Grado en Bioloxía

Memoria do Traballo de Fin de Grao

Crecemento clonal en poboacións de *Zostera marina*

Crecimiento clonal en poblaciones de *Zostera marina*

Clonal growth in *Zostera marina* populations

Ángela Hoces Gutiérrez

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Tutor(es) Académico: Sergio Rodríguez Roiloa

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ABSTRACT

The seagrass meadows are really highly productive habitats and very important for many other species that use to live within these plants. Despite their large abundance, the coverage of seagrass is decreasing due to the human impact. Because of this, we have tried to infer if there are actually differences in some morphological characteristics between two habitats with different level of disturbance caused by human activity. For it, we took samples of 25 clonal fragments in both habitats and we analyzed the differences between internodes distance, leaf length and leaf sheath length, and the amount of plants with developed sexual structures. Against our first expectations, there were only significant differences in the length of the internodes distance and in the production of sexual structures. This can be explained with the escape theory, which says that the plant may produce longer rhizomes in order to avoid the same unfavorable habitat for its offspring.

Key words: *Zostera marina*, clonal growth, disturbance, human impact, escape theory

RESUMEN

Las praderas marinas son hábitats altamente productivos y son muy importante para muchas otras especies que viven entre estas plantas. A pesar de su gran abundancia, la cobertura de estas praderas está disminuyendo debido al impacto humano. Por esto, hemos intentado inferir si realmente hay diferencias en varios caracteres morfológicos entre dos hábitats con niveles de perturbación distintos causados por la actividad humana. Para ello, recogimos muestras de 25 fragmentos clonales de ambos hábitats y analizamos las diferencias entre las distancias internodales, la longitud de la hoja y la longitud de la vaina de la hoja, así como la cantidad de plantas que habían desarrollado estructuras sexuales. En contra de nuestras primeras expectativas, solo había diferencias significativas en la distancia entre nudos del rizoma y también en la producción de estructuras sexuales. Esto puede ser explicado con la teoría de escape, la cual dice que una planta puede producir rizomas más largos para así evitar el mismo hábitat desfavorable para su descendencia.

Palabras clave: *Zostera marina*, crecimiento clonal, perturbación, impacto humano, teoría de escape

INTRODUCTION

What are clonal plants?

In nature, plants can grow in different ways and one of them is the clonal or vegetative growth. Tiffney and Niklas (1985) estimated that clonal growth is present in 70% of Angiosperms, and more frequently in monocots than in dicotyledonous. This growth can be present as an ecological alternative for a plant with sexual reproduction, for instance if the habitat conditions are not good enough to develop sexual structures. This type of growth consists in the development of one ramet, i.e. independent member of the clonal plant, from a vegetative tissue of another one. Some examples of plants with clonal growth are some

species of genus *Festuca*, *Carpobrotus edulis* (see Fig. 1 and Fig. 2) or *Zostera marina*.

Fig. 1 and 2: examples of clonal plants (*Carpobrotus* on the left and *Festuca* on the right).

One of the main characteristics of the clonal growth is that the plant can spread very fast and occupy a lot of space in a short time, so depending on the habitat this plant can become invasive, just like *Zostera japonica* in regions occupied by native *Zostera marina*.

There are different types of clonal growth depending on the plant; we will see in the next paragraph some of these types.

Virtually, all types of shoots are capable of vegetative propagation, some examples are:

- Rhizome: the rhizome is a modified underground stem serving as an organ of vegetative reproduction, *e. g. Zostera marina* (see Fig. 3).

Fig.3: Fragment of *Z. marina*

- Prostrate aerial stems: called stolons are important vegetative reproduction organs in some species, such as the strawberry *Fragaria vesca* (see Fig. 4), numerous grasses, and some ferns.

Fig, 4: *F. vesca* stolons

- Suckering: is the reproduction or regeneration of a plant by shoots that arise from an existing root system (see Fig. 5). Species that characteristically produce suckers include Elm (*Ulmus*).

Fig. 5: tree reproduction throughout roots

Seagrass: Ecology and environmental importance

Seagrasses are clonal monocots and their vegetative growth is throughout rhizomes. They dominate shallow subtidal coastal and estuarine environments worldwide (Kendrick, Duarte and Marbà, 2005). They form a polyphyletic group since they have more than one evolutionary root and despite of being a grass-like higher plant, they are not related to grass families on land. Their first appearance in marine environments was approx 100 million years ago, as genus *Posidonia*.

Seagrass meadows are highly productive ecosystems and cover between 1.5×10^5 and 6×10^5 km^2 worldwide (Duarte & Cebrián 1996, Green & Short 2003). They represent a significant part of the carbon burial in the ocean and support highly diverse and productive food webs (Hemminga & Duarte 2000). However, seagrass populations are experiencing worldwide decline (Duarte 2002) under the effects of global climate change (Marbà & Duarte 1997, Seddon et al. 2000) and human impacts (Short & Wyllie-Echeverria 1996, Duarte 2002). The need to predict both loss and recovery of seagrasses is becoming a major social, yet the time scales involved in seagrass colonization are largely unknown. We need further research about the processes that control the clonal spread of seagrasses, its survival rate and growth and the dynamics of meadows within subtidal landscapes.

In the present study we tried to determine the human impact in order to know if we can do something to maintain these habitats, since they are so important both for ocean organisms and for us (seagrass is an important Carbon sink). Coming up next we can see some of the important facts of the seagrass.

Seagrass ecosystem is very important for the marine environment because of several reasons;

- It is one of the most productive ecosystem in marine environment since it serves as habitat and food for many species, such as fishes, starfishes, sea urchins, turtles, etc. (see Fig. 6, Fig. 7 and Fig. 8), and it is also a source for detrital food webs (see Fig. 9).

- The growing structures of seagrasses provide a complex environment that is used by different species as habitat, refuge and nursery, as well as epiphyte and epifaunal substrate.

- Seagrass help to both settle and remove contaminants from the water column and sediments, improving water quality in the adjacent habitats.

- It is also very important its role in coastal sediment stabilization: the canopy helps to encourage settlement of sediments and prevent resuspension, while the root systems help to bind sediments over the longer term and also favours the oxygenation of the soil.

These are some of the organisms that use to live in the seagrass habitat:

Fig. 6: starfish (Author: Ángela Hoces)　　　Fig. 7: pipefishes (Author: Ángela Hoces)　　　Fig. 8: sea urchin (Author: Ángela Hoces)
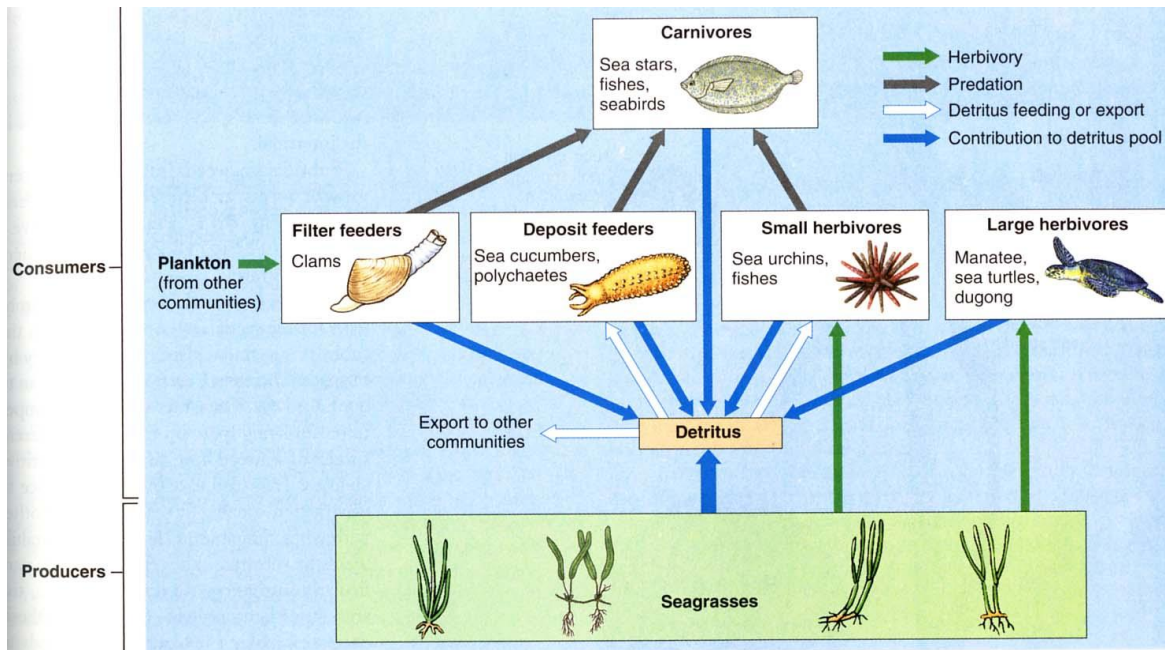
Fig. 9: food web associated to seagrasses.

AIMS

We aim to determine the effect of human perturbation in *Z. marina* growth. We tested the specific hypothesis that perturbation will produce a negative impact in *Z. marina* populations, and as consequence we expect a reduction in internodes distance, leaf and sheath length.

MATERIAL AND METHODS

Study species

This study is focused in the eelgrass *Z. marina*, which has the following morphology: the above ground structure is composed by a horizontal rhizome which bears the strip-like leaves attached to the rhizome by the leaf sheath, separated from the next one by the internodes (see Fig. 10). The underground part is composed by the roots. Thus, a seagrass ramet consists of a leaf-bearing shoot, portion of rhizome and roots (Kendrick, Duarte and Marbà, 2005) (see Fig. 11).

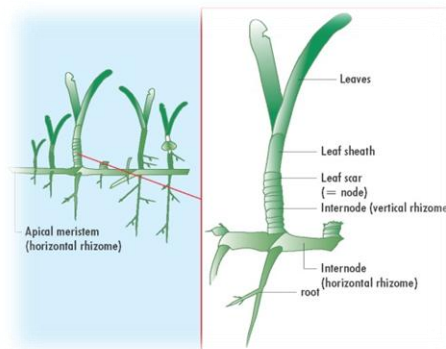Fig. 10: photo of *Z.marina (*Author: Ángela Hoces)

Fig.11: Picture of *Z. marina*

Eelgrass *Z. marina* is the most dominant seagrass species throughout the temperate northern hemisphere except in the polar oceans, and it is found up to 20m of depth. It is present in the northern Pacific and northern Atlantic, being the seagrass species reaching to the highest latitudes, within the Arctic Circle (Fig. 12) (den Hartog, 1970).

Fig. 12: Distribution of *Z. marina* in the Northern hemisphere

Experimental design

The experimental design consisted in a unique factor ("habitat") with two levels: disturbed and no disturbed. For the disturbed treatment we collected plant material at Sætrepollen location (59°40'52.3" N; 10°31'36.4"E, Norway). This area is characterized by the presence of a sailing club, some shops, small restaurants and some industries that could be affecting negatively to the natural conditions of the area. In this perturbed area we observed swans feeding on the seagrass (see Fig. 13, Fig 14. and Fig. 15). For the no disturbed area plant material was collected at Sandspollen (59°40'00.4"N; 10°34'35.0"E, Norway) which is located in a small bay without human presence or impact (Fig. 16 and Fig. 17). Both areas showed sandy/muddy substrate and calm waters. These areas are both located in the county of Buskerud, in the inner part of the Oslofjord (Norway).

The study was conducted in mid-September, the period when eelgrass (*Z. marina* seagrass) is expected to have already reached its maximum seasonal development (Duarte, 1989), and in each location (disturbed and no disturbed) we collected 25 clonal fragments of *Z. marina*.

Fig. 13 and 14: View of the disturbed area in Sætrepollen.

Fig. 15: Aerial picture of the disturbed area Sætrepollen.

Fig. 16: View of the non disturbed area in Sandspollen

Fig. 17: Aerial picture of the disturbed area Sandspollen

Measurements

Clonal fragments were carefully picked up to retrieve the longest possible rhizome pieces (see Fig. 18 and Fig. 19). We used a boat (see Fig. 20) to visit both areas and collect the plants. In both locations, clonal fragments were obtained using a collection net to drag on the sea bottom (see fig. 19). In the disturbed area (Sætrepollen point) the profundity was 3.2 m, and in the no disturbed location (Sandspollen point) the profundity for plant collection was 3.1 m. Collected material was kept in plastic containers of 20L (Fig. 21) and conducted to the laboratory of the Biological Station of the University of Oslo (Fig. 22) located in Drøbak, for plant measurements. The following morphological characteristics were determined: leaf length, sheath length and internodes length (i.e. the distance between two adjacent ramets) (see Fig. 23). For each clonal fragment, internodes distance, leaf length and leaf's sheath length were determined. In addition presence of sexual structures was recorded. A total of 25 clonal fragments were measured in each habitat. 1-5 individual ramets were measured in each clonal fragment.

Fig. 18: collecting plant material

Fig. 19: net to drag on the bottom

Fig. 20: boat used for the vegetal material collection

Fig. 21: plant material in the container

Fig. 22: biological station of the University of Oslo (Author: Ángela Hoces)

Fig. 23: Example of plant material measurements in the lab.

We also look at the number of plants that had developed sexual structures, such as flowers and seeds (see Fig. 26 and 27):

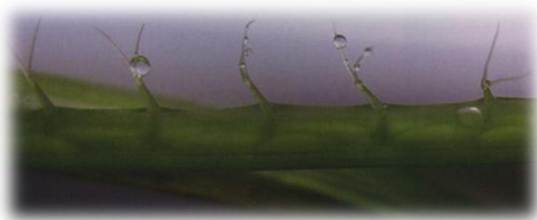Fig. 26: Female flowers in *Z. marina* (Author: Stein Frediksen)

Fig. 24: female flowers in Z. marina (Author: Stein Frediksen)

Fig. 25: Male flowers in *Z.marina* (Author: Stein Frediksen)

Statistical analysis

Prior to analyses, variables were transformed as necessary to meet the assumptions of parametric tests. Thus, the internodes length was log-transformed. We analyzed differences in internodes length, sheath length and leaf length by one-way analysis of variance (ANOVA) with habitat (disturbed, no-disturbed) as fixed effect. Chi-square test was performed to determine differences in sexual structures production between treatments (disturbed and no disturbed). Significance level was set at $P < 0.05$. Statistical tests were performed with SPSS Statistics 22.0 (IBM, Armonk, New York, USA).

RESULTS

Our results showed that there was a statistically significant effect of habitat on internodes length (see Table 1). Thus, the internodes length values were significantly higher in the disturbed than in the no disturbed area (see Fig. 26). Similarly, both the sheath and leaf length were greater in the disturbed in comparison with the no disturbed area (see Fig. 27 and Fig. 28), however these differences were not statically significant (see Table 1). Results showed a significant effect of treatments on sexual structures production. Clonal fragments under disturbed conditions showed a higher allocation to sexual structures than clones in no disturbed areas ($\chi^2_{1, 0.05} = 3.74$ P < 0.05).

Effect	Internodes length			Sheath length			Leaf length		
	d.f.	*F*	*P*	*d.f.*	*F*	*P*	*d.f.*	*F*	*P*
Habitat	1	32.226	**<0.001**	1	1.209	0.274	1	0.467	0.496
Error	76			104			102		

Table 1: ANOVA results for the different measures of the samples collected in the disturbed and no-disturbed areas. In bold, results with significant differences, (p-value < 0.05). See Table 1 for data.
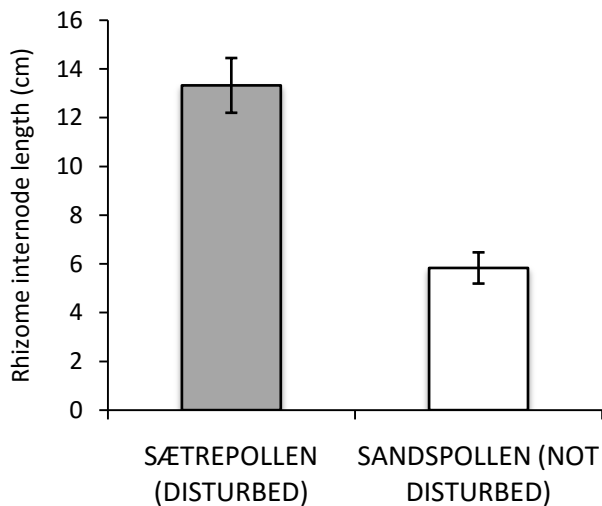

Fig. 26: graph show the significant difference between rhizome internodes distances in the different habitats; the distance is greater in the disturbed area (left bar) than in the no disturbed area (white bar).
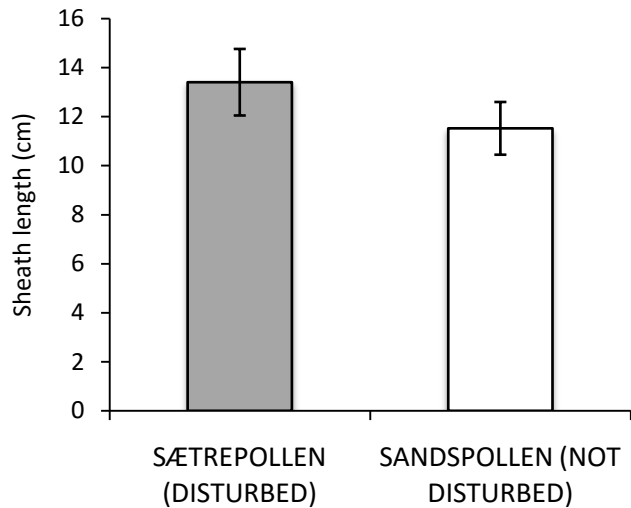
Fig. 27: graph show that there are differences between sheath length of the different habitats but also show that they are not statistically significant.
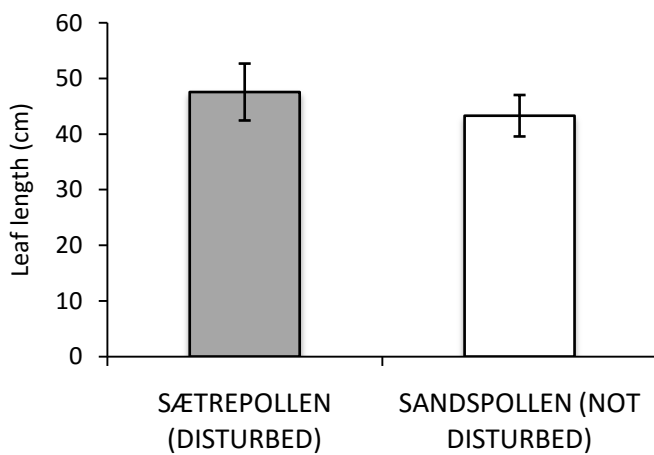

Fig. 28: graph show that there are differences between leaf length of the different habitats but also show that they are not statistically significant..

As we can see, the only morphological character that seems to be affected by the human impact is the rhizome internodes distance, being greater in the disturbed area (ANOVA: $F = 32.226$, $P < 0.001$, for internodes distance. See Table 1). Leaf and sheath length did not show significant differences between both disturbed and not disturbed areas.

DISCUSSION

Contrary to our hypothesis our results did not showed a negative effect on rhizome growth but they showed that internodes distance was significantly greater in the disturbed area than in the no disturbed area. This behavior could be explained with the theory of escape; the length of the rhizome can be longer when the conditions are not good enough, in order to try to 'escape' from this unfavourable ambient and find a better one for the offspring and thus guarantee the survival of these offspring (Roiloa and Retuerto, 2006).

The longer the internodes distance the lower the plant density, so it is reasonable to thinking about the negative effect of the perturbation in these eelgrass populations, since it reduces the plant density, and so the photosynthetic activity and also the whole habitat operation.

However, sheath and leaf length did not show statistically significant difference between the disturbed and no disturbed areas.

Several authors have reported that ramets growing under unfavourable conditions generated longer internodes, but in smaller numbers, than ramets established in favourable areas; they interpreted this response as a strategy for escape from unfavourable conditions (Roiloa and Retuerto, 2006)

Morphological plasticity in stolon length has also been described as a strategy for escaping from unfavourable conditions (Roiloa and Retuerto, 2006).

On the other hand, we also measure the production of sexual structures in the different habitats in order to try to show that sexual reproduction or clonal propagation strategies may be favoured under different conditions. This response can be adaptive, because sexual reproduction would allow the establishment of new plants from seeds at locations relatively distant from the stressful environment in the vicinity of the parent (Roiloa and Retuerto, 2006). We interpret this as a strategy for escape from stressful environments.

CONCLUSIONS

We Plants with clonal growth may invest more energy in sexual structures there where the habitat conditions are not good enough. They also can invest the energy on developing longer rhizomes in order to establish the offspring in a farer environment and thus guarantee a good development.

CONCLUSIONES

Las plantas con crecimiento clonal pueden invertir más energía en el desarrollo de estructuras sexuales allí donde las condiciones ambientales no son lo suficientemente buenas. Por otra parte también pueden invertir la energía en desarrollar rizomas más largos para poder establecer su descendencia en un ambiente más lejano y así asegurar un buen desarrollo.

ACKNOWLEDGMENT

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