacity of seagrass species to sustain burial disturbance.

6.5. Conclusions

Changes of the sediment level related to sediment burial and erosion revealed to be detrimental to seagrasses. The impacts of sediment erosion on seagrass survival and on the plant allometric responses are different than those caused by burial. Whereas burial results in the decrease of the available photosynthetic area, erosion leads to the exposure of the belowground parts of the plants. Such situation affects the anchoring capacity of the species, and consequently the plants may be more easily detached from the substrate. The extent of the effects is species-specific and depends on the magnitude and on the frequency of disturbance. Large seagrass species are less susceptible to burial, whereas small, fast-growing species showed to be very sensitive to burial. *Posidonia australis* was the most tolerant seagrass species to burial, while *Thalassia testudinum* was the most tolerant species to erosion. The capacity of seagrasses to resist sediment burial is strongly size-dependent. The leaf size and the rhizome diameter are the best predictors of the burial impact on seagrasses. Both natural and human-induced changes of the sediment level may seriously impact seagrasses. However, many natural events allow the recovery and/or adaptation of seagrasses to the sediment burial/erosion, as a consequence of the natural sediment redistribution occurring after disturbance. In contrast, most human-induced disturbances related to coastal construction and dredging activities deeply affect the sedimentary dynamics, and also result in increased water turbidity. These often result in the regression and permanent destruction of seagrass meadows. The burial thresholds estimated for seagrass species are generally in accordance with the burial impacts reported by the field descriptive studies. In some cases, the recovery of seagrasses was related to the long-term meadow dynamics, rather than to their specific burial thresholds.

Acknowledgements. This research is a contribution to projects “F-ECTS: feed-backs of estuarine circulation and transport of sediments on phytobenthos” (MAS3-CT97-0145) and “Praderas” funded by the Fundación BBVA. We thank N. Marbà for data on seagrass characteristics. S.C. was supported by a PhD grant from Fundação para a Ciência e a Tecnologia (SFRH/BD/2880/2000).

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CHAPTER 7

General Discussion

7. General Discussion

The anthropogenic activities studied in Ria Formosa lagoon, specifically the urban wastewater discharges, the clam harvesting activity, and the burial and erosion events, revealed to be detrimental to the seagrass *Zostera noltii*. Different levels of impacts were found depending on the type of disturbance. Anthropogenic activities related to physical or mechanical disturbance, such as the clam harvesting and the sediment burial and erosion, resulted in evident plant loss, whereas disturbances related to modifications of the chemical environment, such as the urban wastewater discharges, resulted in changes of *Z. noltii* plants/populations rather than direct seagrass loss.

The primarily potential human threat for *Z. noltii* seems to be related to the activities that result in changes of the sedimentary dynamics, as the critical level of burial tolerated by this species is extremely low. Total shoot loss occurred between 4 cm and 8 cm of burial (Fig. 5.1A), whereas 50% shoot mortality was observed at the burial level of 2 cm (Table 6.3). The small size of this species and the lack of vertical rhizomes make *Z. noltii* very vulnerable to changes of the sediment level. Even though, longer internodes of the horizontal rhizome were observed as a response to experimental burial and erosion (Fig. 5.3), reflecting a plant attempt to relocate the meristems closer to the sediment surface or the plant response in search of sediment avoiding the eroded area, respectively. There was also an internal mobilization of carbon to meet the plants demands (mainly the starch content in rhizomes), and the internal translocation of nitrogen from the senescent photosynthetic tissues (leaves) into the storage organs (rhizomes). About 50% of the N lost by the leaves was recovered by the rhizomes. This resource mobilization may represent a survival strategy that allows *Z. noltii* to recover when facing short-term burial events. The laboratory experiment confirmed that shoots did not survive more than 2 weeks under complete burial. This experiment revealed a mortality threshold of plants between 1 and 2 weeks, when mortality increased one order of magnitude from about 7% to 70%. This fact may be a consequence of the low storage capacity of this species (Brun et al. 2003). The species may survive and recover from short-term burial disturbance of about 1 week, but no survival of the plants is expected when withstanding long-term burial disturbance. *Z. noltii* did not show signs of recovery within the time range of experiment (2 months). Recovery of buried meadows might only occur through vegetative development of plants from undisturbed locations or by seeds produced elsewhere, since no evidence of induced flowering was found for *Z. noltii* under burial or erosion disturbance (Fig. 5.1B). In Ria Formosa lagoon, changes in the sedimentary dynamics resulting from human-induced activities are mainly related to dredging of navigation channels and artificial inlet relocation. Such activities induce dramatic changes in the seagrass landscapes that take many years to recover (Cunha et al. 2005). Hence, coastal activities resulting in changes of the sedimentary dynamics should take into account the high sensitivity of this species to sediment burial and erosion.

In general, seagrasses are highly vulnerable to changes of the sediment level, as indicated by the compilation of the available information regarding the species-specific impacts of sediment burial and erosion. The extent of the effects caused by the changes of the sediment level is species-specific and depends on the magnitude and on the frequency of disturbance. The burial tolerance was more evident in large seagrass species with vertical rhizomes, while the small-size seagrasses lacking vertical rhizomes were very sensitive to burial. *Z. noltii* was amongst the less tolerant species to burial (Fig. 6.1D), as this is one of the smallest seagrasses. On the other hand, the high horizontal elongation rate of this species may allow a fast recovery after burial (Figs. 6.4D & 6.5D). The horizontal elongation rate, which is strongly size-dependent, is the main mechanism involved in space occupation (Marbà & Duarte 1998), providing valuable information on the species recovery capacity. The leaf size and the rhizome diameter revealed to be the best predictors of the burial impact on seagrasses, as they were strongly related to the species burial thresholds (Figs. 6.4 & 6.5). These are morphometric characteristics easy to determine, providing important information on the capacity of each species to sustain burial. In particular, these easily determined architectural traits allow predictions on the vulnerability to sediment burial of yet untested seagrass species (about two-thirds of the seagrass flora).

Recovery of seagrasses seems to occur often from natural disturbances, such as inlet migration and hurricanes (Fourqurean & Rutten 2004, Cunha et al. 2005), than from human-induced disturbances, such as coastal works (Ruiz & Romero 2003). Besides, the indirect effects originated by the changes of the sedimentary dynamics, such as increased water turbidity, also play an important role in the adverse effects related to the burial and erosion events (Table 6.7). The burial thresholds estimated for seagrass species are generally in accordance with the burial impacts reported by the field descriptive studies. However, in some cases, the recovery of seagrasses was related to the long-term meadow dynamics, rather than to their specific burial thresholds. The coupling between the *Z. noltii* patch dynamics and the effects of inlet migration and relocation reported in Ria Formosa lagoon, southern Portugal (Cunha et al. 2005) is one of these cases. The low burial thresholds estimated for this species, of 2 cm (50% mortality) and 8 cm (100% mortality), are in a completely different scale to the dramatic changes caused by the artificial inlet migration and relocation in this landscape. However, the long-term patch dynamics of the species was coupled to the burial caused by the passage of the lagoon inlet over the seagrass meadows (around 10 years).

Clam harvesting activity that represents the most important socio-economic activity in Ria Formosa lagoon can also adversely affect *Z. noltii* meadows. The physical disturbance associated with this activity, which occurs mostly in intertidal areas where *Z. noltii* meadows develop, result in significant reduction of shoot density and biomass of disturbed meadows (Fig. 4.3). The seagrass loss seems to result from the continuous harvesting activity within *Z. noltii* meadows, as experimental harvest consisting of one disturbance event revealed a short-term impact on shoot density, which recovered to control levels within a month (Fig. 4.4). The high growth and production rates of *Z. noltii* (Vermaat & Verhagen 1996, Marbà & Duarte 1998, Laugier et al. 1999) seem to rapidly buffer the long-term effects of isolated disturbances, but not to compensate the effects of cumulative clam harvesting. Meadows under continuous disturbance have a visually fragmented aspect and a lower seagrass cover. The experimental manipulation of rhizome fragmentation of *Z. noltii* plants revealed that recovery of disturbed meadows might occur through vegetative development, as long as modular units with at least 2 rhizome internodes with the respective connected shoots remain on the sediment (Fig. 4.5). Moreover, significant adverse effects on plant survival, rhizome elongation, and production were only found when the apical shoot was damaged/removed (Fig. 4.6). The species seems to be able to sustain the impacts of local clam harvesting, as long as the frequency and the intensity of this activity is moderate and meadows are allowed to recover between disturbances. These results can be directly applied to the management of *Z. noltii* meadows in Ria Formosa lagoon. The sexual reproduction of *Z. noltii* may also contribute to the recovery of disturbed meadows as indicated by the higher reproductive effort of this species under clam harvesting disturbance (Alexandre et al. 2005).

The impact of urban wastewater discharges in *Z. noltii* meadows did not directly result in plant loss, as found for the physical or mechanical damages originated by the clam harvesting activity and by the sediment burial and erosion, but rather lead to changes at both plant- and population-level. In spite of the high water renewal occurring in Ria Formosa in each tidal cycle (Andrade 1990), which contributes to maintain of the water quality in the lagoon, changes in the population structure, plant morphology and leaf N content of *Z. noltii* were found along the gradient originated by the urban wastewater discharge. The multivariate analysis clearly identified the wastewater discharge as an important source of environmental disturbance and nutrients availability in Ria Formosa lagoon. The multiple regression analysis showed that two of the main biotic processes operating within *Z. noltii* populations, i.e. the overall size of the plants and the biomass-density dynamics, were significantly correlated to the abiotic processes clearly related to the effects of the urban wastewater, i.e. to the nutrients and anoxia conditions of the sediment, and to the contrast between water column salinity and nutrient concentration, respectively. The adverse effects of the urban wastewater in *Z. noltii* meadows of Ria Formosa seem to be spatially restricted to areas up to 600 m distant to the WWTW discharge, which is a relatively small spatial impact. On the other hand, the high nutrient concentrations, particularly of ammonium (158.3-663.4 μM), at the site closest (270 m) to the nutrient source showed toxic effects on this species by reducing total biomass and both leaf and internode size (Fig. 2.4). In agreement, Brun et al. (2002) showed that ammonium concentrations of 200 μM had inhibitory toxic effects on *Z. noltii* growth and survival. The higher abundance of macroalgae and epiphytes (Fig. 2.5) at this site reflects the higher availability of nutrients and may also affect *Z. noltii* negatively. At high nutrient levels, opportunistic macroalgae and epiphytes are better competitors than seagrasses, and thus, will proliferate (Duarte 1995). This may shade and suffocate the plants, leading to seagrass declines (Den Hartog 1994, Duarte 1995, Hauxwell et al. 2001, Hughes et al. 2004, Lapointe et al. 2004). This study provides important information to the understanding and monitoring of the effects of the urban wastewater discharge on *Z. noltii* meadows of Ria Formosa. Such knowledge is imperative to the conservation of existent seagrass meadows and to prevent irretrievable losses.

The nutrient enrichment originated by the urban wastewater discharges clearly affect the population structure of *Z. noltii* meadows, as indicated by the variation of the biomass-density relationships along the disturbance gradients (Fig. 3.2 to 3.4). Nutrient-disturbed stations showed significant correlations between the temporal data of biomass and density in *Z. noltii* populations, whereas non-significant correlations were observed in undisturbed populations (Table 3.1). The population parameters derived from the population structure also varied along the nutrient gradients. The slope of the biomass-density relationships, the coefficient of variation of biomass and the ratio of maximum/minimum biomass increased with nutrient availability (Figs. 3.5A & 3.6), whereas the intercept of the biomass-density relationships decreased with nutrient availability (Fig. 3.5B). Differences among the biomass-density relationships were mainly attributable to changes in biomass, as shoot density varied little among stations. The higher biomass variability found in nutrient-disturbed stations might reflect both the beneficial effects (availability) and the detrimental effects (toxicity) of nutrients along the year (Brun et al. 2002). In contrast, the nutrient-undisturbed stations showed a more conservative range of biomass-density data, varying more in density but very little in biomass, with no clear trend of the seasonal variation. The patterns of variation described by the biomass-density relationships and by the parameters derived from the population structure may be used to assess the global nutrient-disturbance level of coastal areas. Validation of these patterns for *Z. noltii* populations elsewhere and for other seagrass species may provide a general, valuable tool to assess the anthropogenic nutrient disturbance.

The high morphological plasticity of *Z. noltii* allows the species to inhabit different environmental conditions (Peralta et al. 2000, Peralta et al. 2005, Peralta et al. 2006) by adapting to changes of the surrounding environment, such as the changes in resources originated by the urban wastewater discharges. The species fast growth and production rates, as well as the high population dynamics (Hemminga & Duarte 2000), allow *Z. noltii* to rapidly respond to environmental changes and/or disturbances. Besides, the low modular integration revealed by the experimental manipulation of the rhizome and shoot fragmentation (Chapter 4) suggests that *Z. noltii* do not depend much on accumulated reserves, but rather on the direct and immediate investment of photosynthates in growth. These species-specific characteristics represent an important advantage when facing environmental disturbances, allowing the species to sustain under disturbance to a certain extent and to rapidly recover from it. In spite of this advantageous life-strategy, the species faces permanent human-induced threats in the Ria Formosa lagoon and along its geographical distribution (e.g. Dutch Wadden Sea), highlighting the need for adequate monitoring and sustainable management of their meadows. The socio-economic perspective concerning local activities, such as clam harvesting, should also be considered, in order to achieve an integrated management of coastal systems. This challenging task may represent the only way to protect seagrasses and this species in particular, and assure all the functions and services provided by their ecosystems to both marine coastal areas and humans.

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CHAPTER 8

Synthesis

8. Synthesis

1. The present study revealed that anthropogenic disturbances such as the urban wastewater discharges, the clam harvesting activity and the burial and erosion events have detrimental effects on the seagrass *Zostera noltii*, representing a serious threat to this species in Ria Formosa lagoon and elsewhere.

2. The level of impact was different depending on the type of disturbance. Activities related to physical or mechanical disturbance, such as the clam harvesting or the sediment burial and erosion, resulted in evident plant loss, whereas disturbances related to changes of the chemical environment, such as the urban wastewater discharges, resulted in changes at both plant- and population-level.

3. Human-induced activities resulting in changes of the sediment level were the primarily potential threat for *Z. noltii*, as the critical level of burial tolerated by this species is extremely low (50% mortality at 2 cm of burial and 100% mortality at 8 cm of burial). The small size of this species and the lack of vertical rhizomes make *Z. noltii* very vulnerable to changes of the sediment level. However, *Z. noltii* may recover from short-term burial of about 1 week, as suggested by the plant survival under burial (70% after 1 week) and the internal resource mobilization (50% of leaf N recovered by the rhizomes).

4. Changes of the sediment level revealed to be detrimental to seagrasses in general. The extent of the burial and erosion effects is species-specific and depends on the magnitude and on the frequency of disturbance. The capacity of seagrasses to resist sediment burial

is strongly size-dependent. Allometric relationships reveal that the leaf length and the rhizome diameter, which are architectural plant characteristics easily determined, are the best predictors of burial impact on seagrasses.

5. Recovery of seagrasses from sediment burial/erosion occurs often from natural events than from human-induced disturbances. The burial thresholds estimated for seagrass species are generally in accordance with the impacts reported by the field descriptive studies. In some cases, the recovery of seagrasses was related to the long-term meadow dynamics rather than to their specific burial thresholds.

6. Clam harvesting activity can also adversely affect *Z. noltii* meadows by significantly reducing shoot density and biomass. This loss seems to result from continuous disturbance, as experimental harvesting consisting of an isolated event revealed a short-term impact on shoot density (1 month). Recovery of disturbed meadows might occur through vegetative development, as long as modular units with at least 2 modules, including the apical shoot remain on the sediment.

7. The nutrient enrichment originated by the urban wastewater discharge affected the population structure, the plant morphology and the leaf N content of *Z. noltii*. The high ammonium concentrations (158.3-663.4 μM) at the site closest (270 m) to the nutrient source showed toxic effects on this species (lower biomass, smaller leaves and internodes). The higher abundance of macroalgae and epiphytes at this site may also affect the species negatively. Two of the main biotic processes operating within *Z. noltii* populations, i.e. the overall size of the plants and the biomass-density dynamics, were significantly correlated to the abiotic processes clearly related to the effects of the urban

wastewater, i.e. to the nutrients and anoxia conditions of the sediment, and to contrast between water column salinity and nutrient concentration, respectively. The adverse effects of the urban wastewater in *Z. noltii* meadows of Ria Formosa seem to be spatially restricted to areas up to 600 m distant to the WWTW discharge.

8. The *Z. noltii* population structure reflected the anthropogenic nutrient disturbance originated by the urban wastewater discharges, as indicated by the different biomass-density relationships found along the disturbance gradients. The nutrient-disturbed stations showed significant correlations between biomass and density, whereas at nutrient-undisturbed stations the biomass-density data were uncorrelated. The parameters derived from the *Z. noltii* population structure, namely the slope of the biomass-density relationships, the coefficient of variation of biomass and the ratio of maximum/minimum biomass increased with nutrient availability, whereas the intercept of the biomass-density relationships decreased with nutrient availability. These patterns may be used to assess global nutrient disturbance in coastal areas.

9. Both descriptive and experimental studies provided evidence of the negative impacts of the anthropogenic activities on the seagrass *Z. noltii*. However, the species fast growth and low modular integration seems to buffer the long-term effects of physical or mechanical disturbances (e.g. clam harvesting). The high plasticity of *Z. noltii* allows the species to adapt to changes of the surrounding environment, such as that caused by the urban wastewater discharges. These species-specific characteristics allow *Z. noltii* to sustain disturbance to a certain extent and to rapidly recover from it.