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The photosynthetic ecology of *Zostera noltii*

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Contents

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Esta é a melhor parte na escrita de qualquer tese. Em primeiro lugar porque marca o final de um processo longo e exigente. Depois porque implica parar um pouco para pensar em quem nos acompanhou ao longo dos últimos anos. E eu tive a sorte de ter sido muito bem acompanhado.

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Ao meu pai,
Por me ter mostrado o mar

Summary

Seagrass photosynthesis is usually measured in laboratory experiments, following oxygen evolution in closed chambers. Pulse amplitude modulated (PAM) fluorometry constitutes an alternative and non-intrusive method of measuring photosynthesis in the field. Validation of electron transport rate (ETR) measurements as reliable estimators of actual photosynthetic production requires that a significant linear relationship between oxygen production and ETR is demonstrated, and also that a 0.25 molar ratio between these two measures is verified. In this work, both parameters were measured simultaneously in laboratory experiments, over a range of light intensities, under well-defined and controlled conditions. A linear relationship was observed between the average rates of oxygen production and the electron transport rates for *Z. noltii* (Hornemann) obtained at several irradiances. The molar ratio found between oxygen production and ETR was 0.15 ± 0.02, lower than the theoretically expected value of 0.25. The use of PAM fluorescence as a valid proxy for photosynthetic production was validated for the range of 35 to 490 μmol photons $m^{-2}s^{-1}$ (PAR), under the assumption that the electron sinks responsible for the molar ratio deviation remain constant in similar experimental conditions.

The photosynthetic productivity of the intertidal communities dominated by the seagrass *Zostera noltii* and the cordgrass *Spartina maritima* was assessed in two contrasting situations during a tidal cycle, i.e., air exposure and water immersion. Two complementary methods were used: infra red gas analysis of CO_2 flux measurements in whole communities and chlorophyll *a* fluorescence measurements of individual plants photosynthetic activity. Higher photosynthetic rates of *Zostera noltii* in air were observed both at the individual plants response level, determined by chlorophyll fluorescence, and at the community level, measured as gas exchange (CO_2 uptake). *Spartina maritima* plants consistently showed low photosynthetic response when immersed. Gross community production (GCP), measured as carbon dioxide uptake, was always higher in air than in water, for both communities. When immersed, the GCP of both communities was similar. However, when exposed to the air, the GCP of the *S. maritima* community was higher than the one of *Z. noltii*'s. The key factor in CO_2 assimilation by air-exposed *Z. noltii* was the leaf water content. During low-tide, depressions in the sediment retain a considerable amount of water, enough to maintain leaf hydration. In these conditions, rapid air-water CO_2 diffusion occurs, making it

readily available to plants. The community gas exchange measurements compared well with the fluorescence indications. Both *Z. noltii* and *S. maritima* were shown to be responsible for the global pattern of photosynthetic carbon fixation within their respective communities, both during submersion and emersion periods. The short-term incubations method described in this report proved to be a valuable tool for field measurements of intertidal lagoon productivity. It provides fast and precise values of carbon dioxide fixation, both in submerged and air-exposed communities.

The relationship between the available photosynthetic active radiation (PAR) and the photosynthetic yield of photosystem II (PSII), measured by chlorophyll *a* fluorescence was assessed in the intertidal seagrasses of Ria Formosa, a coastal lagoon in southern Portugal. Most of the lagoons' intertidal is occupied by a monospecific population of *Zostera noltii*, which extends within a vertical gradient of about 2 meters. The upper limit of this species confines with the *Spartina maritima* salt marsh and the lower limit is defined by the transition to the subtidal, dominated by *Cymodocea nodosa*. The daily changes in the pattern of rapid light curves (RLC's) was investigated with pulse amplitude modulated (PAM) fluorometry in *C. nodosa* and in *Z. noltii* at the lower and upper intertidal. The light reactions of photosynthesis were assessed by fitting photosynthesis-irradiance (*P-I*) models to the RLC's. The photosynthetic parameters of *Z. noltii* indicated that in its lower and upper vertical distribution limits, this species presents, respectively, shade- and sun-type plant behaviour. The initial slopes of all the RLC's were steeper in the lower limit at low light but decreased with increasing irradiances, while in the upper limit values were always significantly lower but unaffected by increasing irradiances. *C. nodosa* presented a typical shade-type plant pattern, evidenced by the daily variation and light dependence of both photosynthetic efficiency and optimal quantum yield. The relationship between the maximum electron transport rate and irradiance, suggests that this species is strongly light-limited. We suggest that attempts to characterize the photosynthetic behaviour of an intertidal meadow should consider both the daily fluctuations in the plants photosynthetic activity, as well as its vertical distribution frequency.

The seasonal pattern of *Zostera noltii* photosynthesis was assessed in Ria Formosa lagoon (southern Portugal). Plants were sampled once a month, at both the upper and lower edges of a selected intertidal *Z. noltii* meadow. Photosynthetic capacity was determined at 320 μmol quanta $m^{-2}s^{-1}$ (PAR) in an oxygen electrode. Photosynthetic pigments, carbon, nitrogen, soluble protein and non-structural carbohydrates were also

determined monthly. The net photosynthetic capacity of *Zostera noltii* had a significant negative correlation with both the average daily PAR dose and the water temperature. It was lowest in the summer and highest in the winter months, at both the upper and lower sites. This pattern was the opposite of the most common response of seagrass photosynthesis to light and temperature, particularly of *Zostera noltii* populations from northern latitudes, where positive correlations of photosynthesis with temperature and irradiance have been described. This contrasting difference is attributed to the species adaptation to southern intertidal conditions, where light and temperature extremes are common in the summer. Our results revealed a clear seasonal productivity strategy. During the summer, the plants strategy is to cope with the extended daily periods of inhibitory irradiance, dissipating excessive energy and reducing the photosynthetic productivity. On the other hand, from October until February plants are exposed most of the day to irradiance levels between the saturation and the inhibition thresholds, which are the ideal conditions for photosynthesis and consequently for growth. The mild winter temperatures on Ria Formosa contribute to the success of this seasonal strategy.

Resumo

A actividade fotossintética das angiospérmicas marinhas é normalmente medida em laboratório, seguindo a evolução do oxigénio em câmaras de incubação fechadas. A fluorescência de pulso modulado (PAM) é uma técnica alternativa, que permite a determinação da actividade fotossintética no campo, de forma não intrusiva. A validação das medidas da taxa de transporte de electrões (ETR) como estimadores da taxa efectiva de fotossíntese requer a demonstração de uma relação linear entre a produção de oxigénio e a ETR, para além da existência de uma razão molar de 0.25 entre as duas medidas. Neste trabalho, ambos os parâmetros foram medidos simultaneamente, a várias intensidades luminosas, em condições bem definidas e controladas. Foi observada a existência de uma relação linear entre a produção de oxigénio e a taxa de transporte de electrões em *Z. noltii*. A razão molar entre as duas medidas foi de 0.15 ± 0.02, inferior à razão teórica de 0.25. O uso da fluorescência modulada como estimador da produção fotossintética foi validado para o intervalo de 35 a 490 μmol $m^{-2}s^{-1}$ (PAR), assumindo que os "sinks" de electrões responsáveis pelo desvio molar permanecem constantes em condições experimentais idênticas.

A produtividade das comunidades intertidais de *Zostera noltii* e de *Spartina maritima* foi avaliada nas duas situações contrastantes que ocorrem ao longo de um ciclo de maré, a exposição ao ar e os períodos de imersão. Foram utilizados dois métodos complementares: medidas do fluxo de CO_2 das comunidades através de análise de gases por infravermelhos (IRGA) e fluorescência da clorofila *a* em plantas individuais. As taxas fotossintéticas de *Z. noltii* e *S. maritima* foram consistentemente mais elevadas durante a exposição ao ar do que em imersão. Este resultado foi obtido tanto ao nível das respostas individuais das plantas como ao nível da comunidade. A produção bruta de ambas as comunidades foi idêntica quando em imersão. Nos períodos de exposição ao ar a produção da comunidade dominada por *S. maritima* foi mais elevada. O teor em água foi o factor chave na assimilação de CO_2 pelas plantas de *Z. noltii*, quando expostas ao ar. Nos períodos de baixa-mar, a microtopografia do sedimento favorece a retenção de água em quantidade suficiente para manter a hidratação das folhas. Nestas condições ocorre uma rápida difusão de CO_2 na interface ar-água, resultando numa elevada disponibilidade desta fonte de carbono para as plantas. As trocas gasosas medidas ao nível das comunidades compararam bem com os resultados da fluorescência. Foi demonstrado que tanto *Z. noltii* como *S. maritima*

foram responsáveis pelos padrões de fixação de carbono dentro das suas comunidades. As incubações curtas provaram ser um bom método para determinação da produtividade de comunidades intertidais, permitindo a rápida obtenção de medidas precisas da fixação de carbono, tanto durante os períodos de submersão como durante a exposição ao ar.

A relação entre a disponibilidade de radiação fotossinteticamente activa (PAR) e o rendimento efectivo do fotossistema II foi avaliada nas angiospérmicas marinhas da Ria Formosa (sul de Portugal). A maior parte da zona intertidal desta lagoa costeira é ocupada por uma população de *Zostera noltii*, que se estende ao longo de um gradiente vertical de cerca de 2 metros. O limite superior dos prados de *Z. noltii* confina com o sapal baixo, dominado por *Spartina maritima*. O limite inferior é determinado pela transição para o subtidal, onde se desenvolvem os prados de *Cymodocea nodosa*. As variações diurnas nos padrões fotossintéticos de *C. nodosa* e de *Z. noltii* nos seus limites inferior e superior foram investigadas pelo método de fluorescência de pulso modulado (PAM). Curvas rápidas de resposta à luz (RLCs) foram determinadas em vários momentos do dia. Os parâmetros fotossintéticos de *Z. noltii* revelaram que esta espécie se comporta como planta de sombra e de sol nos seus limites de distribuição inferior e superior, respectivamente. O declive inicial das curvas rápidas em plantas do limite inferior foi mais acentuado que em plantas do limite superior, mostrando ainda as primeiras uma maior reacção à variação da luz ambiente. *C. nodosa* revelou ter características de uma planta de sombra, com uma elevada dependência da luz e acentuada variação diurna da eficiência fotossintética. A relação entre a taxa máxima de transporte de electrões e a irradiância sugerem que esta planta é fortemente limitada pela luz. Os resultados indicam que a caracterização do comportamento fotossintético de populações intertidais de angiospérmicas marinhas devem ter em conta tanto as variações diurnas da actividade fotossintética como a freqüência de distribuição vertical das espécies.

O padrão de variação da fotossíntese de *Zostera noltii* ao longo de um ano foi estudado na Ria Formosa. Plantas de ambos os extremos da distribuição vertical desta espécie foram amostrados mensalmente. Foi determinada a capacidade fotossintética e os teores em pigmentos fotossintéticos, carbono, azoto, proteína solúvel, açúcares solúveis e amido. A capacidade fotossintética apresentou uma relação negativa com a dose média diária de radiação fotossinteticamente activa (PAR) e com a temperatura da água. Foi mais baixa no verão e mais elevada no inverno, em ambos os sítios de amostragem. Este padrão é oposto ao verificado normalmente noutras angiospérmicas marinhas, particularmente em populações de *Z. noltii* de outras latitudes. Este contraste é atribuído a uma adaptação desta espécie às condições das áreas intertidais do sul da Europa, onde valores extremos de luz e temperatura são freqüentes durante o verão. Os resultados revelam uma clara estratégia estacional. Durante o verão, as plantas suportam prolongados períodos diurnos de radiação fotoinibitória, dissipando a energia em excesso e reduzindo a productividade. Entre Outubro e Fevereiro as plantas dispõem de consideráveis períodos diários de irradiância saturante, mas não inibitória, o que proporciona condições óptimas para a fotossíntese e conseqüentemente para o crescimento. Esta estratégia é favorecida pelas temperaturas amenas, características do inverno na Ria Formosa.

Chapter 1

General introduction

General introduction

Seagrass dominated habitats are among the world's most productive coastal systems, along with mangroves and coral reefs (Duarte & Cebrián 1996, Duarte & Chiscano 1999, Touchette & Burkholder 2000). The ecological role of seagrasses extends from being simple food sources for fish, turtles or marine mammals up to the crucial buffer function in land-margin ecosystems. Seagrasses provide shelter, breeding and feeding grounds for a number of species, promote sediment stabilisation, filter contaminants, remove nutrients and provide the basis for important detrital food chains. Seagrass communities are also extremely valuable from an economic point of view, due to their high productivity and remarkable capacity for nutrient and organic matter cycling (Duarte & Cebrián 1996, Duarte & Chiscano 1999, Costanza et al. 1997).

Throughout the world, an increasing awareness of the seagrass populations' importance has led to the implementation of protective measures in seagrass dominated areas. Restoration programs have been implemented in locations where severe losses occurred. In Portugal, no legislation or protective measures exist, despite the occurrence of several important seagrass populations in ecologically sensitive areas of the coast. One of these areas is the Ria Formosa coastal lagoon (southern Portugal), a protected land-margin system of major ecological and economical relevance.

In the global changes scenario, the increase of atmospheric CO_2 concentration will also result in the rise of its concentration in seawater, particularly in near-shore systems (Beer & Koch 1996). In consequence, seagrass productivity is expected to increase considerably in the future, as the present photosynthetic rates of these plants are strongly CO_2-limited (Beer & Koch 1996, Thom 1996, Beer & Rehnberg 1997). In comparison, most marine macroalgae are almost fully Ci-saturated at the current CO_2 concentration. The relative contribution of seagrasses for carbon sequestration is then likely to increase in the future. Hence, it is important to investigate the mechanisms of carbon acquisition in seagrass species, as a way to establish the carbon sequestration potential of the coastal systems dominated by these plants. Carbon acquisition mechanisms in seagrasses are diverse and may follow either C_3 or C_4 pathways. In addition, a number of species-specific mechanisms occur (reviewed in Touchette & Burkholder 2000).

Zostera noltii (Hornemann) is the most abundant seagrass species in Ria Formosa. This lagoon is a highly-dynamic and very productive system, in a near-pristine condition. *Z. noltii* monospecific meadows dominate the intertidal area. These meadows are distributed along a ca. 2 m vertical gradient, from the lowest intertidal level up to the *Spartina maritima* (Curtis) Fernald saltmarsh (Silva & Santos 2003).

The global aim of this thesis research is the assessment of *Zostera noltii*'s productivity in Ria Formosa and its contribution for carbon sequestration within the system. This implies the acquirement of primary data, through field sampling and laboratory measurements. These primary data, the "small numbers", must be up scaled, i.e. multiplied by time periods and coverage areas, thus generating the global figures, the "big numbers". In the course of such a process, the error associated with the primary data is also propagated in a multiplicative manner. Hence, in order to obtain good "big numbers" it is imperative to start with much better "small numbers", by reducing their associated error.

In this context, it becomes important to develop and validate methodological approaches that enable reliable measurements of photosynthesis, preferably in the field, under natural conditions and in a reproducible manner. On the other hand, it is also important to understand the variations of primary productivity with time, not only the long-term (seasonal) variations but also the diurnal patterns, which are particularly complex and very significant in intertidal meadows (Silva & Santos 2003).

Seagrass photosynthetic activity is most commonly measured in laboratory experiments, following the oxygen evolution in closed incubation chambers with Clark-type electrodes. This method has provided most of the fundamental information about seagrass responses to factors such as light and temperature. However, it is extremely intrusive as it involves plant removal from the natural environment and a high degree of manipulation (Beer et al. 2001). The chlorophyll *a* fluorescence method has proven to be a very useful alternative for the assessment of photosynthetic light responses. This method is based on the principle that the amount of energy quenched through fluorescence is inversely correlated with the photosynthetic efficiency (Krause & Weis 1991).

In situ measurements of photosynthetic activity in seagrasses were made possible after the development of a submersible pulse amplitude modulated (PAM) fluorometer (Beer et al. 1998, Ralph et al. 1998, Beer & Björk 1999, Björk et al. 1999, Schwarz et al. 2000, Seddon & Cheshire 2001, Durako & Kunzelman 2002). The fluorescence method allows the assessment of seagrass photosynthetic rates under natural conditions, through a non intrusive process. Other methods, like CO_2 flux measurements, have also been assayed, either in the field or in the lab. Nevertheless, the comparisons of these methods against each other, although essential for their validation, have not often been carried out.

Light availability is a critical factor for seagrass productivity. Together with species-specific light requirements, it determines the lower depth limits of these plants distribution (Duarte 1991, Dennison et al. 1993, Kenworthy & Fonseca 1996, Bach et al. 1998, Leuschner et al. 1998, Koch & Beer 1996). Due to the physical constraints of its penetration in the water column, light is mostly considered as a limiting factor (Hemminga & Duarte 2000). Nevertheless, its excess can result in stress induction, with negative effects at the physiological level (Krause & Weis 1991, Demmig-Adams 1998, Ensminger et al. 2001, Ort 2001).

The dynamics of intertidal habitats are characterised by pronounced shifts in light and temperature, produced by the combined rhythms of tides and daily irradiance. This demands a high degree of photosynthetic plasticity from the species that inhabit theses areas. Intertidal seagrasses like *Zostera noltii* have adapted to such conditions and exhibit significant diurnal variations in their photosynthetic activity, interpreted as short-term responses to environmental changes (Silva & Santos 2003). Seasonal variations in photosynthetic patterns are also visible, particularly in temperate latitudes, due to the natural cycles of light, temperature and nutrient availability (Pérez & Romero 1992, Terrados & Ros 1995, Zimmerman et al. 1995, Vermaat & Verhagen 1996, Kraemer & Mazzela 1999, Welsh et al. 2000, Plus et al. 2001, Plus et al. 2003). Plants in temperate zones adapt to both the daily and the seasonal environmental changes, compensating them through several physiological mechanisms, a process generically designated as photosynthetic acclimation (Berry & Björkman 1980, Falkowski & LaRoche 1991, Evans & Poorter 2001, Major & Dunton 2002).

Two distinct and complementary sets of objectives were defined for this work: The first concerned the establishment and validation of new field methodologies for seagrass productivity assessment. The second set pursuit the understanding of time-related variations in *Zostera noltii* photosynthesis at Ria Formosa lagoon, southern Portugal. The first two chapters of this thesis report field and laboratory experiments in which different methods of measuring the photosynthetic activity of *Zostera noltii* were compared and validated. Another two chapters describe the variations of *Z. noltii*'s photosynthetic activity with time and discuss their implications in the assessment of this species productivity.

Specific objectives of this thesis were:

(i) To determine the relationship between the oxygen evolution and the electron transport rate for *Zostera noltii* and to assess the molar ratio of oxygen evolved per electrons transported. To achieve this, both the experimental setup and the methodological procedure were optimised.

(ii) To investigate the differences in the photosynthetic behaviour of *Zostera noltii* and *Spartina maritima* communities, in the two contrasting situations they experience during a tidal cycle, i.e., air exposure and water immersion, and to determine the contribution of dominant species to the pattern of photosynthetic carbon fixation within their respective communities, establishing a relationship between individual plants chlorophyll *a* fluorescence and community CO_2 fluxes.

(iii) To assess how the vertical position of seagrasses in the intertidal determines their daily photosynthetic performance. The relationship between the daily changes in available PAR and the photosynthetic yield of PSII, measured by chlorophyll *a* fluorescence, was investigated in *Zostera noltii* plants from Ria Formosa.

(iv) To assess the acclimation of the photosynthetic ecophysiology of *Zostera noltii* plants to the prevailing ambient conditions along the year. The photosynthetic parameters and the biochemical composition of *Z. noltii* were determined monthly at the species lower and upper vertical distribution limits.

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Chapter 2

Can chlorophyll fluorescence be used to estimate photosynthetic production in the seagrass *Zostera noltii*?

Silva J, Santos R (2004)
J Exp Mar Biol Ecol (in press)

Can chlorophyll fluorescence be used to estimate photosynthetic production in the seagrass *Zostera noltii*?

Abstract: Seagrass photosynthesis is usually measured in laboratory experiments, following oxygen evolution in closed chambers. Pulse amplitude modulated (PAM) fluorometry constitutes an alternative and non-intrusive method of measuring photosynthesis in the field. Validation of electron transport rate (ETR) measurements as reliable estimators of actual photosynthetic production requires that a significant linear relationship between oxygen production and ETR is demonstrated, and also that a 0.25 molar ratio between these two measures is verified. In this work, both parameters were measured simultaneously in laboratory experiments, over a range of light intensities, under well-defined and controlled conditions. A linear relationship was observed between the average rates of oxygen production and the electron transport rates for *Z. noltii* (Hornemann) obtained at several irradiances. The molar ratio found between oxygen production and ETR was 0.15 ± 0.02, lower than the theoretically expected value of 0.25. The use of PAM fluorescence as a valid proxy for photosynthetic production was validated for the range of 35 to 490 μmol photons $m^{-2}s^{-1}$ (PAR), under the assumption that the electron sinks responsible for the molar ratio deviation remain constant in similar experimental conditions.

Introduction

The photosynthetic characteristics of seagrasses have traditionally been studied in laboratory experiments, measuring the oxygen evolution in closed incubation chambers, using Clark-type electrodes. This method, which has provided most of the fundamental information about seagrass responses to factors such as light and temperature, is extremely intrusive as it involves plant removal from its natural environment (Beer et al. 1998).

The chlorophyll excitation energy dissipated by fluorescence has an inverse relationship with photosynthetic carbon assimilation (Walker 1988). This complex relationship led to the development of selective measuring techniques, meant to differentiate the several aspects of fluorescence emissions. One of those techniques is the pulse amplitude modulated (PAM) fluorometry, which due to a special emitter-detector unit allows fluorescence measures to be performed under full sunlight (Schreiber et al. 1988).

Theoretically, there is a molar ratio of 0.25 between the oxygen production and the electron transport rate, i.e., four mol of electrons are transported per each mol of oxygen produced (Walker 1987). Validation of ETR measurements as reliable estimators of actual photosynthetic production requires two basic conditions to be met. The first is that a linear relationship between oxygen production and ETR is demonstrated for the natural range of light levels. Secondly, the molar ratio between the two measures should be 0.25. These conditions have not been satisfied so far, and the first difficulty is inherent to the process itself. Although the quantum yield of photosystem II is closely related with the oxygen production, a number of relevant physiological steps occur between these two measurable events. Each step is conditioned by the previous one, and energy losses occur during the process, namely through heat dissipation or electron sinks (Longstaff et al. 2002). It is even possible to establish several intermediate quantum yields in this photosynthetic chain of events (Walker 1988, Kroon et al. 1993).

Comparisons of photosynthetic electron transport with oxygen evolution have been carried out in marine primary producers such as microphytobenthos (Flameling & Kromkamp 1998) and macroalgae (Hanelt & Nultsch 1995, Franklin and Badger 2001, Longstaff et al. 2002). In these reports, non-linear relationships were observed between the two measured variables at saturating irradiances, characterized by an overestimation of ETR with respect to oxygen evolution. In seagrasses, linear relationships between oxygen evolution and electron transport rate have been found for *Cymodocea nodosa* (Ucria) Ascherson (Beer et al. 1998) and *Halophila ovalis* (R. Brown) Hooker (Beer & Björk 2000). Decreases in oxygen production relative to ETR as irradiances increased were observed in *Zostera marina* L., *Halophila stipulacea* (Forsskål) Ascherson (Beer et al. 1998) and *Halodule wrightii* Ascherson (Beer & Björk 2000). *C. nodosa* photosynthesis, involving a C_4-like carbon acquisition mechanism, was not inhibited by high concentrations of ambient oxygen (Beer 1989). Therefore, the linearity of the relationship could not be affected by an increase in the oxygen concentration inside the reaction vessel.

Information about the carbon acquisition process in seagrasses is still far from being exhaustive (reviewed in Touchette & Burkholder 2000). Description of whether C_3 or

C_4 pathways occur is only available and confirmed for a few species, in spite of its crucial importance in the understanding of species-related photosynthetic behaviours. The lack of standardized methodologies for both fluorescence and oxygen production measurements represents a difficulty in the comparison of results among authors. In fact, each of these two types of determination has its own problems and sources of error (reviewed in Beer at al. 2001). Adding to that, a number of methodological details, dealt differently by distinct authors, also induce differences in estimates and additional degrees of uncertainty.

The main objectives of this work were both to determine the relationship between the oxygen evolution and the electron transport rate for the seagrass *Zostera noltii* (Hornemann), and to assess the molar ratio of oxygen evolved per electrons transported. To achieve these objectives, both the experimental setup and the methodological procedure were optimised. In particular, the effect of renovating the water of the reaction vessel prior to each photosynthetic measurement was tested. Procedures were carefully explained to allow putative comparisons with other works.

Materials and methods

Plant material

This study was specifically directed to *Zostera noltii* (Hornemann), a key species in the Ria Formosa lagoon, southern Portugal. In this coastal mesotidal system, most of the intertidal flats are occupied by meadows of this seagrass, which is responsible for a significant portion of the lagoon's primary production. Plants were collected from the intermediate section of an intertidal meadow in Ria Formosa (described in Silva & Santos 2003) in March 2002 and February 2003 and taken to the laboratory, where they were kept overnight in a growth chamber, programmed to emulate the natural conditions of temperature (18-20 °C) and photoperiod (13:11 day:night). All measurements were made after one night in the growth chamber, as a way of standardizing the procedure and allowing the plants to acclimate to lab conditions.

Absorption factor (AF)

The fraction of incident photosynthetic photon flux density (PPFD) absorbed by the leaves, the absorption factor (AF) was determined in a previous experiment (Silva & Santos 2003) following the procedure described by Beer et al. (2000). This method consists in measuring the irradiance that reaches a planar quantum sensor either when it is uncovered or covered by a seagrass leaf. AF is then expressed as the percentage of light absorbed by the leaf. The absorption factor (AF) obtained (0.79 ± 0.02, n=10) was introduced in the fluorometer settings and used instead of the instrument's pre-defined value of 0.84 to compute the electron transport rates (ETR). The use of an experimentally determined absorption factor is an essential condition for the calculation of absolute electron transport rates (μmol e^{-} $m^{-2}s^{-1}$).

Experimental setting

A square section incubation chamber (15 ml volume) coupled to a Clark-type oxygen electrode (DW3/CB1, Hansatech, Norfolk, UK) was used for measurements of oxygen production. Actinic light was provided by a slide projector (Pradovit 150, Leica, Germany) equipped with a halogen lamp (Osram Xenophot 150W). A series of neutral density filters mounted on slide frames were used to achieve different light intensities. Light intensities inside the chamber were measured with the Diving-Pam PAR quantum sensor, previously calibrated against an underwater planar PAR measuring device (Li-192SA underwater quantum sensor connected to a Li-1000 Data Logger, Li-Cor, Lincoln, Nebraska, USA). The light source was installed perpendicularly to a transparent window in the front of the incubation chamber.

For each measure (n=10), four independent segments ($\approx$2cm long, 30-40 mm^2 each) of *Zostera noltii* leaves (2nd-3rd youngest leaves only) were clipped side by side using a section of a slide-mounting frame, and held vertically inside the measuring chamber by two nylon threads. This methodological detail allowed the even exposure of all segments to the incident light. GF/F filtered seawater (35‰) was used in the reaction vessel. The incubation chamber was coupled to a magnetic stirrer which provided water homogenisation. Water temperature was kept constant at 20 °C, controlled by a thermostatic circulator (Raypa, Spain).

Fluorescence measurements were carried out with a pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Heinz Walz, Effeltrich, Germany). The measuring end of the instrument's optic fibre was positioned at a 70° angle with the sample, in front of the chamber's window, using a custom-made adaptor that attached the optic fibre to the slide projector's lens hood. This setting allowed fluorescence measures to be performed in the illuminated side of the leaf segments.

Oxygen and fluorescence measurements

For every sample, 8-10 light levels were applied sequentially by the slide projector, increasing from 35 to 490 μmol photons $m^{-2}s^{-1}$ (PAR), to obtain photosynthetic light response curves. Both oxygen evolution in the chamber and fluorescence in the light (F_s) were continuously measured. Each of the light steps took approximately 7 min., the time necessary to obtain a straight line in the oxygen recording system, assumed as steady-state photosynthesis. At the end of every light step, a 0.6 s saturating light pulse (ca. 5000 μmol photons $m^{-2}s^{-1}$) was applied using the fluorometer's halogen lamp, for maximum fluorescence (F_m') determination. The effective quantum yield of photosystem II [$Y = (F_m' - F_s)/ F_m' = \Delta F/F_m'$] (being F_s the fluorescence in the light) (Genty et al. 1989) and the electron transport rate (ETR = Y x PPFD x AF x 0.5) (Schreiber et al. 1995) were then computed for that specific light level. Both the oxygen evolution and the electron transport rate curves were fitted with the model equation of Smith (1936) and Talling (1957)

$$P = P_m\, [\alpha I / (P_m^2 + (\alpha I)^2)^{1/2}]$$

in which I is the irradiance (or PPFD), α is the ascending slope at limiting irradiances, and P_m is the maximum photosynthetic rate (or the maximum electron transport rate). Curve fitting was performed iteratively using the SigmaPlot software package.

The effect of changing the water in the reaction vessel before each light step was previously tested in a separate experiment, where light response curves were obtained with and without water exchange. A clear limitation of the oxygen production was verified when the water was not changed between the light steps. Therefore, in latter measurements, the water in the reaction vessel was quickly removed before every light step and replaced by new water from the same original stock, previously brought to the measuring temperature. This prevented the onset of supersaturating and potential inhibiting oxygen levels in the chamber and also the occurrence of excessive pH drifts. Net photosynthesis data had to be converted into gross photosynthesis, for comparison with electron transport rates, and therefore respiration measurements were also conducted. The best timing for measuring dark respiration was previously assessed by taking measures after every light step or, alternatively, at just two predefined moments of the light response curve, one in the light-limited section and another one in the end of all light steps. We found that the latter timing produced better results. When dark periods (7-8 min.) were imposed after every light step, the oxygen values tended to be ca. 30% lower. Therefore, after the first three light steps and in the end of the light response curves, light was turned off and respiration was measured (7-8 min. dark periods). After each experiment, the leaf segments area was measured, and the small portions shaded by the support clip were subtracted from the total area.

Results

A linear relationship ($r^2 = 0.95$) was observed between the average rates of oxygen production and the electron transport rates obtained at several irradiances (Fig. 1).

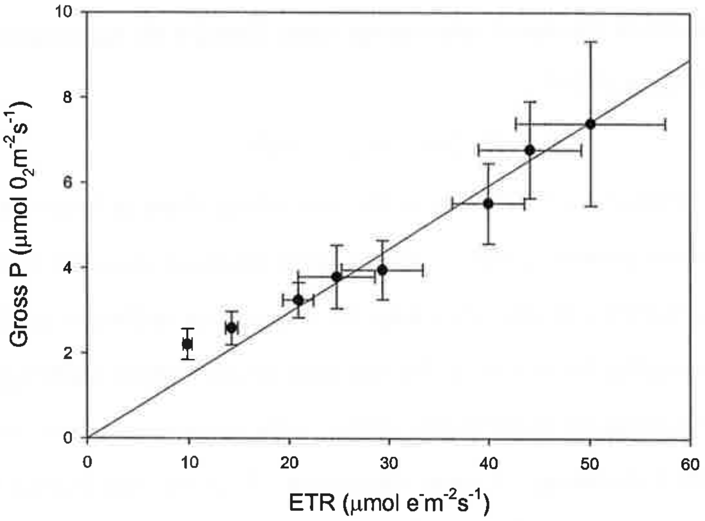

Fig. 1. *Zostera noltii*. Average values (± SE) of gross photosynthesis (Gross P, µmol O_2 m^{-2}s^{-1}) and electron transport rates (ETR, µmol e^{-} m^{-2}s^{-1}) measured in leaf segments at 20°C (n=10, 4 independent segments per replicate), with water exchange between light steps. Irradiance increases from left to right, ranging between 35 to 490 µmol m^{-2}s^{-1}, PAR.

There was an evident increase of the data dispersion with irradiance both in the oxygen and in the fluorescence measurements, indicating a higher physiological variation among plants at higher irradiances. The molar ratio of oxygen production and ETR was 0.15 ± 0.02, different from the theoretically expected value of 0.25 (4 mol electrons per mol oxygen produced).

The effect of changing the water between light steps is very clear in Fig. 2. Without the water exchange, oxygen production became strongly limited, for ETR values higher than 30 μmol e^- $m^{-2}s^{-1}$, near the end of the light-limited part of the response curve.

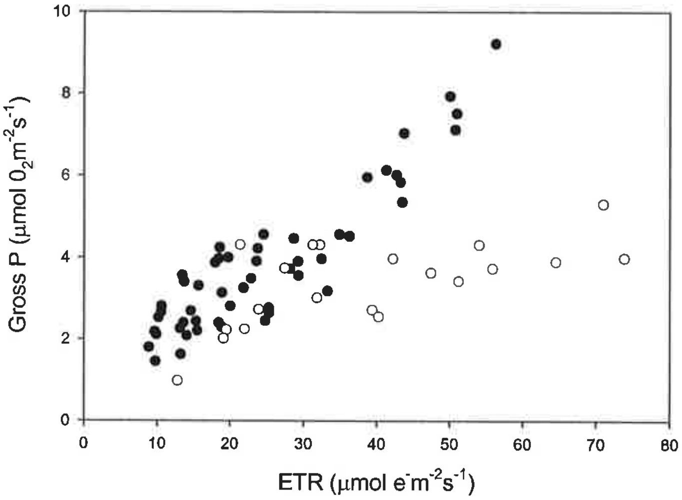

Fig. 2. *Zostera noltii*. Gross photosynthesis (Gross P, μmol O_2 $m^{-2}s^{-1}$) and electron transport rates (ETR, μmol e^- $m^{-2}s^{-1}$), measured with (•) and without (○) water renewal in the reaction chamber between light steps. Measurements were made along an irradiance range of 35 to 700 μmol $m^{-2}s^{-1}$ (PAR) at 20°C.

Different types of light response kinetics between the two photosynthetic measures were visible in the plots of the oxygen evolution and the electron transport rate versus PPFD (Fig. 3). Three aspects of this difference are particularly evident: (i) in the light-limited portion of the curves, the slope of the ETR curve (0.254 ± 0.009) was significantly higher ($p<0.05$) than the slope of the oxygen evolution curve (0.042 ± 0.003), (ii) the light saturation point (expressed as $I_k = P_m/\alpha$) was also higher in the ETR curve (217 μmol photons $m^{-2}s^{-1}$) than in the oxygen evolution curve (175 μmol photons $m^{-2}s^{-1}$) and (iii) the data dispersal was higher in the oxygen data when compared with the fluorescence measures, both increasing with irradiance.

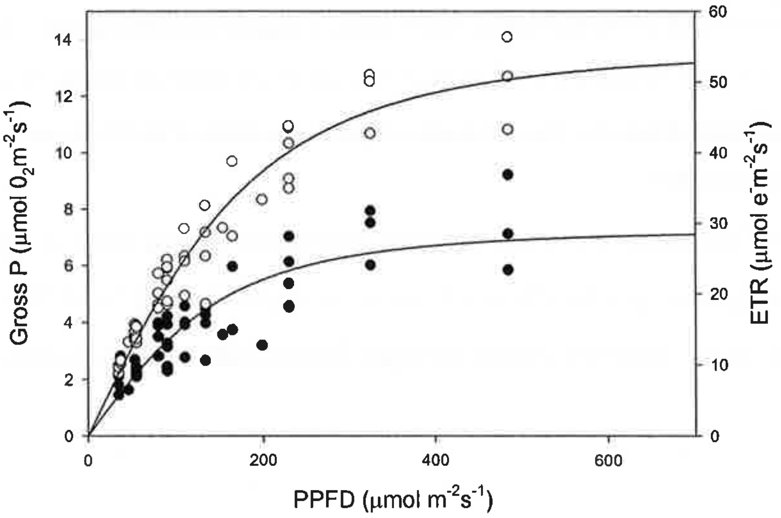

Fig. 3. *Zostera noltii*. Light response curves of oxygen evolution (•) and electron transport rates (○), determined in leaf segments at 20°C and fitted with the model equation of Smith (1936) and Talling (1957).

Discussion

A linear relationship was found between the oxygen evolution and the electron transport rate for *Zostera noltii*. The linearity of this relationship is one of the key aspects in the validation of fluorescence measures for estimates of primary productivity. Non-linear relationships are usually characterized by a drop in the oxygen production with regard to the electron transport rate, at higher irradiances. Increases in processes like the Mehler-ascorbate-peroxidase reaction and photorespiration, have been pointed as possible causes for the discrepancies between electron transport and oxygen evolution (Barranguet & Kromkamp 2000, Longstaff et al. 2002). Other authors suggest that electron cycling around PSII and non-photochemical quenching in PSII reaction centres are the likeliest causes for the loss of correlation (Franklin & Badger 2001) at saturating light levels.

The occurrence of photorespiration in seagrasses is poorly described. We speculate that plants which are well adapted to both high light and high temperature conditions, or possess high physiological plasticity, can also be less sensitive to a build-up of the oxygen in the electrode incubation chamber. This is the case of *Halophila ovalis* (Ralph 1999) and the intertidal *Z. noltii* (Silva & Santos 2003) used in our experiments. These plants may eventually work as C_4-like plants, with a carbon concentrating mechanism, which would be ecological advantageous in their intertidal habitat.

The molar ratio of oxygen production and ETR found in this experiment (0.15 ± 0.02) was lower than the theoretical value of 0.25 (4 mol electrons per mol oxygen produced). The reasons for this difference must obviously be found in either the photosynthetic oxygen evolution measurements or in the calculation of the electron transport rate.

Most seagrasses possess a considerable volume of aerenchyma tissue, which causes imprecision in photosynthesis measurements, since a considerable portion of the evolved oxygen is released into this aerenchyma and partially recycled. Considering that approximately 60% of *Z. noltii*'s leaf volume is occupied by aerenchyma, and that oxygen release into its vessels is a known phenomenon, this could be a potential source of systematic oxygen underestimation. However, at all irradiances, oxygen fluxes were measured at steady-state, which means the aerenchyma had enough time to reach equilibrium with the medium.

Another possible source of error in the calculation of oxygen production has to do with the correction of net to gross photosynthesis. This correction requires that, in addition to net photosynthesis, respiration must also be measured. For this purpose, dark periods are usually imposed after every light step and respiration is measured. Its value is then added to the previous net photosynthetic measure, to calculate a gross photosynthesis value. From the theoretical point of view, this procedure appears to be the most correct, as it allows a better estimate of respiration on the presence of light, and therefore a more accurate determination of gross photosynthesis. When respiration was measured after every light step, *Z. noltii* gross photosynthesis tended to be lower than when respiration was measured in only two moments. We found that one respiration measurement in the light-limited part of the curve and another one in the saturated part are enough to provide a good indication of how respiration evolves with light, and allows a reliable calculation of gross photosynthesis. Additionally, the constant light switching does not occur in natural conditions and it constitutes an experimental artifact. A gradual light increase provides a better emulation of the natural conditions. This suggests that the conversion of net into gross photosynthesis was not a source of oxygen underestimation in our results.

Alteration of the incubation medium during photosynthesis measurements also induces error (Walker 1987). Typically, these alterations consist of: (i) an increase in the oxygen concentration inside the chamber, (ii) a reduction of inorganic carbon (C_i) and (iii) pH drifts. Common ways of dealing with such problems include reduction of oxygen by nitrogen bubbling, addition of bicarbonate to prevent C_i limitation and buffering the medium to prevent pH drifts. However, all these solutions contribute to add more experimental artifacts.

Since this work was partially focused in the assessment of the validity of chlorophyll fluorescence as a proxy for production estimates, we chose to keep the experimental conditions as close as possible to the field environment. Therefore, instead of bubbling nitrogen, adding bicarbonate or buffering the water, we simply changed the water in the reaction chamber before every light step. This produced a significant effect in the oxygen evolution. Water exchange between light steps prevents oxygen inhibition of carbon uptake (Littler 1979), and provides equivalent medium conditions for the whole light range, emulating the natural water renewal that occurs in field conditions. On the other hand, major sources of error in the estimation of electron transport rates include inappropriate calculation of the leaf absorption factor (AF) or inaccurate PAR measurements (Perkins et al. 2002). Discarding light reflection by the leaf surface, which is considered negligible in marine plants (Beer et al. 2001), absorption may be somewhat overestimated due to light absorption by internal leaf structures other than the chloroplasts. However, this possibility appears to be more important in thick-leaved seagrasses (Beer et al. 2000).

An accurate measure of the photosynthetic active radiation (PAR) that actually reaches the leaf surface is also crucial for a correct calculation of the electron transport rate. The accuracy of PAR measurements, apart from the sensor quality, depend on how close to the leaf position can the sensor be placed. In this experiment, PAR measurements were done placing the calibrated sensor in the exact same position as the leaf segments inside the measuring chamber. The use of leaf segments instead of whole leaves allowed an even illumination of the sample. Therefore, PAR measurements were not a likely source of error in the calculation of electron transport rates. Preliminary measures showed no noticeable differences in the short-term photosynthetic response of leaf segments and whole leaves.

In simultaneous measurements of oxygen and ETR, the positioning of the PAM's optic fibre is an important methodological point. The fibre may be positioned either on the top, the rear or the front of the reaction vessel. However, fluorescence values are likely to vary, depending on the fibre's position. When the fibre is positioned in front of the irradiated surface of the leaf, as in our experiment, the fluorescence yield is lower than when measured in a shaded portion of the same leaf. All these experimental details may affect results directly, and should therefore be looked in carefully.

Other methodological details previously tested included finding the ideal amount of leaf segments to use and their positioning inside the reaction vessel. Provided that a good signal/noise ratio is obtained, the lowest possible number of leaf segments was preferred, since less oxygen per water volume is produced and less C_i is consumed. This resulted in fairly constant conditions in the chamber for the light step measuring period. Mounting the leaf segments side by side allowed a more uniform distribution of the incident actinic light through the whole leaf area.

Assuming that a 0.25 molar ratio between oxygen evolution and electron transport must be verified, the use of chlorophyll fluorescence for production estimates depends fundamentally on proper methodological validation. Therefore, being a methodological question, it is essential that full details are given whenever such experiments are described, to allow comparisons of results among authors.

Considering the problems that result from the use of each method, we found that chlorophyll fluorescence presents less experimental problems, and probably less sources of error than oxygen evolution. A similar conclusion was reported by Barranguet & Kromkamp (2000), who found PAM fluorometry to be more robust than carbon fixation methods on the estimation of microphytobenthos productivity. The use of PAM fluorescence as a valid proxy for photosynthetic production must be validated for a determined light range, over which a linear relationship between electron transport and oxygen evolution must exist. Validation of PAM fluorescence for *Zostera noltii*'s photosynthetic production estimates at higher irradiances is still needed, as the natural range of irradiances extends up to ca. 2000 μmol photons $m^{-2}s^{-1}$. On the other hand, we believe that a molar ratio lower than the theoretical value of 0.25 may still be used for unit conversion purposes, assuming that the electron sinks responsible for the ratio deviation remain constant in similar experimental conditions. This requires however a careful control of the experimental conditions and methodological steps, somewhat difficult to achieve under field conditions.

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Chapter 3

Submerged versus air-exposed intertidal lagoon productivity: from Chl *a* fluorescence to CO_2 fixation

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to be submitted to J Exp Mar Biol Ecol

Submerged versus air-exposed intertidal lagoon productivity: from Chl *a* fluorescence to CO_2 fixation

Abstract: The photosynthetic productivity of the intertidal communities dominated by the seagrass *Zostera noltii* and the cordgrass *Spartina maritima* was assessed in two contrasting situations during a tidal cycle, i.e., air exposure and water immersion. Two complementary methods were used: infra red gas analysis of CO_2 flux measurements in whole communities and chlorophyll *a* fluorescence measurements of individual plants photosynthetic activity. Higher photosynthetic rates of *Zostera noltii* in air were observed both at the individual plants response level, determined by chlorophyll fluorescence, and at the community level, measured as gas exchange (CO_2 uptake). *Spartina maritima* plants consistently showed low photosynthetic response when immersed. Gross community production (GCP), measured as carbon dioxide uptake, was always higher in air than in water, for both communities. When immersed, the GCP of both communities was similar. However, when exposed to the air, the GCP of the *S. maritima* community was higher than the one of *Z. noltii*'s. The key factor in CO_2 assimilation by air-exposed *Z. noltii* was the leaf water content. During low-tide, depressions in the sediment retain a considerable amount of water, enough to maintain leaf hydration. In these conditions, rapid air-water CO_2 diffusion occurs, making it readily available to plants. The community gas exchange measurements compared well with the fluorescence indications. Both *Z. noltii* and *S. maritima* were shown to be responsible for the global pattern of photosynthetic carbon fixation within their respective communities, both during submersion and emersion periods. The short-term incubations method described in this report proved to be a valuable tool for field measurements of intertidal lagoon productivity. It provides fast and precise values of carbon dioxide fixation, both in submerged and air-exposed communities.

Introduction

The productivity of intertidal lagoon and salt marsh communities is usually assessed following the evolution of biomass with time (Short & Duarte 2001). Although not so widespread, gas exchange methods, based on infra red gas analysis (IRGA) of carbon dioxide (CO_2) fluxes, have also been employed, either using flow-through systems or closed chambers (Streever et al. 1998). Studies involving CO_2 flux measurements are more common in salt marsh communities than in seagrass meadows. In the former systems, carbon fluxes have been measured either in individual leaves, incubated on special leaf-chambers with temperature control and artificial light, or in whole plants, using larger incubation chambers (Streever et al. 1998 and references therein). These chambers may be more or less complex, with or without temperature and/or humidity control, and usually use natural light.

Carbon flux measurements using infra red gas analysis in seagrasses are scarce, particularly on *Zostera noltii*. Leuschner & Rees (1993), Pérez-Lloréns & Niell (1994) and Leuschner et al. (1998) measured apparent rates of *Zostera noltii* CO_2 uptake in air, in laboratorial experiments. These measurements were performed with minicuvette-type IRGA systems, with individual leaves being incubated in temperature-controlled leaf chambers.

In salt marsh plants and in seagrasses, all CO_2 uptake measurements reported so far were done in air-exposed plants. Considering the long submersion periods that both communities experience in every tide, it is important to measure CO_2 fluxes also underwater. Here we present a method to measure CO_2 uptake rates of the cordgrass *Spartina maritima* and the seagrass *Zostera noltii*, either when exposed to the air or during submersion.

Streever et al. (1998) described an apparatus which included a closed transparent incubation chamber, fitted to the sediment, and an external CO_2 analyser. This system proved to be rather efficient in estimating salt marsh productivity in the air. One advantage of this kind of apparatus is that both the plants and the soil beneath them are included in the incubations, which allows a more realistic measure of productivity per ground area. We followed the same basic approach of Streever et al. (1998), i.e., to use large incubation chambers fitted to sediment, and to route the air through an external gas analyser for continuous carbon dioxide flux measurements. This was done both in submersed and emerged conditions. These fluxes represent the metabolism of the entire community dominated by the target plant species, both in the water and in the air. The simultaneous measurement of the individual plants photosynthetic activity by chlorophyll fluorescence allowed us to assess how the community metabolism (CO_2 fluxes) compared with plant photosynthesis.

Ria Formosa is a coastal lagoon in the South coast of Portugal, characterised by large intertidal flats with a salt marsh community that covers ca. 43% of the lagoon's area (Andrade 1990). The lower intertidal zone is dominated by meadows of the seagrass *Zostera noltii*, distributed within a vertical gradient of ca. 2 m. Confining with the upper limit of *Z. noltii* distribution, the lower marsh is dominated by a monospecific stand of the cordgrass *Spartina maritima*, regularly flooded during high tides. Both species experience alternate daily periods of immersion and air exposure, but while *Z. noltii* is an aquatic plant capable of withstanding periods of air exposure, *S. maritima* is a halophyte plant, withstanding regular submersion periods.

The main aim of this work was to assess the photosynthetic productivity of the intertidal communities dominated by the seagrass *Zostera noltii* and the cordgrass *Spartina maritima*. Specific objectives were (i) to investigate the differences in the photosynthetic behaviour of *Zostera noltii* and *Spartina maritima* communities, in the two contrasting situations they experience during a tidal cycle, air exposure and submersion and (ii) to determine the contribution of dominant species to the pattern of photosynthetic carbon fixation within their respective communities, establishing a relationship between individual plants chlorophyll *a* fluorescence and community CO_2 fluxes.

Materials and methods

Sampling strategy

The metabolism of *Zostera noltii* (Hornemann) and *Spartina maritima* (Curtis) Fernald dominated communities was assessed through gas (CO_2 and O_2) exchange measurements in incubation chambers, using an infra red gas analyser (IRGA). Simultaneously, the photosynthetic performance of individual plants was determined by chlorophyll *a* fluorescence measurements, with a pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Walz, Germany). Both types of measurements were performed in air and in water, at different times of the day. *Z. noltii* was sampled in the morning and in the afternoon. *S. maritima* was sampled in the morning, at midday and in the afternoon. Dark incubations were also performed for community respiration assessment, allowing the calculation of gross community production (GCP) within the community. All measurements were performed *in situ*, at ambient temperatures. Independently of the tide and the time of the day, sampling was always carried out at the same vertical level of the communities' distribution, to isolate possible zonation effects, previously reported for this *Z. noltii* population (Silva & Santos 2003, Alexandre et al. in press).

Aerial photosynthetic active radiation (PAR) was measured in continuum during the experiment, using a planar PAR measuring device (Li-190, Li-Cor, USA). Underwater PAR inside and outside the incubation chambers was measured with the Diving-Pam PAR quantum sensor, previously calibrated against an underwater planar PAR measuring device (Li-192SA, Li-Cor, USA).

Gas exchange measurements

Cylindrical incubation chambers made of transparent plexiglass (172mm in diameter, 400mm high) were used to incubate community samples of both *Zostera noltii* and *Spartina maritima*. Three individual incubations were performed for every sampling point. Each incubation lasted no longer than 20 minutes. The chambers were fitted to the sediment so that no air exchange was possible. The height of the air column inside the chambers was measured in order to calculate the effective incubation volume. Following every incubation, the plant material contained within the chamber was collected, for volume determination and weighting.

On air-exposure incubations, the gas stream was routed to a non-dispersive infrared gas analyser that measures CO_2 concentrations in a range from 0 to 2000ppm (μmol mol^{-1}) (EGM-4, PP-systems, USA). A desiccation column filled with anhydrous calcium sulphate (Drierite, USA) was placed before the gas entry into the analyser, to remove humidity from the air and to avoid water molecular interferences in the carbon dioxide molecules infra-red measurements. The gas within the chamber was continuously monitored over 10-20min, being the CO_2 concentration recorded every minute. Linear gas concentration evolution through time was monitored.

To perform the underwater immersion incubations, chambers were placed in the sediment as described above, using SCUBA-diving. The chambers were flooded and air bubbles were completely removed. For gas measurements, the water was pumped with a peristaltic pump (Dinko Instruments, Spain) through a gas exchange column (Mini-Module Membrane Contactor, Celgard, USA) where air was flowing. After reaching equilibrium with the water coming from the chamber, the gas was then routed to the gas analyser and the CO_2 concentration was recorded as described above.

The net flux (F) of CO_2 (mmol C $m^{-2}h^{-1}$) was then computed as:

$$F = s \times mv \times V / A \times 60/1000$$

where s (ppm CO_2 min^{-1} = (μmol CO_2 mol air^{-1}) min^{-1}) is the slope of the change in concentration of CO_2 over time during the incubation period, V (L) is the chamber volume, A (m^2) is the benthic exchange area, and mv (mol L^{-1}) is the molar volume or number of gas mol per volume unit, calculated from the ideal gases states law ($mv = P / (R \times T)$), where P (atm) is the gas pressure, T (K) is the temperature, and R (0.082 atm L K^{-1} mol^{-1}) is the universal gas constant.

The net CO_2 flux is a measure of the net community production (NCP), and the CO_2 flux measured during the night represents the community respiration (CR= - night CO_2 flux). Thus the gross community production (GCP) is given by NCP + R.

Fluorescence measurements

Rapid light curves (RLC's) were measured in selected leaves (2^{nd}-3^{rd} youngest) of both *Zostera noltii* and *Spartina maritima*. Leaves were submitted to a series of eight increasing irradiance levels, supplied by the halogen light source incorporated in the fluorometer. During this period, samples were shaded to prevent superimposition of ambient light on the light supplied by the instrument. At the end of each irradiance level (15 s periods), a 0.6 s saturating light pulse (ca. 4000 μmol photons $m^{-2}s^{-1}$) was applied for maximum fluorescence (F_m') determination. The effective quantum yield of photosystem II [$Y = (F_m' - F_s)/ F_m' = \Delta F/F_m'$] (Genty et al. 1989) was computed and the electron transport rate (ETR) was calculated for each pre-set irradiance level (Schreiber et al. 1995) as

$$ETR = Y \times \text{Irradiance } (I) \times \text{AF} \times 0.5$$

where AF is the absorption factor, i.e. the fraction of incident photosynthetic photon flux absorbed by the leaves. The *Zostera noltii* absorption factor was previously determined (0.79, Silva & Santos, 2003). The absorption factor of *Spartina maritima*, was kept at 0.84, which is the instrument's default value, determined for terrestrial plants. The model equation of Bannister (1979) was fitted to the ETR vs. I data plots

$$ETR = ETR_m \, [\alpha I / (ETR_m^{\,c} + (\alpha I)^c)^{1/c}]$$

where I is irradiance, a is the ascending slope at limiting irradiances, and ETR_m is the maximum electron transport rate. The half-saturation irradiance, I_k, was calculated as the ratio between ETR_m and a.

In parallel with rapid light curves, the effective quantum yield (Y) was determined at ambient light. A saturating light pulse was applied to light-adapted leaves, identical to the one used in the RLC's, for maximum fluorescence (F_m') determination. The quantum yield value was used to calculate the instantaneous electron transport rate (ETR) at ambient irradiance, using the ETR calculation formula described above.

Results

Zostera noltii presented higher photosynthetic rates in air than underwater (Fig. 1).

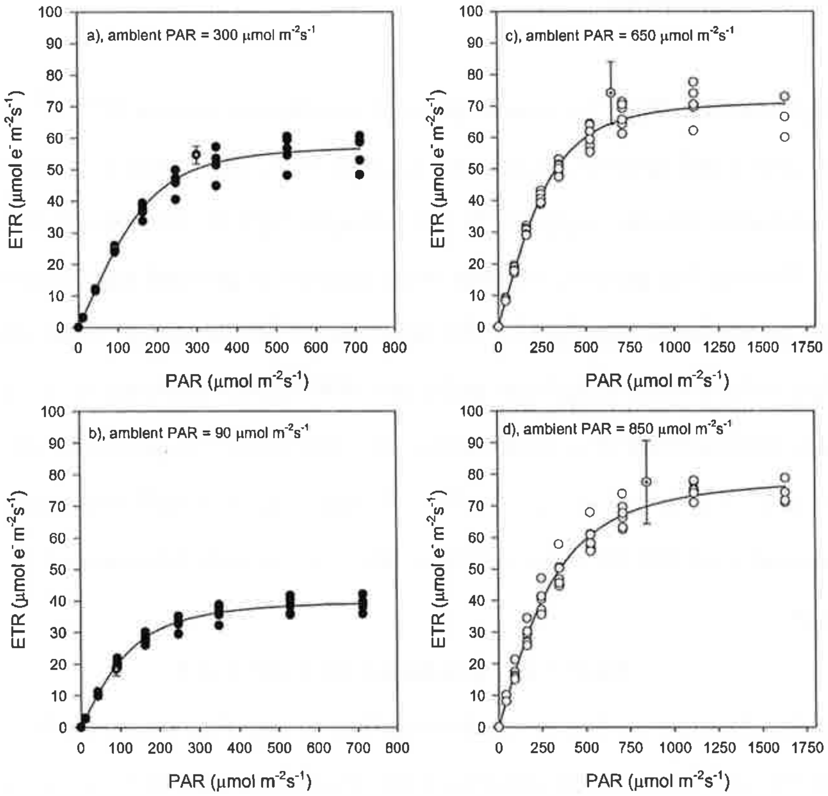

Fig. 1. *Zostera noltii*. Rapid light curves measured in water (closed circles) and in air (open circles), at different times during the day. Cross-haired symbols with standard error bars represent instantaneous electron transport rates (ETR) determined in individual plants at ambient PAR.

Table 1
Photosynthetic parameters derived from the model adjusted to rapid light curves

Species (condition)	ETRm (μmol e$^-$m^{-2}s^{-1})	α	Ik (μmol m^{-2}s^{-1})
Z. noltii (underwater)	40.2 (0.9)	0.249 (0.015)	161.4 (10.3)
	57.6 (1.7)	0.273 (0.019)	211.8 (15.8)
Z. noltii (in air)	71.8 (1.7)	0.191 (0.010)	375.3 (20.7)
	79.2 (2.5)	0.189 (0.011)	417.9 (28.4)
S. maritima (underwater)	30.5 (1.4)	0.275 (0.059)	110.8 (24.6)
	29.8 (1.2)	0.243 (0.077)	122.2 (40.2)
	26.7 (0.9)	0.203 (0.027)	131.1 (18.6)
S. maritima (in air)	60.2 (1.5)	0.250 (0.023)	240.5 (23.6)
	59.2 (1.9)	0.258 (0.025)	229.2 (24.2)
	86.2 (1.6)	0.223 (0.009)	385.3 (17.3)

Values are means (SE)

The maximum rates of electron transport (ETR$_m$) were obtained in air exposed plants, during the morning period and near midday (Fig.1 c and d), reaching a maximum of 79.2 μmol e$^-$ m^{-2}s^{-1}, at 850 μmol m^{-2}s^{-1} (Table 1). The model adjustment to the rapid light curves (RLCs) revealed significantly lower initial slopes (α) in air-exposed plants (Table 1), and consequently higher values for the half-saturation irradiances (Table 1), when compared to submerged plants. Instantaneous electron transport rates (ETR) determined in individual plants at ambient PAR fitted well in the light response curves. These instantaneous values varied directly with ambient PAR, both in air exposed and in submerged plants.

Spartina maritima plants consistently showed low photosynthetic response during the day when submerged (Fig. 2 a to c). Maximum electron transport rates varied between 26.7 and 30.5 μmol e$^-$ m^{-2}s^{-1} (Table 1) showing little or no dependence of ambient PAR, which varied between 40 and 350 μmol m^{-2}s^{-1}. The half-saturation irradiances were lower than in air exposed plants, respectively 111-131 μmol m^{-2}s^{-1} against 229-385 μmol m^{-2}s^{-1} (Table 1). When in air (Fig. 2 d to f), these plants exhibited a very different photosynthetic response, with ETR$_m$ varying directly with ambient

PAR, up to a maximum of 86.2 µmol e$^-$ m^{-2}s^{-1}, obtained at 900 µmol m^{-2}s^{-1}. Instantaneous electron transport rates (ETR) determined in individual plants at ambient PAR also fitted well in the light response curves, particularly in immersed plants.

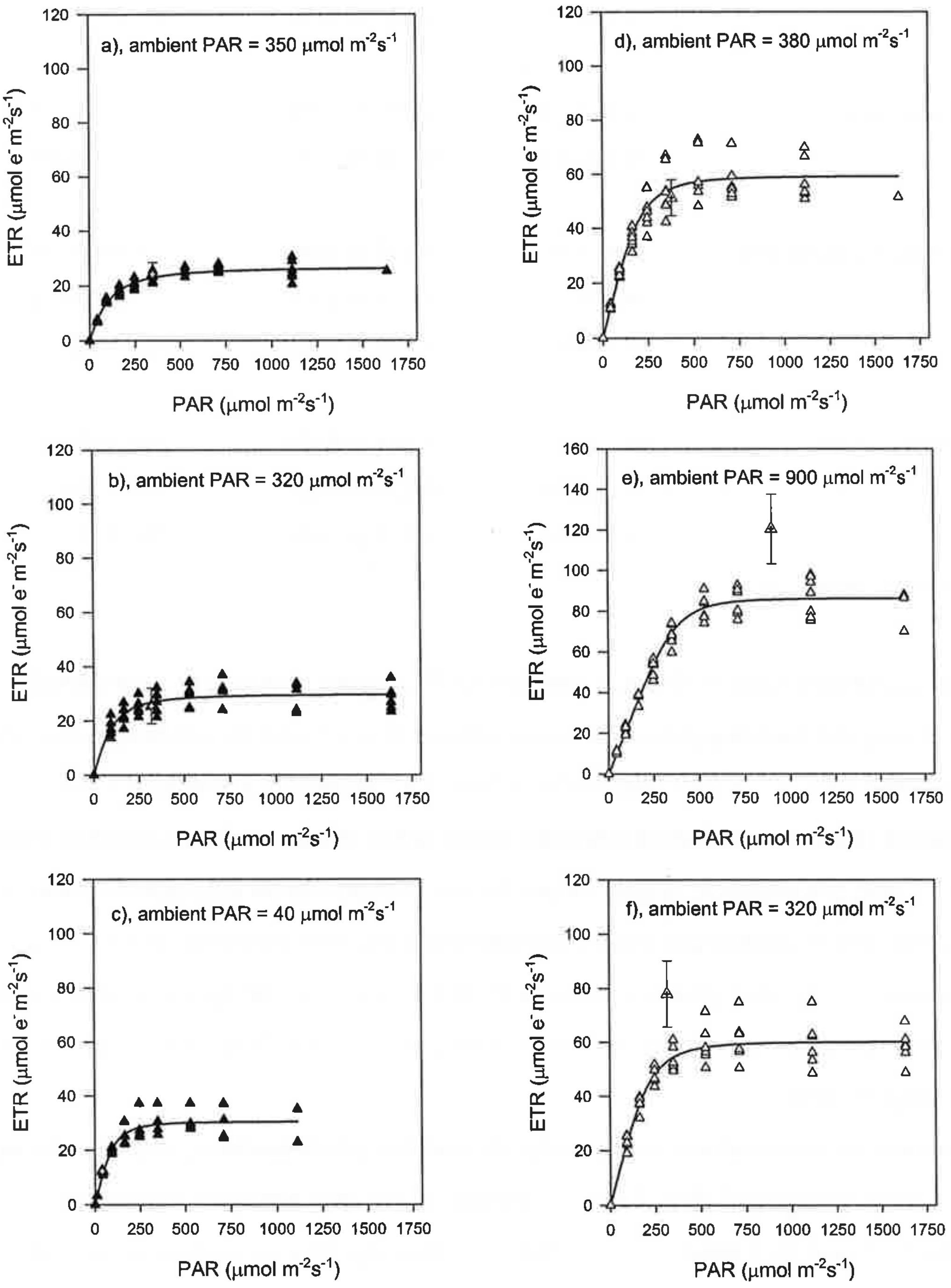

Fig. 2. *Spartina maritima*. Rapid light curves measured in water (closed triangles) and in air (open triangles), at different times during the day. Cross-haired symbols with standard error bars represent instantaneous electron transport rates (ETR) determined in individual plants at ambient PAR.

Gross community production (GCP), measured as carbon dioxide fixation, was always higher in air than in water, both for *Z. noltii* and *S. maritima* communities (Fig. 3). When exposed to the air, the GCP of the *S. maritima* (Fig. 3 b) community was higher than the one of *Z. noltii*'s (Fig. 3 a). When submerged, the GCP of both communities was similar. In both communities, dark respiration was higher in air than in water. Although the data sets are not extensive, apparently GCP tended to saturate in water, in both communities, at PAR levels between 300 and 400 µmol $m^{-2}s^{-1}$, whereas in air such saturation was not visible.

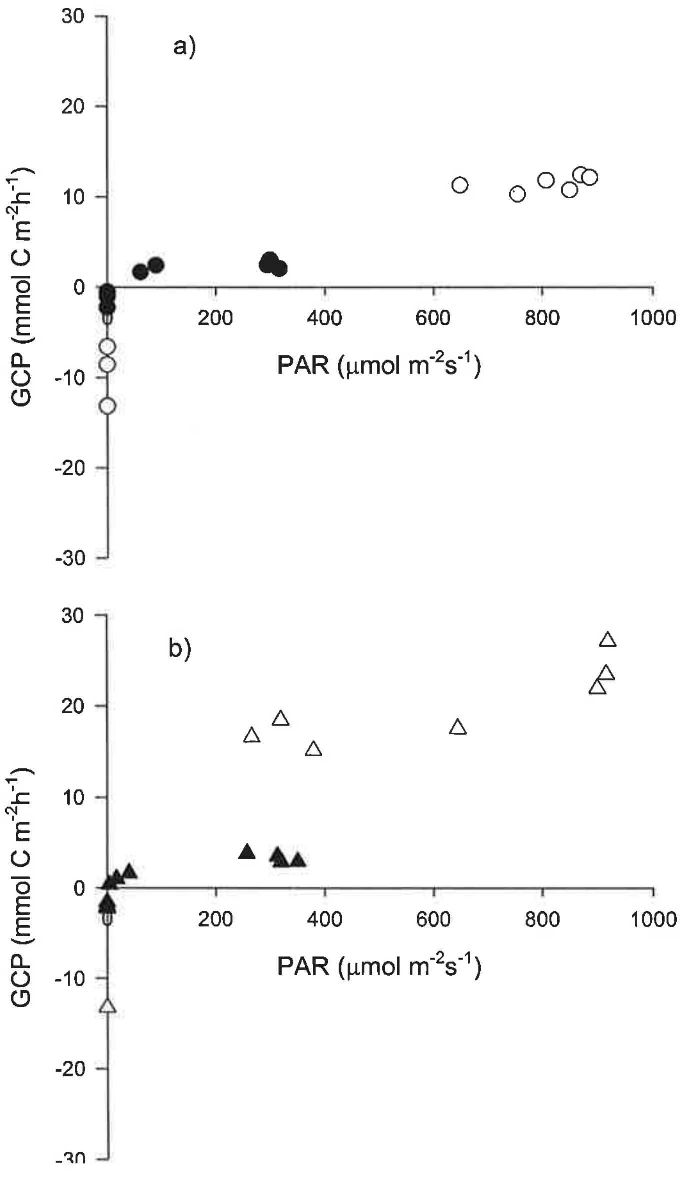

Fig. 3. *Zostera noltii* (a) and *Spartina maritima* (b). Gross community production (GCP) as a function of ambient photosynthetic active radiation (PAR), measured in water (closed symbols) and in air (open symbols).

GCP was significantly related to individual plants electron transport rates (Fig. 4, GCP = -1.296 + 0.053 ETR, $r^2 = 0.77$, $p = 0.0008$). The molar ratio between GCP and ETR was ≈0.04.

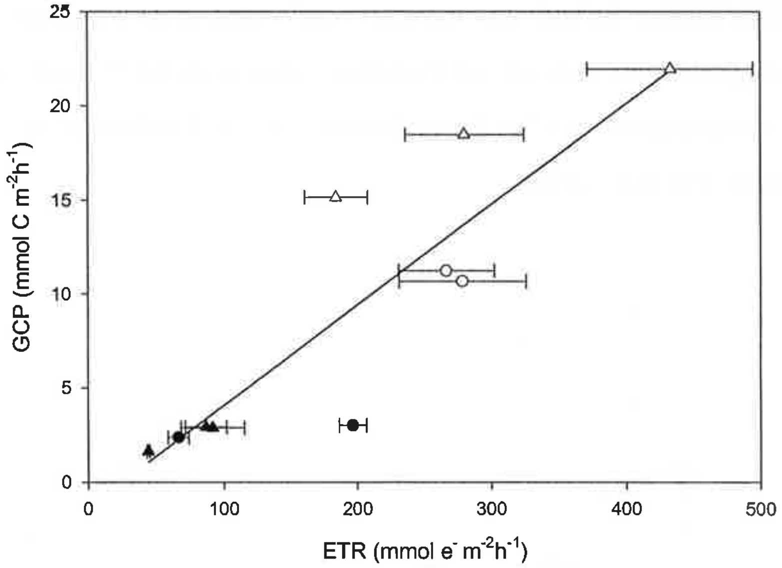

Fig. 4. *Zostera noltii* (circles) and *Spartina maritima* (triangles). Relationship between gross community production (GCP), expressed as carbon fixation, and instantaneous electron transport rates (ETR) (mean ± standard error) determined in individual plants, both in water (closed symbols) and in air (open symbols).

Discussion

Both the chlorophyll fluorescence of individual plants and the CO_2 uptake of the community indicated that the photosynthetic rates of *Zostera noltii* in air were higher than the underwater rates. The available information on the aerial versus submerged photosynthesis of *Zostera noltii* is not consistent. While Leuschner & Rees (1993) and Leuschner et al. (1998) measured identical or even higher rates of CO_2 assimilation in air than in water, Pérez-Lloréns & Niell (1994) found CO_2 uptake rates in air 10 to 20 times lower than in water. The discrepancy of these results suggests that the key factor determining CO_2 assimilation by air-exposed *Z. noltii* is the leaf water content. Leuschner et al. (1998) demonstrated a linear relationship between the leaf water content of *Z. noltii* and its net photosynthetic rate. On a study involving eight tropical seagrass species, Björk et al. (1999) also found a direct relationship between the plants hydration level and the photosynthetic yield. Interestingly, upper intertidal species were less tolerant to desiccation than lower intertidal or shallow subtidal ones, but did not suffer desiccation during low tide periods, due to morphological traits and sediment characteristics. In the intertidal meadows of *Z. noltii* in Ria Formosa, desiccation is never severe. The high leaf density (Silva & Santos 2003) traps the water, maintaining the plants wet during low tide. The micro topography of the sediment surface also plays an important role, as the small but numerous depressions in the sediment retain a considerable amount of water, maintaining hydration for the whole emersion period between tides. In these conditions, it is likely that rapid air-water CO_2 diffusion occurs, making it readily available to plants. This CO_2 availability, together with high light levels, creates the ideal conditions for high photosynthetic rates of hydrated plants during low tide.

When low tide coincides with middle-day periods, particularly during the summer, irradiance and temperature levels rise considerably during the air-exposure period, up to potentially photoinhibitory conditions. Our present results were obtained in late summer (September), and thus they may not entirely reflect the differences between *Z. noltii*'s photosynthesis in air and in water along the year. In a previous study (Silva & Santos unpublished), winter photosynthetic rates of *Z. noltii*, measured as net oxygen production in a oxygen electrode, were ca. five-fold higher than summer rates, due to downregulation of photosynthesis during the summer months. This observation raises the hypothesis that the photosynthetic rates of air-exposed *Z. noltii* in Ria Formosa could be even higher during the winter.

Overall, both the fluorescence and the CO_2 exchange revealed a clear dependence of photosynthesis on light, independently of the medium (air or water). The maximum electron transport rate (ETR_m) and the half-saturation intensity obtained with the rapid light curves (RLCs), increased generally with ambient PAR, showing no limitations during air-exposure. In fact, the two sets of RLCs measured in air further confirm the described photosynthetic plasticity of *Z. noltii* (Silva & Santos 2003), as they showed the highest measured values of half-saturation irradiance ($I_k = 417\ \mu mol\ m^{-2}s^{-1}$, Fig. 1 d) and the lowest initial slopes ($\alpha = 0.191$ and 0.190, Fig. 1 c and d, respectively) of light response curves for this species.

The community gas exchange measurements compared well with the fluorescence indications. During incubations in air, the rates of CO_2 assimilation reached ca. three-fold higher values than underwater, although obtained at much higher irradiances. Once again, when the values obtained in water and in air were plotted together, a clear dependence of light was visible. The instantaneous photosynthetic rates (ETR), determined at ambient irradiance, fitted well within the RLCs data points, suggesting that these two measures provide comparable information about the instantaneous photosynthetic performance of these plants.

Spartina maritima, a terrestrial plant, was expected to perform much better in air than in water, and both fluorescence and gas exchange measurements confirmed it. However, some photosynthetic activity was observed in completely immersed plants. The gas exchange measurements clearly show that, for the same irradiance level, the *S. maritima* dominated community produces ca. four times more in air than in water. The low level of CO_2 fixation measured in water could be attributed to the other primary producers of the community, particularly the red alga *Bostrichia scorpioides*, the brown alga *Fucus vesiculosus* and microphytobenthos. However, chlorophyll fluorescence measurements of individual *S. maritima* plants showed that they remain photosynthetically active, even when submerged. To the best of our knowledge, these were the first measurements of underwater photosynthesis of *S. maritima*. We have no explanation for the observation that the underwater light response was not dependent of ambient PAR, rather being constant along the day.

The molar relationship between the community gross community production and the photosynthetic rates of individual plants shows that both *Z. noltii* and *S. maritima* are responsible for the global pattern of photosynthetic carbon fixation within their respective communities, both during submersion and emersion periods. The molar ratio found between GCP and ETR (≈0.04) is approximately six-fold lower than the theoretical molar ratio of 0.25 that exists between the gross oxygen production and the electron transport rate (Walker 1987). This ratio means that per each mol of oxygen produced, four mol of electrons are transported. In a previous report, Silva & Santos (in press) determined a ratio of 0.15 for *Zostera noltii*, lower than the theoretical value, essentially due to the existence of electron sinks other than the photochemical pathway. A deviation from the theoretical value, in the same order of magnitude, may be considered for *Spartina maritima* as well. This physiological deviation explains only part of the difference between the theoretical and the observed molar ratio. Hence, the remaining difference must result from the metabolism of the animals within the community. This raises the possibility of using this method also to quantify the metabolism of the animal component of intertidal communities.

For both species, the light response curves in air and underwater were different. This feature has to be considered for the long-term modelling of productivity, as both communities experience considerable submersion periods in every tidal cycle. Since some photosynthetic activity occurs during the submersion periods of *S. maritima*, these must also be accounted for, and treated separately. It is interesting that the photosynthetic rates of *Z. noltii* in air are higher than underwater, as this is an aquatic plant. This reveals a Ci-limitation to photosynthesis in water, a common feature in seagrasses and probably a remaining of their terrestrial past (Beer & Koch 1996). Overall, the short-term incubations method described in this report proved to be a powerful tool for field measurements of intertidal lagoon productivity. It provides fast and precise values of community carbon dioxide fixation, both in submerged and air-exposed conditions. An important detail in this approach is the use of small chambers and short incubation periods, so that the temperature and humidity conditions remain fairly constant during the incubations.

This method allows repeatable *in situ* measurements of carbon uptake with minimum community disturbance. For these reasons, we believe that this could be the choice method to assess the different communities' contribution to the global carbon budget of coastal systems like the Ria Formosa lagoon.

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Chapter 4

Daily variation patterns of seagrass photosynthesis along a vertical gradient

Silva J, Santos R (2003)
Mar Ecol Prog Ser 257: 37-44

Daily variation patterns of seagrass photosynthesis along a vertical gradient

Abstract: The relationship between the available photosynthetic active radiation (PAR) and the photosynthetic yield of photosystem II (PSII), measured by chlorophyll *a* fluorescence was assessed in the intertidal seagrasses of Ria Formosa, a coastal lagoon in southern Portugal. Most of the lagoons' intertidal is occupied by a monospecific population of *Zostera noltii*, which extends within a vertical gradient of about 2 meters. The upper limit of this species confines with the *Spartina maritima* salt marsh and the lower limit is defined by the transition to the subtidal, dominated by *Cymodocea nodosa.* The daily changes in the pattern of rapid light curves (RLC's) was investigated with pulse amplitude modulated (PAM) fluorometry in *C. nodosa* and in *Z. noltii* at the lower and upper intertidal. The light reactions of photosynthesis were assessed by fitting photosynthesis-irradiance (*P-I*) models to the RLC's. The photosynthetic parameters of *Z. noltii* indicated that in its lower and upper vertical distribution limits, this species presents, respectively, shade- and sun-type plant behaviour. The initial slopes of all the RLC's were steeper in the lower limit at low light but decreased with increasing irradiances, while in the upper limit values were always significantly lower but unaffected by increasing irradiances. *C. nodosa* presented a typical shade-type plant pattern, evidenced by the daily variation and light dependence of both photosynthetic efficiency and optimal quantum yield. The relationship between the maximum electron transport rate and irradiance, suggests that this species is strongly light-limited. We suggest that attempts to characterize the photosynthetic behaviour of an intertidal meadow should consider both the daily fluctuations in the plants photosynthetic activity, as well as its vertical distribution frequency.

Introduction

Intertidal seagrasses colonize one of the harshest marine habitats, in which conditions change very rapidly and in a very significant manner. Stress factors are imposed and relieved at the combined rhythm of tides and daily irradiance. Plants are compelled to maintain a permanent and very dynamic trade-off between photosynthetic efficiency and photoprotection.

Just like their terrestrial relatives, seagrasses perform real-time adjustments in their photosynthetic activity, in response to daily changes in the available photosynthetic active radiation (PAR) (Ralph et al. 1998). Although photosynthesis usually follows directly the daily irradiance, a midday depression in photosynthetic activity may occur under high irradiances. This depression is common among marine plants, together with the real time photoacclimation capacity expressed by rapid adjustments in pigment contents and ratios (Dennison & Alberte 1986, Abal et al. 1994).

Light availability is considered to be the most important factor regulating the depth distribution of seagrass beds and has been widely used in predicting the lower vertical limits of a number of species (Duarte 1991, Dennison et al. 1993, Kenworthy & Fonseca 1996, Bach et al. 1998). The interaction between high nutrient loads, concentration of phytoplankton and turbidity determines the light attenuation in the water column (Abal & Dennison 1996, Koch 2001). These parameters may present considerable variations, either on long-term (e.g. seasonal) or on short-term (e.g. daily, tidal) scales, particularly in meadows with an important intertidal component, where physical, chemical and biological parameters tend to vary more dramatically than in the subtidal environment.

Intertidal meadows are regularly exposed to oversaturating irradiances that induce light stress and enhance conditions for thermal and desiccation stress. However, due to morphological traits, intertidal plants usually deal well with desiccation (Leuschner et al. 1998, Björk et al.1999). In this specific type of environment, the ability to cope with irradiances that can be up to 7-8 times the saturating values, appear to be the major constraint to plants survival.

Plants have the capacity to adapt to their light environment in a number of ways, from the morphological level down to the biochemical one (Falkowski & LaRoche 1991, Evans & Poorter 2001). From a physiological point of view, plants are able to regulate photosynthesis in a highly dynamic way in response to several external factors. When the factor is high irradiance, the response is usually photoprotection, involving non-photosynthetic thermal dissipation of energy, through a process often called non-photochemical quenching (Krause & Weis 1991, Demmig-Adams 1998, Ort 2001). This kind of response enables the maintenance of the crucial balance between energy absorption and photosynthetic light utilization by carbon fixation, preventing photo-oxidative damages in photosystem II (Ensminger et al. 2001).

Chlorophyll fluorescence measurements have proven to be very useful in the assessment of photosynthetic light responses, as the amount of energy dissipated through this way is inversely correlated with photosynthetic efficiency (Krause & Weis 1991). The description of a linear relationship between the chlorophyll *a* fluorescence parameter (F_m' - F_s)/ F_m' (where F_s is the fluorescence in the light, when only part of the reaction centres are closed and F_m' is the maximal fluorescence of a light adapted leaf immediately after the closure of all reaction centres, by a saturating pulse) and the quantum yield of photosynthesis (Genty et al. 1989), opened the way for photosynthetic measurements using fluorometry based instruments (Seaton & Walker 1990). Since then, the pulse amplitude modulated (PAM) fluorescence method has been widely explored and improved (Schreiber et al. 1995).

In situ measurements of chlorophyll fluorescence in seagrasses were made possible after the development of a submersible PAM fluorometer (Beer et al. 1998, Ralph et al. 1998, Beer & Björk 1999, Björk et al. 1999, Schwarz et al. 2000, Seddon & Cheshire 2001, Durako & Kunzelman 2002). PAM fluorescence has been used on the evaluation of seagrass responses to specific environmental stresses like desiccation, elevated temperatures or high irradiances. In parallel, it has also provided valuable insights on the dynamic behaviour of the photosynthetic apparatus of seagrass species under fluctuating field conditions. Chlorophyll fluorescence has proven to be particularly useful in the assessment of several aspects of the daily pattern of photosynthetic activity in seagrasses (Ralph et al. 1998, Enríquez et al. 2002, Figueroa et al. 2002), as it allows a considerable number of measures along the day under field conditions.

The existence of considerable alterations on the photosynthetic performance during a daily cycle is widely recognized and must be taken into account when long-term measures or estimates of photosynthesis are required. On the other hand, in south-western Europe, where tides are semi-diurnal with daily changes in amplitude and time, the daily light regime in intertidal seagrass meadows depends both on the time of the day and the tide schedule. When a third factor, the vertical distribution of plants within the meadow is introduced, it becomes impossible to predict global photosynthetic performance, without considering that a number of distinct light regimes occurs simultaneously at different levels of a same meadow.

The aim of this work was to assess how the vertical position of seagrasses in the intertidal determines their daily photosynthetic performance. The relationship between the daily changes in available PAR and the photosynthetic yield of PSII, measured by chlorophyll *a* fluorescence, was investigated in the intertidal zone of Ria Formosa, southern Portugal, which is dominated by monospecific meadows of the seagrass *Zostera noltii*. The transition to the subtidal is colonized by a band of the seagrass *Cymodocea nodosa*.

Materials and methods

Study site and plant distribution

Ria Formosa is a shallow mesotidal lagoon in the South coast of Portugal. The tidal amplitude varies between 3.50 m in spring tides and 1.30 m in neap tides. *Zostera noltii* (Hornemann) meadows are distributed within a vertical gradient of ca. 2 m. The upper limit confines with the *Spartina maritima* (Curtis) Fernald saltmarsh and the lower limit is defined by the transition to the subtidal, dominated by *Cymodocea nodosa* (Ucria) Ascherson. This transition zone is only emerged at the lowest water level of spring tides, while the upper limit of *Z. noltii* meadows has an average emersion of 6 to 8 hours per tidal cycle (12 hours). Environmental conditions change notoriously along the intertidal seagrass distribution, particularly light. In the winter, the lower limit receives an average daily PAR dose of 4 mol photons m^{-2}, whereas the upper limit receives around 15 mol photons m^{-2}. The amplitude of pH changes is also higher at the upper site (from 7.4 at low tide up to 8.8 at high tide) than at the lower site (from 7.9 to 8.3).

In southern Portugal (37° N, 008° W), where tides are semidiurnal (two low- and two high-tides every 24 hours), low tide occurs at early morning and late afternoon during spring tides and at midday during neap tides (Fig. 1). The coupling of this tidal rhythm with the daily changes in solar radiation leads to strong differences in the light environment at the upper and lower limits of the seagrass distribution. This experiment was conducted under the conditions show in Fig. 1.b. Low tide was coincident with solar noon, allowing lower plants to receive the maximum possible PAR.

Sampling strategy

Rapid light curves (RLC's) were obtained by PAM fluorometry (Diving-PAM, Walz, Effeltrich, Germany) every 2-3 hours from pre-dawn until sunset, for *Cymodocea* *nodosa* (transition to subtidal) and for two *Zostera noltii* sites, at its lowest and highest intertidal positions. All measurements were performed *in situ*, at ambient temperature (16-18°C). RLC's were obtained from five replicates for each point.

An underwater planar PAR measuring device (Li-192SA Underwater Quantum Sensor connected to a Li-1000 Data Logger, Li-Cor, Lincoln, Nebraska, USA) mounted on a telescopic tower was installed in the transition between *Cymodocea nodosa* and *Zostera noltii*, at the exact location where samples of both species were collected. PAR radiation at the upper site of *Z. noltii* was measured with an identical set of instruments. Both devices were calibrated against each other.

Absorption factor (AF)

The absorption factor (AF), which represents the fraction of incident PAR that is absorbed by the leaves, was determined according to Beer et al. (2000) by placing seagrass leaves on top of the fluorometer PAR quantum sensor and irradiating them perpendicularly at a fixed distance with the instruments' own optic fibre. Irradiance reaching the sensor with and without leaf was recorded underwater in ten replicates of each species, and AF was calculated as the percentage of light absorbed by the leaves. The use of this specific absorption factor instead of the instruments' predefined and commonly used value of 0.84, allows the calculation of absolute (μmol $e^- m^{-2}s^{-1}$) instead of relative electron transport rates.

Fluorescence measurements

Seagrass leaves (3rd youngest for *Cymodocea nodosa* and 2nd-3rd youngest for *Zostera noltii*) were placed in the fluorometer leaf distance clip and shaded from ambient light for the duration of each curve, to prevent superimposition of ambient light on the light supplied by the instruments' optic fibre. Samples were irradiated with a series of eight increasing pre-set irradiance steps, supplied by the fluorometer's halogen lamp. At the end of each irradiance step (15 s periods), a 0.6 s saturating light pulse (ca. 4000 μmol photons $m^{-2}s^{-1}$) was applied for maximum fluorescence (F_m') determination. Effective quantum yield of photosystem II [$Y = (F_m' - F_s)/ F_m' = \Delta F/F_m'$] (Genty et al.

1989) was computed and electron transport rate (ETR = Y x Irradiance (I) x AF x 0.5) (Schreiber et al. 1995) was calculated for each irradiance step.

The light reactions of photosynthesis were assessed by the parameters obtained directly through PAM fluorometry and by the parameters derived from the equation model fitted to the RLC's. The curves (ETR-I) were fitted with the adapted hyperbolic tangent model equation of Jassby & Platt (1976), $\mathrm{ETR} = \mathrm{ETR_m} \times \tanh(a \times \mathrm{I}/\mathrm{ETR_m})$, where I is irradiance, a is the ascending slope at limiting irradiances, and $\mathrm{ETR_m}$ is the maximum electron transport rate. The half-saturation irradiance, $\mathrm{I_k}$, was calculated as the ratio between $\mathrm{ETR_m}$ and a.

In parallel with rapid light curves, potential quantum yield ($F_m - F_o/ F_m = F_v/F_m$) (where F_o is the fluorescence in the dark, when all the reaction centres are open) was also assessed for *Cymodocea nodosa* and both *Zostera noltii* types along the day. For this purpose, dark leaf clips were used to dark-adapt portions of attached functional leaves. Leaves were left for 15 min (enough time for full oxidation of reaction centres, previously determined) in the dark, after which a saturating light pulse, identical to the one used in the RLC's, was applied for determination of the potential quantum yield, expressed as F_v/F_m.

All results are presented as mean values ± standard error. One or two way ANOVA was used to test for the significant differences among variables ($\alpha = 0.05$) (Sokal & Rohlf 1981). The Tukey test was used to establish significant differences between individual means.

Results

The absorption factor (AF) determined for *Zostera noltii* (0.79 ± 0.02) was lower than the standard value preintroduced in the PAM (0.84), whereas *Cymodocea nodosa*'s (0.88 ± 0.01) was higher. These values differed from each other and from the standard value and were therefore used to calculate the absolute electron transport rates of the respective species. AF values for *Z. noltii* were identical along its vertical distribution in the intertidal.

The ambient photosynthetic active radiation (PAR) reaching the two sampling sites followed a similar trend along the day, yet with very different absolute values (Fig. 2). In the lower site, where both *Cymodocea nodosa* and *Zostera noltii* were sampled, PAR peaked at ca. 800 µmol $m^{-2}s^{-1}$, while in the upper site it reached 1400 µmol $m^{-2}s^{-1}$. Considering the fact that low tide was around solar noon, the PAR measured in the lower site reached its highest possible value, which was almost half the irradiance measured at the upper site.

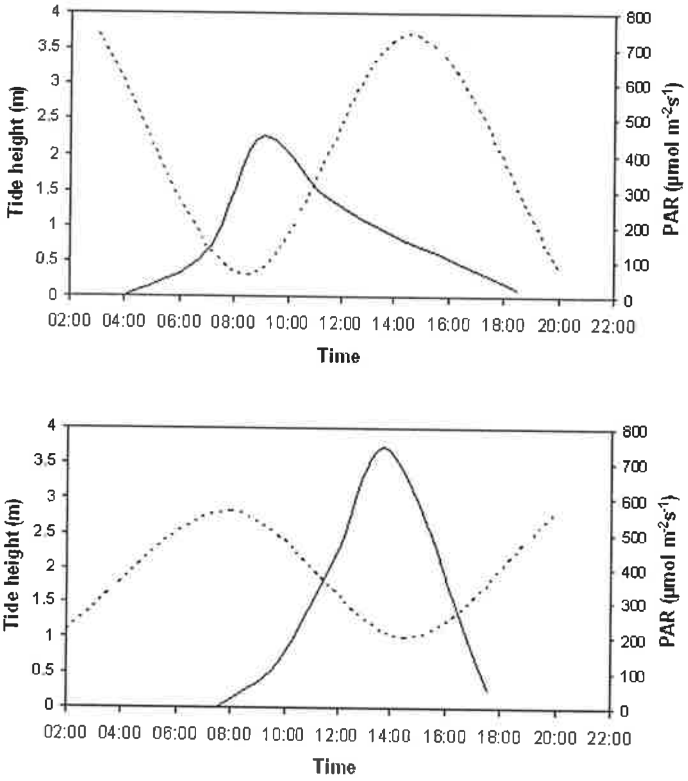

Fig. 1. Typical patterns of tidal cycle (······) and available PAR (—) at the lower end of a *Zostera noltii* meadow, during spring (a) and neap tides (b).

In the daily course of photosynthesis, *Cymodocea nodosa* reached the highest value of the maximum electron transport rate (ETR_m), 69.9 ± 9.2 µmol $e^- m^{-2}s^{-1}$, at solar noon (14:00) (Fig. 2.c). At the same time, upper *Zostera noltii* reached a similar value (68.9 ± 11.3 µmol $e^- m^{-2}s^{-1}$, Fig. 2.a). The ETR_m of *Z. noltii* at the lower site was significantly lower (55.4 ± 11.6 µmol $e^- m^{-2}s^{-1}$, Fig 2.b), and was attained earlier, at 10:30. No significant changes were observed until solar noon.

The half-saturation irradiances (I_k) were always significantly higher for the upper *Zostera noltii* when compared with plants from the lower site, more notoriously from 12:00 to 16:00, the period of higher irradiance (Fig. 2). *Cymodocea nodosa*'s I_k remained below the ones of the two *Z. noltii* types, except at 13:00, when it was significantly higher (278 μmol m^{-2}s^{-1}). From solar noon to the end of the day all values decreased in parallel.

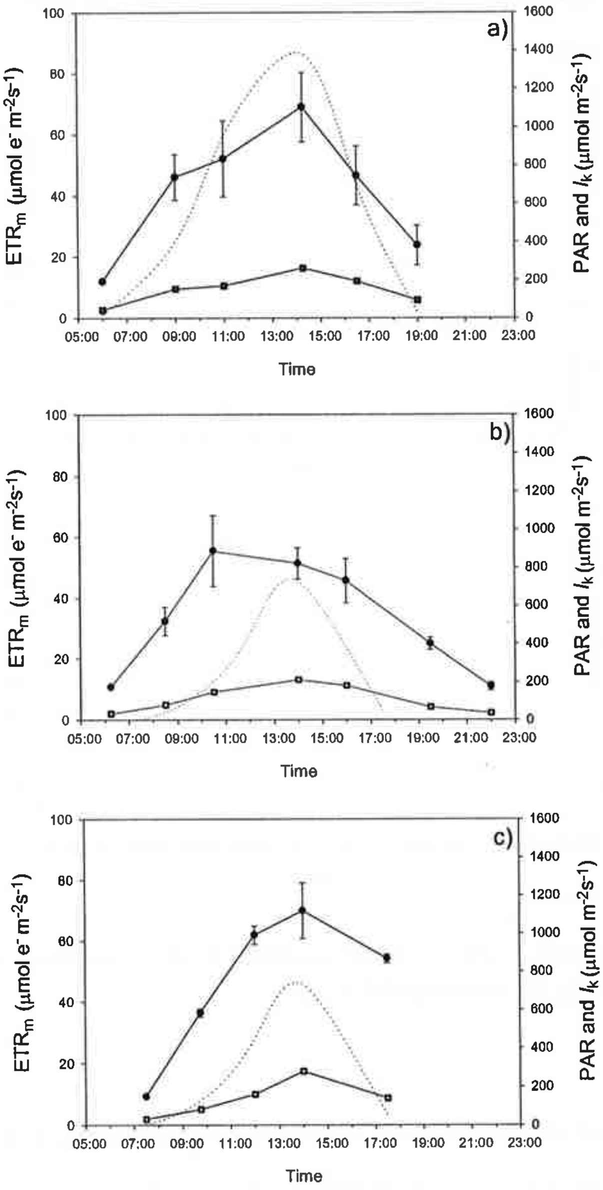

Fig. 2. *Zostera noltii* and *Cymodocea nodosa*. Daily variation of maximum electron transport rate (ETR$_m$) (•), half-saturation irradiance (I_k) (■) and available photosynthetically active radiation (PAR) (······) for upper (a) and lower (b) *Z. noltii* and for *C. nodosa* (c).

The initial slopes of *Z. noltii* RLC's (α, Fig. 3) were always significantly higher in the significantly lower than in the upper site. In *Z. noltii* plants from both sites, α remained fairly unaltered along the day, in contrast with *Cymodocea nodosa*, whose α showed a very strong oscillatory tendency. It peaked in early morning, with significantly higher values than for *Z. noltii*, and decayed strongly at solar noon. There were no significant changes of lower *Zostera noltii* F_v/F_m during the whole day (Fig. 3.a). At the upper site, two depressions were observed, one in the early morning and another in the early afternoon (Fig. 3.b). In *Cymodocea nodosa*, F_v/F_m followed the same pattern as α, with a notorious midday depression and full recovery towards the afternoon (Fig. 3.c).

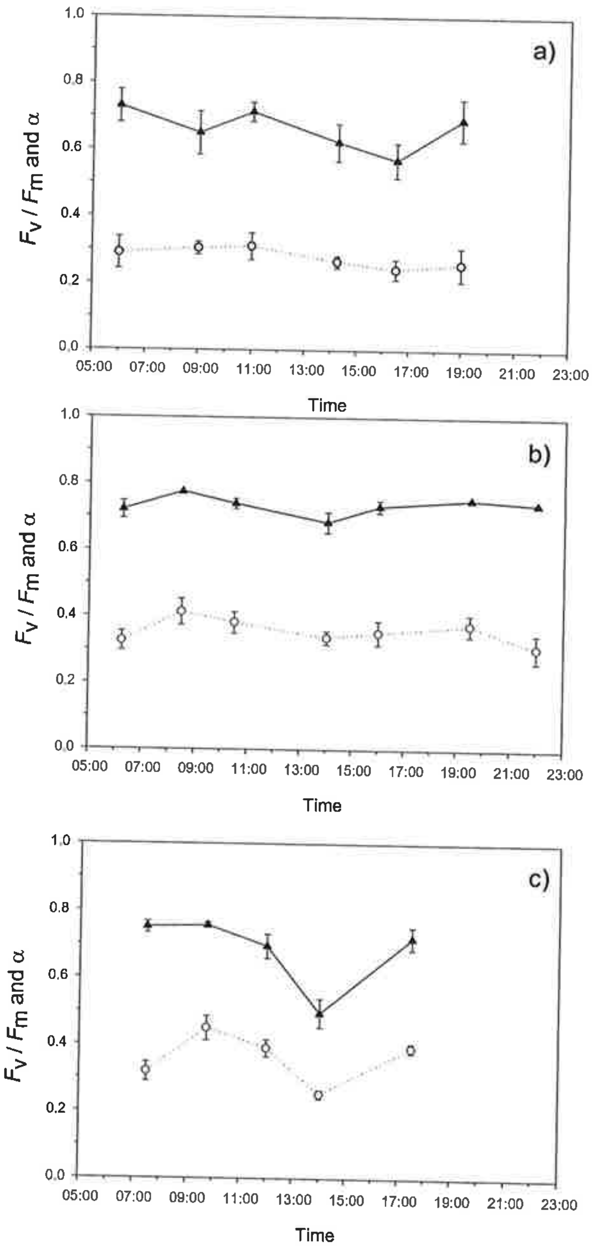

Fig. 3. *Zostera noltii* and *Cymodocea nodosa*. Daily variation of optimum quantum yield (F_v/F_m) (▲) and initial slope of rapid light curves (α) (○) for upper (a) and lower (b) *Z. noltii* and for *C. nodosa* (c).

For identical PAR values, the electron transport rates of *Cymodocea nodosa* were always significantly higher than those of both *Zostera noltii* samples (Fig. 4). Both *C. nodosa* and *Z. noltii* from the upper site did not show a saturation tendency of ETR_m, but on the contrary, there was a clear tendency to increase with irradiance. The ETR_m of the lower *Z. noltii* appears to have saturated at a medium irradiance, of about 500 µmol $m^{-2}s^{-1}$, decreasing afterwards.

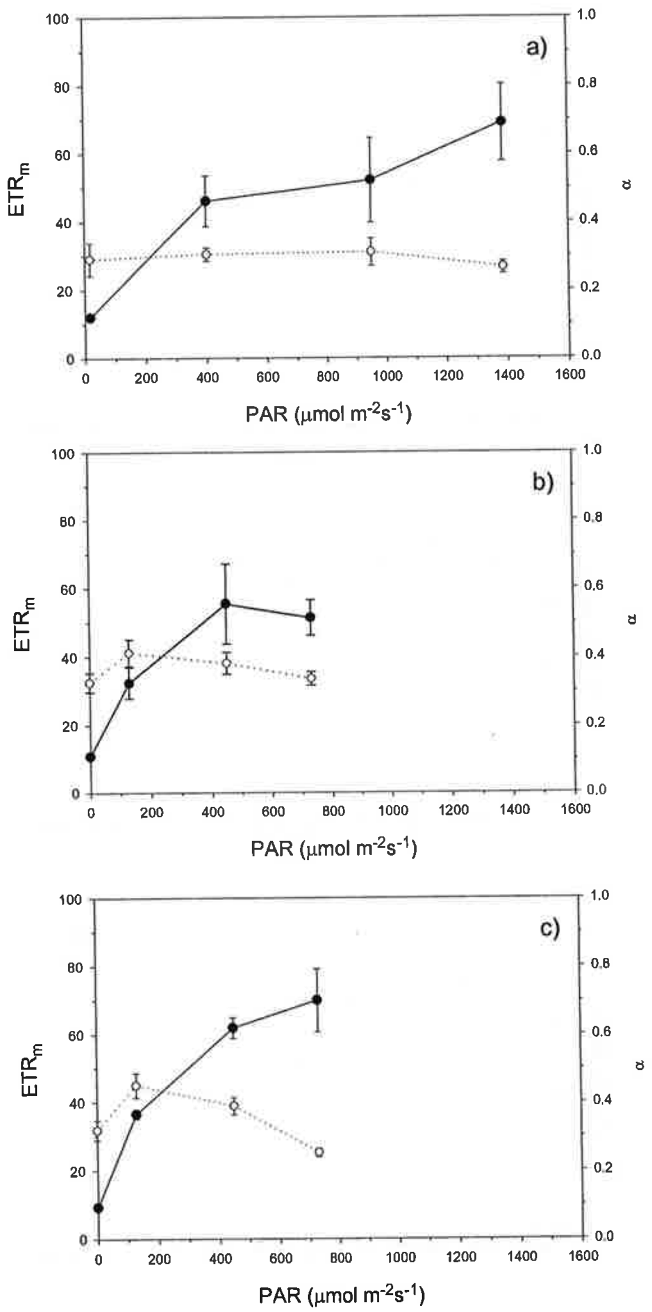

Fig. 4. *Zostera noltii* and *Cymodocea nodosa*. Relationships between PAR and both maximum electron transport rate (ETR_m) (•) and initial slope of rapid light curves (α) (○), for upper (a) and lower (b) *Z. noltii* and for *C. nodosa* (c).

The upper *Z. noltii* plants were less efficient at low light than both the lower *Z. noltii* and *Cymodocea nodosa*, but α remained constant with increasing PAR (Fig. 4). Both the lower *Z. noltii* and *C. nodosa* were significantly more efficient in the use of low light. For these plants, the initial slope of the RLC's, α, peaked before 200 μmol $m^{-2}s^{-1}$ and decreased afterwards, with increasing irradiance. At higher irradiances (750 μmol $m^{-2}s^{-1}$), the photosynthetic efficiency of the upper *Z. noltii* was about the same as the one of both the lower *Z. noltii* and *C. nodosa*.

Discussion

The amount of light that can be absorbed by a leaf depends mostly of its pigment content (Beer et al. 2000). This implies that all environmental factors capable of influencing the pigment content of a plant are also responsible for changes in the absorption factor (AF) values. Such factors include geographical location, time of the year, depth, leaf age and nutrient status. It is therefore expected that AF values, namely in seagrasses, will vary considerably, either interspecifically or even intraspecifically. One example of this variation is the difference found between the AF value determined by Beer et al. (1998) for *Cymodocea nodosa* collected in the Eastern Mediterranean (Israel) (0.72 ± 0.11) and the value determined in the present work (0.88 ± 0.01), for plants of the same species collected in South Portugal. The measured AF for *Cymodocea nodosa* (0.88 ± 0.01) was higher than any published value for a seagrass species. In Ria Formosa, *C. nodosa* leaves are large, considerably thick and highly pigmented (data not shown) which can account for its high optical density. The absorption factor determined for *Zostera noltii* (0.79 ± 0.02), although lower than the standard value, was identical to the highest published values for seagrasses, namely *T. testudinum* (Durako & Kunzelman 2002).

Previously published AF values for seagrasses appear to have two common features: the considerable differences between them (from 0.44 in *Zostera marina* to 0.78 in *Thalassia testudinum*, Beer et al. 1998, Durako & Kunzelman 2002) and the fact that all of them are lower than the standard value of 0.84, which was originally described for terrestrial plants. Determining the leaf AF is thus essential to calculate the absolute electron transport rates, particularly to relate ETR to oxygen production.

Although this was not the case in the present work, we believe it is good practice to measure AF, because considerable differences are likely to be found and published values are scarce.

Cymodocea nodosa presented a typical shade-type plant pattern, evidenced by the light dependence of both photosynthetic efficiency and optimal quantum yield (Fig. 3 c). These plants revealed a very efficient use of low light and were very sensitive to higher irradiances. The relationship between the maximum electron transport rate and irradiance (Fig. 4.c), suggests that this species is strongly light-limited.

In the upper and lower ends of its vertical distribution, *Zostera noltii* revealed, respectively, clear patterns of sun- and shade-type plants, during the daily course of photosynthesis. Upper *Z. noltii* plants reached significantly higher ETR_m (Fig. 2.a and b), and the light response curves saturated at higher irradiances than those of lower plants. This saturation resulted mainly from the consistently lower initial slopes of the curves, expressed by the α parameter, which in itself constitutes a useful indicator in distinguishing sun-adapted plants from shade-adapted ones (Henley 1993). The plot of ETR_m vs. ambient PAR for different moments of the day (Fig. 4) constitutes a global light response curve for a day's length. It allowed an overall analysis of how the two *Z. noltii* types reacted to increasing irradiances, and showed that the lower plants were more efficient in the use of limiting irradiances, whereas upper plants responded better at higher PAR values. This kind of intraspecific differences has been previously described for subtidal seagrass species growing at different depths (Schwarz et al. 2000) or acclimated to different irradiances (Major & Dunton 2002), but this is the first time these differences are described for an intertidal seagrass.

The fact that a particular *Z. noltii* type growing in the upper intertidal revealed a sun-adapted plant pattern is not particularly relevant in itself. The interesting issue is the particular conditions to which *Z. noltii* adapted to in Ria Formosa. On a day in which low tide occurs at noon, plants are emersed for ca. 10 hours, from 09:00 to 19:00, being exposed to direct sun light for the whole day. Plants in the Ria Formosa intertidal are never under severe desiccation, due the species high density of small packed leaves that retain water. In such conditions, *Z. noltii* is able to maintain some degree of photosynthetic carbon uptake, due to the rapid diffusion of CO_2 from the air (Leuschner et al. 1998). For this carbon uptake to take place, it must be pulled by the light-driven reactions of photosynthesis, which we found to occur during the whole period of near-air exposure. The photosynthetic yield of upper *Z. noltii* was significantly lower than the one of low intertidal plants, indicating that in spite of being adapted to a high light environment, the plants need to dissipate some energy, channelling it trough a non-photochemical quenching pathway. This appears to be common amongst seagrasses (Ralph et al. 1998). Our observations support the existence of such a process on *Z. noltii*, involving the thermal dissipation of energy, and a consequent reduction of the fluorescence emission yield.

When the high tide occurs at noon, plants receive the highest light intensities during the first morning hours and remain submersed for most of the day. In this type of situation, a similar pattern of the *Zostera noltii* daily light responses of photosynthesis was observed (data not shown), indicating that the sun- and shade-adaptation is not related to the low tide timing, but instead depends on the vertical position of the plants. Photosynthesis measures are usually obtained with traditional methods, such as gas exchange techniques, but care must be taken regarding the time of the day at which samples are processed, as their recent light exposure history has a significant effect on photosynthetic responses. Repeated measures of photosynthetic oxygen production are difficult to achieve over a daily cycle, particularly in underwater conditions. On the other hand, the chlorophyll fluorescence method provides information about light-driven reactions only and therefore cannot be used as a stand alone method of assessing absolute photosynthetic production, because processes like photorespiration and changes in rates of dark respiration are not possible to account for (Beer et al. 1998). Another important aspect to consider is that the fluorometer's halogen lamp has a different spectral composition than the ambient light, which can influence the ETR values, as a result of possible chromatic regulation, with effects on charge separation at the PSII level (Kroon at al. 1993). In balance, weighting the constraints and the advantages, PAM fluorometry remains the choice method for field estimates of photosynthesis, especially in aquatic environments.

The vertical distribution of seagrasses on the intertidal is associated with a diversity of level-dependent environmental conditions that lead to pronounced differences in the plants ecophysiology, even within a same species as shown here for *Zostera noltii*. We suggest that attempts to characterize the photosynthetic production of an intertidal meadow, should consider both the daily fluctuations in the plants photosynthetic activity as well as its vertical distribution frequency. The need of considering daily changes in photosynthesis is well evidenced by the fact that ETR_m (which in theory is directly correlated with gross photosynthesis) varies along the day within a range of 3-4 times its lower value, taken after the onset of photosynthesis. In an intertidal system like Ria Formosa, for example, a productivity estimate based solely on ETR_m values obtained around noon would yield highly overestimated results. Therefore, for productivity estimation purposes, daily integrals have to be used, in order to account for the dynamics of real-time adjustments in photosynthesis. A possible modelling approach would be to establish differentiated and vertically limited "productivity bands" in the intertidal meadows, according to specific local conditions, and calculate daily integrals of photosynthesis for each of those bands.

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Chapter 5

The seasonal photosynthetic strategy of the seagrass *Zostera noltii* in Southern Europe

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submitted to Mar Ecol Prog Ser

The seasonal photosynthetic strategy of the seagrass *Zostera noltii* in Southern Europe

Abstract: The seasonal pattern of *Zostera noltii* photosynthesis was assessed in Ria Formosa lagoon (southern Portugal). Plants were sampled once a month, at both the upper and lower edges of a selected intertidal *Z. noltii* meadow. Photosynthetic capacity was determined at 320 μmol quanta $m^{-2}s^{-1}$ (PAR) in an oxygen electrode. Photosynthetic pigments, carbon, nitrogen, soluble protein and non-structural carbohydrates were also determined monthly. The net photosynthetic capacity of *Zostera noltii* had a significant negative correlation with both the average daily PAR dose and the water temperature. It was lowest in the summer and highest in the winter months, at both the upper and lower sites. This pattern was the opposite of the most common response of seagrass photosynthesis to light and temperature, particularly of *Zostera noltii* populations from northern latitudes, where positive correlations of photosynthesis with temperature and irradiance have been described. This contrasting difference is attributed to the species adaptation to southern intertidal conditions, where light and temperature extremes are common in the summer. Our results revealed a clear seasonal productivity strategy. During the summer, the plants strategy is to cope with the extended daily periods of inhibitory irradiance, dissipating excessive energy and reducing the photosynthetic productivity. On the other hand, from October until February plants are exposed most of the day to irradiance levels between the saturation and the inhibition thresholds, which are the ideal conditions for photosynthesis and consequently for growth. The mild winter temperatures on Ria Formosa contribute to the success of this seasonal strategy.

Introduction

In the temperate and sub-tropical regions, seagrass populations experience seasonal variations in their productivity patterns, essentially due to the natural cycles of light, temperature and nutrient availability (Pérez & Romero 1992, Terrados & Ros 1995, Zimmerman et al. 1995, Vermaat & Verhagen 1996, Kraemer & Mazzela 1999, Welsh et al. 2000, Plus et al. 2001, Plus et al. 2003). Species growing in these regions are considered to be adapted to more or less pronounced periodic changes in environmental parameters (Major & Dunton 2002). These plants possess the ability to compensate such changes by the action of several physiological mechanisms, a process designated as photosynthetic acclimation (Berry & Björkman 1980, Falkowski & LaRoche 1991, Evans & Poorter 2001).

Light and temperature are commonly regarded as the main factors influencing the photosynthetic performance of seagrasses. Although light is mostly considered as a limiting factor, due to the physical constraints of its penetration in the water column (Hemminga & Duarte 2000), its excess can also result in stress induction with negative effects at the physiological level (Silva & Santos 2003). Temperature increases tend to favour photosynthesis, but can also contribute, when coupled to high irradiances, to the onset of photoinhibition and oxidative stress reactions (Ensminger et al. 2001).

The combined effects of light and temperature are particularly noticeable in intertidal seagrass meadows, where plants are exposed to full sunlight and high temperatures for considerable periods during low tides (Silva & Santos 2003). Some species are particularly well adapted to this intertidal environment, like *Zostera noltii*, which has developed the ability to photosynthesise in air, making use of the exposition period to continue producing (Leuschner et al. 1998).

The light requirements of seagrasses are highly species-specific (Hemminga & Duarte 2000). Within the *Zostera* genus, for example, Vermaat at al. (1996) found very significant differences in the light requirements of the co-occuring species *Zostera marina* and *Zostera noltii*. These two species showed photosynthetic rates and saturation irradiances that differed up to a fourfold factor. In addition to these inter-specific differences, intra-specific variations of photosynthetic behaviour were also observed, revealing a strong site-dependency. Therefore, the exportation of conclusions drawn for a given species in a given site is not easy and can be misleading. When comparing the results of several seasonal productivity studies made for *Zostera noltii* in different European populations (Vermaat & Verhagen 1996, Kraemer & Mazzela, 1999, Welsh et al. 2000, Plus et al. 2001), considerable differences are visible, both in absolute values and in time synchronicity.

Concerning nutrient availability, seagrass populations are generally considered to be limited by inorganic nitrogen availability (Touchette & Burkholder 2000). Compared to terrestrial plants, seagrasses possess a relatively short leaf lifespan, which makes nutrient conservation difficult (Hemminga & Duarte 2000). Some species, particularly those with longer-lived leaves, appear to make use of clonal integration to conserve nutrients, and examples of nutrient resorption before leaf senescence are described (Pedersen & Borum 1993). Species with short-lived leaves seem to be less clonally integrated (Kraemer & Mazzela 1999) and mechanisms of nutrient conservation are poorly described. However, these species are generally more productive than species with longer-lived leaves, uptaking nutrients at higher rates. *Zostera noltii*, one of these species, uptakes nitrogen at a very high rate, including during dark periods. This species does not discriminate sources of inorganic nitrogen (Welsh et al. 2000), which compensates the reduced capacity of nutrient conservation by clonal integration.

Considering that the seagrass communities are among the most productive marine systems in the world (Duarte & Chiscano 1999), it is of great importance to know the details of the seasonal productivity variations and its dependence on local environmental conditions. Such knowledge allows a sound assessment of the annual productivity of specific systems and provides the scientific basis for proper management decisions.

Zostera noltii is the most abundant seagrass species in the Ria Formosa coastal lagoon (southern Portugal). This lagoon is a highly-dynamic and very productive system, in a near-pristine condition. *Z. noltii* monospecific meadows dominate the intertidal area of the lagoon. In a previous work (Silva & Santos 2003), significant differences were found on the daily physiological performance of *Z. noltii* plants collected at the upper and lower limits of the species vertical distribution. Higher and lower intertidal plants were considered respectively as sun- and shade-adapted ecotypes. Here we assess the acclimation of the photosynthetic ecophysiology of *Zostera noltii* plants to the prevailing ambient conditions, along one year. The photosynthetic parameters and the biochemical composition of *Z. noltii* were determined monthly at both vertical distribution limits.

Materials and methods

Study site and plant material

Zostera noltii (Hornemann) intertidal meadows at Ria Formosa occupy a vertical gradient of ca. 2 m. The lower limit of these meadows defines the transition to the subtidal, where this species confines with the seagrass *Cymodocea nodosa* (Ucria) Ascherson. The upper limit confines with the cordgrass *Spartina maritima* (Curtis) Fernald salt marsh. A detailed characterisation of the ecological conditions on these meadows was provided by Silva & Santos (2003).

Sampling strategy

Plants were collected monthly, at both the upper and lower edges of an intertidal meadow of *Zostera noltii*. Sampling was always carried out at early morning (from 08:00 to 10:00) and low tide conditions, to isolate possible effects of diurnal photosynthetic patterns, known to exist in these plants (Silva & Santos 2003). For photosynthesis measurements, whole healthy plants without epiphytes were transported to the laboratory under low temperature (5-6 °C) in a dark and moist container, in order to minimise metabolic activity. Plants were cleaned from excessive sediment and maintained immersed in seawater overnight in a growth chamber, set to emulate the temperature and photoperiod values measured in the field. For biochemical analysis, leaf and rhizome samples were collected and immediately frozen in liquid nitrogen. All photosynthetic measurements and biochemical analysis were performed on samples from the 2nd-3rd youngest leaves of *Zostera noltii* shoots. Air, water and sediment temperature were recorded during field trips. Surface photosynthetically active radiation (PAR) was measured continuously along the whole sampling period (Li-190 Quantum Sensor and Li-1000 Data Logger, Li-Cor, USA).

Photosynthetic capacity

Photosynthetic capacity (mg O_2 g DW^{-1} h^{-1}) was determined at 320 µmol quanta $m^{-2}s^{-1}$ (PAR). Segments of *Zostera noltii* (n = 5, 4 segments per replicate, each ≈2cm long and 2-3 mg DW) were incubated in 15 ml GF/F filtered seawater in a Hansatech DW3 measuring chamber mounted on a Clark type oxygen electrode (Hansatech Instruments, UK) coupled to a magnetic stirrer. Light was provided by a slide projector (Pradovit 150, Leica, Germany) equipped with a halogen lamp (Osram Xenophot 150W), installed in front of a transparent window on one side of the incubation chamber. Dark respiration of each sample was measured after a 7-8 min. dark period following light exposure. The temperature of the reaction vessel was set and maintained at field measured values by a circulating water bath (Raypa, Spain).

Photosynthetic pigments

Lipid soluble pigments from leaf samples (n = 5, 30-40 mg FW each) were extracted overnight in N,N-Dimethyl-formamide and determined spectrophotometrically (Beckman DU-650, Beckman Coulter, USA) using the equations of Wellburn (1994).

Carbon and nitrogen

Leaf and rhizome samples (n = 5, 5-8 mg DW each) were oven dried at 60°C for 48 hours. Carbon and nitrogen contents were determined through elemental analysis (Macler, 1988) (Carlo Erba CHNSO EA1108, Carlo Erba, Italy).

Soluble protein

Leaf and rhizome samples (n = 5, 30-60 mg FW each) were ground in a protein extraction buffer (50 mM Hepes, pH 7.5, 10 mM EDTA, 0.5 mM PMSF, 0.1% (v/v) Triton-X). The extract was centrifuged at 15000 g for 10 min. at 4°C and the supernatant was collected. Soluble protein concentration was determined by a dye binding assay (Coomassie Brilliant Blue G-250 dye) (Bradford 1976), against a Bovine Serum Albumin standard.

Non-structural carbohydrates

Dried samples of leaves and rhizomes (n = 5-10 mg DW each) were ground to powder on a ball mill, extracted in ethanol at 80°C for 10 min. and centrifuged at 2000 g for 5 min. (adapted from Burke at al. 1996). The supernatant was collected and the pellet was ressuspended in ethanol for additional extraction. This procedure was repeated a third time to allow full extraction of soluble sugars (glucose, sucrose and fructose). The supernatants from the three step extraction were all mixed together and the amount of soluble sugars was determined by a phenol-sulphuric assay (adapted from Dubois et al. 1956) using glucose standards.

For starch quantification, the pellet was washed in deionised water and centrifuged, ressuspended again in water (repeated three times) and autoclaved for 15 min. Starch was hydrolysed to glucose in the presence of an enzymatic complex (1.4U amyloglucosidase and 2U α-amylase per sample) and determined as glucose equivalents following the phenol-sulphuric assay described above.

Statistical analysis

All results are presented as mean values ± standard error. When not stated otherwise, one or two way ANOVAs were applied to test the significance of the results ($\alpha = 0.05$). Tukey's HSD test was used to reveal significant differences between individual means (Sokal & Rohlf, 1981). Spearman Rank Order Correlation correlation tests were also used to assess the strength of association between variables. All data treatment and statistical analysis was performed using the SigmaStat/SigmaPlot (SPSS Inc.) software package.

Results

The average daily dosage of the photosynthetically active radiation (PAR) available at the water surface (Fig. 1) at Ria Formosa peaked in June, with ca. 50 mol quanta m^{-2} day^{-1}, while the lowest value was observed in December (ca. 10 mol quanta m^{-2} day^{-1}). This results in a five fold amplitude in the annual variation of the available daily PAR dosage. While this dosage is valid for the upper site of the *Zostera noltii* meadow, the lower site receives about one third of this radiation. This estimate was based on discrete measurements regularly made throughout the year at both sites. Water temperature variation along the year followed the PAR dosage trend (Fig. 1).

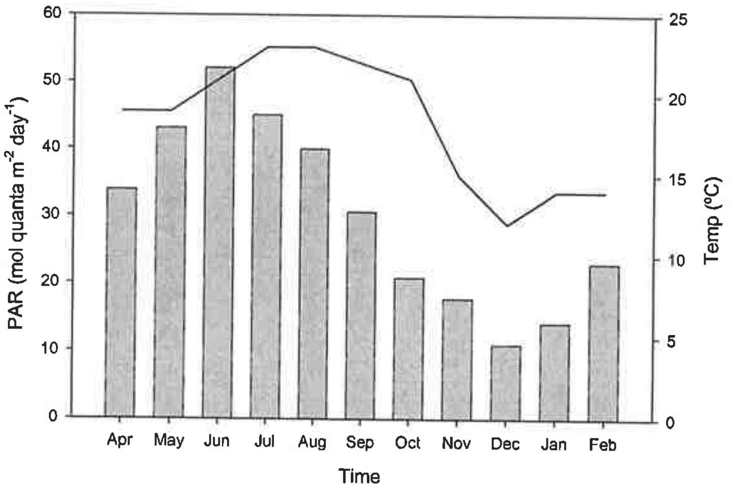

Fig. 1. Monthly averages of the daily dose of photosynthetically active radiation (PAR) at the surface (bars) and water temperature (line) in the Ria Formosa

The net photosynthetic capacity of *Zostera noltii* was lowest in June, at both the upper and lower sites (Fig. 2), and highest in the winter months, from December to February. Apart from July and September, when the plants from the upper site had a higher photosynthetic capacity than the ones from the lower site, no significant site differences were observed along the year. The net photosynthetic capacity showed a significant negative correlation ($p < 0.05$) with the average daily PAR dosage (Fig. 3). This correlation was verified in plants from both sites, with no significant differences between them. A negative correlation was also observed between the net photosynthetic capacity and the water temperature.

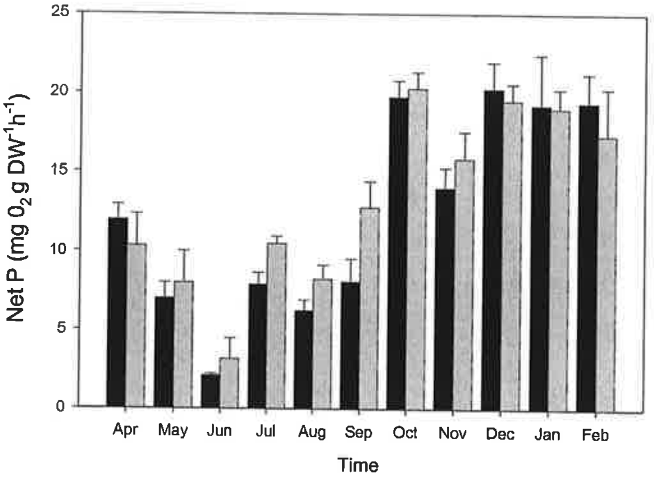

Fig. 2. *Zostera noltii*. Monthly variation of the net photosynthetic capacity, at the lower (black) and upper (gray) ends of an intertidal meadow

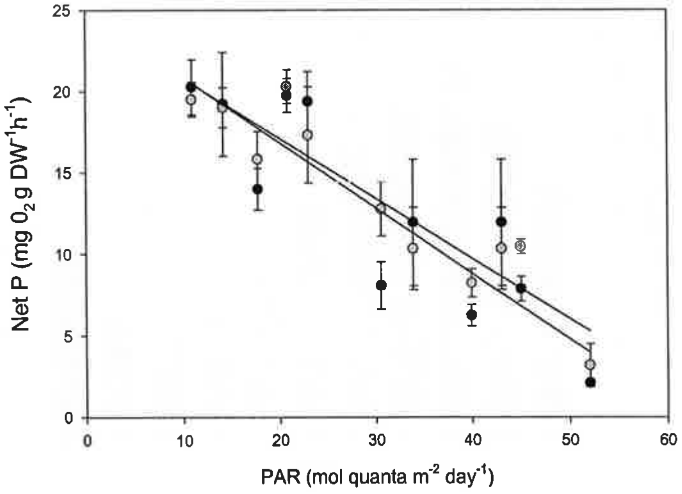

Fig. 3. *Zostera noltii.* Relationship between the daily dose of surface PAR and the net photosynthetic capacity, at the lower (black) and upper (gray) ends of an intertidal meadow

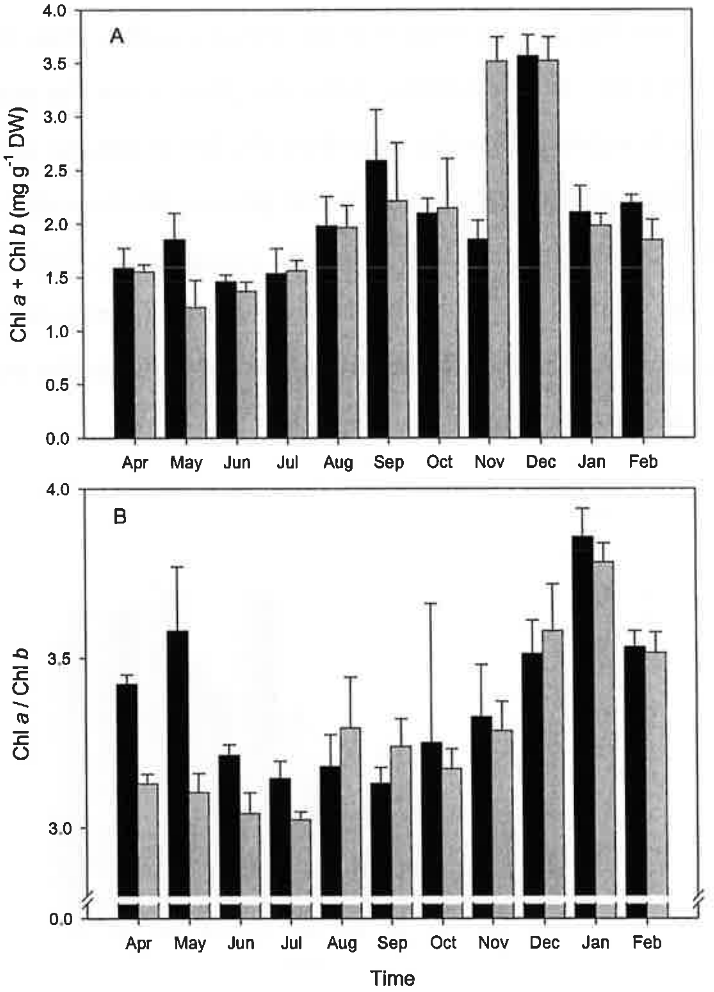

Fig. 4. *Zostera noltii.* Monthly variation of the Chl *a* + Chl *b* contents (A) and of the Chl *a* / Chl *b* ratio (B) in leaves, at the lower (black) and upper (gray) ends of an intertidal meadow

The total chlorophyll content (Chl *a* + Chl *b*, Fig. 4 A) of *Zostera noltii* leaves varied in a similar manner in both sites along the year. There were no significant differences between sites, except in May and September, when the plants from the lower site had higher chlorophyll content. The general seasonal trend was an increase in chlorophylls from June to December, followed by a decrease on the remaining period. The ratio between chl *a* and chl *b* (Fig. 4 B) was lowest in the summer and highest in the winter. During the summer months, from June to September, the ratio chl *a* / chl *b* remained low and constant. No significant differences were observed between sites, except from April to June, when the plants from the lower site showed a higher chl *a* / chl *b* ratio, due to an increase in chl *a* during these months. Chlorophylls decreased with PAR in a non-linear relationship (Fig. 5). During the autumn and winter months (from October to February), chlorophyll levels were in general higher than during spring and early summer.

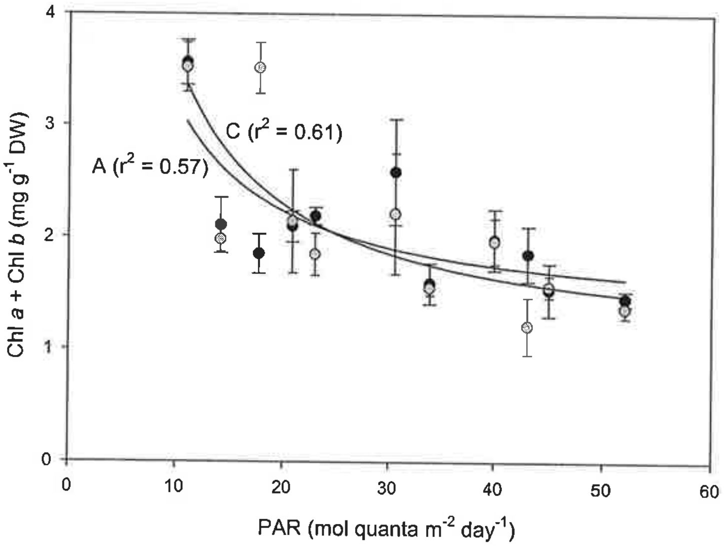

Fig. 5. *Zostera noltii*. Relationship between the daily dose of surface PAR and the Chl *a* + Chl *b* contents in leaves, at the lower (black) and upper (gray) ends of an intertidal meadow

The total organic nitrogen content of *Zostera noltii* leaves remained considerably low (around 2% DW) from May to July, increasing onwards and peaking above 3.5% in December-January (Fig. 6 A). No significant differences were observed between sites. The C:N ratio (Fig. 6 B) reflected an inverse pattern, mainly conditioned by the nitrogen values, as the leaf carbon content remained fairly constant throughout the year.

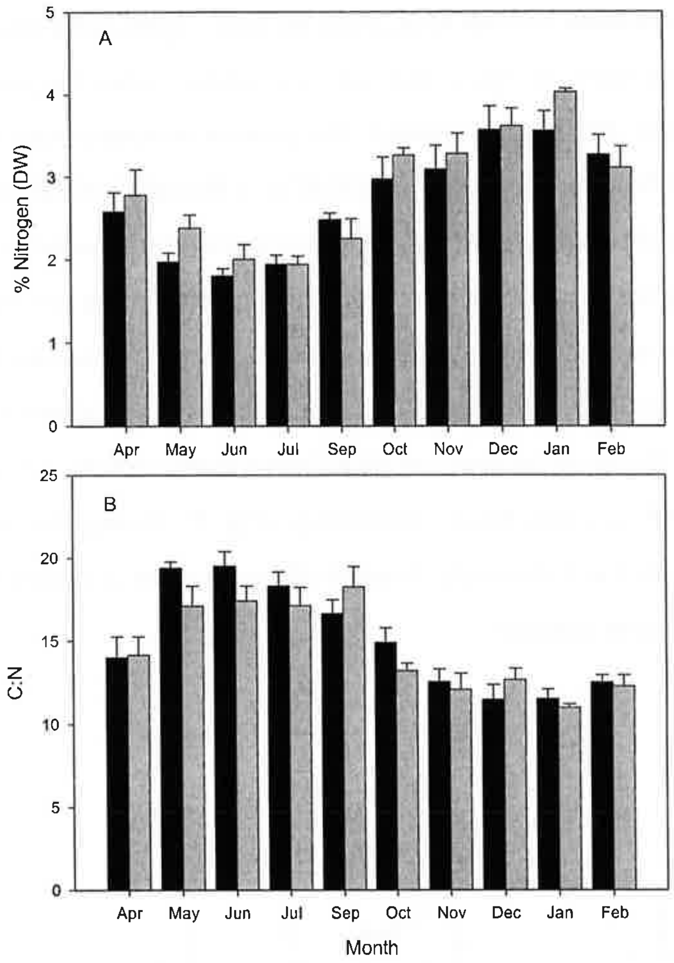

Fig. 6. *Zostera noltii*. Monthly variation of the nitrogen contents (A) and of the C:N ratio (B) in leaves, at the lower (black) and upper (gray) ends of an intertidal meadow

In general, the leaf soluble protein content (Fig. 7) of plants from both sites increased during spring and summer, until September, and decreased afterwards. The rhizome soluble protein content increased in spring and early summer to peak in July, decreasing afterwards to peak again in November. In general, the *Z. noltii* leaves from both sites had higher protein content than rhizomes (ca. 30% more in the lower site and 50% in the upper site, on annual average).

Soluble sugars in leaves and rhizomes (Fig. 8) presented no clear seasonal pattern in any site. The sugar content of leaves and rhizomes from the upper site was always significantly higher than the lower site, except in November. Overall, the rhizomes had much higher sugar content than the leaves (at least two-fold).

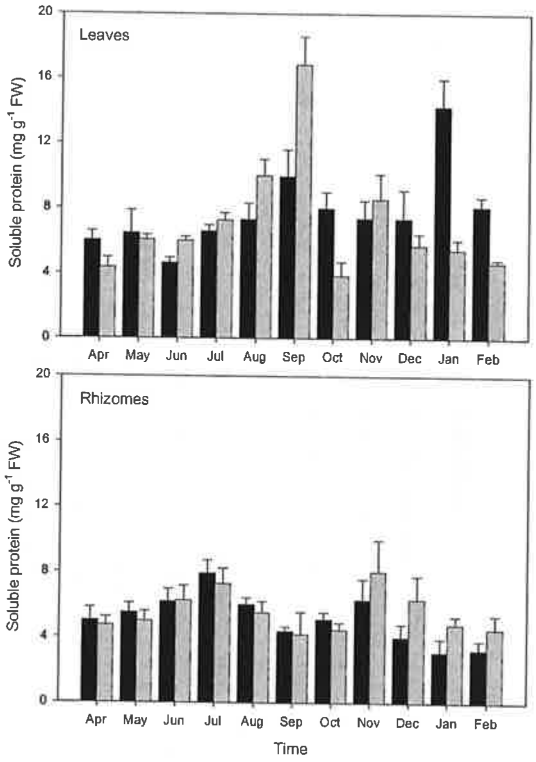

Fig. 7. *Zostera noltii*. Monthly variation of the soluble protein contents of leaves and rhizomes, at the lower (black) and upper (gray) ends of an intertidal meadow

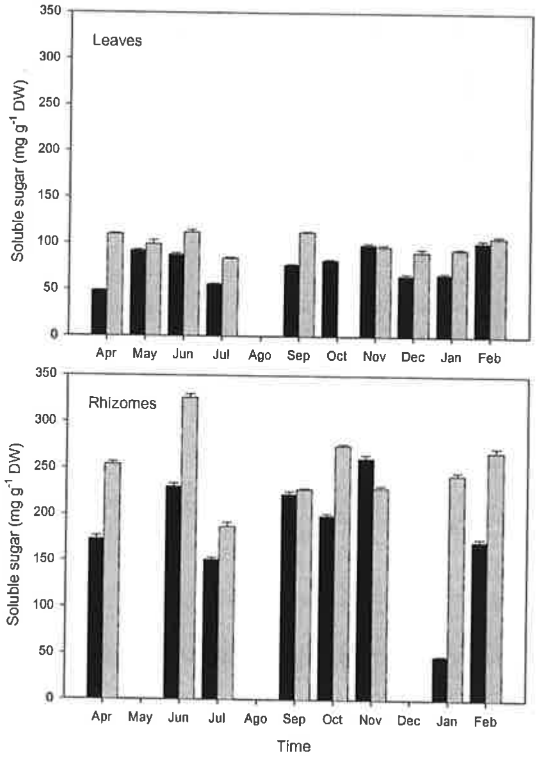

Fig. 8. *Zostera noltii*. Monthly variation of the soluble sugar contents in leaves and rhizomes, at the lower (black) and upper (gray) ends of an intertidal meadow

As well there was no visible seasonal pattern of starch in leaves, except in September when starch content was very low (Fig. 9). In the rhizomes of both sites, a decrease in the starch content was evident between April and September (Fig. 9). The rhizome starch content in September was also lowest.

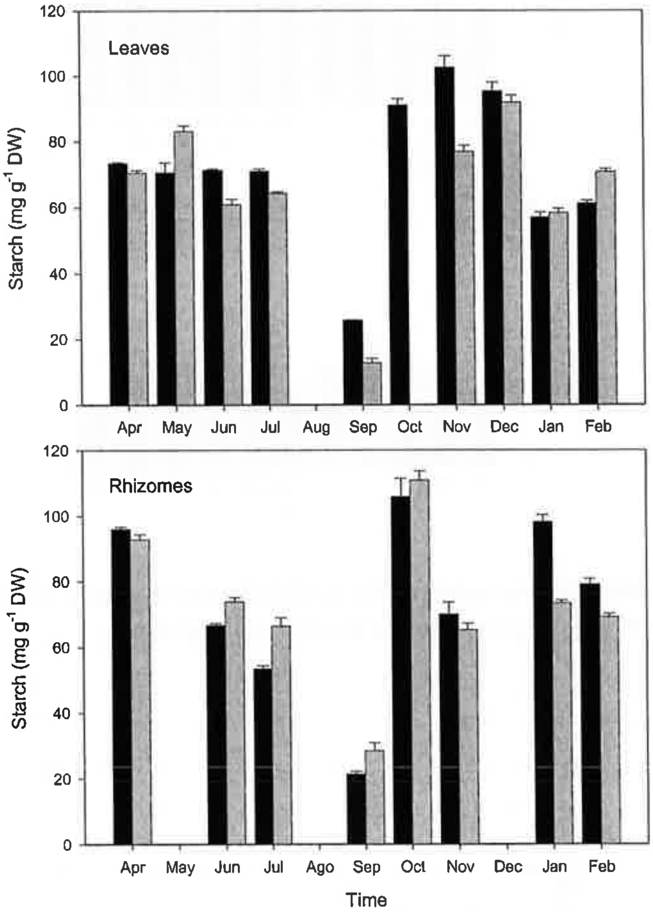

Fig. 9. *Zostera noltii*. Monthly variation of the starch contents in leaves and rhizomes, at the lower (black) and upper (gray) ends of an intertidal meadow

Discussion

The net photosynthetic capacity of the intertidal *Zostera noltii* plants of Ria Formosa was negatively related to both light and water temperature. This pattern was not the result of an increase in respiration with temperature during the summer period. In fact, the respiration rates were quite constant all year round and independent of temperature fluctuations, not showing the commonly described exponential increase with temperature (Plus et al. 2003 and references therein). In a previous study concerning the seasonality of *Zostera noltii* growth in Ria Formosa, Peralta (2000) also reported higher net photosynthetic rates in autumn and winter compared to lower values in spring and summer.

This pattern is the opposite of the most common response of seagrass photosynthesis to light and temperature, and particularly of other *Zostera noltii* populations. In the Mediterranean French coast (Thau lagoon), Plus et al. (2001) reported positive correlations of *Z. noltii* gross photosynthesis with both temperature and irradiance. A summer increase in the shoot growth rate was described by Kraemer & Mazzela (1999) for a subtidal *Z. noltii* population in the Gulf of Naples. In the Bassin d'Arcachon, on the Atlantic French coast, Welsh et al. (2000) verified a ca. 4 fold increase in the oxygen production from winter to mid-spring. Further north, in a Dutch *Z. noltii* population (Zandkreek estuary), Vermaat & Verhagen (1996) described an even more visible contrast, with photosynthesis being much higher in mid-summer than in the winter.

These latitudinal differences in the seasonal photosynthetic behaviour of *Zostera noltii* reveal the high adaptation capacity of this species or eventually some genetic differences, as suggested by Vermaat et al. (2000). In fact, genetic differences were found by Diekmann et al. (submitted) between the northern and southern Portuguese populations of *Z. noltii*. An inter-specific comparison study, involving seagrass species from different oceans, revealed *Zostera noltii* as the species with the highest variation in its light requirements along the geographical distribution range (Vermaat et al. 1996). The adaptation to different light regimes results in different long-term patterns, which can be completely opposite, as those described between the southern Ria Formosa population (this study) and the northern Zandkreek estuary population in the Netherlands (Vermaat & Verhagen 1996).

Despite the different seasonal productivity strategies found among *Z. noltii* populations, the highest photosynthetic rates are always coincident with the growing season, which usually occurs between spring and summer. The reproductive season for this species also goes from spring to summer, which means that the reproductive effort is made during the period of maximum productivity, when more biochemical energy is available. However, this is not the case of *Z. noltii* at Ria Formosa, as its reproductive season also occurs in spring and summer (Alexandre et al. submitted), coincident with the least productive time of the year. Considering that flowering is a major sink for photosynthates, we speculate that the *Z. noltii* populations of the Ria Formosa, will have less energy available to invest in sexual reproduction. In this case, flowering will depend on the mobilisation of reserves and will therefore be limited. Our data showed a significant decrease in the rhizomes starch content from April to September, indicating that during this period the plants mobilised starch reserves, part of which could have been used to support flowering during spring and summer.

The photosynthetic plasticity of *Z. noltii*, assessed by chlorophyll fluorescence, was greatly evidenced by real-time responses to short-term PAR variations (Silva & Santos 2003). On the course of a diurnal cycle, plants from the upper and lower intertidal sites revealed distinct adaptation patterns, in response to different light field dynamics, behaving respectively as sun- and shade-adapted plants. On a longer time-scale (this report), with monthly measures being carried out simultaneously on plants from both sites, in an oxygen electrode, these light-adaptation patterns were not visible, and the overall photosynthetic capacity showed no significant differences between sites. Despite the identical photosynthetic capacity of plants from both sites, the total time-integrated productivity will certainly be higher in the upper site, given the higher amount of radiation received by these plants along the time. This different productivity is well expressed in the leaf growth data of Cabaço et al. (in prep.), where, despite a similar growth pattern along the year, leaves from the upper site grow up in size to almost the double of lower site leaves. This difference in growth is crudely proportional to the difference between the two sites in the PAR dosage received by plants.

Following Peralta et al. (2002) classification of limiting, reduced, saturating and inhibitory light levels for *Z. noltii*, between April and September the plants from the upper intertidal studied in this work were under inhibitory levels of irradiance for most of the day. This classification is based on the assumption of a saturation irradiance (I_k) of 330 μmol quanta $m^{-2}s^{-1}$ and a inhibitory irradiance region beyond 1000 μmol quanta $m^{-2}s^{-1}$. However, on *Zostera noltii* plants of Ria Formosa, both the saturation and the inhibitory thresholds are usually attained at lower values (Silva & Santos 2003 and other unpublished data). During spring and summer, plants are daily exposed to levels of irradiance that inhibit photosynthesis and induce stress.

The described seasonality of the *Zostera noltii* photosynthesis in Ria Formosa was reflected both in the growth analysis of Cabaço et al. (in prep.) and on the monthly evolution of the biochemical parameters of the plants. The observations of Cabaço et al. (in prep.), made in parallel with this study, show that the leaf elongation rates were minimum in May and June, increasing thereafter until maximum values between

October and December. Rhizome growth, expressed by internodal length, was also highest during the winter months. Peralta (2000) also reported a marked seasonality in *Zostera noltii* growth parameters, with highest leaf length values in autumn and winter and lowest in summer.

Z. noltii plants show a very high leaf renovation rate (up to 0.15 d^{-1}; Peralta 2000). When sampling is carried every month along the year, each leaf sampled was developed under different historical environmental background. Therefore, the variation of the leaves biochemical composition will directly reflect the environmental variation. On the other hand, the high leaf turnover may explain why the storage of soluble sugar was made in the rhizomes, which appears to be a common feature of this species (Vermaat & Verhagen 1996). The plants will not accumulate reserves in a structure that will soon shed. Translocation of photoassimilates from the leaves into the rhizomes and their remobilisation requires energy, but it is necessary as a way of insuring a permanently available source of energy.

The total soluble sugar content of *Z. noltii* throughout the year was always higher than the starch content, particularly in the rhizomes. This pattern appears to be common within the Zosteraceae (Drew 1978, Zimmerman et al. 1989, Burke et al. 1996), suggesting a low level of sugar to starch conversion. This can be interpreted as an energy saving strategy, as no energy is spent in converting sugar to starch when the production exceeds the demand. As well when the plant needs to use carbohydrate reserves, no energy is spent in starch re-conversion into soluble sugar (Burke et al. 1996).

The nitrogen content of *Zostera noltii* leaves followed the photosynthetic production pattern, with the lowest values in June and July (1.9-2.0 % DW), yet above the median leaf value of 1.8% DW for seagrasses (Duarte 1990). The summer decrease in N contents was also observed in other *Zostera noltii* populations (Vermaat & Verhagen 1996, Kraemer & Mazzela 1999, Plus et al. 2001, Plus et al. 2003). The maximum values, near 4% DW, were measured in the winter, during December and January, just after the leaf growth peak, registered in November by Cabaço et al. (in prep.).

As in most seagrass communities in the temperate regions, the nutrient availability in Ria Formosa changes throughout the year, with maximum values during the autumn and winter and lower values in spring and summer (Falcão & Vale 2003). The general pattern in which seagrass growth occurs during spring and summer, implies that plants use reserve pools to support growth (Invers et al. 2002 and references therein). In Ria Formosa, *Zostera noltii* grows in the autumn and winter, when nutrient availability is higher. The nutrient demand for growth is then met by the external nutrient availability. Moreover, as the availability of inorganic nitrogen exceeds growth requirements, plants may simultaneously store nitrogen, by uptaking more than required for growth and building reserves (Invers et al. 2002). Together with the year-round low values of the C:N ratio, this supports the hypothesis that inorganic nitrogen is not a limiting factor for *Z. noltii* growth in Ria Formosa, and that the nitrogen storage during autumn and winter can be crucial to meet the demands of both flowering and below-ground tissues growth during spring. The increase in leaf nitrogen content from September to January could not be explained by soluble protein or chlorophyll content, indicating that the main nitrogen storage pool was probably the free amino acids.

The nutrient conservation strategy appears to be related to leaf longevity. In species whose leaves have a long lifespan, partial leaf nutrient resorption appears to be a way of preserve tissue nitrogen pools (Pedersen & Borum 1993, Hemminga et al. 1999). However, in species with short-lived leaves such as *Zostera noltii*, it does not appear advantageous to store nitrogen pools in the leaves in excess of short-term needs. Internal nitrogen redistribution within the meadows could play a more important role, with pools being transferred from leaves to rhizomes and roots, as suggested by Plus et al. (2003).

In conclusion, our results revealed a clear seasonal productivity pattern for the Ria Formosa *Zostera noltii* intertidal population. During the summer period, the plants strategy is to cope with the extended daily periods of inhibitory irradiance, many times coincident with air exposure due to low tide, dissipating considerable amounts of energy and reducing the photosynthetic productivity. In addition, during spring and summer, flowering occurs, which represents an important extra sink for photosynthates, forcing the use of reserves. In contrast, in the period from October until February, plants are exposed to irradiance levels between the saturation and the inhibition thresholds most of the day. Thee are the ideal conditions for photosynthesis and consequently for growth. During this period of the year, daily irradiance only sporadically attains inhibitory levels, which reduces stress and provides the conditions for the plants to commit on photosynthetic productivity, energy fixation and storage and, ultimately, on growth. The mild winter temperatures on Ria Formosa contribute to the success of this seasonal strategy.

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Chapter 6

Final synthesis

Final synthesis

1. Pulse amplitude modulated (PAM) fluorescence is a valid proxy for *Zostera noltii* photosynthetic production in the light range between 35 and 490 µmol photons $m^{-2}s^{-1}$ (PAR). A linear relationship exists between the oxygen evolution and the electron transport rate for *Zostera noltii* over this light range. The molar ratio between oxygen production and the electron transport rate (ETR) is 0.15 ± 0.02.

2. The leaf absorption factor (AF) of *Zostera noltii* is 0.79 ± 0.02. This factor is required to calculate absolute electron transport rates, used in the comparison between chlorophyll fluorescence measurements and oxygen production.

3. The chlorophyll fluorescence method presents less experimental problems and less sources of error than the oxygen evolution method. In balance, weighting the constraints and the advantages, PAM fluorometry remains the choice method for field estimates of photosynthesis, especially in aquatic environments.

4. *Zostera noltii* plants have higher photosynthetic rates during air-exposure periods of low tide than when submerged. This was observed both at the individual plants response level, determined by chlorophyll fluorescence, and at the gross community production level, measured as gas exchange (CO_2 uptake).

5. The key factor determining CO_2 assimilation by air-exposed *Z. noltii* is the leaf water content. The high leaf density and the sediment micro topography retain water during low tide, maintaining a fair hydration level for the whole emersion period between tides. In these conditions, CO_2 diffusion in the air-water interface insures Ci availability for photosynthesis.

6. The underwater photosynthetic rates of *Zostera* noltii are Ci-limited. The underwater gross community production of *Z. noltii* saturates at PAR levels between 300 and 400 µmol $m^{-2}s^{-1}$, whereas in air such saturation is not visible.

7. *Zostera noltii* is responsible for the global pattern of photosynthetic carbon fixation within its community, both during submersion and emersion periods. The gross community production (GCP) is linearly related to the individual plants electron transport rate (ETR). The molar ratio between GCP and ETR is ≈ 0.04.

8. CO_2 flux measurements in short-term incubations are a valuable tool for field assessment of intertidal communities' productivity. This method provides fast and precise values of carbon dioxide fixation, both in submerged and air-exposed communities.

9. The analysis of the molar ratio between GCP and ETR, namely by the difference between the observed value and the theoretical one, raises the possibility of using this method also to quantify the metabolism of the animal component of intertidal communities.

10. In the upper and lower ends of its vertical distribution, *Zostera noltii* clearly reveals sun- and shade-type plant patterns, respectively, during the daily course of photosynthesis. Lower plants are more efficient in the use of limiting irradiances, whereas upper plants respond better at higher PAR values.

11. *Zostera noltii* has a high photosynthetic plasticity. In different experiments, the photosynthetic parameters derived from rapid light response curves (maximum electron transport rate, initial slope and half-saturation irradiance) varied within considerable ranges, revealing the species ability to adapt rapidly to changing environmental conditions.

12. The net photosynthetic capacity of *Zostera noltii* is lowest in the summer and highest in the winter months, at both the upper and lower sites. This pattern is the opposite of other *Z. noltii* populations from northern latitudes and results from the species adaptation to southern intertidal conditions, where light and temperature extremes are common in the summer.

13. The long-term productivity of *Zostera noltii* in Ria Formosa follows a clear seasonal strategy. During the summer, the plants cope with the extended daily periods of inhibitory irradiance, dissipating excessive energy and reducing the photosynthetic productivity. From October until February plants are exposed most of the day to irradiance levels between the saturation and the inhibition thresholds, which are the ideal conditions for photosynthesis and consequently for growth. The mild winter temperatures on Ria Formosa contribute to the success of this strategy.