

MESTRADO EM CIÊNCIAS DO MAR - RECURSOS MARINHOS
ESPECIALIZAÇÃO EM BIOLOGIA E ECOLOGIA MARINHAS

Growth dynamics and sexual reproduction of *Zostera noltei* in the Ria de Aveiro lagoon

Manuel Ankel

M
2022



Manuel Ankel

Growth dynamics and sexual reproduction of *Zostera noltei* in the Ria de Aveiro lagoon

Dissertation for the Master Degree in Marine Sciences - Marine Resources submitted to the Abel Salazar Institute of Biomedical Sciences from the University of Porto

Supervisor - Doctor Laura Guerrero-Meseguer

Category - Auxiliary researcher

Affiliation - Interdisciplinary Centre of Marine and Environmental Research - CIIMAR

Co-Supervisor - Doctor Marcos Rubal Garcia

Category - Auxiliary researcher

Affiliation - Interdisciplinary Centre of Marine and Environmental Research - CIIMAR

Co-Supervisor - Doctor Maria Purificación Veiga Sánchez

Category - Auxiliary researcher

Affiliation - Interdisciplinary Centre of Marine and Environmental Research - CIIMAR

“When we investigate the reality, our mind should be calm.”

The 14th Dalai Lama

Acknowledgements

It has been a long journey since the beginning of the master's course until the writing of these last words. Of course, I would not be where I am today without the support and help of others.

First of all, I would like to thank my supervisor Doctor Laura Meseguer-Guerrero for guiding me the last two years. She received me with open arms and patience throughout the whole thesis project. There has always been room for clearing doubts, answering questions and suggestions. Thank you very much for all the support. I learned a lot about working in the scientific community and it has been a pleasure to share the fascination for seagrasses. I wish all the best for your future and all the upcoming seagrass projects!

Another big thank-you goes to my co-supervisors Doctor Marcos Rubal Garcia and Doctor Maria Purificación Veiga Sánchez. Thank you for helping with the field trips to Ria de Aveiro, introducing me to the granulometry, the help with the statistics and the feedback during the writing process. At this point, I also would like to thank Doctor Leandro Sampaio for the company during the laboratory work and all the good conversations.

To the organizers of the 20-21's BYTplus program, to the director of the master's course Marine Sciences/Marine Resources at ICBAS, Professor Eduardo Rocha as well as all the other professors that accompanied us students through the course.

At last, to my family, my mother and my brother, for being a safe harbor that I always can return to. To my friends, both here in Portugal and back home in Germany. Thank you for your support and being here for me. To my girlfriend Deborah, for staying with me since the last year and a half, for holding my back and tolerating my "grumpiness".

This research was developed under Project No. 029818, co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF, and by the Portuguese Foundation for Science and Technology (FCT) through national funds.



Declaração de Honra

Declaro que a presente dissertação é de minha autoria e não foi utilizada previamente noutro curso ou unidade curricular, desta ou de outra instituição. As referências a outros autores (afirmações, ideias, pensamentos) respeitam escrupulosamente as regras da atribuição, e encontram-se devidamente indicadas no texto e nas referências bibliográficas, de acordo com as normas de referência. Tenho consciência de que a prática de plágio e auto-plágio constitui um ilícito académico.

20 de junho de 2022

O estudante

Manuel Ankel

Manuel Ankel

Abstract

Zostera noltei is a small seagrass species that has a great ability to adapt to variable environmental conditions such as those of coastal lagoons. The Ria de Aveiro lagoon hosts the second largest population of this seagrass species in Portugal. As all seagrasses, *Z. noltei* can reproduce asexually through clonal, vegetative growth, and sexually by the formation of flowers, fruits and seeds. Sexual reproduction is important to increase the resilience of their meadows to environmental drivers and to form seedbanks that can facilitate recolonization after disturbances and the formation of new seagrass meadows at long distance from the parent plant. However, data on the annual growth cycle and the sexual reproduction of *Z. noltei* for Ria de Aveiro meadows are non-existent. Since this data can help understanding the conservation status and predicting the population development of *Z. noltei* in Ria de Aveiro, two studies were conducted in this thesis (Chapter 2 and Chapter 3) to address this knowledge gap.

In the first study (Chapter 2), the vegetative growth cycle of *Z. noltei* was monitored in six meadows for one year. In addition, environmental parameters were recorded to explore the influence on the development of *Z. noltei*. In the second study (Chapter 3), the sexual reproduction of *Z. noltei* was recorded in four meadows with high flowering activity. Moreover, the reproductive phenology of *Z. noltei* in Ria de Aveiro was described, for the first time, by defining five stages of sexual reproduction. Finally, the seed production per meadow and the *in-vitro* seed germination ability were recorded.

Results of the first study showed that the environmental conditions of the *Z. noltei* meadows from the Ria de Aveiro lagoon had a high spatio-temporal variability. The vegetative growth dynamics did not show clear differences among meadows. Flowering effort was the only parameter that differed strongly among meadows and revealed to be influenced by the sediment characteristics. The results of the second study did not show differences in the flowering effort among the *Z. noltei* meadows from Ria de Aveiro with values similar to other European populations. The reproductive stages followed a temporal pattern with two peaks of fertilization and seed formation. Moreover, the germination ability of seeds was independent of the meadow of origin.

The similar growth dynamics of *Z. noltei* found in the biometrics measured in this thesis suggest that this seagrass is adapted to the high spatio-temporal variability of the environmental conditions of the Ria de Aveiro lagoon. Nevertheless, flowering effort seems to be influenced by the sediment characteristics and could be influenced by additional factors not measured in this thesis project. The similar flowering effort and germination ability in the study on sexual reproduction suggest that all four meadows produce seeds of similar quantity and quality. In addition, the two peaks of fertilization

and seed production could suggest that *Z. noltei* is able to form seedbanks in Ria de Aveiro.

The present study is essential for the management and conservation of seagrass meadows both in the Ria de Aveiro and in other places with similar conditions where the species is present. Future research is needed to monitor the spatial development of *Z. noltei* in Ria de Aveiro and to finish understanding the natural recolonization capacity of the species in the lagoon. This knowledge will help to enhance the quality of coastal ecosystems, improving their biodiversity and productivity.

Keywords: seagrass; *Zostera*; growth dynamics; sexual reproduction; conservation

Resumo

Zostera noltei é uma pequena espécie de erva marinha que possui uma grande capacidade de se adaptar a condições ambientais variáveis, tais como as que existem nas lagunas costeiras. Em Portugal, a segunda maior população desta espécie encontra-se na Ria de Aveiro. Como todas as ervas marinhas, *Z. noltei* pode reproduzir-se assexualmente através do crescimento clonal e vegetativo, e sexualmente a partir da formação de flores, frutos e sementes. A reprodução sexual é deveras importante para o aumento de resiliência das pradarias aos fatores ambientais adversos, para a formação dos bancos de sementes que podem facilitar a recolonização após perturbações, e para a formação de novas pradarias longe da planta-mãe. Todavia, os dados sobre o ciclo anual de crescimento e da reprodução sexual de *Z. noltei* nas pradarias da Ria de Aveiro são inexistentes. Uma vez que estes dados poderiam ajudar a compreender o estado de conservação e a prever o desenvolvimento populacional de *Z. noltei* na Ria de Aveiro, foram realizados dois estudos na presente tese (Capítulo 2 e Capítulo 3), de modo a colmatar esta grande lacuna de conhecimento.

No primeiro estudo (Capítulo 2), monitorizou-se, durante um ano, o ciclo do crescimento vegetativo de *Z. noltei* em seis pradarias da Ria de Aveiro, e registou-se os parâmetros ambientais, de forma a investigar influência sobre o desenvolvimento da espécie. No segundo estudo (Capítulo 3), registou-se a reprodução sexual de *Z. noltei* em quatro pradarias com alta atividade de floração. Neste sentido, descreveu-se, pela primeira vez, a fenologia reprodutiva de *Z. noltei* na Ria de Aveiro, definindo ainda cinco fases de reprodução sexual. Por último, registou-se a produção de sementes por pradaria e a capacidade de germinação de sementes *in vitro*.

Os resultados do primeiro estudo, mostraram que as condições ambientais das pradarias de *Z. noltei* da Ria de Aveiro apresentavam uma elevada variabilidade espaço-temporal. A dinâmica de crescimento vegetativo não mostrou diferenças nítidas entre as pradarias. O esforço da floração foi o único parâmetro que diferiu fortemente entre as pradarias e que revelou ser influenciado pelas características do sedimento. Os resultados do segundo estudo não mostraram diferenças no esforço de floração entre as pradarias de *Z. noltei* da Ria de Aveiro, com valores semelhantes aos das outras populações europeias. Os estádios reprodutivos seguiram um padrão temporal com dois picos de fertilização e de formação de sementes. Ademais, a capacidade germinativa das sementes foi independente da pradaria de origem.

A dinâmica de crescimento semelhante de *Z. noltei* encontrada na biometria medida nesta tese, sugere que esta erva marinha se encontra adaptada à elevada variabilidade espaço-temporal das condições ambientais da Ria de Aveiro. No entanto,

o esforço da floração parece ser influenciado pelas características do sedimento, mas também pode ser influenciado por fatores adicionais não-medidos neste projeto. Deste modo, o esforço da floração e capacidade de germinação semelhantes no estudo sobre reprodução sexual sugerem que todas as quatro pradarias produzem sementes de quantidade e qualidade semelhantes. Adicionalmente, os dois picos de fertilização e produção de sementes podem sugerir que *Z. noltei* é capaz de formar bancos de sementes na Ria de Aveiro.

A presente tese é essencial para a gestão e conservação que deverão ser realizadas nas pradarias de ervas marinhas, tanto na Ria de Aveiro como noutros locais com condições semelhantes onde esta espécie está presente. Futuras investigações são necessárias para monitorizar o desenvolvimento espacial de *Z. noltei* na Ria de Aveiro e para concluir a compreensão da capacidade natural de recolonização da espécie na laguna. Este conhecimento ajudará a melhorar a qualidade dos ecossistemas costeiros, ao promover a biodiversidade e produtividade.

Palavras-chave: erva marinha; *Zostera*; dinâmica de crescimento; reprodução sexual; conservação

Table of contents

Abstract	V
Resumo	VII
List of figures	XI
List of tables	XII
1. Chapter 1 - General introduction	1
1.1. Seagrasses - an overview	1
1.2. Seagrass services	1
1.3. Worldwide seagrass decline	3
1.4. Seagrass development and sexual reproduction	3
1.5. Thesis outline and objectives.....	6
2. Chapter 2 - Growth dynamics of <i>Zostera noltei</i> meadows in the Ria de Aveiro	7
2.0. Abstract.....	7
2.1. Introduction	7
2.2. Material and Methods	10
2.2.1. Study area	10
2.2.2. Environmental conditions.....	11
2.2.3. Seagrass biometric parameters	11
2.2.4. Data analysis	12
2.3. Results	13
2.3.1. Spatio-temporal variability in the environmental conditions	13
2.3.2. Seagrass growth dynamics	19
2.3.3. Relationship of environmental and biometric parameters of <i>Z. noltei</i>	24
2.4. Discussion.....	26
3. Chapter 3 - Reproductive cycle of the seagrass <i>Zostera noltei</i> in the Ria de Aveiro lagoon.....	30
3.0. Abstract.....	30
3.1. Introduction	30
3.2. Material and Methods	32
3.2.1. Study area	32
3.2.2. Flowering effort and reproductive phenology.....	33
3.2.3. Germination ability	35
3.2.4. Data analyses	35
3.3. Results	37
3.3.1. Spatio-temporal variability in environmental conditions	37
3.3.2. General reproductive phenology of <i>Z. noltei</i> in the Ria de Aveiro lagoon ...	38

3.3.3. Spatio-temporal variability in the flowering effort and reproductive phenology	39
3.3.4. Germination ability	41
3.4. Discussion	41
4. Chapter 4 - Final conclusions and considerations	45
Supplementary material	47
References	49

List of figures

Figure 1. Ramet (A) and single, flowering shoot (B) of the seagrass <i>Zostera noltei</i> . R, roots; Rh(h), horizontal rhizome; Vs, vertical shoot; L, leaves; Sp; sexual spathes (carrying female and male flowers).	5
Figure 2. Location of the three delimited areas and the six <i>Z. noltei</i> meadows (M1-M6) selected in the Mira channel to study the growth dynamics of the seagrass over the year 2020. The dredging sediment depositing area is marked in dark red on the map.....	10
Figure 3. Spatio-temporal variability in the seawater temperature (A) and salinity (B) measured at the six <i>Z. noltei</i> meadows over time (mean \pm SE; n = 4). Solid lines indicate significant prototypical trajectories of the variables over time.	13
Figure 4. Spatio-temporal variability in the environmental parameters measured at the six <i>Z. noltei</i> meadows over time (mean \pm SE; n = 4). Solid lines indicate significant prototypical trajectories of the variables over time.....	18
Figure 5. Development of seagrass biometrics in the six <i>Z. noltei</i> meadows over the time (mean \pm SE; n = 4). Solid lines indicate significant prototypical trajectories of the variables along the time.	23
Figure 6. Study area (A) and location of the four studied <i>Z. noltei</i> meadows (M1-M4) in the Mira channel, Ria de Aveiro (B).	33
Figure 7. Morphology of the five sexual reproductive stages (I–V) determined to study the reproductive phenology of the <i>Z. noltei</i> meadows at the Ria de Aveiro lagoon.....	34
Figure 8. Salinity (A), organic matter content (B) and percentages of each sediment grain size (C-E) (mean \pm SE; n = 2) amidst the four studied <i>Z. noltei</i> meadows (M1-M4) before, during and after the flowering period.....	38
Figure 9. General reproductive phenology of the studied <i>Z. noltei</i> meadows of the Ria de Aveiro lagoon.....	39
Figure 10. Flowering effort (A), percentages of spathes in each reproductive stage (B, C, D, E and F) and percentage of aborted spathes (G) over time (mean \pm SE, n = 4). 40	

List of tables

Table 1. Results of the Akaike information criterion (AICc) for environmental parameters. Values in bold indicate the best fitting model. An asterisk above the variable indicates the model that was selected in cases of similar (difference < 2) AICc values.	14
Table 2. Coefficients (mean \pm SE) of the regression models for describing the development of different environmental parameters in each <i>Z. noltei</i> meadow along the study period. Significant effects ($p < 0.05$) of the factor meadow are indicated in bold. .	15
Table 3. Results of the Akaike information criterion (AICc) for the biometric measurements. Values in bold indicate the best fitting model.	20
Table 4. Coefficients of the regression models for describing the development of different <i>Z. noltei</i> biometrics in each meadow along the study period. Significant effects ($p > 0.05$) of the factor meadow are indicated in bold.	20
Table 5. Results of the sequential tests of the DISTLM analysis between seagrass biometrics and environmental parameters. For each variable the five most influential parameters are shown. Cum. % explains the cumulative percentage of each variable. Significant results are in bold.	24
Table 6. Description of the five sexual reproductive stages used to study the reproductive phenology of <i>Z. noltei</i> in Ria de Aveiro. The morphology of each reproductive stage is shown in Figure 7.	34
Table 7. Summary of the results obtained in the two-way ANOVA analyses applied for the environmental descriptors. Results are expressed by the <i>F</i> -values and associated <i>p</i> -values (in parenthesis). Significant results are in bold. Asterisks above the variables indicate that significance level (α) was lowered to 0.01 because data did not fulfil the ANOVA assumptions	37
Table 8. Results obtained in the two-way ANOVA analyses for FE and stages of sexual reproduction. Results are expressed as <i>F</i> -values and associated <i>p</i> -values (in parenthesis). Significant results are in bold. The significance level in all tests was $\alpha = 0.01$	39
Table 9. Percentage of germinated and no germinated seeds. Comparison among meadows was done through Fisher's exact test of independence.	41

1. Chapter 1 - General introduction

1.1. Seagrasses - an overview

Seagrasses are submerged, marine angiosperms that inhabit coastal and estuarine environments on every continent except Antarctica (Green & Short, 2003; McKenzie et al., 2020). Covering only up to 0.2% of the ocean's surface (Duarte, 2002), seagrasses form extensive, homospecific or heterospecific beds mainly on soft bottom substrate (den Hartog, 1970) into depths up to 60 m (Green & Short, 2003). Worldwide, 72 species from six families of seagrasses are known, distributing over six bioregions: the temperate North Atlantic, the Tropical Atlantic, the Mediterranean, the Temperate North Pacific, the Tropical Indo-Pacific and the Temperate Southern Oceans (Short et al., 2011; Short et al., 2007). The diversity in seagrass species differs among bioregions with an increasing number of species towards the equatorial regions and the highest number of species existing in the Indo-Pacific (Hemminga & Duarte, 2000; Short et al., 2007).

1.2. Seagrass services

Seagrasses provide a huge variety of ecological and economical services and are considered to be among the most important ecosystems worldwide (Costanza et al., 1997; Hemminga & Duarte, 2000; Nordlund et al., 2016). Costanza et al. (1997) estimated an annual value of 19.000 US dollars per hectare for the services that these ecosystems provide.

Seagrass meadows sequester large quantities of CO₂ at rates that surpass those of terrestrial ecosystems, thus being important carbon sinks (Duarte et al., 2013a; Duarte et al., 2013b; Duarte et al., 2005; Fourqurean et al., 2012; Mcleod et al., 2011). Nearly 10% to the yearly carbon burial capacity of the oceans can be attributed to seagrass meadows (Duarte et al., 2005). The sequestered carbon can be buried for several thousand years (Duarte et al., 2005; Mateo et al., 2006), which emphasizes the importance of these ecosystems in the current scenario of global warming. This capacity is amplified by a multiple through sediments and associated organic particles that enter the meadows and are captured by the spatial complexity of seagrass systems (Kennedy et al., 2010; Mcleod et al., 2011). The burial of this so called allochthonous carbon and the burial deriving from seagrass production increases to an estimated, worldwide burial capacity of 48 to 112 Mt carbon per year (Kennedy et al., 2010).

Being highly productive systems, seagrass meadows play a major role for the nutrient cycling in coastal ecosystems (Costanza et al., 1997). The retention of

sediments and the uptake of dissolved nutrients through seagrass meadows improve the water quality of coastal waters (Moore, 2004; Short & Short, 1984) and impede the excessive growth of bloom-forming algae, thereby preventing eutrophication (McGlathery et al., 2007).

Furthermore, seagrass meadows sustain a high faunal biomass and diversity of many vertebrates and invertebrates (Hemminga & Duarte, 2000; Unsworth & Cullen, 2010), providing shelter for many vulnerable or threatened species such as the dwarf seahorse (*Hippocampus zosterae*) or the fan mussel (*Pinna nobilis*) (Hughes et al., 2009). At the same time, seagrass meadows are the nursery area for economically important fish species, thereby sustaining part of the worldwide production of fisheries (Lilley & Unsworth, 2014; Unsworth et al., 2019). Especially traditional (small-scale) fisheries in Africa and the Indo-Pacific region strongly depend on seagrass meadows (Nordlund et al., 2016; Unsworth et al., 2019) as they are easily accessible fishing grounds (Unsworth et al., 2019) that enhance the occurrence of specific commercial fish species (Jones et al., 2021). Moreover, the existence of seagrass meadows can contribute to the tourism sector. Flagship species such as turtles or dugongs which attract diving tourists are associated with seagrass meadows (Cullen-Unsworth et al., 2014; McKenzie et al., 2021). Hence, seagrass meadows build up the livelihood for various human communities and their socio-economic and cultural value must not be underestimated (Cullen-Unsworth et al., 2014).

Besides their importance for climate mitigation and being a biodiversity hotspot, seagrass meadows are important for the protection of the coasts (Gracia et al., 2018; Ondiviela et al., 2014). Nowadays, erosion and floods are serious threats for nearby coastal communities (Gracia et al., 2018; Temmerman et al., 2013). The presence of seagrass beds attenuates the energy of waves which in return decreases the mobilization of sediments (Reidenbach & Thomas, 2018). In addition, the root and rhizome system stabilizes the sediment, thus inhibiting its degradation (Christianen et al., 2013). Conventional, artificial engineering measures that are applied to mitigate coastal erosion often have negative side effects, but the usage of seagrass meadows as complementary mean for coastal protection is a natural alternative with many additional, positive effects (Gracia et al., 2018).

1.3. Worldwide seagrass decline

A worldwide decline in seagrasses with an accelerating rate of loss since the 1990s has been reported (Waycott et al., 2009). Already over a decade ago, Short et al. (2011) classified 25% of all seagrass species as threatened or near threatened and a 14 % at higher risk to become extinct by applying the IUCN (International Union for the Conservation of Nature) criteria. Numerous environmental and anthropogenic stressors are responsible for this trend, leading to a net loss of nearly 30% in the extent of seagrass meadows compared to the late 19th century, which corresponds to an area of over five million hectares (Orth et al., 2006a; Waycott et al., 2009). The greatest part of all seagrass species is affected by human activities (Short et al., 2011). Poor water quality has been identified as one of these main threats, especially in areas with a high rate of urbanization and adjacent agricultural land use (Bowen & Valiela, 2001; Burkholder et al., 2007; Orth et al., 2006a). The resulting eutrophication of the seawater promotes algae blooms and enhances the growth of epiphytes (Ralph et al., 2007; Smith et al., 1999) which attenuate the light available for the seagrass, affecting its metabolism and lowering the chance of survival (Brodersen et al., 2015; Brun et al., 2003a; Brush & Nixon, 2002). Coastal development also has deleterious consequences for seagrasses. The mobilization and relocation of sediment through dredging change the hydromorphological characteristics of seagrass habitats, increase the turbidity of the water and carry the risk of burial of seagrass meadows (Erftemeijer & Robin Lewis, 2006). Intensive tourism, shellfish harvesting and mooring put additional anthropogenic pressure onto seagrasses, leading to the fragmentation and loss of the meadows through their physical destruction (Cabaço et al., 2005; Herrera-Silveira et al., 2010; Milazzo et al., 2004; Neckles et al., 2005; Unsworth et al., 2017). Moreover, as result of global warming, extreme weather events have become more frequent in the last decades, turning storms or heat waves into serious hazards for seagrasses (Congdon et al., 2019; Strydom et al., 2020).

1.4. Seagrass development and sexual reproduction

As aquatic angiosperms, seagrasses use two main modes to grow and colonize new areas or those that have been negatively affected because of disturbances, namely: 1) asexual, vegetative growth and 2) sexual reproduction (Hemminga & Duarte, 2000; Philbrick & Les, 1996).

Seagrasses are formed by clonal ramets which can duplicate for vegetative growth. The rhizome, located in the sediment, forms the base of each individual ramet. Rhizomes can either be horizontal (plagiotropic) or vertical (orthotropic), but seagrasses

that develop both forms exist as well (dimorphic rhizomes). Attached to the rhizome, a root system and several leaves extend vertically into opposite directions (Figure 1A) (Hemminga & Duarte, 2000). The horizontal, clonal extension of rhizomes allows seagrasses to colonize neighbouring areas at rates that differ among species from a few centimetres to more than one meter per year (Hemminga & Duarte, 2000; Marbà & Duarte, 1998). Detached rhizome fragments or viviparous propagules can allow seagrasses to colonize new areas at long distances when dispersed by currents and winds (Marbà et al., 2004; Orth et al., 2006b; Thomson et al., 2014). Like their terrestrial counterparts, seagrasses form flowers, fruits and seeds through sexual reproduction which are released into the marine environment (Orth et al., 2006b). Negatively buoyant seeds remain close to their meadow of origin, dispersing only up to a few meters (Orth et al., 1994). However, seeds can be dispersed over long distances on detached reproductive shoots (Erftemeijer et al., 2008; Källström et al., 2008) or in the interior of buoyant fruits (Guerrero-Meseguer et al., 2018; Kaldy & Dunton, 1999; Lacap et al., 2002). Moreover, megafauna such as dugongs and turtles which grazes on seagrass meadows also has the potential to disperse huge amounts of seeds over hundreds of kilometres (Tol et al., 2017).

The vegetative, clonal growth is thought to be the main mode of expansion in seagrasses (Hemminga & Duarte, 2000) with some populations existing of nearly genetically identical clones (e.g., Reusch et al., 1999). Nonetheless, sexual reproduction plays an important role for the maintenance and resilience of seagrass meadows by enhancing their genetic variability (Kendrick et al., 2012). After extreme events, such as heat waves or storms, the recovery of seagrass meadows is facilitated through the emergence of seedlings, especially at the early stage of recovery (Jarvis & Moore, 2010; Paulo et al., 2019). Genotypic diversity has shown to be positive related to the resilience in various seagrasses species (Salo & Gustafsson, 2016) and to different types of disturbances in *Posidonia oceanica* (L.) (Jahnke et al., 2015). Examples of *Posidonia australis* (L.) and *Zostera* sp. demonstrated that an elevated, genotypic diversity increases the resistance towards different environmental drivers (Evans et al., 2017; Hughes & Stachowicz, 2004; Massa et al., 2013).

However, growth and physiology in seagrasses are strongly influenced by environmental conditions (Maxwell et al., 2014; Udy & Dennison, 1997) and the life history (vegetative versus sexual reproduction) of one species can differ among different sites and geographic regions (Blok et al., 2018; Ito et al., 2021; Kim et al., 2014; McDonald et al., 2016). Thus, the growth dynamics and life history strategies in seagrasses also can be understood as reflection of their environment.

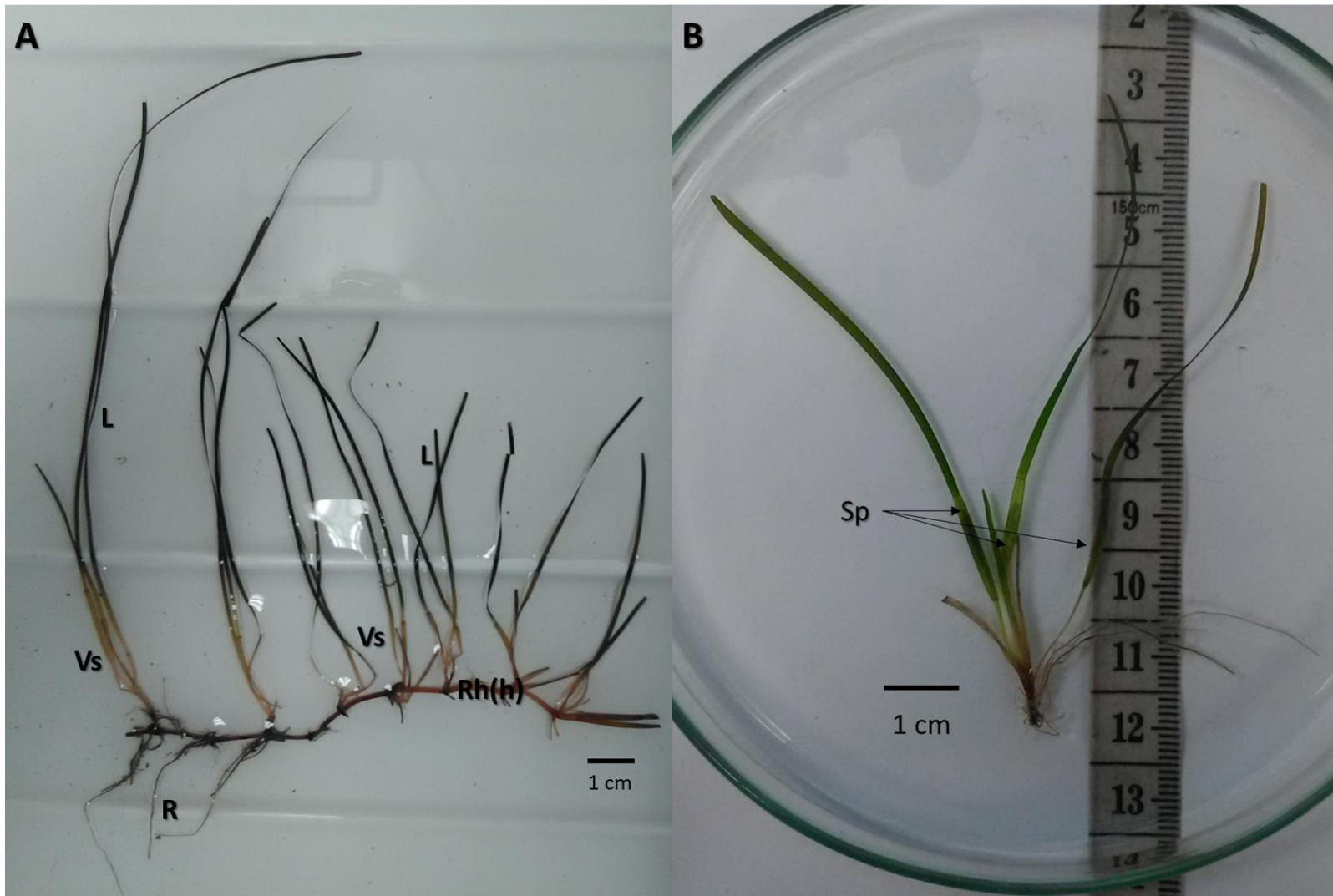


Figure 1. Ramet (A) and single, flowering shoot (B) of the seagrass *Zostera noltei*. R, roots; Rh(h), horizontal rhizome; Vs, vertical shoot; L, leaves; Sp; sexual spathes (carrying female and male flowers).

1.5. Thesis outline and objectives

Portugal hosts a great biodiversity of seagrasses with three of the four native European seagrass species inhabiting its coast: *Cymodocea nodosa* (Ucria) Asch., *Zostera marina* (L.) and *Zostera noltei* (Hornemann) (Borum & Greve, 2004; Cunha et al., 2013). Geographically, the Portuguese coast is interesting since in the Eastern Atlantic it forms the southern distributional limit for *Z. marina* (Cabaço & Santos, 2010) and the northern distributional limit for *C. nodosa* (Borum & Greve, 2004; Cunha et al., 2013). For *Z. noltei*, Portugal's coast builds the centre of its latitudinal distribution limits, being the most abundant seagrass species in this country (Cunha et al., 2013).

In Portugal a huge loss in the extent of seagrass meadows over the last decades has been registered (Cunha et al., 2013; Paulo et al., 2019). Consequently, studies which investigate the effects of environmental and anthropogenic stressors on the growth and reproductive cycle of seagrasses (e.g., Cabaço et al., 2012; Cunha & Duarte, 2007; Paulo et al., 2019) are necessary to understand the response of seagrass systems towards external impacts, helping further to conserve and manage these ecosystems in Portugal.

Considering the ecological importance and the threatened conservation status of these ecosystems, this thesis aims to contribute to the knowledge of seagrasses in Portugal by studying the dynamics of growth and sexual reproduction of *Z. noltei* meadows in the Ria de Aveiro lagoon. This area is a highly dynamic environment and is under constant anthropogenic pressure, hosting the second largest population of *Z. noltei* in Portugal. Although the effect of certain stressors on the coverage and distribution of *Z. noltei* is known, to date the annual growth cycle and the stages of sexual reproduction of this species in the Ria de Aveiro lagoon have not been studied in detail. To address this problem, the objectives of the following two chapters are:

Chapter 2. To evaluate the influence of the temporal and spatial variability of environmental drivers that occur in the Ria de Aveiro lagoon on the growth dynamics of *Z. noltei* meadows

Chapter 3. To monitor for the first time the sexual reproductive cycle of *Z. noltei* in the Ria de Aveiro lagoon (published in *Plants* 2021: Ankel, M., Rubal, M., Veiga, P., Sampaio, L. & Guerrero-Meseguer, L. (2021). Reproductive cycle of the Seagrass *Zostera noltei* in the Ria de Aveiro lagoon. *Plants*, 10 (11))

2. Chapter 2 - Growth dynamics of *Zostera noltei* meadows in the Ria de Aveiro

2.0. Abstract

The seagrass *Zostera noltei* possesses a high morphological and physiological plasticity, allowing this species to adapt to the huge variety of environmental conditions of coastal lagoons. In Ria de Aveiro, a very dynamic and anthropized coastal lagoon, *Z. noltei* forms the second largest seagrass population in Portugal. However, data on the growth dynamics and on how the morphological development of this seagrass is influenced by the variable environment of the lagoon are lack. To get a better understanding of the growth dynamics of *Z. noltei* under the spatio-temporal variability of environmental conditions in Ria de Aveiro, together with the high anthropogenic influence of the area, different biometric and physicochemical parameters were monitored for one year in six different *Z. noltei* meadows. Although the environmental parameters showed a high spatio-temporal variability and a dredging operation was conducted during the study, only flowering effort differed strongly among meadows. The similar dynamics of the remaining biometric parameters (biomass, shoot density, leaf area, root and vertical shoot length) observed in all the meadows suggest that vegetative growth in *Z. noltei* is not influenced by the different environmental conditions and stressors in Ria de Aveiro. Therefore, the *Z. noltei* meadows demonstrated a certain resilience in the Ria de Aveiro lagoon. Nonetheless, further studies are needed to explore the contribution of sexual reproduction to the development of the *Z. noltei* meadows and to compare the growth dynamics of this seagrass at the long-term. This will help to better protect this seagrass species in the Ria de Aveiro lagoon.

Keywords: seagrass, vegetative growth; growth dynamics; environmental drivers; conservation

2.1. Introduction

Coastal lagoons are shallow ecosystems which are sheltered from the open sea and under influence of strong physicochemical gradients, sharing characteristics of both estuaries and coastal waters (Conde et al., 2015; Pérez-Ruzafa et al., 2011). The Ria de Aveiro is a large coastal lagoon in Portugal, extending up to 45 km in length and 10 km in width with an average depth of 1 m (Dias et al., 2000). Four main channels (Mira, S. Jacinto, Espinheiro and Ílhavo) stretch out into opposite directions, forming the complex morphology of this lagoon (Dias et al., 1999). The area that is covered by water differs between 66 km² at low tide and a maximum of 83 km² at high tide (Dias et al., 2000). The tidal regime

averages 2 m and is the main force of water circulation (Dias et al., 1999), whereby the Vouga and Antuã rivers are the main source of freshwater in the lagoon (Dias et al., 2000).

The environmental conditions in Ria de Aveiro are very variable in space and time (Dias et al., 1999; Leandro et al., 2014; Lopes et al., 2007; Lopes et al., 2001; Rodrigues et al., 2012). Physicochemical parameters such as seawater temperature and salinity, for instance, vary daily between low and high tide as well as among seasons (Rodrigues et al., 2012). On a spatial scale, both parameters form a longitudinal gradient, with higher seawater temperatures and lower salinities at the upper ends of the channels (Dias et al., 1999; Leandro et al., 2014), whereby the gradient in salinity is mainly caused by the tidal driven inflow of seawater and the inflow of freshwater from the adjacent rivers (Dias et al., 1999). Similarly, in the Ria de Aveiro lagoon, the resuspension of sediments is also strongly influenced by the tidal cycle and the river inflow, resulting in varying concentrations of suspended sediment in the different parts of the lagoon (Lopes et al., 2001).

Moreover, the present salinity regime and sediment composition in Ria de Aveiro are to a great part the result of anthropogenic activities. The lagoon has been subjected to major hydromorphological alterations which increased the tidal influence and enhanced the tidal currents, accompanied by an altered salinity regime (Silva & Duck, 2006). In addition, a shift from fine to coarser, sandier sediments which sustain less biological activity (Silva & Duck, 2006) and contain less water during tidal emersion (Azevedo et al., 2016) occurred. As consequence of these alterations, a great part of the submerged aquatic vegetation, such as the seagrass *Zostera noltei* (Hornemann), disappeared on the tidal flats (Silva & Duck, 2006; Silva et al., 2004), favouring the establishment of invasive macroalga such as *Gracilaria* sp. (Abreu et al., 2011; Silva et al., 2004).

Nowadays, *Z. noltei* is the dominant seagrass in Ria de Aveiro, forming homospecific meadows on the intertidal flats (Azevedo et al., 2013; Sousa et al., 2019). This seagrass has a high horizontal rhizome elongation rate (Duarte, 1991; Hemminga & Duarte, 2000) which allows the formation of single clones that cover areas of several square meters (Brun et al., 2007; Zipperle et al., 2009a). Hence, the dynamics of *Z. noltei* meadows largely depend on vegetative growth (Marbà & Duarte, 1998), but sexual reproduction has shown to play also an important role for the population development (Zipperle et al., 2009b). Studies in the past decade revealed that *Z. noltei* adapts to the variable environmental conditions and different stressors in Ria de Aveiro and even has achieved to recover lost areas (Azevedo et al., 2016; Sousa et al., 2019; Sousa et al., 2017). These studies used the biomass and physiology of *Z. noltei* as proxies to describe its development under variable environmental conditions, whereby the data mostly was collected seasonally. However, *Z. noltei* also responses to different stressors in its morphology (Cabaço et al., 2009; de los Santos et al., 2010; Peralta et al., 2006). Hence, the occurrence of different

morphotypes reflects the spatio-temporal variability of the numerous stressors in coastal lagoons such as Ria de Aveiro (Peralta et al., 2005). Furthermore, environmental drivers on seagrasses co-occur (Stockbridge et al., 2020) and thus can have different effects on the growth of *Z. noltei* as when considered individually (e.g., Brun et al., 2008; Suykerbuyk et al., 2018; Vieira et al., 2020).

Until today, studies that investigated the growth dynamics of *Z. noltei* in Ria de Aveiro and its relationship to the spatio-temporal variability of the environmental conditions are scarce. In addition, relevant, biometric measurements other than biomass (e.g., leaf area, root length) that are influenced by these stressors are lack. This data is important to get a better understanding of the conservation status of *Z. noltei* in Ria de Aveiro and for understanding the growth dynamics of this seagrass in relation to its variable environment. Knowing the variability of these biometric parameters can help to predict the impact level of different stressors on the growth dynamics. This can facilitate both the monitoring of meadows and the detection of changes in the growth dynamics.

To fill this knowledge gap, the growth of *Z. noltei* was monitored throughout one year in six different meadows. Therefore, several biometric parameters and the environmental conditions in each meadow were recorded for one year. The resulting data were used to model the spatio-temporal variability of the seagrass growth dynamics and the environmental conditions of their meadows. In addition, the relationship of the seagrass biometrics with the environmental drivers of Ria de Aveiro was explored.

2.2. Material and Methods

2.2.1. Study area

To study the growth dynamics of *Z. noltei* in Ria de Aveiro, three areas were delimited in the Mira channel (Figure 2). In each area, two homospecific *Z. noltei* meadows were selected to check the spatio-temporal variability in the growth dynamics among areas (M1, M2, M3, M4, M5 and M6; Figure 2). The meadows were visited nine times throughout the year 2020 to measure environmental conditions and seagrass biometrics of each meadow.

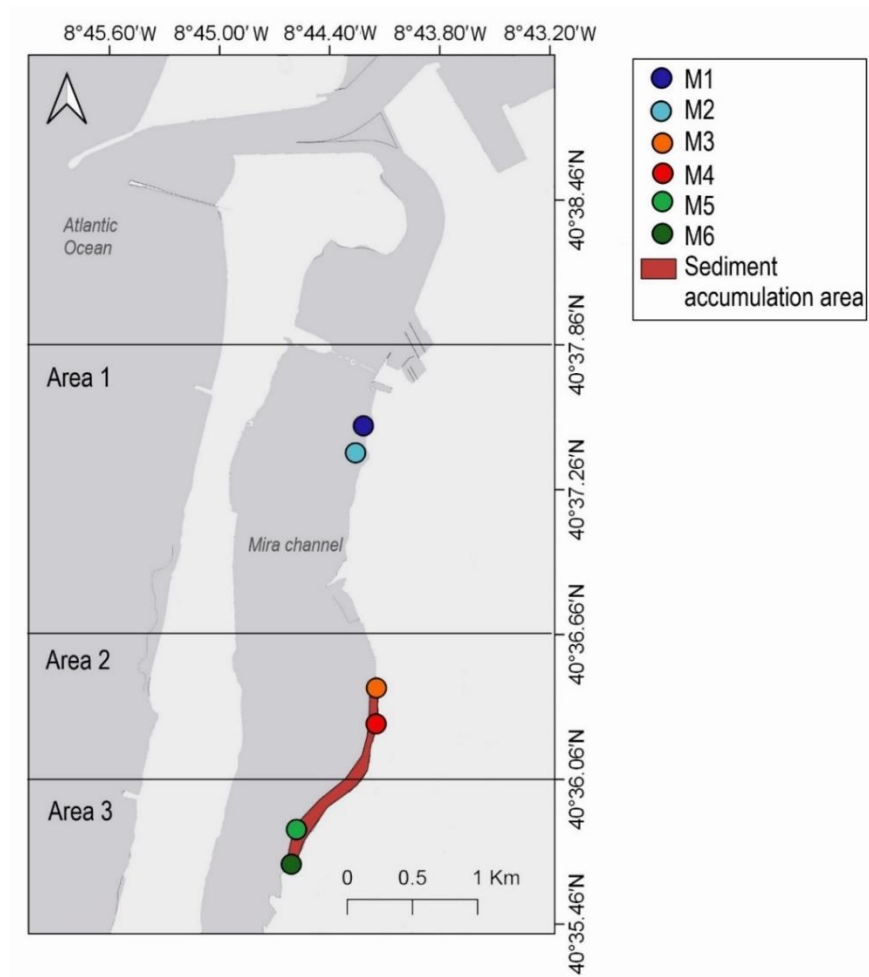


Figure 2. Location of the three delimited areas and the six *Z. noltei* meadows (M1-M6) selected in the Mira channel to study the growth dynamics of the seagrass over the year 2020. The dredging sediment depositing area is marked in dark red on the map.

2.2.2. Environmental conditions

Seawater temperature and salinity were recorded at each visit (January, February, May, June, July, August, September, November and December), whereas organic matter content (OM) and grain sizes of the sediments were monitored once per season (January, May, August and December).

Seawater temperature and salinity were measured with a multi-sonde (HQ40d, Hach®, Germany) at two randomly selected points in each meadow and date. To determine the OM and the grain sizes of the sediment in each meadow, sediment corers (n = 2, 5.5 cm of diameter of, 7 cm of length) were taken and dried for 72 h at 65°C. OM of the dry sediment was determined by loss on ignition (1.0 g of sediment at 450°C for 4.5 h). The different grain sizes of the sediment were classified following Wentworth (1922) by separating into seven different grain sizes (CISA# SIEVING TECHNOLOGIES BA 200N; t = 20 min, amplitude = 1.2 mm): fine gravel (2-4 mm), very coarse sand (1-2 mm), coarse sand (0.5-1 mm), medium sand (0.25-0.5 mm), fine sand (0.125-0.25 mm), very fine sand (0.063-0.125 mm) and silt and clay (< 0.063 mm).

It is important to mention that a dredging operation (Polis Litoral, 2017) was started in the lagoon in April of the studied year, thus being a possible factor for influencing the growth dynamics of *Z. noltei*. For this operation the upstream area of the Mira channel was selected for sediment depositing. Hence, A2 and A3 were inside the sediment depositing area, while A1 was downstream to the disturbance (Figure 2).

2.2.3. Seagrass biometric parameters

To study the growth dynamics of *Z. noltei* in Ria de Aveiro, seagrass corers (n=4, 10.5 cm of diameter, 15 cm of length) were collected in each meadow and visit. At the laboratory, corers were cleaned from sediments and fauna and stored in a freezer until seagrass biometrics were measured.

The biometric measurements consisted of the vegetative shoot density, flowering effort, leaf area, vertical shoot length and maximum root length. Additionally, the above- and below-ground biomass of the seagrass and the biomass of associated macroalgae species (*Ulva* spp. and *Gracilaria* spp.) were measured. These two macroalgae were recorded since they can have deleterious effects on *Zostera* sp. (e.g., Brun et al., 2003a; Thomsen et al., 2013).

To determine the vegetative shoot density and the flowering effort of the seagrass meadows, the vertical shoots and sexual spathes of each corer were counted. Four vertical, vegetative shoots with apparently healthy leaves and roots were randomly chosen to measure the maximum root length, vertical shoot length and leaf area. To determine the

maximum root length per shoot, the length of all roots in each shoot was measured from the basis of the rhizome until the end of the root and then, the maximum value was calculated. The maximum value was used for the analyses since the roots of *Z. noltei* are very fragile and broke easily during the cleaning. In this way, reliable measures of the root length were ensured. The vertical shoot length was measured from the insertion into the horizontal rhizome until the furthest leaf ligule. The leaf area was calculated by dividing the total leaf area per shoot (leaf length x leaf width) by the number of leaves per shoot.

The leaves and vertical shoots of each seagrass corer were separated from horizontal rhizomes and roots to determine the above- and below-ground biomass, respectively. The macroalgae which were present in each seagrass corer were separated to determine their biomass. The different fractions of the seagrass and macroalgae were cleaned and dried in the oven at 65°C for 72 h and subsequently, the dry weight of each fraction was determined. Since the corers differed in shoot density and quantity of macroalgae and thus in dry weight, the biomass of each variable was extrapolated by expressing each fraction as percentage of the total dry weight.

2.2.4. Data analysis

The trends of all the measured parameters (environmental and biometrics) over time were analysed separately by adjusting the data to the best fitted regression models using the Akaike information criterion (AICc). Subsequently, ANCOVA analyses were used to compare the trends among *Z. noltei* meadows over time, using the factor *time* as the co-variable. For each parameter, the model with the lowest AICc value was chosen to run the ANCOVA analyses, except when the difference among AICc values was smaller than two units. In this case, the models with similar AICc were compared visually to select the one that best adjusted to the results. To test for significant differences among meadows over the time, the intercept of the regression model was set up to M1. Before models were ran, data was checked for normality and homoscedasticity of variances, using Q-Q plots and Bartlett tests, respectively. Tests were run with the statistical software R Version 4.0.0 (R Core Team, 2021) and the packages “AICcmodavg” and “stringr” (RStudio Team, 2021) with a significance level of $\alpha = 0.05$. Following results are given as mean \pm SE.

To investigate relationships between seagrass biometrics and the environmental conditions in Ria de Aveiro for January, May, August and December, non-parametric multivariate regression analyses (McArdle & Anderson, 2001) were done. Models for the biometric measurements were obtained by subjecting both environmental and biometric parameters to a subsequential selection process. Euclidean similarity matrices were used to do analyses and *p*-values were obtained with 9999 residual permutations for the reduced model (Anderson, 2001). The program DISTLM (Anderson, 2004) was used to do non-

parametric multivariate multiple regressions. Draftsman plots were created beforehand to check the skewness of environmental and biometric variables. Since strong correlations ($R > 0.85$) for two pairs of sediment fractions were found (i.e. fine gravel with very coarse sand and fine sand with very fine sand), fine gravel and fine sand were removed from the analyses, whereas very coarse sand and very fine sand were maintained to exclude redundancy from the analysis (Anderson, 2004). Distance-based redundancy analysis (dbRDA) (Legendre & Anderson, 1999) was then used to explore the relationship between environmental parameters and biometrics.

2.3. Results

2.3.1. Spatio-temporal variability in the environmental conditions

Seawater temperature evolved similarly among meadows during the entire study period, following a second-polynomial degree trend in which seawater temperature increased from January to June and decreased from August to December (Table 1 and Table 2; Figure 3A). In contrast, seawater salinity varied among meadows over time following a second-polynomial degree trend (Table 1 and Table 2). M1, M2, M3 and M5 maintained similar values of salinity over time, but the salinity of M4 and M6 significantly increased from January to August and decreased from August to December (Table 2, Figure 3B).

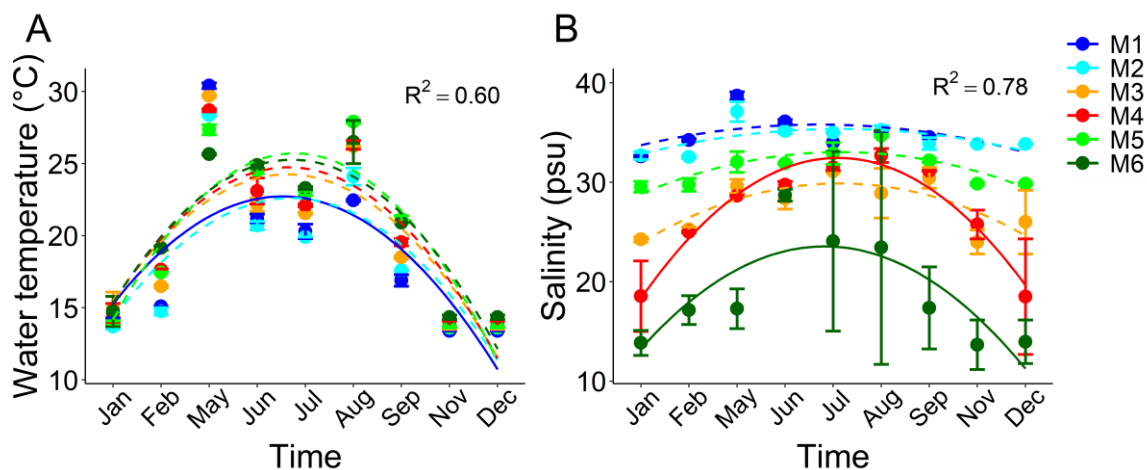


Figure 3. Spatio-temporal variability in the seawater temperature (A) and salinity (B) measured at the six *Z. noltei* meadows over time (mean \pm SE; $n = 4$). Solid lines indicate significant prototypical trajectories of the variables over time.

Table 1. Results of the Akaike information criterion (AICc) for environmental parameters. Values in bold indicate the best fitting model. An asterisk above the variable indicates the model that was selected in cases of similar (difference < 2) AICc values.

	First-degree polynomial	Second-degree polynomial	Inverse polynomial	Exponential
Seawater temperature	685.4	583.6	656.5	684.8
Salinity	641.0	585.9	628.8	625.2
Organic matter	397.8*	395.6	401.0	411.1
Fine gravel	490.6	423.2	486.0	474.6
Very coarse sand	553.1	441.3	552.7	532.2
Coarse sand	379.3	378.8	398.6	417.6
Medium sand	748.1	693.9	727.9	786.8
Fine sand	223.8	215.6	220.1	229.3
Very fine sand	510.6	400.8	404.8	546.1
Silt and Clay	681.3	621.6	543.7	740.3

The OM of the sediments showed a high spatio-temporal variability over the study period following linear trends (Table 1 and Table 2, Figure 4A). M1, M2 and M5 increased in OM from January to December, while M3, M4 and M6 significantly decreased over time (Figure 4A).

Regarding the sediment grain size, all the meadows showed great spatio-temporal variability (Table 2, Figure 4B-H). All grain sizes evolved following a second-degree polynomial trend, less silt and clay which followed an inverse polynomial (Table 1). In M1, M3 and M4, both the fine gravel and the very coarse sand contents, were maintained similar over time. In M2, the content of both grain sizes decreased significantly from January to December, whereas in M5 it decreased only until August and then increased until December (Table 2, Figure 4B and C). In addition, the fine gravel and very coarse sand contents of M6 significantly increased between January and June (Table 2, Figure 4B and C). All the meadows showed similar values or slightly increased in the content of coarse sand along the study, less in M5 whose content decreased over time (Table 2, Figure 4D). Regarding the content of medium sand all the meadows followed different dynamics over time (Table 2). The medium sand content of M1 decreased from January to August but increased from August to December, whereas in M2 the opposite situation occurred (Figure 4E). Medium sand content in M3, M4 and M5 decreased slightly over time, while in M6 it increased continuously throughout the study (Figure 4E). Regarding the fine sand content, only M2 varied over time by decreasing in its content from January to August (Table 2, Figure 4F).

All the meadows showed different dynamics to M1 in the content of very fine sand and silt/clay over time (Table 2). The content of very fine sand in M1 increased over time, whereas in M2 it decreased. In the remaining four meadows the content of very fine sand was stable (Figure 4G). The silt and clay content in M1 increased slightly throughout the year, while in M2 and M6 it decreased and remained stable in M3, M4 and M5 (Table 2, Figure 4H).

Table 2. Coefficients (mean \pm SE) of the regression models for describing the development of different environmental parameters in each *Z. noltei* meadow along the study period. Significant effects ($p < 0.05$) of the factor meadow are indicated in bold.

		Intercept (M1)	Time linear trend	Time quadratic trend
Seawater				
temperature^b	M1	10.44 \pm 2.982	5.430 \pm 1.369	-0.600 \pm 0.133
	M2	-1.327 \pm 4.217	0.312 \pm 1.936	-0.011 \pm 0.189
	M3	-0.430 \pm 4.217	0.726 \pm 1.936	-0.065 \pm 0.189
	M4	-0.906 \pm 4.217	1.108 \pm 1.936	-0.103 \pm 0.189
	M5	-3.181 \pm 4.217	2.298 \pm 1.936	-0.209 \pm 0.189
	M6	-1.170 \pm 4.217	1.346 \pm 1.936	-0.118 \pm 0.189
Salinity^b	M1	32.46 \pm 2.896	1.423 \pm 1.330	-0.151 \pm 0.130
	M2	-0.982 \pm 4.096	0.091 \pm 1.881	0.004 \pm 0.183
	M3	-11.80 \pm 4.096	2.173 \pm 1.881	-0.199 \pm 0.183
	M4	-21.88 \pm 4.096	7.156 \pm 1.881	-0.690 \pm 0.183
	M5	-5.868 \pm 4.096	1.075 \pm 1.881	-0.091 \pm 0.183
	M6	-25.28 \pm 4.096	5.367 \pm 1.881	-0.553 \pm 0.183
Organic matter^a	M1	4.014 \pm 0.815	0.314 \pm 0.145	
	M2	-2.268 \pm 1.152	-0.201 \pm 0.204	
	M3	0.767 \pm 1.152	-0.622 \pm 0.204	
	M4	0.542 \pm 1.152	-0.564 \pm 0.204	
	M5	-1.987 \pm 1.152	0.045 \pm 0.204	
	M6	-0.660 \pm 1.152	-0.609 \pm 0.204	
Fine gravel^b	M1	-0.892 \pm 1.419	1.174 \pm 0.715	-0.055 \pm 0.069
	M2	5.077 \pm 2.006	-2.357 \pm 1.011	0.147 \pm 0.098
	M3	1.860 \pm 2.006	-0.427 \pm 1.011	-0.028 \pm 0.098
	M4	1.131 \pm 2.006	-0.858 \pm 1.011	0.038 \pm 0.098
	M5	5.780 \pm 2.006	-3.226 \pm 1.011	0.284 \pm 0.098

Table 2 continued

	M6	-2.662 ± 2.006	5.288 ± 1.011	-0.625 ± 0.098
Very coarse				
sand^b	M1	< 0.001 ± 1.558	0.841 ± 0.786	0.002 ± 0.076
	M2	9.138 ± 2.205	-3.739 ± 1.111	0.236 ± 0.108
	M3	1.971 ± 2.205	-0.646 ± 1.111	0.010 ± 0.108
	M4	2.178 ± 2.205	-0.679 ± 1.111	0.057 ± 0.108
	M5	10.14 ± 2.205	-4.705 ± 1.111	0.451 ± 0.108
	M6	-6.108 ± 2.205	8.990 ± 1.111	-1.014 ± 0.108
Coarse sand^b				
	M1	5.102 ± 1.126	0.612 ± 0.567	-0.014 ± 0.055
	M2	5.102 ± 1.592	-1.486 ± 0.802	0.099 ± 0.078
	M3	4.976 ± 1.592	-0.590 ± 0.802	0.085 ± 0.078
	M4	3.251 ± 1.592	1.434 ± 0.802	-0.094 ± 0.078
	M5	13.91 ± 1.592	-1.881 ± 0.802	0.1172 ± 0.078
	M6	7.693 ± 1.592	0.639 ± 0.802	-0.105 ± 0.078
Medium sand^b				
	M1	69.51 ± 5.809	-18.97 ± 2.928	1.482 ± 0.284
	M2	-69.48 ± 8.215	41.96 ± 4.140	-3.311 ± 0.402
	M3	0.995 ± 8.215	14.85 ± 4.140	-1.17 ± 0.402
	M4	3.430 ± 8.215	15.58 ± 4.140	-1.33 ± 0.402
	M5	-24.11 ± 8.215	28.00 ± 4.140	-2.543 ± 0.402
	M6	-62.56 ± 8.215	21.29 ± 4.140	-1.031 ± 0.402
Fine sand^b				
	M1	9.218 ± 0.481	0.221 ± 0.243	-0.028 ± 0.024
	M2	-0.081 ± 0.681	-0.923 ± 0.343	0.083 ± 0.033
	M3	-5.523 ± 0.681	0.397 ± 0.343	-0.035 ± 0.033
	M4	-6.133 ± 0.681	0.171 ± 0.343	-0.008 ± 0.033
	M5	-5.875 ± 0.681	-0.069 ± 0.343	0.023 ± 0.033
	M6	-6.711 ± 0.681	0.482 ± 0.343	-0.037 ± 0.033
Very fine sand^b				
	M1	4.497 ± 1.263	8.255 ± 0.636	-0.721 ± 0.062
	M2	21.67 ± 1.786	-14.94 ± 0.9	1.243 ± 0.087
	M3	-0.758 ± 1.786	-7.296 ± 0.9	0.628 ± 0.087
	M4	-1.355 ± 1.786	-8.028 ± 0.9	0.700 ± 0.087
	M5	-0.929 ± 1.786	-8.426 ± 0.9	0.760 ± 0.087
	M6	-2.627 ± 1.786	-6.861 ± 0.9	0.586 ± 0.087
Silt and clay^c				
	M1	35.40 ± 1.418	-16.40 ± 2.643	
	M2	-28.48 ± 2.005	41.77 ± 3.738	
	M3	-23.72 ± 2.005	16.11 ± 3.738	

Table 2 continued

M4	-25.55 ± 2.005	17.62 ± 3.738
M5	-21.92 ± 2.005	14.37 ± 3.738
M6	-31.10 ± 2.005	82.10 ± 3.738

Regression models (according to the AICc) are ^aFirst-degree polynomial, ^bSecond-degree polynomial, ^cInverse polynomial

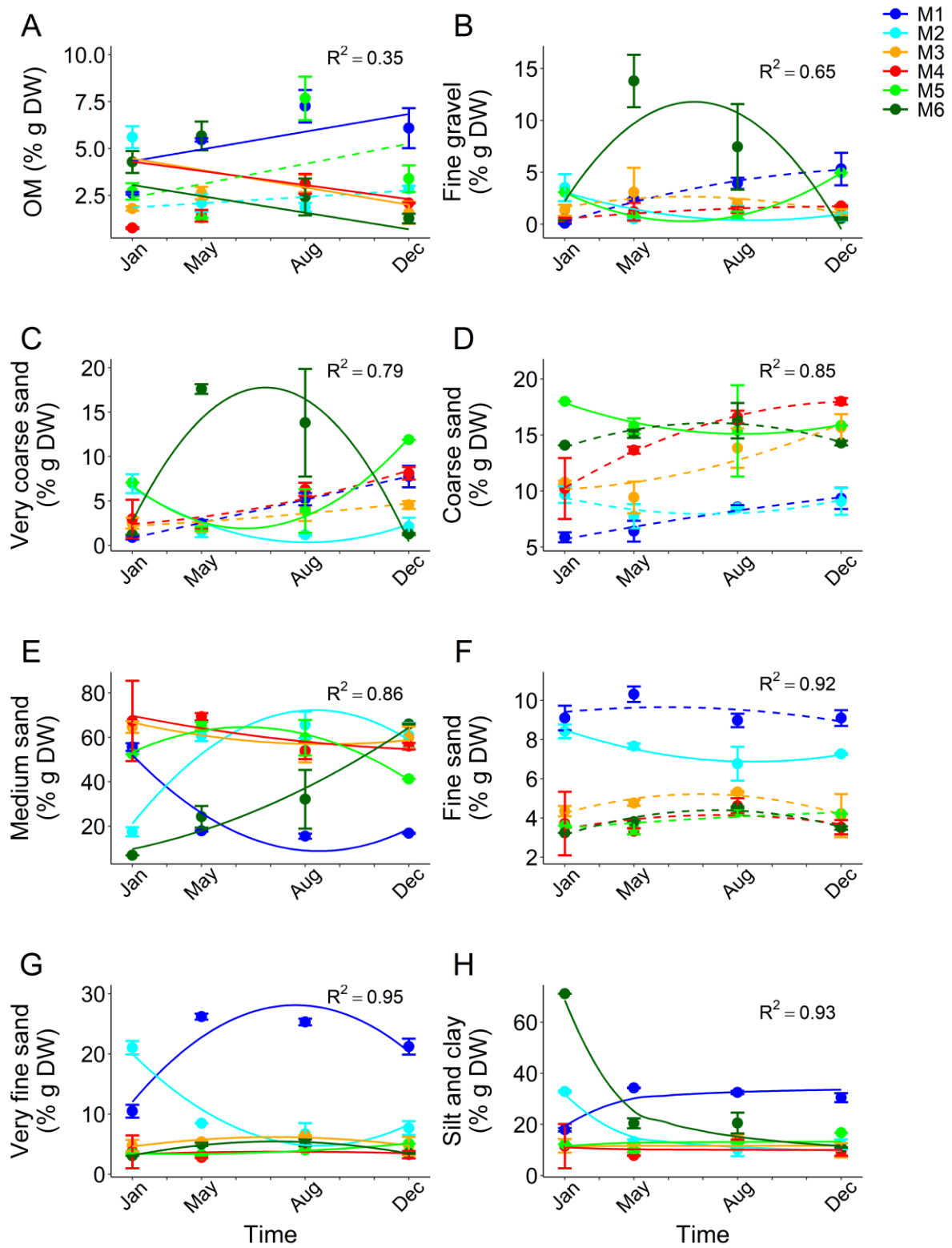


Figure 4. Spatio-temporal variability in the environmental parameters measured at the six *Z. noltei* meadows over time (mean \pm SE; $n = 4$). Solid lines indicate significant prototypical trajectories of the variables over time.

2.3.2. Seagrass growth dynamics

Flowering effort, leaf area, vertical shoot length and the above- and below-ground biomass of *Z. noltei* followed a second-degree polynomial trend over time (Table 3). The maximum root length and the biomass of *Gracilaria* spp. followed an exponential trend, whereby the vegetative shoot density and the biomass of *Ulva* spp. followed an inverse polynomial trend over time (Table 3).

M1 and M2 showed a similar dynamic of flowering effort over time and differed significantly to the other meadows (Table 4, Figure 5A). In M1 and M2, the number of sexual spathes increased from May until August/September and decreased from November to December, whereas the other four meadows showed scarce values of flowering effort over time (Figure 5A). The vegetative shoot density was maintained around the same values in all the meadows over time (Table 4, Figure 5B). The leaf area in M3 was significantly different to the other meadows over time with increasing values from January to July and decreasing in the following months, whereas in the remaining meadows values remained relatively stable (Table 4, Figure 5C). The dynamic of the vertical shoot length was similar in M3 and M5 with increasing values towards summer, whereas in the remaining four meadows this variable did not change significantly over time (Table 4, Figure 5D). No differences were found among meadows in the root length over time (Table 4, Figure 5E). Analogously, the above-ground biomass followed a similar trend in all the meadows, increasing from January to July and decreasing in the following months (Table 4, Figure 5F). The below-ground biomass in M4 increased throughout the study period, which was in contrast to the other meadows, in which it declined towards the summer and increased towards the winter months (Table 4, Figure 5G).

The biomass of *Gracilaria* spp. in M3 evolved significantly different to the other meadows over time (Table 4). In M3, the biomass of this macroalga increased strongly from August to December, whereas in the other four meadows the biomass was maintained constant over time (Table 4, Figure 5H). Similarly, the biomass of *Ulva* spp. in M4 was significantly different to the other meadows over time (Table 4, Figure 5I). From January towards May a drastic decline in the biomass of *Ulva* spp. was recorded in M4, while in the remaining meadows the biomass was constant throughout the year (Figure 5I).

Table 3. Results of the Akaike information criterion (AICc) for the biometric measurements. Values in bold indicate the best fitting model.

	First-degree polynomial	Second-degree polynomial	Inverse polynomial	Exponential
Flowering effort	880.0	864.9	874.3	881.4
Vegetative shoot density	2058.6	2060.7	2052.4	2055.4
Leaf area	103.7	91.33	105.2	102.2
Vertical shoot length	418.7	396.1	409.9	440.4
Maximum root length	952.8	962.1	951.2	948.7
Above-ground biomass	1733.7	1683.6	1745.6	1721.3
Below-ground biomass	1690.1	1649.7	1696.7	1696.8
Biomass of <i>Ulva spp.</i>	1192.2	1187.7	1170.7	1198.8
Biomass of <i>Gracilaria spp.</i>	1408.0	1408.3	1414.3	1387.0

Table 4. Coefficients of the regression models for describing the development of different *Z. noltei* biometrics in each meadow along the study period. Significant effects ($p > 0.05$) of the factor *meadow* are indicated in bold.

		Intercept (M1)	Time linear trend	Time quadratic trend
Flowering effort^b				
M1		-3.405 ± 1.080	2.529 ± 0.496	-0.238 ± 0.048
M2		1.423 ± 1.528	-1.323 ± 0.702	0.139 ± 0.068
M3		3.464 ± 1.528	-2.503 ± 0.702	0.234 ± 0.068
M4		3.369 ± 1.528	-2.488 ± 0.702	0.234 ± 0.068
M5		3.351 ± 1.528	-2.499 ± 0.702	0.236 ± 0.068
M6		2.958 ± 1.528	-2.236 ± 0.702	0.214 ± 0.068
Vegetative shoot density^c				
M1		78.32 ± 6.925	-19.75 ± 16.74	
M2		22.14 ± 9.794	-19.98 ± 23.68	
M3		-7.180 ± 9.794	23.02 ± 23.68	
M4		36.96 ± 9.794	-26.42 ± 23.68	

Table 4 continued

	M5	8.485 ± 9.794	18.16 ± 23.68	
	M6	9.641 ± 9.794	-0.802 ± 23.68	
Leaf area^b	M1	0.599 ± 0.180	0.118 ± 0.083	-0.006 ± 0.008
	M2	-0.247 ± 0.255	< 0.001 ± 0.117	-0.004 ± 0.011
	M3	0.051 ± 0.255	0.193 ± 0.117	-0.025 ± 0.011
	M4	0.169 ± 0.255	-0.025 ± 0.117	-0.010 ± 0.011
	M5	-0.037 ± 0.255	0.055 ± 0.117	-0.013 ± 0.011
	M6	0.236 ± 0.255	-0.089 ± 0.117	-0.002 ± 0.011
Vertical shoot length^b	M1	2.108 ± 0.365	0.286 ± 0.168	-0.008 ± 0.016
	M2	-0.978 ± 0.516	0.156 ± 0.237	-0.024 ± 0.023
	M3	-0.385 ± 0.516	0.388 ± 0.237	-0.046 ± 0.023
	M4	-0.183 ± 0.516	0.094 ± 0.237	-0.037 ± 0.023
	M5	-0.516 ± 0.516	0.292 ± 0.237	-0.047 ± 0.023
	M6	0.023 ± 0.516	-0.051 ± 0.237	-0.022 ± 0.023
Maximum root length^d	M1	6.347 ± 0.401	< 0.001 ± < 0.001	
	M2	-1.193 ± 0.568	< 0.001 ± < 0.001	
	M3	-1.451 ± 0.568	< 0.001 ± < 0.001	
	M4	-2.227 ± 0.568	< 0.001 ± < 0.001	
	M5	-1.050 ± 0.568	< 0.001 ± < 0.001	
	M6	-1.924 ± 0.568	< 0.001 ± < 0.001	
Above-ground biomass^b	M1	38.90 ± 7.189	11.99 ± 3.301	-1.177 ± 0.322
	M2	0.463 ± 10.17	-4.998 ± 4.669	0.517 ± 0.455
	M3	-0.134 ± 10.17	3.840 ± 4.669	-0.546 ± 0.455
	M4	19.92 ± 10.17	-7.944 ± 4.669	0.459 ± 0.455
	M5	-3.208 ± 10.17	-1.373 ± 4.669	0.073 ± 0.455
	M6	10.35 ± 10.17	-6.498 ± 4.669	0.422 ± 0.455
Below-ground biomass^b	M1	62.48 ± 6.647	-13.39 ± 3.052	1.277 ± 0.298
	M2	-1.095 ± 9.401	5.625 ± 4.316	-0.557 ± 0.421
	M3	-13.66 ± 9.401	3.713 ± 4.316	-0.327 ± 0.421
	M4	-39.76 ± 9.401	15.33 ± 4.316	-1.034 ± 0.421
	M5	-5.317 ± 9.401	3.203 ± 4.316	-0.214 ± 0.421

Table 4 continued

	M6	-13.12 ± 9.401	6.523 ± 4.316	-0.392 ± 0.421
Biomass of				
<i>Ulva</i> spp.^c	M1	0.011 ± 0.900	0.067 ± 2.175	
	M2	1.188 ± 1.272	-1.319 ± 3.076	
	M3	1.606 ± 1.272	-1.732 ± 3.076	
	M4	-2.680 ± 1.272	13.45 ± 3.076	
	M5	0.526 ± 1.272	-0.289 ± 3.076	
	M6	-0.101 ± 1.272	0.887 ± 3.076	
Biomass of				
<i>Gracilaria</i> spp.^d	M1	2.221 ± 1.107	$< 0.001 \pm < 0.001$	
	M2	-1.803 ± 1.566	$< 0.001 \pm 0.001$	
	M3	-0.264 ± 1.566	0.002 ± 0.001	
	M4	0.164 ± 1.566	$< 0.001 \pm 0.001$	
	M5	3.817 ± 1.566	$< 0.001 \pm 0.001$	
	M6	2.195 ± 1.566	$< 0.001 \pm 0.001$	

Regression models (according to the AICc) are ^aFirst-degree polynomial, ^bSecond-degree polynomial, ^cInverse polynomial and ^dExponential

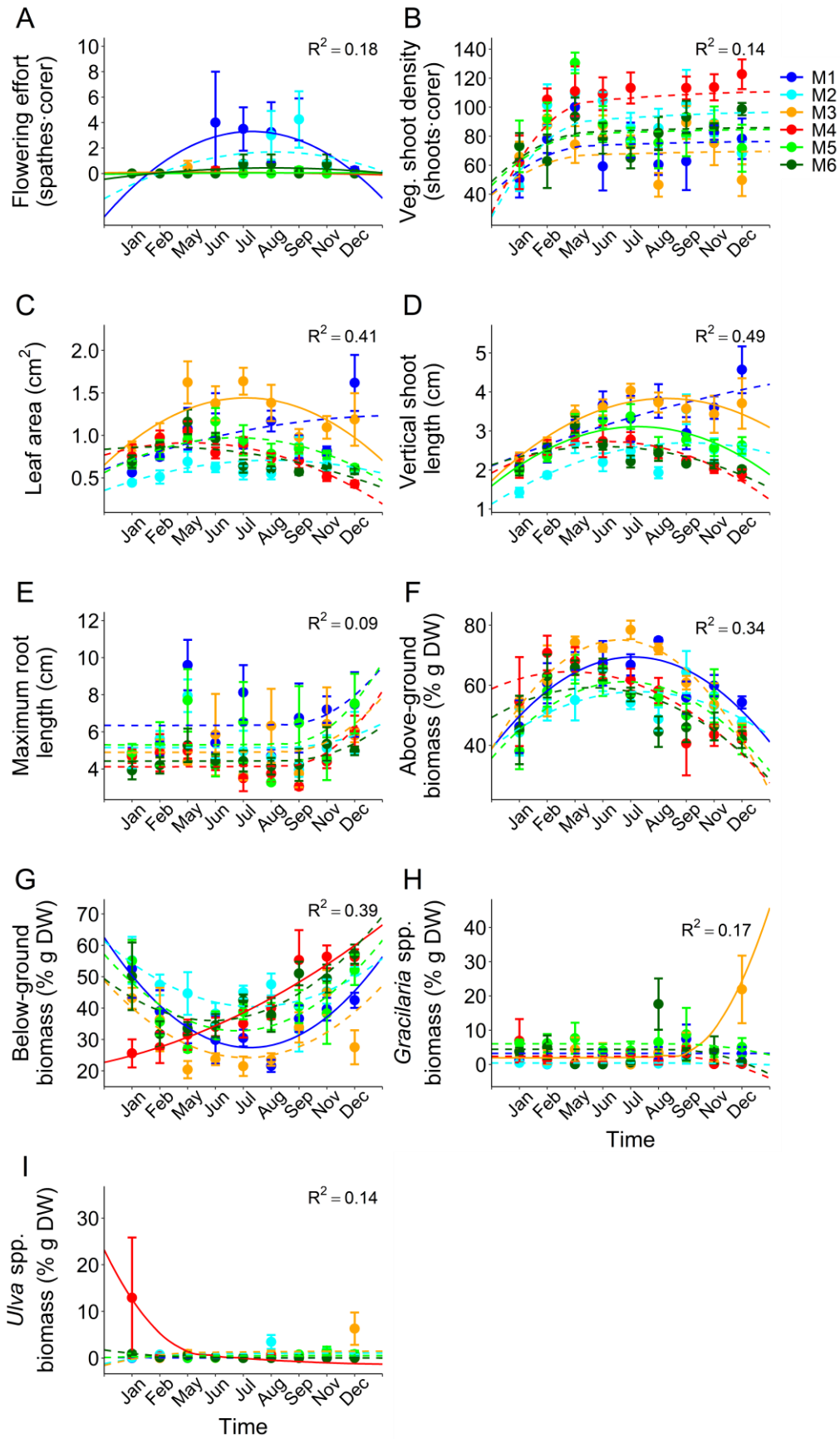


Figure 5. Development of seagrass biometrics in the six *Z. noltei* meadows over the time (mean \pm SE; $n = 4$). Solid lines indicate significant prototypical trajectories of the variables along the time.

2.3.3. Relationship of environmental and biometric parameters of *Z. noltei*

The sequential tests of the DISTLM revealed that fine sand/very fine sand and temperature significantly explained a 12.72% of the variability in the flowering effort with both parameters contributing with similar percentages (Table 5). Temperature was the only parameter that significantly contributed to the density of vegetative shoots, explaining a 6.82% of its variability (Table 5). OM and temperature explained the total variation in the leaf area with a 19.87%, whereby OM contributed the major part (Table 5). A combination of OM, temperature, fine sand/very fine sand and very coarse sand/fine gravel was found to significantly explain the variability of the vertical shoot length in a 22.36%. OM explained the greatest part of the total variability, while temperature, fine sand/very fine sand and very coarse sand explained equal parts (Table 5). For the maximum root length, fine sand/very fine sand significantly explained the total variation of 6.75% (Table 5). Regarding the above-ground biomass, temperature and fine sand/very fine sand significantly explained the total variation of a 35.43% (Table 5). Similarly, temperature and OM significantly explained the total variation of a 28.29% for the below-ground biomass (Table 5). For both variables, temperature contributed the major part to the total variability (Table 5).

For the biomass of *Ulva* spp. and *Gracilaria* spp., medium sand and coarse sand significantly explained the variation in biomass of a 4.65% and 6.49%, respectively (Table 5).

Table 5. Results of the sequential tests of the DISTLM analysis between seagrass biometrics and environmental parameters. For each variable the five most influential parameters are shown. Cum. % explains the cumulative percentage of each variable. Significant results are in bold.

	Variable	Variance %	F	<i>p</i>	Cum. %
Flowering effort	Sequential tests				
	Very fine sand	7.640	7.776	0.011	7.64
	Seawater temperature	5.080	5.412	0.023	12.72
	OM	1.390	1.485	0.244	14.11
	Salinity	0.292	0.311	0.559	14.40
	Coarse sand	0.069	0.072	0.792	14.47
Vegetative shoot density	Sequential tests				
	Seawater temperature	6.820	68.83	0.012	6.820
	Coarse sand	3.530	36.67	0.052	10.36
	OM	1.210	12.64	0.256	11.57

Table 5 continued

	Very coarse sand	0.682	0.707	0.406	12.25
	Salinity	0.628	0.649	0.433	12.90
Leaf area	Sequential tests				
	OM	15.36	17.05	0.001	15.36
	Seawater temperature	4.510	5.232	0.024	19.87
	Very coarse sand	3.400	4.119	0.051	23.27
	Coarse sand	2.570	3.186	0.077	25.84
	Salinity	1.410	1.652	0.216	27.25
Vertical shoot length	Sequential tests				
	OM	9.580	9.954	0.002	9.580
	Seawater temperature	4.390	4.743	0.033	13.97
	Very fine sand	4.330	4.872	0.030	18.30
	Very coarse sand	4.060	4.806	0.031	22.36
	Coarse sand	1.530	1.735	0.198	23.89
Max. root length	Sequential tests				
	Very fine sand	6.750	6.802	0.011	6.750
	Salinity	1.840	1.876	0.175	8.590
	OM	1.220	1.248	0.246	9.810
	Silt and clay	0.356	0.361	0.539	10.17
	Very coarse sand	0.142	0.142	0.733	10.30
Above-ground biomass	Sequential tests				
	Seawater temperature	31.35	42.93	0.001	31.35
	Very fine sand	4.080	5.870	0.019	35.43
	OM	1.590	2.321	0.143	37.02
	Salinity	1.270	18.70	0.178	38.29
	Medium sand	1.120	1.657	0.197	39.40
Below-ground biomass	Sequential tests				
	Seawater temperature	24.95	31.25	0.001	24.95
	OM	3.340	43.34	0.037	28.29
	Salinity	0.397	0.512	0.444	28.69
	Silt and clay	0.566	0.727	0.360	29.25

Table 5 continued

	Very fine sand	1.090	1.410	0.229	30.30
<hr/>					
Biomass of					
<i>Ulva</i> spp.	Sequential tests				
	Medium sand	4.650	4.581	0.018	4.650
	Seawater temperature	2.240	2.237	0.107	6.890
	Coarse sand	1.380	1.387	0.269	8.270
	Salinity	1.330	1.341	0.288	9.600
	OM	0.969	0.975	0.320	10.57
<hr/>					
Biomass of					
<i>Gracilaria</i> spp.	Sequential tests				
	Coarse sand	6.490	6.524	0.010	6.490
	Salinity	0.481	0.480	0.531	6.970
	Seawater temperature	0.318	0.316	0.583	7.290
	Medium sand	0.276	0.272	0.642	7.570
	Silt and clay	0.026	0.026	0.845	7.590
<hr/>					

2.4. Discussion

The results of this study demonstrated a great resilience in the *Z. noltei* meadows towards the different environmental conditions through the Mira channel of the Ria de Aveiro lagoon. Despite the great spatio-temporal variability of the environmental conditions, *Z. noltei* achieved to maintain its vegetative development in the Ria de Aveiro lagoon. In general, all the meadows developed similarly and no clear differences in the growth dynamics were found: the vegetative shoot density, maximum root length and the above-ground biomass showed similar growth dynamics in all the meadows, whereas the flowering effort, leaf area, vertical shoot length and below-ground biomass developed differently in only a few cases (cf. Figure 5). This suggests that *Z. noltei* in the Ria de Aveiro shows a high adaptability to changes in environmental parameters such as the content of organic matter or the changes in the sediment granulometry.

Regarding the flowering effort, only the meadows M1 and M2 showed high values with a strong occurrence of sexual spathes during the summer. Along with seawater temperature, the content of fine sand/very fine sand significantly explained the variability in flowering effort among meadows, whereas the content of fine and very fine sand was

highest in M1 and M2 throughout the year. Guerrero-Meseguer et al. (2021) recently showed that the flowering effort of *Z. noltei* in Ria de Aveiro is strongly influenced by the sediment characteristics, whereby fine, rich in organic matter sediments favoured the development of sexual spathes. Hence, the here encountered relationship between fine sand/very fine sand and the flowering effort align with this finding.

The above-ground biomass showed a seasonal pattern in all the meadows with higher values during summer and lower values during winter. This seasonality suggests that *Z. noltei* invests in the formation of photosynthetic tissues when seawater temperatures rise and days last longer. Similar seasonal patterns with minimal and maximal biomass during winter and summer, respectively, have been reported for other *Z. noltei* populations along the European west coast and the Mediterranean (Auby & Labourg, 1996; Cabaço et al., 2008b; Peralta et al., 2005; Pérez-Lloréns & Niell, 1993; Vermaat & Verhagen, 1996). In addition, a positive correlation between the above-ground biomass and seawater temperature in *Z. noltei* was found earlier (Pergent-Martini et al., 2005). Vermaat & Verhagen (1996) showed that the mean air temperature could best explain the variation of *Z. noltei* in biomass. Hence, the results of the DISTLM, in which seawater temperature explained the major part in the variability in both the above- and below-ground biomass, are consistent with those findings.

Analogously, the belowground biomass of the studied *Z. noltei* meadows followed the contrary pattern (except meadow M4), showing higher values during winter and lower values in summer. The values here were extrapolated and represent the ratios between the different fractions (shoots, root/rhizomes and macroalgae). Therefore, the dynamics of the below-ground biomass complement the explanation above for the development of the above-ground biomass very well: during the winter months, when conditions are less favourable for photosynthesis and *Z. noltei* invests less in the formation of leaves, plant nutrients (e.g., nitrogen and phosphorus) and energy in form of starch are allocated into the rhizomes and stored to facilitate the regrowth after overwintering (Govers et al., 2015; Pérez-Lloréns & Niell, 1993; Romero et al., 2006; Soissons et al., 2018). In general, temperature and light are considered as two limiting factors for seagrass growth since irradiance drives photosynthesis and physiological processes are accelerated through temperature (Greve & Binzer, 2004).

In *Z. noltei*, the development of biomass has shown to be related to changes in the shoot density (Vermaat & Verhagen, 1996) with higher densities during summer (Sfriso & Francesco Ghetti, 1998). In Ria de Aveiro, however, in all the meadows the vegetative shoot density did not change in time. This, together with the similar development of the remaining growth parameters in all the meadows could indicate that *Z. noltei* in Ria de Aveiro is at a stable state. In the Cádiz Bay Natural Park, Spain, for instance, shoot densities at the centre

of *Z. noltei* patches did not change significantly in time, whereas under colonizing conditions at the edge of patches, differences in shoot densities among seasons were found (Brun et al., 2003b). Another example from Ria Formosa, Portugal, shows that the maximum biomass and shoot density of an established *Z. noltei* meadow were not coupled temporally (Cabaço et al., 2012). This finding could be reflected in the different dynamics of the biomass and shoot density found in Ria de Aveiro. Furthermore, the established meadow in Ria Formosa showed a high above- to below-ground biomass ratio (>1) (Cabaço et al., 2012) which is consistent with the meadows in the Ria de Aveiro lagoon: in all the meadows, the above-ground biomass was $>50\%$ (corresponding to a ratio >1) during most time of the study period. Generally, an increase in the extent of *Z. noltei* in Ria de Aveiro has been observed (Sousa et al., 2019), indicating that *Z. noltei* could be in a stable state although environmental and anthropogenic stressors are present in this lagoon (Silva & Duck, 2006). However, the seagrass corers in this study were predominantly taken from the centres of the meadows, therefore not representing the growth dynamics on the edges and providing no information about the colonization rate of *Z. noltei* in Ria de Aveiro. Therefore, more studies are needed to monitor the expansion rate of this seagrass in relation to the different stressors to get a better understanding of the spatial development of *Z. noltei* in the lagoon.

Overall, four of the six studied meadows were inside the sediment deposition area and potentially under the direct influence of the dredging operation that was conducted during the study year. Partially, the increase in the concentration of coarser sediments in some meadows (e.g., fine gravel and very coarse sand in M6) overlapped with the initiation of the dredging in April and thus could be attributed to this operation. Cabaço & Santos (2007) suggested that *Z. noltei* could react to burial with the formation of horizontal rhizomes to locate its meristems (leaf production) closer to the surface. In M4, the below-ground biomass increased throughout the study, suggesting that this meadow could increase the formation of horizontal rhizomes due to burial. However, this variable was not measured in our study to confirm this theory. Moreover, M3 and M5 increased in the vertical shoot length towards summer, which could be an additional response to burial as found for several seagrass species that enhanced vertical growth after experimental sediment disturbances (reviewed in Cabaço et al., 2008a). The vertical shoot length was the only biometric parameter which showed a significant relationship with coarse sediments, namely very coarse sand/fine gravel. Nonetheless, no spatio-temporal coupling between the dynamic of sediments and the development of the biometric parameters was visible. For *Z. noltei*, decreases in biomass, shoot density, leaf length and the vertical growth (sheath length) have been observed after experimental sediment disturbances, whereby these effects increased with intensity and duration of the disturbance (Cabaço & Santos, 2007; Han et al., 2012; Vieira et al., 2020). In Ria de Aveiro, no similar effects were observed, suggesting

that the impact of the dredging operation was too low to have severe effects on *Z. noltei* or that other factors such as the tidal driven resuspension and relocation of sediments (Lopes et al., 2001) could have mitigated negative effects.

Medium and coarse sand were found to significantly explain the variability in the biomass of *Ulva* spp. and *Gracilaria* spp., respectively. Abreu et al. (2011) suggested that fluctuations of *Gracilaria* spp. in Ria de Aveiro could be due to redistribution of sediments caused by dredging and spring tides. The high spatio-temporal dynamic found both for coarse and medium sand thus might explain why in M3 and M4 the biomass of *Gracilaria* spp. and *Ulva* spp. developed differently at the beginning and the end of the study period, respectively. However, in the remaining meadows the sediment dynamics were also very variable and no differences in the development of macroalgae over time were found. In general, the biomass of both macroalgae genera was maintained nearly constant and relatively low throughout the year. Therefore, it is likely that the density of both macroalgae genera was too low to have deleterious effects on the development of the studied *Z. noltei* meadows (Vieira et al., 2020).

The data here present the first, detailed insight of the growth dynamics of *Z. noltei* in the Ria de Aveiro lagoon, covering the development of six different meadows throughout a whole year. Although environmental drivers were variable both in space and time, *Z. noltei* achieved to develop in the lagoon. The similar development of all the meadows underlines the resilience of *Z. noltei* and the general adaptability of this species towards different environmental conditions. The clonal, vegetative growth of *Z. noltei* not seems to be influenced negatively by the presence of environmental drivers. Contrary, sexual reproduction of *Z. noltei* seems to be influenced stronger by the variability of environmental drivers in the Ria de Aveiro lagoon. Further studies that investigate the development of *Z. noltei* at the long-term and other parts of the lagoon are needed. Studying interannual differences in vegetative growth and the sexual reproduction will help to get a better understanding of spatial development and the conservation status of this seagrass in the lagoon.

3. Chapter 3 - Reproductive cycle of the seagrass *Zostera noltei* in the Ria de Aveiro lagoon

3.0. Abstract

Sexual reproduction in seagrasses is essential to increase their resilience towards environmental stressors, but its phenology is still unknown in some regions, limiting our knowledge about the recovery capacity of these ecosystems. In this study, the flowering effort, reproductive phenology, seed production and potential of germination of *Zostera noltei* was studied for the first time in the Ria de Aveiro lagoon, Portugal. Flowering of *Z. noltei* in the Ria de Aveiro lasts from June to November, reaching a peak between July and August. All the meadows showed similar flowering effort and phenology over time. Comparing with other European populations, the flowering effort of *Z. noltei* in Ria de Aveiro lasted for a longer period, which could be related with the milder temperatures in summer and autumn and the great anthropogenic stress to which the meadows are subjected in the lagoon. The number of seeds produced and their ability of germination were similar among meadows and sampling periods, reaching levels similar to those of other European regions. Nevertheless, future studies are needed to determine the fate of the produced seeds in the field to have a better understanding about the natural recovery capacity of the species.

Keywords: sexual reproduction; flowering; conservation; seeds; germination

3.1. Introduction

Seagrasses are marine angiosperms that form extensive meadows worldwide, hosting a large number of aquatic organisms (Short et al., 2016). Since seagrass meadows support a high biodiversity and provide many ecological services, they are considered to be among the most important ecosystems worldwide (Hemminga & Duarte, 2000). Despite this, a global decline in seagrasses with an accelerating rate of loss has been reported since the 1990s (Waycott et al., 2009). Anthropogenic stressors, such as poor water quality, coastal development and dredging, have been identified as main drivers for this seagrass decline (Orth et al., 2006a), but extreme weather events derived from global warming are also negatively affecting seagrasses and their sexual reproduction (Guerrero-Meseguer et al., 2020; Oprandi et al., 2020; Qin et al., 2020).

In general, the main growth pattern of seagrasses is through asexual cloning of their rhizomes, but as angiosperms, they can reproduce sexually through the formation of flowers, fruits and seeds (Hemminga & Duarte, 2000). Seedling recruitment enhances their genetic diversity and in consequence, strengthens the resistance and resilience of the

seagrass meadows towards environmental stressors (Hughes & Stachowicz, 2004; Reusch et al., 2005). Seagrasses follow two strategies when reproducing sexually: the dispersal of seeds by the sea surface and the formation of seedbanks by accumulation of dormant seeds in the sediment (Inglis, 1999; Orth et al., 2006b). For instance, the dwarf eelgrass *Zostera noltei* (Hornemann) produces non-dormant seeds and forms seedbanks in the sediment that can be both annual and persistent (Hootsmans et al., 1987; Zipperle et al., 2009b). The existence of a persistent seedbank guarantees the survival of the seagrass meadows (Zipperle et al., 2009a), facilitating their recovery after negative impacts (Short, 1983).

Zostera noltei often occurs in sheltered environments such as lagoons and estuaries. This seagrass mainly grows on muddy and sandy sediments on intertidal areas, forming extensive beds (Green & Short, 2003). *Z. noltei* adapts to a wide range of environmental conditions (i.e., different sediment types, nutrient levels, tidal ranges or current velocities), which is reflected in its plasticity on morphological, physiological and population levels (Cabaço et al., 2009; Peralta et al., 2006). The timing of sexual reproduction in *Z. noltei* differs between latitudes. In southern European populations, sexual reproduction usually starts in March/April and lasts until autumn (October/November) (e.g., Alexandre et al., 2005), whereas at higher latitudes it starts later at the end of June (e.g., Hootsmans et al., 1987). Sexual reproduction in *Z. noltei* also differs when exposed to different environmental conditions: flowering is enhanced in areas exposed to environmental stressors such as increased hydrodynamics and organic matter enrichment, whereas stable and sheltered areas lead to reduced flowering effort (Cabaço et al., 2012; Guerrero-Meseguer et al., 2021). Thus, flowering seems to be variable in this species and influenced by many environmental factors.

The Ria de Aveiro lagoon holds the second largest *Z. noltei* population in Portugal (Cunha et al., 2013), covering around 2.3 km² in 2014 (Sousa et al., 2019). In the last decade, some studies have addressed the vegetative growth of *Z. noltei* under different environmental conditions (Azevedo et al., 2016; Sousa et al., 2017), as well as its role as blue carbon sink in the Ria de Aveiro lagoon (Sousa et al., 2019). Nevertheless, the reproductive capacity of the species has never been taken in consideration when evaluating its conservation status in the lagoon. A recent study suggests that there is a relationship between the reproductive effort of this species and the content of organic matter and silt in the sediment (Guerrero-Meseguer et al., 2021). However, there are no baseline data on the phenology and germination ability of *Z. noltei* in Ria de Aveiro which allows us to compare the reproductive capacity of the species over time. This lack of knowledge limits our understanding of the natural colonization capacity of this seagrass in the area and, in consequence, the understanding on how future disturbances, such as climate change, could affect its sexual reproduction.

The aim of this study was to describe for the first time the phenology and reproductive capacity of *Z. noltei* in the Ria de Aveiro lagoon. To achieve this goal, we monitored the flowering effort, reproductive phenology and germination ability of four reproductive meadows throughout the flowering period of the species.

3.2. Material and Methods

3.2.1. Study area

To study the sexual reproduction cycle of *Z. noltei* in Ria de Aveiro (40°38'N, 8°45'W), four monospecific seagrass meadows that showed reproductive capacity were visited along the Mira Channel while their flowering persisted in the lagoon. The Mira channel is a shallow arm with 20 km in length (Dias et al., 1999) which shows characteristics of a seasonally poikilohaline estuary with salinity ranges from 0 to 35 psu (practical salinity unit) (Moreira et al., 1993). Nearly one fifth of the tidal water volume diverts into the Mira channel, whereas at its upper end, a small network of lagoons and streams constantly delivers freshwater. To assess spatial variability in sexual reproduction, the sampling was carried out using two spatial scales, meters and kilometers. Thus, meadows M1 and M2 were about 200 meters apart, the same distance between M3 and M4, and M1-M2 were 3 km away from M3-M4 (Figure 6).

In this channel, all the studied *Z. noltei* meadows had a similar seawater temperature before (May), during (August) and after (December) the flowering period ($27.94 \pm 0.65^{\circ}\text{C}$, $25.24 \pm 0.85^{\circ}\text{C}$ and $13.75 \pm 0.14^{\circ}\text{C}$, respectively; Table S3), but salinity, grain size and sediment organic matter were variable over time. Thus, those three parameters were recorded in each meadow over the study period to analyse differences among meadows.

Seawater salinity was recorded at two randomly selected points at each sampling date and meadow, using a multi sonde (Hach® HQ 40d, Germany). To analyse organic matter content and sediment grain size, sediment corers (n = 2, 5.5 cm diameter, 7 cm of length) were taken before, during and after the sexual reproduction period. Sediment corers were dried for 72 h at 60°C to measure the organic matter content of the sediment (OM) by loss on ignition in 1 g of sediment (450°C, 4.5 h). Then, sediment was separated into seven sizes using a mechanical sieve shaker (CISA# SIEVING TECHNOLOGIES BA 200N; t = 20 min, amplitude = 1.2 mm), and subsequently classified following the Wentworth scale (Wentworth, 1922): fine gravel (2–4 mm), very coarse sand (1–2 mm), coarse sand (0.5–1 mm), medium sand (0.25–0.5 mm), fine sand (0.125–0.25 mm), very fine sand (0.063–0.125 mm) and silt and clay (< 0.063 mm).

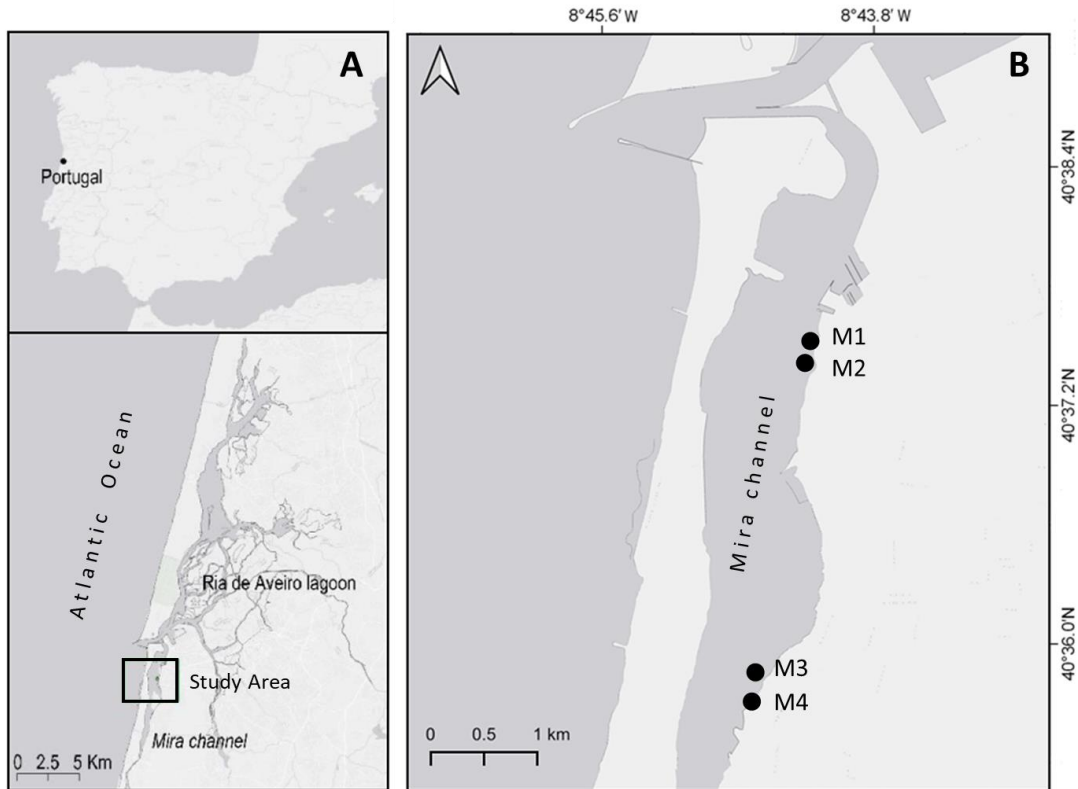


Figure 6. Study area (A) and location of the four studied *Z. noltei* meadows (M1-M4) in the Mira channel, Ria de Aveiro (B).

3.2.2. Flowering effort and reproductive phenology

To study the flowering effort and the reproductive phenology of *Z. noltei* in the Ria de Aveiro lagoon, seagrass corers ($n = 4$; 9 cm of diameter, 6.5 cm of length) were randomly collected at five dates (June, July, August, September and November) in each meadow as the flowering period persisted. At the laboratory, seagrass corers were cleaned from fauna and sediment with artificial seawater (30 psu). Then, the sexual spathes contained in each corer were counted for recording the flowering effort.

The reproductive phenology of *Z. noltei* in Ria de Aveiro was assessed by analysing the spathes under a dissecting microscope and assigning them a stage of development based on their morphological characteristics. In this way, five different stages of sexual reproduction were defined (Table 6, Figure 7). Stages I, II and III corresponded to the period of flowering formation, while stages IV and V coincided with the period of seed formation and maturation. Broken and necrotic spathes were quantified as abortions.

The total number of spathes collected in each reproductive stage during the study period was calculated to describe the general reproductive phenology of *Z. noltei* in the Ria

de Aveiro lagoon. To test differences among meadows in the reproductive phenology, the percentage of spathes in each reproductive stage was estimated by meadow and date.

Table 6. Description of the five sexual reproductive stages used to study the reproductive phenology of *Z. noltei* in Ria de Aveiro. The morphology of each reproductive stage is shown in Figure 7.

Period	Stage	Description
Flowering	I	Yellow-green spathe, sheath closed; pistils and stamina are visible, aligned onto the stem
	II	Pistils (IIa) and/or stamina erected (IIb); styles and stigma and/or anthers are outside the sheath
	III	Stigma brown, start to depart from spathe; often with stamina already detached from the spathe
Seed formation	IV	Green spathe with immature seeds; sheath closed
	V	Green/brown spathe, dark brown seeds are visible, sheath open

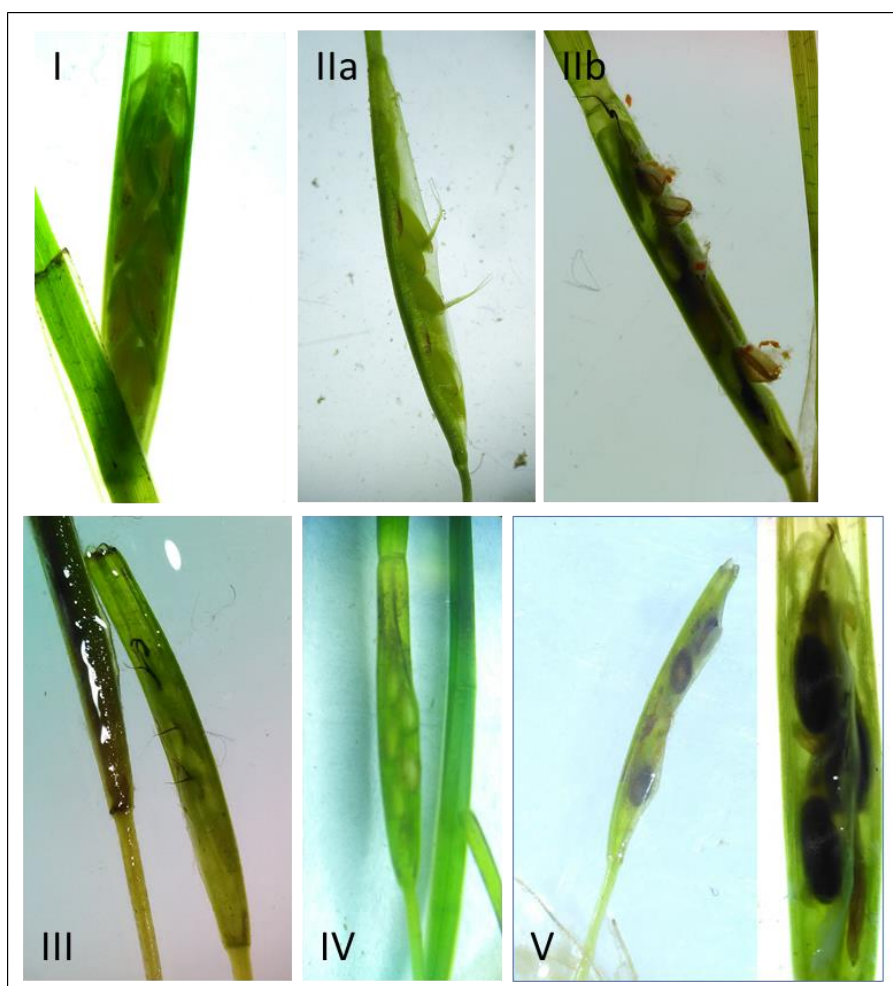


Figure 7. Morphology of the five sexual reproductive stages (I–V) determined to study the reproductive phenology of the *Z. noltei* meadows at the Ria de Aveiro lagoon.

3.2.3. Germination ability

To test the germination ability of the *Z. noltei* meadows, all collected sexual spathes, regardless of their stage of sexual reproduction, were separated per meadow and sampling date and cultured in aquaria (10 cm of diameter, filled with 600 ml artificial seawater) until mature seeds were obtained to apply for germination tests.

The salinity in each aquarium was adjusted every 72 h to the mean seawater salinity obtained in each *Z. noltei* meadow in the corresponding sampling date (Table S1). Aquaria were aerated (Sera® Air 550 R Plus, O₂ flux >5 mg/l) and maintained at room temperature (from 18.35 ± 0.20°C to 23.66 ± 0.12°C) under natural photoperiod (from 14:10 to 12:12 light:dark hours, from July 2019 to March 2020). Every week, mature seeds, with hard, dark brown seed coat, were collected, counted and stored at salinities above 33 psu in 50 ml tubes filled with artificial seawater to avoid natural seed germination before the test. The seeds were kept stored in the tubes until March 2020, when the germination tests were carried out, coinciding with the beginning of spring and the natural germination period of the species (Loques et al., 1990). Spathes that suffered senescence were removed from the aquaria to avoid their decomposition. To prevent overgrowth of epiphytes, artificial seawater was renewed, and the aquaria and aeration system were cleaned once a week. Seed production was expressed as the number of seeds per spathe.

Germination tests were conducted by exposing mature seeds to a low salinity shock to induce germination (Hootsmans et al., 1987). Seeds were introduced into aerated tubes (50 ml) filled with 20 ml of 1 psu artificial seawater and 20 ml of agar (5% at 1 psu) as neutral substrate and placed inside an environmental chamber with controlled temperature and photoperiod (25.00 ± 1.026°C, 12:12 light:dark hours, respectively). Then, the number of germinated seeds (with broken seed coat and visible cotyledon) was recorded every week for four weeks to calculate the ability of germination. The germination ability was expressed as percentage of germinated seeds per meadow. Seeds from M4 were not considered for this experiment since they were not mature or died during the storage. Aeration of the tubes was provided as described above. Salinity was monitored weekly in four randomly selected tubes and adjusted when 3 psu were surpassed.

3.2.4. Data analyses

To test if the environmental conditions (salinity, OM and sediment grain size), flowering effort and reproductive phenology differed among meadows and through the reproductive period of *Z. noltei*, two-way ANOVAs were done using *meadow* as fixed factor with four levels (M1, M2, M3 and M4) and *time* as random factor with three levels for environmental conditions (before, during and after sexual reproduction period) and four

levels for flowering effort and reproductive phenology (July, August, September and November). Prior to the analyses, data were checked for normality and homogeneity of variances and transformed when necessary to fulfil ANOVA assumptions. If transformed data did not meet the assumptions, the significance level (α) was lowered to 0.01 (Underwood, 1997).

To test if seed production was different among meadows, t-tests were separately applied. Fisher's exact test of independence was used to explore significant differences on germination ability among meadows.

All the tests were performed with the statistical software R Version 4.0.0 (R Core Team, 2021). All the results throughout this article have been expressed as mean \pm standard error (SE).

3.3. Results

3.3.1. Spatio-temporal variability in environmental conditions

Seawater salinity was significantly lower in M4 than in the remaining meadows before, during and after the flowering period (Figure 8A, Table 7). The organic matter content was very variable in space and time in the *Z. noltei* meadows (Figure 8B, Table 7). The sediment grain sizes also revealed a high spatio-temporal variability in the *Z. noltei* meadows, except in coarse and very fine sand content, which only showed differences among meadows (Table 7). The percentages of coarse sand in M1 and M2 were significantly lower than in M3 and M4 along the flowering period (Figure 8C-E). Regarding very fine sand, M1 and M2 showed higher values than the other meadows throughout the flowering period (Figure 8C-E).

Table 7. Summary of the results obtained in the two-way ANOVA analyses applied for the environmental descriptors. Results are expressed by the *F*-values and associated *p*-values (in parenthesis). Significant results are in bold. Asterisks above the variables indicate that significance level (α) was lowered to 0.01 because data did not fulfil the ANOVA assumptions

Variable	Meadow (df = 3)	Time (df= 2)	Meadow x Time (df = 6)
Salinity	16.59 (<0.001)	1.632 (0.236)	0.474 (0.815)
OM	18.87 (<0.001)	4.820 (0.029)	10.33 (<0.001)
Fine gravel	17.42 (<0.001)	0.211 (0.812)	13.38 (<0.001)
Very coarse sand	12.50 (<0.001)	0.368 (0.699)	11.79 (<0.001)
Coarse sand	25.76 (<0.001)	0.516 (0.609)	0.460 (0.830)
Medium sand*	42.99 (<0.001)	0.677 (0.527)	7.480 (0.002)
Fine sand	196.5 (<0.001)	0.808 (0.467)	3.506 (0.031)
Very fine sand*	446.1(<0.001)	3.187 (0.078)	4.176 (0.017)
Silt and clay	52.27 (<0.001)	0.488 (0.626)	5.631 (0.005)

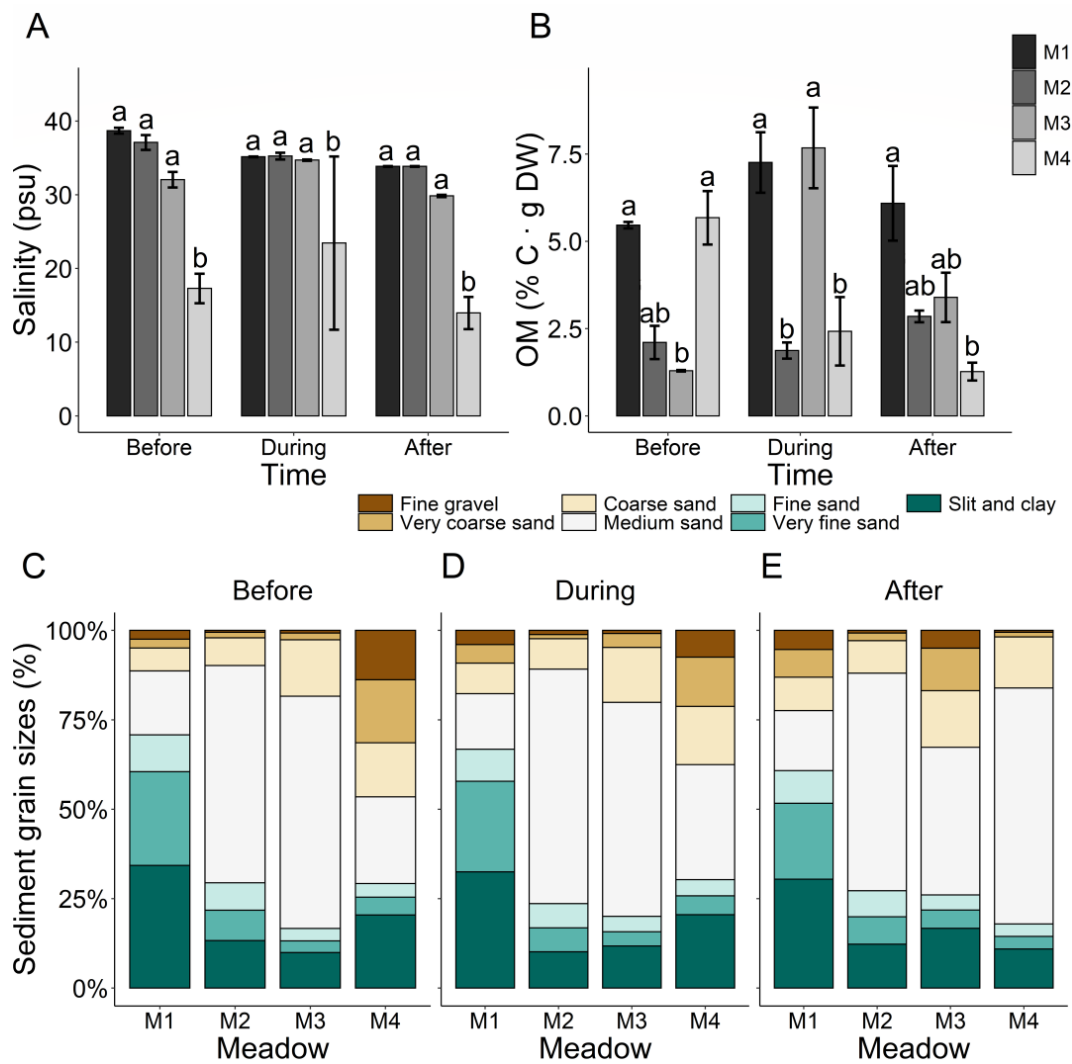


Figure 8. Salinity (A), organic matter content (B) and percentages of each sediment grain size (C-E) (mean \pm SE; n = 2) amidst the four studied *Z. noltei* meadows (M1-M4) before, during and after the flowering period.

3.3.2. General reproductive phenology of *Z. noltei* in the Ria de Aveiro lagoon

The flowering period of *Z. noltei* in the Ria de Aveiro lagoon lasted approximately from June to November 2020. Since spathes with seeds were found in June and there were still spathes with flowers in formation in November, the flowering of *Z. noltei* in the lagoon could begin a little before June and end sometime after November. In general, the number of spathes reached a peak in July and decreased in the following months (Figure 9). The number of Stage I spathes was similarly maintained over the flowering period, while Stage II spathes reached maximal values in August (Figure 9). The number of Stage III spathes peaked in June and September, while Stage IV reached the highest values in July and Stage V values increased slightly from July to September (Figure 9). Moreover, the number of aborted spathes peaked in July and declined in the following months (Figure 9).

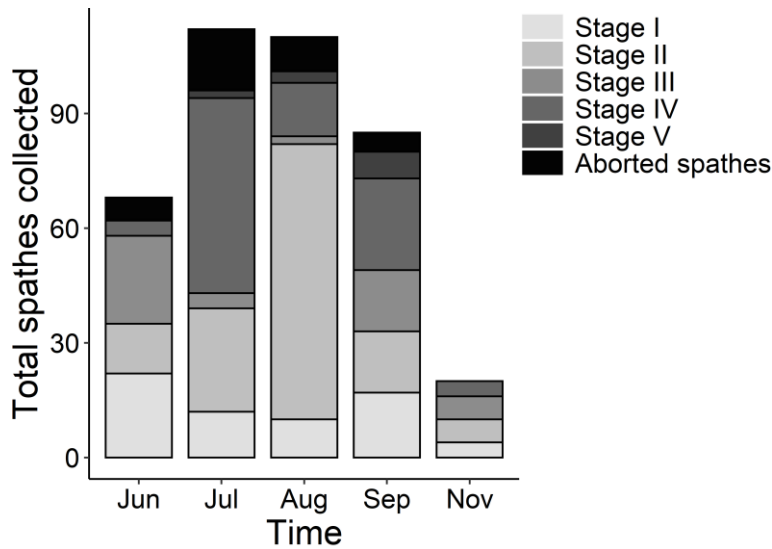


Figure 9. General reproductive phenology of the studied *Z. noltei* meadows of the Ria de Aveiro lagoon.

3.3.3. Spatio-temporal variability in the flowering effort and reproductive phenology

Flowering effort did not significantly differ among meadows and time (Table 8; Figure 10A), showing an average of 781 ± 157 spathes \cdot m² per *Z. noltei* meadow. Similarly, there were no significant differences among meadows and time regarding the aborted spathes and the percentages of spathes in stages I, III, and V (Table 8; Figure 10B, 10D, 10F and 10G). However, the percentage of spathes in stages II and IV were significantly variable over time (Table 8; Figure 10C and 10E).

Table 8. Results obtained in the two-way ANOVA analyses for FE and stages of sexual reproduction. Results are expressed as *F*-values and associated *p*-values (in parenthesis). Significant results are in bold. The significance level in all tests was $\alpha = 0.01$.

Variable	Meadow (df = 3)	Time (df = 3)	Meadow \times Time (df = 9)
Flowering effort	2.853 (0.047)	2.495 (0.071)	0.708 (0.699)
Stage I	1.274 (0.294)	2.290 (0.090)	1.782 (0.097)
Stage II	0.408 (0.748)	10.37 (> 0.001)	1.010 (0.446)
Stage III	0.287 (0.834)	1.470 (0.235)	2.282 (0.032)
Stage IV	1.150 (0.339)	10.92 (> 0.001)	0.807 (0.612)
Stage V	1.266 (0.297)	1.595 (0.203)	1.047 (0.418)
Aborted spathes	0.774 (0.514)	3.375 (0.026)	0.534 (0.842)

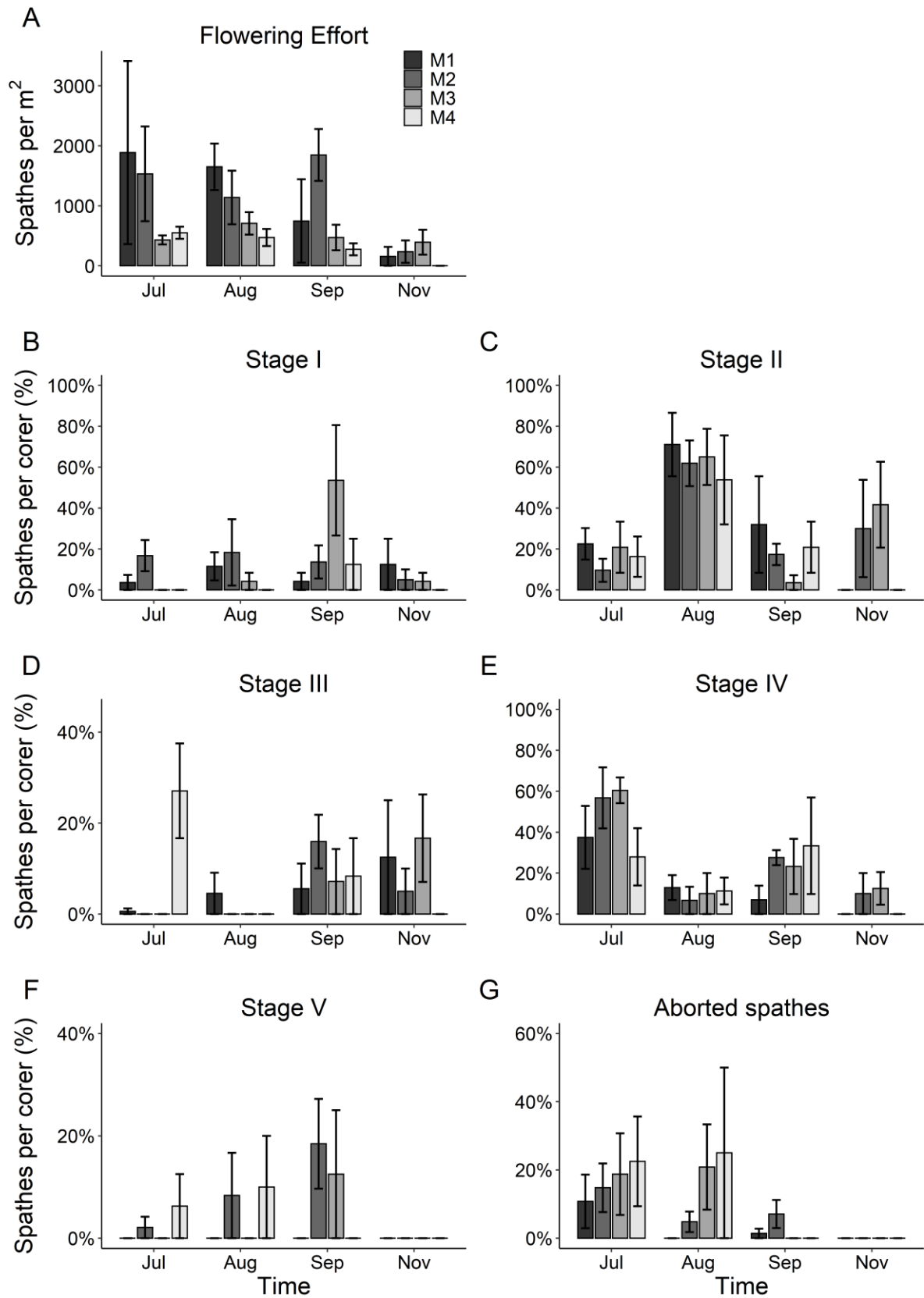


Figure 10. Flowering effort (A), percentages of spathes in each reproductive stage (B, C, D, E and F) and percentage of aborted spathes (G) over time (mean \pm SE, n = 4).

3.3.4. Germination ability

A total of 158 seeds were obtained from the cultured spathes. Seed production did not differ significantly among meadows (M1-M2, $t = 0.443$, $P = 0.669$; M1-M3, $t = 0.482$, $P = 0.643$ and M2-M3, $t = 0.786$, $P = 0.455$), averaging 0.422 ± 0.109 seeds per collected spathe. Amid these seeds, 51.26% (81 seeds) were fully matured and used for the germination test (Table S2). Similarly, germination capacity did not show significant differences among meadows (Table 9), germinating 33.73% of mature seeds. The average germination time of the seeds formed in M1, M2 and M3 was 1.000 ± 0.001 , 1.389 ± 0.164 and 2.200 ± 0.490 weeks, respectively.

Table 9. Percentage of germinated and no germinated seeds. Comparison among meadows was done through Fisher's exact test of independence.

Meadow	Germinated (%)	No Germinated (%)
M1	24	76
M2	43.9	56.1
M3	33.3	66.7
Fisher's exact test of independence	2.69	$p = 0.253$

3.4. Discussion

This study shows the first data on reproductive phenology of *Zostera noltei* in the Ria de Aveiro lagoon. The flowering period of *Z. noltei* in Ria de Aveiro started around June and lasted until near November, peaking between July and August. Flowering effort and reproductive phenology were similar among meadows and over time, suggesting that all meadows evolved similarly over the flowering period. The proportion of newly formed spathes (Stage I), apparently fertilized spathes (Stage III) and spathes with mature seeds (Stage V) was constant over time. In contrast, the percentages of spathes in Stage II and Stage IV differed over time, which suggests that the development of these stages could be prompted under certain environmental conditions in *Z. noltei*. The production of seeds and the capacity and time of germination of the cultured spathes did not differ among meadows, showing 34% of germination ability. These results suggest that *Z. noltei* meadows of the Ria de Aveiro lagoon can produce seedlings similarly and with a potential of reproduction comparable to other European populations.

Flowering effort of *Z. noltei* in Ria de Aveiro did not show spatio-temporal differences, ranging from 0-157 to a maximum of 6445 spathes · m⁻². This range is slightly higher than that found for *Z. noltei* in the south of Portugal (Alexandre et al., 2005; Cabaço & Santos, 2007) and in the Mediterranean (Buia & Mazzella, 1991; Loques et al., 1988) but lower than in other European areas further north to Ria de Aveiro (Auby & Labourg, 1996). Latitudinal differences in the flowering effort of *Z. noltei* appear to be due to the fact that the southern populations were more stable than the Northern-Europe populations and do not have to recolonize the environment each year in the same manner as those further north (Van Tussenbroek et al., 2016). On the other hand, the timing of flowering in the *Z. noltei* meadows of Ria de Aveiro, which persisted from June to November, was similar to that reported in the south of Portugal (Alexandre et al., 2005) and for European areas further north from our study area (Auby & Labourg, 1996; Zipperle et al., 2009b) but longer than in the Mediterranean (Buia & Mazzella, 1991; Loques et al., 1988). Therefore, these data could suggest that the timing of flowering in *Z. noltei* is strongly linked to seawater temperature, starting when temperatures rise in the area and persisting as long as warm temperatures remain, but are not too extreme for the development of spathes, as reported in the Mediterranean (Buia & Mazzella, 1991). However, in Ria de Aveiro, factors other than temperature could have influenced the seagrass reproductive effort since flowering in *Z. noltei* continued even though seawater temperature dropped to 14°C in November.

Greater reproductive efforts and longer flowering periods in seagrass meadows are also indicators of stress due to disturbances in the area, which can occur naturally or as result of anthropogenic activities (Cabaço & Santos, 2012). Seagrasses react with increased flowering under stressful situations as an adaptation strategy to ensure the recolonization of impacted areas through the formation of seedbanks (Lee et al., 2007; Short, 1983). Since, the Ria de Aveiro lagoon is under high anthropogenic influence, numerous stressors could have influenced the flowering of *Z. noltei* in our study, explaining the similarity between the flowering effort observed in Ria de Aveiro and other stressed *Z. noltei* meadows of Portugal. For instance, shellfish and bait harvesting can extend the reproductive period and induce greater production of spathes in *Z. noltei* meadows (Alexandre et al., 2005) and we found traces of this activity along the entire Mira channel (Figure S1). Furthermore, a longer flowering period in *Z. noltei* can also be associated with sandy sediments (Alexandre et al., 2005; Auby & Labourg, 1996). Ria de Aveiro has historically been subjected to major hydromorphological alterations which resulted in changes from finer to sandier or coarser sediments within the lagoon (Silva & Duck, 2006). Moreover, in the year of our study, dredging activities were initiated upstream of the Mira channel, resulting in the mobilization of fine gravel and very coarse sand along the meadows (see Figure 8C-E). On the other hand, the flowering effort of human-dominated *Z. noltei*

meadows of Ria de Aveiro is strongly linked to silty sediments which carry high contents of organic matter (Guerrero-Meseguer et al., 2021). Although in our study the grain size and organic matter content of the sediments were variable, relatively high values (above 2% · g DW) were found in all *Z. noltei* meadows throughout the study period. Mineralization of organic matter is the main process that supplies inorganic nitrogen and phosphorus to the porewater of marine sediments (Hemminga & Duarte, 2000; Middelburg et al., 1997). Consequently, a high content of organic matter in the sediments of Ria de Aveiro during the flowering period could have been responsible for a higher nutrient uptake in *Z. noltei* meadows, extending the period of sexual spathes formation in the lagoon.

The continuous formation of Stage I spathes during the study period supports the fact that sexual spathes can be continually developed in Ria de Aveiro throughout the summer and part of the autumn (Alexandre et al., 2005). Moreover, Stage II corresponded to the moment when the spathes opened to expose the female and male organs to facilitate pollination, and the percentage of Stage II spathes reached a peak in August, suggesting that in *Z. noltei*, longer exposure to high temperatures could be responsible for the opening of spathes. Since pollination results in the formation of seeds, the high proportion of Stage II spathes observed in August could be the origin of the great number of Stage IV spathes in September, which already carry immature, green seeds in their interior. Following this assumption, the peak of Stage IV spathes in July could be explained by the first cohort of spathes in Ria de Aveiro.

Other environmental stressors in Ria de Aveiro could have also influenced flowering in *Z. noltei*. Desiccation of the seagrass meadows could be one additional factor since *Z. noltei* in Ria de Aveiro is exposed daily for several hours to high temperature and light incidence during low tide. In addition, although the four studied *Z. noltei* meadows had similar values of temperature during the flowering period, they had different ranges of seawater salinity. Stormwater runoffs can transport freshwater from the storm drain outlets to the Mira channel, reducing salinity in certain areas (Dias et al., 2003; Dias et al., 1999). This effect was noticed in M4 in our study, which showed lower salinities than in the rest of the meadows. However, reproductive effort and phenology in this meadow were similar to others that were exposed to salinities above 30 psu. Therefore, salinity seems to have no effect on the flowering in *Z. noltei* in Ria de Aveiro. Nevertheless, it should be noted that the spathes collected in this meadow produced less mature seeds in the laboratory than the others, which suggests that salinity could negatively affect the formation of seeds in *Z. noltei*.

Cultured spathes did not show significant differences among meadows and sampling dates in the production of mature seeds. The production of seeds per spathe reported here (0.42 seeds · collected spathe) was within the range of other studies (around

0.2 to 0.7 seeds per spathe) (Hootsmans et al., 1987; Loques et al., 1990). Similarly, the germination potential of the *Z. noltei* seeds from Ria de Aveiro (34%) was comparable to those reported in other studies that induced germination at similar environmental conditions found in our experiment (30 - 42%) (Hootsmans et al., 1987; Loques et al., 1990; Zipperle et al., 2009b). The interplay between environmental factors that enhance germination in *Z. noltei* is still poorly understood and therefore, more studies are needed to improve the *in-vitro* germination and especially the survival rates of the seagrass seedlings. This knowledge could help to understand which conditions benefit the natural colonization of this species, and at the same time improve the management for its conservation. In addition, refining the culture of *Z. noltei* seedlings in laboratory would allow us to promote the environmental restoration of their meadows through efficient seeding with a higher survival rate of transplants and an accelerated rate of recovery (Reynolds et al., 2016).

This study presents the first baseline data addressing the sexual reproduction of *Z. noltei* in the Ria de Aveiro lagoon. Flowering effort of *Z. noltei* in Ria de Aveiro was similar among meadows and resulted in the successful production of seeds. The flowering period was longer than in other further north European areas and in the Mediterranean, probably due to long lasting mild temperatures in summer and autumn in the Ria de Aveiro and because this area is subjected to several anthropogenic activities and local environmental stressors. The occurrence of different reproductive stages in *Z. noltei* followed a temporal pattern in which a peak in the pollination stage was followed by a peak in the seed formation stage. *Z. noltei* in Ria de Aveiro produced seeds with similar germination ability compared to other populations. Future studies in Ria de Aveiro are needed to investigate factors that influence flowering, the *in-situ* germination of seeds as well as the existence of seedbanks. Furthermore, studies in different parts of Ria de Aveiro are needed for the comparison of the reproductive effort and phenology among different sites of the lagoon, and also for assessing the connectivity of the *Z. noltei* meadows. A complete understanding of the sexual reproduction will help future decision makers to conserve and restore this species in the lagoon.

4. Chapter 4 - Final conclusions and considerations

This thesis aimed to investigate the vegetative growth and sexual reproduction of the seagrass *Zostera noltei* in relation to its dynamic environment, the Ria de Aveiro lagoon. In both studies of this thesis *Z. noltei* demonstrated to have great morphological plasticity and the ability to adapt to several environmental conditions of coastal systems. Overall, the following main conclusions arise from this thesis:

Chapter 2: Growth dynamics of *Zostera noltei* meadows in the Ria de Aveiro lagoon

- 1) *Z. noltei* demonstrated a strong capacity to develop in highly dynamic environments, as in the Ria de Aveiro lagoon. The simultaneous presence of multiple stressors in the Mira channel did not reveal a clear effect on the annual growth cycle of this species, providing an example for the adaptability of this seagrass to different environmental conditions.
- 2) The production of sexual spathes was highest during the summer months, confirming that irradiance and temperature are two main factors controlling sexual reproduction in this seagrass species.
- 3) The intensity of sexual reproduction of *Z. noltei* depended on the sediment characteristics, emphasizing that the life history strategy of a seagrass species can strongly be influenced by the environmental conditions of the area.

Chapter 3: Reproductive cycle of the seagrass *Zostera noltei* in the Ria de Aveiro lagoon

- 4) Flowering effort of *Z. noltei* in the Mira channel was within the range reported for other European populations, showing that at this site sexual reproduction could play a similar role for the population dynamics.
- 5) The similar flowering effort indicates that all the meadows could contribute equally through seed production to the population dynamics of *Z. noltei* in Ria de Aveiro.
- 6) The flowering period of *Z. noltei* lasted longer than reported for other European areas which implies that factors other than seawater temperature and organic matter content could influence the formation of spathes in the Mira channel.
- 7) The sexual reproduction stages of *Z. noltei* showed a temporal pattern with two peaks of fertilization followed by two peaks of seed formation.

- 8) The production of mature seeds and the germination ability of *Z. noltei* in the Mira channel was similar to that obtained in other European regions, indicating that this seagrass is able to form seedbanks in the lagoon.

The foundation for future research on the growth dynamics and in particularly the sexual reproduction of *Z. noltei* in Ria de Aveiro was built in this thesis. Since the data generated here represent just one growth season and one part of the Ria de Aveiro lagoon, future studies are remaining. In the best case, yearly surveys that include the remaining regions of the lagoon would help to monitor the spatial development of *Z. noltei* with a higher resolution than previous studies. Parallely, research that investigates the diversity of *Z. noltei* on a genetic level in Ria de Aveiro could help to assess the geneflow among different regions of the lagoon and therefore the contribution of sexual reproduction to the population dynamics. In addition, monitoring the seed formation until the *in-situ* germination could help to identify recruitment areas and to assess the role of seedlings for the colonization of the lagoon. Complementary mesocosm experiments that simulate the different environmental conditions of Ria de Aveiro could help to understand which parameters favour seed germination, seedling establishment and the development of seedbanks, respectively. This knowledge would allow to better predict the resilience of *Z. noltei* towards extreme events, which will be more and more important, especially in times of global warming.

Within the scientific community the relevance of seagrasses has been widely recognized. Meanwhile, research on seagrasses has developed to an important scientific field, considering the ecological, socio-economic and cultural value of these ecosystems. The future of seagrass meadows is uncertain with their status remaining seriously endangered in many regions of the world. At the same time, there exist many examples for the recovery of seagrass meadows due to environmental management improving the water quality and restoration projects. In Ria de Aveiro, the designation of protected areas, a better regulation of visitors and habitat restoration could be appropriate means to preserve and even improve the status of this seagrass. Finally, communicating the importance of seagrasses like *Z. noltei* to the general public and professionals in areas related to these ecosystems such as fishing, shellfish harvesting, aquaculture farms or ports will be essential to create more awareness of their ecological and economical role and the need for their protection.

Supplementary material



Figure S1. Area impacted by shellfish and bait harvesting in the Mira channel of the Ria de Aveiro.

Table S1. Seawater salinity (psu) of the aquaria where the sexual spathes were cultured (mean \pm SE) of the salinities measured in each meadow.

	June	July	August	September	November
M1	36.10 \pm	34.00 \pm	35.15 \pm	34.55 \pm	33.85 \pm
	0.200	0.000	0.050	0.150	0.050
M2	35.15 \pm	35.00 \pm	35.25 \pm	33.90 \pm	33.85 \pm
	0.250	0.000	0.450	0.600	0.050
M3	31.90 \pm	32.90 \pm	34.70 \pm	32.20 \pm	29.85 \pm
	0.100	1.100	0.100	0.200	0.150
M4	28.70 \pm	24.07 \pm	23.45 \pm	17.37 \pm	13.67 \pm
	0.600	9.030	11.750	4.130	2.490

Table S2. Number of mature seeds collected from aquaria per meadow and date of seed production.

	June	July	August	September	November	Total
M1	1	10	12	2	0	25
M2	4	0	8	28	1	41
M3	3	0	3	7	2	15
M4	0	0	0	0	0	0
Total	8	10	23	37	3	81

Table S3. Seawater temperatures (mean \pm SE) among the four studied *Z. noltei* meadows before (May), during (August) and after (December) sexual reproduction period.

	Before	During	After
M1	27.91 \pm 0.20	25.15 \pm 0.05	13.40 \pm 0.01
M2	27.91 \pm 0.05	24.75 \pm 0.60	13.60 \pm 0.10
M3	28.09 \pm 0.35	25.22 \pm 0.10	13.65 \pm 0.05
M4	27.82 \pm 0.05	25.78 \pm 1.50	14.35 \pm 0.15
Average	27.94 \pm 0.65	25.24 \pm 0.85	13.75 \pm 0.14

References

- Abreu, M.H., Pereira, R., Sousa-Pinto, I. & Yarish, C. (2011). Ecophysiological studies of the non-indigenous species *Gracilaria vermiculophylla* (Rhodophyta) and its abundance patterns in Ria de Aveiro lagoon, Portugal. *European Journal of Phycology*, 46 (4), 453-464. doi:10.1080/09670262.2011.633174
- Alexandre, A., Santos, R. & Serrão, E. (2005). Effects of clam harvesting on sexual reproduction of the seagrass *Zostera noltii*. *Marine Ecology Progress Series*, 298, 115-122. doi:10.3354/meps298115
- Anderson, M.J. (2004). *DISTLM v.5: A FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model*. Department of Statistics, University of Auckland, Auckland, New Zealand.
- Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26 (1), 32-46. doi:10.1111/j.1442-9993.2001.01070.pp.x
- Auby, I. & Labourg, P.-J. (1996). Seasonal dynamics of *Zostera noltii* Hornem. in the bay of Arcachon (France). *Journal of Sea Research*, 35 (4), 269-277. doi:10.1016/s1385-1101(96)90754-6
- Azevedo, A., Dias, J.M. & Lillebø, A.I. (2016). Thriving of *Zostera noltei* under intertidal conditions: Implications for the modelling of seagrass populations. *Marine Biology*, 163 (5), 114. doi:10.1007/s00227-016-2887-x
- Azevedo, A., Sousa, A., Lencart e Silva, J.D., Dias, J. & Lillebø, A.I. (2013). Application of the generic DPSIR framework to seagrass communities of Ria de Aveiro: A better understanding of this coastal lagoon. *Journal of Coastal Research*, 65, 19-24. doi:10.2112/SI65-004.1
- Blok, S.E., Olesen, B. & Krause-Jensen, D. (2018). Life history events of eelgrass (*Zostera marina* L.) populations across gradients of latitude and temperature. *Marine Ecology Progress Series*, 590, 79-93. doi:10.3354/meps12479
- Borum, J. & Greve, T.M. (2004). The four European seagrass species. In J. Borum, C. M. Duarte, D. Krause-Jensen & T. M. Greve (Eds.). *European seagrasses: An introduction to monitoring and management* (pp. 1-7). The M&MS Project, Copenhagen.

- Bowen, J.L. & Valiela, I. (2001). The ecological effects of urbanization of coastal watersheds: Historical increases in nitrogen loads and eutrophication of Waquoit Bay estuaries. *Canadian Journal of Fisheries and Aquatic Sciences*, 58 (8), 1489-1500. doi:10.1139/f01-094
- Brodersen, K.E., Lichtenberg, M., Paz, L.-C. & Kühl, M. (2015). Epiphyte-cover on seagrass (*Zostera marina* L.) leaves impedes plant performance and radial O₂ loss from the below-ground tissue. *Frontiers in Marine Science*, 2. doi:10.3389/fmars.2015.00058
- Brun, F.G., Olivé, I., Malta, E., Vergara, J.J., Hernández, I. & Pérez-Lloréns, J.L. (2008). Increased vulnerability of *Zostera noltii* to stress caused by low light and elevated ammonium levels under phosphate deficiency. *Marine Ecology Progress Series*, 365, 67-75. doi:10.3354/meps07512
- Brun, F.G., Cummaudo, F., Olivé, I., Vergara, J.J. & Pérez-Lloréns, J.L. (2007). Clonal extent, apical dominance and networking features in the phalanx angiosperm *Zostera noltii* Hornem. *Marine Biology*, 151 (5), 1917-1927. doi:10.1007/s00227-007-0627-y
- Brun, F.G., Hernández, I., Vergara, J.J. & Pérez-Lloréns, J.L. (2003a). Growth, carbon allocation and proteolytic activity in the seagrass *Zostera noltii* shaded by *Ulva* canopies. *Functional Plant Biology*, 30 (5), 551-560. doi:10.1071/FP03010
- Brun, F.G., Pérez-Lloréns, J., Hernández, I. & Vergara, J.J. (2003b). Patch distribution and within-patch dynamics of the seagrass *Zostera noltii* Hornem. in Los Toruños salt-marsh, Cádiz Bay, Natural Park, Spain. *Botanica Marina*, 46, 513-524. doi:10.1515/BOT.2003.053
- Brush, M., J. & Nixon, S.W. (2002). Direct measurements of light attenuation by epiphytes on eelgrass *Zostera marina*. *Marine Ecology Progress Series*, 238, 73-79. doi:10.3354/meps238073
- Buia, M.C. & Mazzella, L. (1991). Reproductive phenology of the Mediterranean seagrasses *Posidonia oceanica* (L.) Delile, *Cymodocea nodosa* (Ucria) Aschers., and *Zostera noltii* Hornem. *Aquatic Botany*, 40 (4), 343-362. doi:10.1016/0304-3770(91)90080-O
- Burkholder, J.M., Tomasko, D.A. & Touchette, B.W. (2007). Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology*, 350 (1-2), 46-72. doi:10.1016/j.jembe.2007.06.024

- Cabaço, S. & Santos, R. (2012). Seagrass reproductive effort as an ecological indicator of disturbance. *Ecological Indicators*, 23, 116-122. doi:10.1016/j.ecolind.2012.03.022
- Cabaço, S., Santos, R. & Sprung, M. (2012). Population dynamics and production of the seagrass *Zostera noltii* in colonizing versus established meadows. *Marine Ecology*, 33 (3), 280-289. doi:10.1111/j.1439-0485.2011.00494.x
- Cabaço, S. & Santos, R. (2010). Reproduction of the eelgrass *Zostera marina* at the species southern distributional limit in the Eastern Atlantic. *Marine Ecology*, 31 (2), 300-308. doi:10.1111/j.1439-0485.2009.00331.x
- Cabaço, S., Machás, R. & Santos, R. (2009). Individual and population plasticity of the seagrass *Zostera noltii* along a vertical intertidal gradient. *Estuarine, Coastal and Shelf Science*, 82 (2), 301-308. doi:10.1016/j.ecss.2009.01.020
- Cabaço, S., Santos, R. & Duarte, C.M. (2008a). The impact of sediment burial and erosion on seagrasses: A review. *Estuarine, Coastal and Shelf Science*, 79 (3), 354-366. doi:10.1016/j.ecss.2008.04.021
- Cabaço, S., Machás, R., Vieira, V. & Santos, R. (2008b). Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*). *Estuarine, Coastal and Shelf Science*, 78 (1), 1-13. doi:10.1016/j.ecss.2007.11.005
- Cabaço, S. & Santos, R. (2007). Effects of burial and erosion on the seagrass *Zostera noltii*. *Journal of Experimental Marine Biology and Ecology*, 340 (2), 204-212. doi:10.1016/j.jembe.2006.09.003
- Cabaço, S., Alexandre, A. & Santos, R. (2005). Population-level effects of clam harvesting on the seagrass *Zostera noltii*. *Marine Ecology Progress Series*, 298, 123-129. Retrieved from <https://www.int-res.com/abstracts/meps/v298/p123-129/>
- Christianen, M.J.A., van Belzen, J., Herman, P.M.J., van Katwijk, M.M., Lamers, L.P.M., van Leent, P.J.M. et al. (2013). Low-canopy seagrass beds still provide important coastal protection services. *PLOS ONE*, 8 (5), e62413. doi:10.1371/journal.pone.0062413
- Conde, D., Vitancurt, J., Rodríguez-Gallego, L., de Álava, D., Verrastro, N., Chreties, C. et al. (2015). Solutions for sustainable coastal lagoon management: From conflict to the implementation of a consensual decision tree for artificial opening. In J. Baztan,

- O. Chouinard, B. Jorgensen, P. Tett, J.-P. Vanderlinden & L. Vasseur (Eds.). *Coastal Zones: Solutions for the 21st century* (pp. 217-250). Elsevier.
- Congdon, V.M., Bonsell, C., Cuddy, M.R. & Dunton, K.H. (2019). In the wake of a major hurricane: Differential effects on early vs. late successional seagrass species. *Limnology and Oceanography Letters*, 4 (5), 155-163. doi:10.1002/lol2.10112
- Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B. et al. (1997). The value of the world's ecosystem services and natural capital. *Nature*, 387 (6630), 253-260. doi:10.1038/387253a0
- Cullen-Unsworth, L.C., Nordlund, L.M., Paddock, J., Baker, S., McKenzie, L.J. & Unsworth, R.K.F. (2014). Seagrass meadows globally as a coupled social-ecological system: Implications for human wellbeing. *Marine Pollution Bulletin*, 83 (2), 387-397. doi:10.1016/j.marpolbul.2013.06.001
- Cunha, A.H., Assis, J.F. & Serrão, E.A. (2013). Seagrasses in Portugal: A most endangered marine habitat. *Aquatic Botany*, 104, 193-203. doi:10.1016/j.aquabot.2011.08.007
- Cunha, A.H. & Duarte, C.M. (2007). Biomass and leaf dynamics of *Cymodocea nodosa* in the Ria Formosa lagoon, South Portugal. *Botanica Marina*, 50 (1), 1-7. doi:10.1515/BOT.2007.001
- de los Santos, C.B., Brun, F.G., Bouma, T.J., Vergara, J.J. & Pérez-Lloréns, J.L. (2010). Acclimation of seagrass *Zostera noltii* to co-occurring hydrodynamic and light stresses. *Marine Ecology Progress Series*, 398, 127-135. doi:10.3354/meps08343
- den Hartog, C. (1970). *The Sea-grasses of the world*. North-Holland Publishing Company, Amsterdam, London.
- Dias, J.M., Lopes, J.F. & Dekeyser, I. (2003). A numerical system to study the transport properties in the Ria de Aveiro lagoon. *Ocean Dynamics*, 53 (3), 220-231. doi:10.1007/s10236-003-0048-5
- Dias, J.M., Lopes, J.F. & Dekeyser, I. (2000). Tidal propagation in Ria de Aveiro Lagoon, Portugal. *Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere*, 25 (4), 369-374. doi:10.1016/S1464-1909(00)00028-9

- Dias, J.M., Lopes, J.F. & Dekeyser, I. (1999). Hydrological characterisation of Ria de Aveiro, Portugal, in early summer. *Oceanologica Acta*, 22 (5), 473-485. doi:10.1016/S0399-1784(00)87681-1
- Duarte, C.M., Losada, I.J., Hendriks, I.E., Mazarrasa, I. & Marbà, N. (2013a). The role of coastal plant communities for climate change mitigation and adaptation. *Nature Climate Change*, 3 (11), 961-968. doi:10.1038/nclimate1970
- Duarte, C.M., Kennedy, H., Marbà, N. & Hendriks, I. (2013b). Assessing the capacity of seagrass meadows for carbon burial: Current limitations and future strategies. *Ocean & Coastal Management*, 83, 32-38. doi:10.1016/j.ocecoaman.2011.09.001
- Duarte, C.M., Middelburg, J.J. & Caraco, N. (2005). Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences*, 2 (1), 1-8. doi:10.5194/bg-2-1-2005
- Duarte, C.M. (2002). The future of seagrass meadows. *Environmental Conservation*, 29 (2), 192-206. doi:10.1017/S0376892902000127
- Duarte, C.M. (1991). Allometric scaling of seagrass form and productivity. *Marine Ecology Progress Series* 77, 289-300. doi:10.3354/meps077289
- Erfteemeijer, P.L.A., van Beek, J.K.L., Ochieng, C.A., Jager, Z. & Los, H.J. (2008). Eelgrass seed dispersal via floating generative shoots in the Dutch Wadden Sea: A model approach. *Marine Ecology Progress Series*, 358, 115-124. doi:10.3354/meps07304
- Erfteemeijer, P.L.A. & Robin Lewis, R.R. (2006). Environmental impacts of dredging on seagrasses: A review. *Marine Pollution Bulletin*, 52 (12), 1553-1572. doi:10.1016/j.marpolbul.2006.09.006
- Evans, S.M., Vergés, A. & Poore, A.G.B. (2017). Genotypic diversity and short-term response to shading stress in a threatened seagrass: Does low diversity mean low resilience? *Frontiers in Plant Science*, 8, 1417. doi:10.3389/fpls.2017.01417
- Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A. et al. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience*, 5 (7), 505-509. doi:10.1038/ngeo1477
- Govers, L.L., Suykerbuyk, W., Hoppenreijns, J.H.T., Giesen, K., Bouma, T.J. & van Katwijk, M.M. (2015). Rhizome starch as indicator for temperate seagrass winter survival. *Ecological Indicators*, 49, 53-60. doi:10.1016/j.ecolind.2014.10.002

- Gracia, A., Rangel-Buitrago, N., Oakley, J.A. & Williams, A.T. (2018). Use of ecosystems in coastal erosion management. *Ocean & Coastal Management*, 156, 277-289. doi:10.1016/j.ocecoaman.2017.07.009
- Green, E.P. & Short, F.T. (2003). *World atlas of seagrasses*. University of California Press, Berkeley, USA.
- Greve, T.M. & Binzer, T. (2004). Which factors regulate seagrass growth and distribution? In J. Borum, C. M. Duarte, D. Krause-Jensen & T. M. Greve (Eds.). *European seagrasses: An introduction to monitoring and management* (pp. 19-23). The M&MS project, Copenhagen.
- Guerrero-Meseguer, L., Veiga, P., Sampaio, L. & Rubal, M. (2021). Sediment characteristics determine the flowering effort of *Zostera noltei* meadows inhabiting a human-dominated lagoon. *Plants*, 10 (7), 1387. doi:10.3390/plants10071387
- Guerrero-Meseguer, L., Marín, A. & Sanz-Lázaro, C. (2020). Heat wave intensity can vary the cumulative effects of multiple environmental stressors on *Posidonia oceanica* seedlings. *Marine Environmental Research*, 159, 105001. doi:10.1016/j.marenvres.2020.105001
- Guerrero-Meseguer, L., Sanz-Lázaro, C. & Marín, A. (2018). Understanding the sexual recruitment of one of the oldest and largest organisms on Earth, the seagrass *Posidonia oceanica*. *PLOS ONE*, 13 (11), e0207345. doi:10.1371/journal.pone.0207345
- Han, Q., Bouma, T., Brun, F., Suykerbuyk, W. & Katwijk, M. (2012). Resilience of *Zostera noltii* to burial or erosion disturbances. *Marine Ecology Progress Series*, 449, 133-143. doi:10.3354/meps09532
- Hemminga, M.A. & Duarte, C.M. (2000). *Seagrass ecology*. Cambridge University Press, Cambridge.
- Herrera-Silveira, J.A., Cebrian, J., Hauxwell, J., Ramirez-Ramirez, J. & Ralph, P. (2010). Evidence of negative impacts of ecological tourism on turtlegrass (*Thalassia testudinum*) beds in a marine protected area of the Mexican Caribbean. *Aquatic Ecology*, 44 (1), 23-31. doi:10.1007/s10452-009-9260-9
- Hootsmans, M.J.M., Vermaat, J.E. & Van Vierssen, W. (1987). Seed-bank development, germination and early seedling survival of two seagrass species from The

- Netherlands: *Zostera marina* L. and *Zostera noltii* hornem. *Aquatic Botany*, 28 (3-4), 275-285. doi:10.1016/0304-3770(87)90005-2
- Hughes, A.R., Williams, S.L., Duarte, C.M., Heck Jr, K.L. & Waycott, M. (2009). Associations of concern: Declining seagrasses and threatened dependent species. *Frontiers in Ecology and the Environment*, 7 (5), 242-246. doi:10.1890/080041
- Hughes, A.R. & Stachowicz, J.J. (2004). Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences, USA*, 101 (24), 8998-9002. doi:10.1073/pnas.0402642101
- Inglis, G.J. (1999). Variation in the recruitment behaviour of seagrass seeds: Implications for population dynamics and resource management. *Pacific conservation biology*, 5 (4), 251-259. doi:10.1071/PC000251
- Ito, M.A., Lin, H.J., O'Connor, M.I. & Nakaoka, M. (2021). Large-scale comparison of biomass and reproductive phenology among native and non-native populations of the seagrass *Zostera japonica*. *Marine Ecology Progress Series*, 675, 1-21. doi:10.3354/meps13884
- Jahnke, M., Olsen, J.L. & Procaccini, G. (2015). A meta-analysis reveals a positive correlation between genetic diversity metrics and environmental status in the long-lived seagrass *Posidonia oceanica*. *Molecular Ecology*, 24 (10), 2336-2348. doi:10.1111/mec.13174
- Jarvis, J.C. & Moore, K.A. (2010). The role of seedlings and seed bank viability in the recovery of Chesapeake Bay, USA, *Zostera marina* populations following a large-scale decline. *Hydrobiologia*, 649 (1), 55-68. doi:10.1007/s10750-010-0258-z
- Jones, B.L., Nordlund, L.M., Unsworth, R.K.F., Jiddawi, N.S. & Eklöf, J.S. (2021). Seagrass structural traits drive fish assemblages in small-scale fisheries. *Frontiers in Marine Science*, 8, 354. doi:10.3389/fmars.2021.640528
- Kaldy, J.E. & Dunton, K.H. (1999). Ontogenetic photosynthetic changes, dispersal and survival of *Thalassia testudinum* (turtle grass) seedlings in a sub-tropical lagoon. *Journal of Experimental Marine Biology and Ecology*, 240 (2), 193-212. doi:10.1016/S0022-0981(99)00058-1

- Källström, B., Nyqvist, A., Åberg, P., Bodin, M. & André, C. (2008). Seed rafting as a dispersal strategy for eelgrass (*Zostera marina*). *Aquatic Botany*, 88 (2), 148-153. doi:10.1016/j.aquabot.2007.09.005
- Kendrick, G.A., Waycott, M., Carruthers, T.J.B., Cambridge, M.L., Hovey, R., Krauss, S.L. et al. (2012). The central role of dispersal in the maintenance and persistence of seagrass populations. *Bioscience*, 62 (1), 56-65. doi:10.1525/bio.2012.62.1.10
- Kennedy, H., Beggins, J., Duarte, C.M., Fourqurean, J.W., Holmer, M., Marbà, N. et al. (2010). Seagrass sediments as a global carbon sink: Isotopic constraints. *Global Biogeochemical Cycles*, 24 (4). doi:10.1029/2010GB003848
- Kim, S.H., Kim, J.H., Park, S.R. & Lee, K.S. (2014). Annual and perennial life history strategies of *Zostera marina* populations under different light regimes. *Marine Ecology Progress Series*, 509, 1-13. doi:10.3354/meps10899
- Lacap, C.D.A., Vermaat, J.E., Rollon, R.N. & Nacorda, H.M. (2002). Propagule dispersal of the SE Asian seagrasses *Enhalus acoroides* and *Thalassia hemprichii*. *Marine Ecology Progress Series*, 235, 75-80. doi:10.3354/meps235075
- Leandro, S.M., Tiselius, P. & Queiroga, H. (2014). Spatial and temporal scales of environmental forcing of *Acartia* populations (Copepoda: Calanoida) in the Canal de Mira (Ria de Aveiro, Portugal). *ICES Journal of Marine Science*, 71 (3), 585-596. doi:10.1093/icesjms/fst008
- Lee, K.S., Park, J.I., Kim, Y.K., Park, S.R. & Kim, J.H. (2007). Recolonization of *Zostera marina* following destruction caused by a red tide algal bloom: The role of new shoot recruitment from seed banks. *Marine Ecology Progress Series*, 342, 105-115. doi:10.3354/meps342105
- Legendre, P. & Anderson, M.J. (1999). Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs*, 69 (1), 1-24. doi:10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2
- Lilley, R.J. & Unsworth, R.K.F. (2014). Atlantic Cod (*Gadus morhua*) benefits from the availability of seagrass (*Zostera marina*) nursery habitat. *Global Ecology and Conservation*, 2, 367-377. doi:10.1016/j.gecco.2014.10.002

- Lopes, C., Pereira, M., Vale, C., Lillebø, A.I., Pardal, M. & Duarte, A.C. (2007). Assessment of spatial environmental quality status in Ria de Aveiro (Portugal). *Scientia Marina*, 71, 293-304.
- Lopes, J.F., Dias, J.M. & Dekeyser, I. (2001). Influence of tides and river inputs on suspended sediment transport in the Ria de Aveiro lagoon, Portugal. *Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere*, 26 (9), 729-734. doi:10.1016/S1464-1909(01)00077-6
- Loques, F., Caye, G. & Meinesz, A. (1990). Germination in the marine phanerogam *Zostera noltii* Hornemann at Golfe Juan, French Mediterranean. *Aquatic Botany*, 38 (2-3), 249-260. doi:10.1016/0304-3770(90)90009-A
- Loques, F., Caye, G. & Meinesz, A. (1988). Flowering and fruiting of *Zostera noltii* in Golfe Juan (French Mediterranean). *Aquatic Botany*, 32 (4), 341-352. doi:10.1016/0304-3770(88)90106-4
- Marbà, N., Duarte, C., Alexandre, A. & Cabaço, S. (2004). How do seagrasses grow and spread? In J. Borum, C. M. Duarte, D. Krause-Jensen & T. M. Greve (Eds.). *European seagrasses: An introduction to monitoring and management* (pp. 11-18). The M&MS project, Copenhagen.
- Marbà, N. & Duarte, C.M. (1998). Rhizome elongation and seagrass clonal growth. *Marine Ecology Progress Series*, 174, 269-280. Retrieved from <http://www.jstor.org/stable/24831772>
- Massa, S.I., Paulino, C.M., Serrão, E.A., Duarte, C.M. & Arnaud-Haond, S. (2013). Entangled effects of allelic and clonal (genotypic) richness in the resistance and resilience of experimental populations of the seagrass *Zostera noltii* to diatom invasion. *BMC Ecology*, 13 (1), 39. doi:10.1186/1472-6785-13-39
- Mateo, M.A., Cebrián, J., Dunton, K. & Mutchler, T. (2006). Carbon flux in seagrass ecosystems. In A. W. D. Larkum, R. J. Orth & C. M. Duarte (Eds.). *Seagrasses: Biology, ecology and conservation* (pp. 159-192). Springer, Dordrecht, The Netherlands.
- Maxwell, P.S., Pitt, K.A., Burfeind, D.D., Olds, A.D., Babcock, R.C., Connolly, R.M. et al. (2014). Phenotypic plasticity promotes persistence following severe events: physiological and morphological responses of seagrass to flooding. *Journal of Ecology*, 102 (1), 54-64. doi:10.1111/1365-2745.12167

- McArdle, B.H. & Anderson, M.J. (2001). Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology*, 82 (1), 290-297. doi:10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2
- McDonald, A.M., Prado, P., Heck, K.L., Fourqurean, J.W., Frankovich, T.A., Dunton, K.H. et al. (2016). Seagrass growth, reproductive, and morphological plasticity across environmental gradients over a large spatial scale. *Aquatic Botany*, 134, 87-96. doi:10.1016/j.aquabot.2016.07.007
- McGlathery, K.J., Sundbäck, K. & Anderson, I.C. (2007). Eutrophication in shallow coastal bays and lagoons: The role of plants in the coastal filter. *Marine Ecology Progress Series*, 348, 1-18. doi:10.3354/meps07132
- McKenzie, L.J., Yoshida, R.L., Aini, J.W., Andréfouet, S., Colin, P.L., Cullen-Unsworth, L.C. et al. (2021). Seagrass ecosystem contributions to people's quality of life in the Pacific island countries and territories. *Marine Pollution Bulletin*, 167, 112307. doi:10.1016/j.marpolbul.2021.112307
- McKenzie, L.J., Nordlund, L.M., Jones, B.L., Cullen-Unsworth, L.C., Roelfsema, C. & Unsworth, R.K.F. (2020). The global distribution of seagrass meadows. *Environmental Research Letters*, 15 (7), 074041. doi:10.1088/1748-9326/ab7d06
- Mcleod, E., Chmura, G.L., Bouillon, S., Salm, R., Björk, M., Duarte, C.M. et al. (2011). A blueprint for blue carbon: Toward an improved understanding of the role of vegetated coastal habitats in sequestering CO₂. *Frontiers in Ecology and the Environment*, 9 (10), 552-560. doi:10.1890/110004
- Middelburg, J.J., Soetaert, K. & Herman, P.M.J. (1997). Empirical relationships for use in global diagenetic models. *Deep Sea Research (Part I, Oceanographic Research Papers)*, 44 (2), 327-344. doi:10.1016/S0967-0637(96)00101-X
- Milazzo, M., Badalamenti, F., Ceccherelli, G. & Chemello, R. (2004). Boat anchoring on *Posidonia oceanica* beds in a marine protected area (Italy, western Mediterranean): Effect of anchor types in different anchoring stages. *Journal of Experimental Marine Biology and Ecology*, 299 (1), 51-62. doi:10.1016/j.jembe.2003.09.003
- Moore, K.A. (2004). Influence of seagrasses on water quality in shallow regions of the lower Chesapeake Bay. *Journal of Coastal Research*, 10045 (10045), 162-178. doi:10.2112/SI45-162.1

- Moreira, M.H., Queiroga, H., Machado, M.M. & Cunha, M.R. (1993). Environmental gradients in a southern Europe estuarine system: Ria de Aveiro, Portugal implications for soft bottom macrofauna colonization. *Netherlands Journal of Aquatic Ecology*, 27 (2-4), 465-482. doi:10.1007/BF02334807
- Neckles, H., A., Short, F.T., Barker, S. & Kopp, B.S. (2005). Disturbance of eelgrass *Zostera marina* by commercial mussel *Mytilus edulis* harvesting in Maine: Dragging impacts and habitat recovery. *Marine Ecology Progress Series*, 285, 57-73. Retrieved from <https://www.int-res.com/abstracts/meps/v285/p57-73/>
- Nordlund, L.M., Koch, E.W., Barbier, E.B. & Creed, J.C. (2016). Seagrass ecosystem services and their variability across genera and geographical regions. *PLOS ONE*, 12 (1), e0169942. doi:10.1371/journal.pone.0169942
- Ondiviela, B., Losada, I.J., Lara, J.L., Maza, M., Galván, C., Bouma, T.J. et al. (2014). The role of seagrasses in coastal protection in a changing climate. *Coastal Engineering*, 87, 158-168. doi:10.1016/j.coastaleng.2013.11.005
- Oprandi, A., Mucerino, L., De Leo, F., Bianchi, C.N., Morri, C., Azzola, A. et al. (2020). Effects of a severe storm on seagrass meadows. *Science of the Total Environment*, 748, 141373. doi:10.1016/j.scitotenv.2020.141373
- Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L. et al. (2006a). A global crisis for seagrass ecosystems. *Bioscience*, 56 (12), 987-996. doi:10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2
- Orth, R.J., Harwell, M.C. & Inglis, G. (2006b). Ecology of seagrass seeds and seagrass dispersal processes. In A. W. D. Larkum, R. J. Orth & C. M. Duarte (Eds.). *Seagrasses: Biology, ecology and conservation* (pp. 111-133). Springer, Dordrecht, The Netherlands.
- Orth, R.J., Luckenbach, M. & Moore, K.A. (1994). Seed dispersal in a marine macrophyte: Implications for colonization and restoration. *Ecology*, 75 (7), 1927-1939. doi:10.2307/1941597
- Paulo, D., Diekmann, O., Ramos, A.A., Alberto, F. & Alvares Serrão, E. (2019). Sexual reproduction vs. clonal propagation in the recovery of a seagrass meadow after an extreme weather event. *Scientia Marina*, 83 (4), 357-363. doi:10.3989/scimar.04843.06A

- Peralta, G., Brun, F.G., Pérez-Lloréns, J.L. & Bouma, T.J. (2006). Direct effects of current velocity on the growth, morphometry and architecture of seagrasses: A case study on *Zostera noltii*. *Marine Ecology Progress Series*, 327, 135-142. doi:10.3354/meps327135
- Peralta, G., Brun, F.G., Hernández, I., Vergara, J.J. & Pérez-Lloréns, J.L. (2005). Morphometric variations as acclimation mechanisms in *Zostera noltii* beds. *Estuarine, Coastal and Shelf Science*, 64 (2-3), 347-356. doi:10.1016/j.ecss.2005.02.027
- Pérez-Lloréns, J.L. & Niell, F.X. (1993). Seasonal dynamics of biomass and nutrient content in the intertidal seagrass *Zostera noltii* Hornem. from Palmones River estuary, Spain. *Aquatic Botany*, 46 (1), 49-66. doi:10.1016/0304-3770(93)90064-4
- Pérez-Ruzafa, A., Marcos, C., Pérez-Ruzafa, I.M. & Pérez-Marcos, M. (2011). Coastal lagoons: "Transitional ecosystems" between transitional and coastal waters. *Journal of Coastal Conservation*, 15 (3), 369-392. doi:10.1007/s11852-010-0095-2
- Pergent-Martini, C., Pasqualini, V., Ferrat, L., Pergent, G. & Fernandez, C. (2005). Seasonal dynamics of *Zostera noltii* Hornem. in two Mediterranean lagoons. *Hydrobiologia*, 543 (1), 233-243. doi:10.1007/s10750-004-7454-7
- Philbrick, C.T. & Les, D.H. (1996). Evolution of aquatic angiosperm reproductive systems: What is the balance between sexual and asexual reproduction in aquatic angiosperms? *Bioscience*, 46 (11), 813-826. doi:10.2307/1312967
- Polis Litoral Ria de Aveiro - Sociedade para a Requalificação e Valorização da Ria de Aveiro, S.A. (2017). Projeto de execução de transposição de sedimentos para otimização do equilíbrio hidrodinâmico na Ria de Aveiro - Canal de Mira - Relatório de conformidade ambiental do Projeto de execução. Vol.I - Resumo Não Técnico. Retrieved from <https://siaia.apambiente.pt/RECAPE.aspx?ID=459>
- Qin, L.-Z., Kim, S.H., Song, H.-J., Kim, H.G., Suonan, Z., Kwon, O. et al. (2020). Long-term variability in the flowering phenology and intensity of the temperate seagrass *Zostera marina* in response to regional sea warming. *Ecological Indicators*, 119, 106821. doi:10.1016/j.ecolind.2020.106821
- R Core Team (2021). R: A language and environment for statistical computing (Version 4.0.0). Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>

- Ralph, P.J., Durako, M.J., Enríquez, S., Collier, C.J. & Doblin, M.A. (2007). Impact of light limitation on seagrasses. *Journal of Experimental Marine Biology and Ecology*, 350 (1-2), 176-193. doi:10.1016/j.jembe.2007.06.017
- Reidenbach, M.A. & Thomas, E.L. (2018). Influence of the seagrass, *Zostera marina*, on wave attenuation and bed shear stress within a shallow coastal bay. *Frontiers in Marine Science*, 5. doi:10.3389/fmars.2018.00397
- Reusch, T.B.H., Ehlers, A., Hämmerli, A. & Worm, B. (2005). Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences, USA*, 102 (8), 2826-2831. doi:10.1073/pnas.0500008102
- Reusch, T.B.H., Boström, C., Stam, W.T. & Olsen, J.L. (1999). An ancient eelgrass clone in the Baltic. *Marine Ecology Progress Series*, 183, 301-304. doi:10.3354/meps183301
- Reynolds, L.K., Waycott, M., McGlathery, K.J. & Orth, R.J. (2016). Ecosystem services returned through seagrass restoration. *Restoration Ecology*, 24 (5), 583-588. doi:10.1111/rec.12360
- Rodrigues, M., Oliveira, A., Queiroga, H. & Brotas, V. (2012). Seasonal and diurnal water quality and ecological dynamics along a salinity gradient (Mira channel, Aveiro lagoon, Portugal). *Procedia Environmental Sciences*, 13, 899-918. doi:10.1016/j.proenv.2012.01.084
- Romero, J., Lee, K.-S., Pérez, M., Mateo, M.A. & Alcoverro, T. (2006). Nutrient dynamics in seagrass ecosystems. In A. W. D. Larkum, R. J. Orth & C. M. Duarte (Eds.). *Seagrasses: Biology, ecology and conservation* (pp. 227-254). Springer, Dordrecht, The Netherlands.
- RStudio Team (2021). RStudio: Integrated Development Environment for R (Version 1.4.1106). Boston, MA: RStudio, PBC. <http://www.rstudio.com>
- Salo, T. & Gustafsson, C. (2016). The effect of genetic diversity on ecosystem functioning in vegetated coastal ecosystems. *Ecosystems*, 19 (8), 1429-1444. doi:10.1007/s10021-016-0014-y
- Sfriso, A. & Francesco Ghetti, P. (1998). Seasonal variation in biomass, morphometric parameters and production of seagrasses in the lagoon of Venice. *Aquatic Botany*, 61 (3), 207-223. doi:10.1016/S0304-3770(98)00064-3

- Short, F.T., Short, C.A. & Novak, A.B. (2016). Seagrasses. In C. M. Finlayson, G. R. Milton, R. C. Prentice & N. C. Davidson (Eds.). *The wetland book: II: Distribution, description and conservation* (pp. 1-19). Springer, Dordrecht, Netherlands.
- Short, F.T., Polidoro, B., Livingstone, S.R., Carpenter, K.E., Bandeira, S., Bujang, J.S. et al. (2011). Extinction risk assessment of the world's seagrass species. *Biological Conservation*, 144 (7), 1961-1971. doi:10.1016/j.biocon.2011.04.010
- Short, F.T., Carruthers, T., Dennison, W. & Waycott, M. (2007). Global seagrass distribution and diversity: A bioregional model. *Journal of Experimental Marine Biology and Ecology*, 350 (1-2), 3-20. doi:10.1016/j.jembe.2007.06.012
- Short, F.T. & Short, C.A. (1984). The seagrass filter: Purification of estuarine and coastal waters. In V. S. Kennedy (Ed.). *The estuary as a filter* (pp. 395-413). Academic Press.
- Short, F.T. (1983). The response of interstitial ammonium in eelgrass (*Zostera marina* L.) beds to environmental perturbations. *Journal of Experimental Marine Biology and Ecology*, 68 (2), 195-208. doi:10.1016/0022-0981(83)90159-4
- Silva, J.F. & Duck, R.W. (2006). Changing use and hydromorphological adjustment in a coastal lagoon-estuarine system, the Ria de Aveiro, Portugal. In J. S. Rowan, R. W. Duck & W. Alan (Eds.). *Sediment Dynamics and the Hydromorphology of Fluvial Systems* (pp. 197-204). IAHS Publication 306, Dundee, UK.
- Silva, J.F., Duck, R.W. & Catarino, J.B. (2004). Seagrasses and sediment response to changing physical forcing in a coastal lagoon. *Hydrology and Earth System Sciences Discussions*, 8 (2), 151-159. doi:10.5194/hess-8-151-2004
- Smith, V.H., Tilman, G.D. & Nekola, J.C. (1999). Eutrophication: Impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution*, 100 (1-3), 179-196. doi:10.1016/S0269-7491(99)00091-3
- Soissons, L.M., Haanstra, E.P., van Katwijk, M.M., Asmus, R., Auby, I., Barillé, L. et al. (2018). Latitudinal patterns in European seagrass carbon reserves: Influence of seasonal fluctuations versus short-term stress and disturbance events. *Frontiers in Plant Science*, 9 (88). doi:10.3389/fpls.2018.00088

- Sousa, A.I., da Silva, J.F., Azevedo, A. & Lillebø, A.I. (2019). Blue Carbon stock in *Zostera noltei* meadows at Ria de Aveiro coastal lagoon (Portugal) over a decade. *Scientific Reports*, 9 (1), 14387. doi:10.1038/s41598-019-50425-4
- Sousa, A.I., Calado, R., Cleary, D.F.R., Nunes, C., Coimbra, M.A., Serôdio, J. et al. (2017). Effect of spatio-temporal shifts in salinity combined with other environmental variables on the ecological processes provided by *Zostera noltei* meadows. *Scientific Reports*, 7 (1), 1336. doi:10.1038/s41598-017-01359-2
- Stockbridge, J., Jones, A.R. & Gillanders, B.M. (2020). A meta-analysis of multiple stressors on seagrasses in the context of marine spatial cumulative impacts assessment. *Scientific Reports*, 10 (1), 11934. doi:10.1038/s41598-020-68801-w
- Strydom, S., Murray, K., Wilson, S., Huntley, B., Rule, M., Heithaus, M. et al. (2020). Too hot to handle: Unprecedented seagrass death driven by marine heatwave in a world heritage area. *Global Change Biology*, 26 (6), 3525-3538. doi:10.1111/gcb.15065
- Suykerbuyk, W., Govers, L.L., van Oven, W.G., Giesen, K., Giesen, W.B.J.T., de Jong, D.J. et al. (2018). Living in the intertidal: Desiccation and shading reduce seagrass growth, but high salinity or population of origin have no additional effect. *PeerJ*, 6, e5234. doi:10.7717/peerj.5234
- Temmerman, S., Meire, P., Bouma, T.J., Herman, P.M.J., Ysebaert, T. & De Vriend, H.J. (2013). Ecosystem-based coastal defence in the face of global change. *Nature*, 504 (7478), 79-83. doi:10.1038/nature12859
- Thomsen, M., Staehr, P., Nejrup, L. & Schiel, D. (2013). Effects of the invasive macroalgae *Gracilaria vermiculophylla* on two co-occurring foundation species and associated invertebrates. *Aquatic Invasions*, 8 (2), 133-145. doi:10.3391/ai.2013.8.2.02
- Thomson, A.C.G., York, P.H., Smith, T.M., Sherman, C.D.H., Booth, D.J., Keough, M. et al. (2014). Seagrass viviparous propagules as a potential long-distance dispersal mechanism. *Estuaries and Coasts*, 38, 927-940. doi:10.1007/s12237-014-9850-1
- Tol, S.J., Jarvis, J.C., York, P.H., Grech, A., Congdon, B.C. & Coles, R.G. (2017). Long distance biotic dispersal of tropical seagrass seeds by marine mega-herbivores. *Scientific Reports*, 7 (1), 4458. doi:10.1038/s41598-017-04421-1
- Udy, J.W. & Dennison, W.C. (1997). Growth and physiological responses of three seagrass species to elevated sediment nutrients in Moreton Bay, Australia. *Journal of*

Experimental Marine Biology and Ecology, 217 (2), 253-277. doi:10.1016/S0022-0981(97)00060-9

Underwood, A.J. (1997). *Experiments in ecology: Their logical design and interpretation using analysis of variance*. Cambridge University Press, Cambridge, UK.

Unsworth, R.K.F., Nordlund, L.M. & Cullen-Unsworth, L.C. (2019). Seagrass meadows support global fisheries production. *Conservation Letters*, 12 (1), e12566. doi:10.1111/conl.12566

Unsworth, R.K.F., Williams, B., Jones, B.L. & Cullen-Unsworth, L.C. (2017). Rocking the boat: Damage to eelgrass by swinging boat moorings. *Frontiers in Plant Science*, 8, 1309. doi:10.3389/fpls.2017.01309

Unsworth, R.K.F. & Cullen, L.C. (2010). Recognising the necessity for Indo-Pacific seagrass conservation. *Conservation Letters*, 3 (2), 63-73. doi:10.1111/j.1755-263X.2010.00101.x

Van Tussenbroek, B.I., Soissons, L.M., Bouma, T.J., Asmus, R., Auby, I., Brun, F.G. et al. (2016). Pollen limitation may be a common allee effect in marine hydrophilous plants: Implications for decline and recovery in seagrasses. *Oecologia*, 182 (2), 595-609. doi:10.1007/s00442-016-3665-7

Vermaat, J.E. & Verhagen, F.C.A. (1996). Seasonal variation in the intertidal seagrass *Zostera noltii* Hornem.: Coupling demographic and physiological patterns. *Aquatic Botany*, 52 (4), 259-281. doi:10.1016/0304-3770(95)00510-2

Vieira, R., Martin, A., Engelen, A.H., Thomsen, M.S. & Arenas, F. (2020). Interactive effects of co-occurring anthropogenic stressors on the seagrass, *Zostera noltei*. *Ecological Indicators*, 109, 105780. doi:10.1016/j.ecolind.2019.105780

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S. et al. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences , USA*, 106 (30), 12377-12381. doi:10.1073/pnas.0905620106

Wentworth, C.K. (1922). A scale of grade and class terms for clastic sediments. *The Journal of Geology*, 30 (5), 377-392. Retrieved from <http://www.jstor.org/stable/30063207>

Zipperle, A.M., Coyer, J.A., Reise, K., Gitz, E., Stam, W.T. & Olsen, J.L. (2009a). Clonal architecture in an intertidal bed of the dwarf eelgrass *Zostera noltii* in the Northern Wadden Sea: Persistence through extreme physical perturbation and the importance of a seed bank. *Marine Biology*, 156 (10), 2139-2148. doi:10.1007/s00227-009-1244-8

Zipperle, A.M., Coyer, J.A., Reise, K., Stam, W.T. & Olsen, J.L. (2009b). Evidence for persistent seed banks in dwarf eelgrass *Zostera noltii* in the German Wadden Sea. *Marine Ecology Progress Series*, 380, 73-80. doi:10.3354/meps07929