Universidade do Algarve

Faculdade de Ciências do Mar e do Ambiente

Impact of clam harvesting activity in the flowering and sexual reproduction effort of *Zostera noltii* Hornemann in Ria Formosa (Southern Portugal)

Ana Isabel Delfim dos Santos Alexandre

Faro
2004

This research is part of the EU project Monitoring and Managing of European Seagrass Beds (EVK3-CT-2000-00044).

AGRADECIMENTOS

Ao Prof. Doutor Rui Santos e à Prof. Doutora Ester Serrão, pela orientação científica deste estudo, pela ajuda na análise dos dados e pelo constante incentivo ao longo de todo o trabalho.

Ao Projecto Europeu “M&MS - Monitoring and Managing of European Seagrass Beds”, do qual este estudo faz parte, e o qual forneceu todo o apoio financeiro necessário.

À Ana Quaresma, pela preciosa ajuda no trabalho de campo e no laboratório, e nos assuntos burocráticos!, pela sua incondicional disponibilidade e boa disposição, que proporcionaram um importante apoio técnico e psicológico.

À Susana Cabaço, pela colaboração e disponibilidade ao longo de todo o projecto, quer no campo, quer no laboratório, pelas sugestões e informações pessoais enriquecedoras na redacção desta tese, e pelos belos momentos de descontracção que se proporcionaram com a sua boa disposição e sentido de humor!

Ao Sr. Sousa, que gentilmente autorizou a realização dos trabalhos de campo na sua área de concessão, no Marim, fornecendo uma zona de trabalho não perturbada, fundamental para a realização de grande parte das amostragens necessárias para a realização deste estudo.

Ao Luís Dias, à Alexandra Cunha, ao João Silva, e à Raquel Machás, pelos diversos apoios cedidos, e a todas as outras pessoas que contribuíram de forma importante para a realização dste trabalho.

Aos meus amigos do “EMOCA”, pelo apoio, pelo convívio e pelos deliciosos momentos que vivemos todos juntos durante a parte lectiva do mestrado, e que ainda vamos continuando a viver... Foi muito bom conhecer-vos e partilhar o meu dia-a-dia convosco!

E por fim, mas não em último!, aos meus pais, que sempre me incentivaram e apoiaram na progressão dos meus estudos, e a quem devo tudo isto e muito mais...
MUITO OBRIGADA!!!

ABSTRACT

The seagrass *Zostera noltii* develops and reproduces sexually along the intertidal areas of Ria Formosa lagoon (Southern Portugal). The flowering season occurs from March until November. The development of the flowers along the flowering season, from the inflorescence formation to the seed production was observed and organized into a maturation scale. The male to female flower ratio determined for *Z. noltii* was 1:1 (4±0.14 S.E. male flowers to 4±0.13 S.E. female flowers). The impact of the clam harvesting activity in the flowering and in the sexual reproductive effort of *Z. noltii* was investigated. Higher values of reproductive effort (6.38±3.91%), as well as flowering shoot density (2200 flowering shoots per m^2), were observed in the clam harvested meadows in comparison to the control meadows (1.85±1.14% reproductive effort; 1062 flowering shoots per m^2).

The results obtained demonstrate that *Z. noltii* increases its sexual reproduction effort as a response to the disturbance by clam harvesting activity. However, owing the high clonal growth rate of the species and the constraints of its reproductive cycle, the recovery of *Z. noltii* meadows is probably made through vegetative development.

Key-words: *Zostera noltii*, flowering, disturbance, clam harvesting, sexual rerpoduction, Ria Formosa.

1. General Introduction

Seagrasses are specialized marine plants that have adapted to the nearshore life. These aquatic angiosperms comprise about 60 species, grouped into 12 genera and 2 families (Phillips & Meñez 1988). In contrast to other aquatic plants, seagrasses are able to live fully immersed, presenting a hydrophilic pollination. They are distributed along the coastal areas of the world except in the Antarctic (Den Hartog 1970), surviving in a wide range of salinities encompassing freshwater, estuarine, and marine or hypersaline environments (Short *et al.* 2001).

The ecological role of seagrass habitats has become increasingly recognized and is well described (Dawes 1981; Short 1987; Phillips & Meñez 1988; Adam 1990; Alongi 1998; Hemminga & Duarte 2000). These complex ecosystems provide food and shelter for many resident and transient adult and juvenile marine organisms, as well as nursery areas for some commercially important fish and shellfish species. Beside their contribution to stabilize sediments, these plants have also high primary production rates, being one of the world's most productive ecosystems (along with marshes and mangroves) (Duarte & Chiscano 1999). Their great capacity to recycle nutrients, due to the associated bacterial communities, lead seagrass beds to be recognized as one of the natural systems with higher economic value (Constanza *et al.* 1997). Therefore, the importance of the seagrass ecosystem concerns the services that seagrass vegetation provide to the overall functioning of coastal zone systems, playing an essential role in improving coastal water quality and increasing biodiversity.

Zostera noltii Hornemann is a small species belonging to the genus *Zostera*, one of the three seagrass genera with the largest number of species (i.e. *Halophila*, *Zostera* and *Posidonia*). This species extends through the coasts of Western Europe and North-Western Africa, from Southern Norway to Mauritania, occurring also in the Mediterranean Sea, the Black Sea and the Caspian Sea (Den Hartog 1970). It has a fast clonal growth rate (Vermaat *et al.* 1987; Peralta 2000) which is typical of colonizing species. Few seagrass species are able to withstand exposure to air. However, *Z. noltii* is particularly widespread along the intertidal areas of Ria Formosa tidal lagoon (Southern Portugal), forming important populations of dense monospecific meadows.

As other seagrasses, *Z. noltii* spreads through vegetative development (rhizome elongation), especially when colonizing new habitats or recovering from disturbance. Besides vegetative development, these plants can reproduce sexually by producing flowering shoots and seeds. Flowering is considered a rare event for most seagrasses (Gallegos *et al.* 1992; Marbà & Walker 1999). However, seed production and other events related to this process (flowering, seed release, seed germination and dispersal) are valuable to maintain genetic diversity and may be, in certain cases, the only significant mechanism for seagrass colonization of bare sediment areas (Duarte & Sand-Jensen 1990). Coupling both vegetative and sexual reproductive patterns may therefore constitute an excellent survival strategy in adverse and disturbed environments or in the establishment of new areas.

Commercial clam harvesting is a very traditional and significant economic activity in the Ria Formosa lagoon, representing up to 90% of the national clam production (Morais & Carvalho 1992). The intertidal meadows of *Z. noltii* provide exceptional suitable conditions for the existence of a variety of clam species. About 40% more clams were recovered live from large patches of *Z. noltii* than from unvegetated areas (Irlandi 1997).

Certain mariculture practices have been pointed out as the major factor responsible for the reduction of seagrass cover as well as above- and below-ground biomass (de Jonge & de Jong 1992; Everett *et al.* 1995; Boese 2002), particularly in shallow water areas (estuaries or tidal lagoons). At Ria Formosa, a large number of fishermen harvest the clams daily during low tide, removing them from both vegetated and unvegetated areas. Recreational clam harvesting represents an additional impact over *Z. noltii* meadows, especially during the summer, when tourists harvest the clams for self consumption. Both activities disturb these meadows and may result in severe local reductions of seagrass cover, namely in the shoot density and plant biomass (personal observation). The shoots and rhizomes are damaged or completely removed from the sediment, not only manually (using a clam harvesting knife) but also mechanically (using a small tractor). In Ria Formosa, particularly in the eastern sector, there are a large number of private concession areas where *Z. noltii* plants are completely removed with a small tractor to create clam culture plots for seeding and harvesting the clams. This procedure, in which both rhizomes and roots are completely removed from the sediment, is responsible for a significant *Z. noltii* habitat loss (De Jonge & De Jong 1992).

Destruction and loss of seagrass habitats have been reported from most parts of the world, mainly due to human-induced disturbances such as eutrophication, mechanical damage (dredging, boat mooring and propeller scarring, fishing practices) and the release of toxic compounds into coastal waters (Hemminga & Duarte 2000). When the disturbance is intense and persistent (natural or anthropogenic), vegetative development may not be sufficient to maintain the meadows and recolonization from seeds must play an essential role (Duarte & Sand-Jensen 1990). The successful germination of the seeds inside the meadow will originate new recruits. Some studies have demonstrated the existence of a relationship between different types of disturbance (burial derived from hurricanes and subaqueous dune migration) and the enhancement of seagrass flowering (Gallegos *et al.* 1992; Marbà & Duarte 1995).

2. Objectives

The reproductive biology of seagrasses is well studied, but the detailed description of the reproductive structures and the sexual reproduction pattern of *Zostera noltii* is only referred in studies in the Mediterranean Sea (Buia *et al.* 1985; Hootsmans *et al.* 1987; Loques *et al.* 1988; Loques *et al.* 1990; Buia & Mazzella 1991; Goubin & Loques 1991; Curiel *et al.* 1996). The quantification of flowering, fruiting, seed production and the sexual reproduction effort is essential to understand the success of the species dispersal and recruitment mechanisms and to evaluate the potential of the sexual reproduction in seagrass colonization processes, especially in the re-establishment of declining populations (Orth *et al.* 1994) and in recovering from disturbance.

This study investigates the flowering process of the seagrass *Zostera noltii* in Ria Formosa and assesses the sexual reproduction effort of this species in face of the disturbance by clam harvesting. In the first chapter, the specific objectives are to identify the reproductive structures and to describe in detail the developmental stages of the flowers, arranging them into a maturation scale. In the second chapter, the objective is to describe the sexual reproductive effort of *Z. noltii* under disturbance by clam harvesting activity in two sectors of the Ria Formosa. In the third chapter, the objective is to evaluate experimentally the reproductive effort of *Z. noltii* after an induced clam harvesting treatment.

3. Description of the reproductive structures

3.1 Introduction

As aquatic plants, seagrasses must have the ability to reproduce when submerged in the marine environment. Thus, their reproductive structures and pollination process are fully adapted to underwater reproduction. An entirely submerged pollination is referred as the usual form for the genus *Zostera* (Phillips & Meñez 1988). Despite the different environments, the sexual reproduction cycle of seagrasses is very similar to the land angiosperms, except in the dispersal processes (Figure 1).

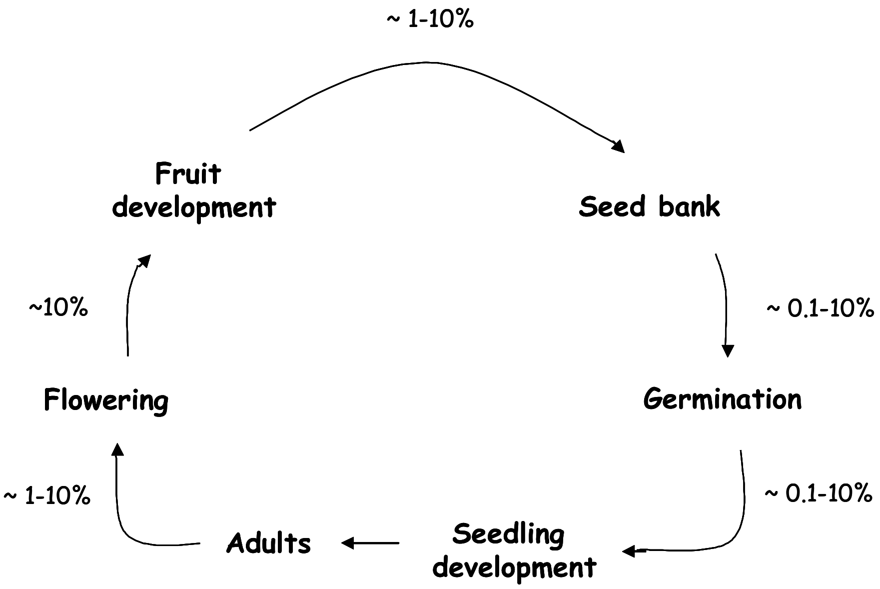

Figure 1 – Transition rates between sexual reproductive stages in seagrasses (adapted from Hemminga & Duarte 2000).

The process starts with the flowering event, i.e. the production of floriferous shoots which form fruits inside. After being mature, the fruits become seeds, fall off and may form a seed bank in the sediment. The process culminates with seed germination and further development of the seedlings to adults (Hemminga & Duarte 2000). Percentage values showed in Figure 1 were derived from the literature and indicate the probability of success expected for each reproduction step for seagrasses. The low flowering probability and the low seed survival rate suggests that these may constitute the major bottlenecks in the sexual reproduction pattern among seagrasses in general.

In spite of the references on the inefficiency of the sexual reproduction among seagrass species in general, there are several evidences of the success of the germination process and seedling survival *in situ* for *Zostera noltii* (Goubin & Loques 1991), for *Z. marina* (Orth & Moore 1983) and for *Cymodocea nodosa* (Pirc *et al.* 1986). Sexual reproduction is considered crucial not only to maintain genetic diversity but also as a species dispersal mechanism to colonize new areas (Buia & Mazzella 1991).

Zostera noltii is the most abundant seagrass species in the Ria Formosa lagoon and is widely spread along the intertidal areas. This small species exhibits both vegetative and sexual reproduction. In spite of being able to develop through clonal growth (rhizome elongation), floriferous shoots have also been observed. The flowers are inconspicuous and morphologically simple since they do not rely on animals for pollination. *Z. noltii* is a monoecious species, which means that both male and female flowers lie together inside the same inflorescence. The flowers appear and die within a single season (Loques *et al*. 1988). *Z. noltii* has potential to form a seed bank (Hootsmans *et al.* 1987; Loques *et al.* 1990) but the existence of seed dormancy is only referred in Buia & Mazzella (1991).

Previous studies on the reproductive biology of *Z. noltii* involving the phenology of its flowers and fruits ascend to the nineteenth century. In spite of recent contributions to improve the knowledge on the reproductive and germinating aspects of this species (Buia *et al.* 1985; Hootsmans *et al.* 1987; Loques *et al.* 1988; Loques *et al.* 1990; Buia & Mazzella 1991; Goubin & Loques 1991; Curiel *et al.* 1996), there is no detailed information on the description and evolution of the developmental stages of the flowers throughout the flowering season. Furthermore, the studies published so far on the aspects of the sexual reproduction biology of *Z. noltii* refer only to Mediterranean coasts. This chapter aims to investigate this subject in the Atlantic coast, specifically in the Ria Formosa tidal lagoon.

The specific objectives of the present chapter are to define the flowering season of *Zostera noltii* in the Ria Formosa, to identify its reproductive structures, as well as to describe the developmental stages of the flowers and their evolution throughout the flowering season in order to construct a maturation scale. The ratio of male to female flowers is also assessed.

3.2 Methodology

3.2.1 Study site

Ria Formosa is a coastal tidal lagoon extending for 55 km along the south coast of Portugal. During low tide, an extensive lagoon area is exposed and the tidal flats dominated by the seagrass *Zostera noltii* become emerged. Water temperature in the lagoon varies between 12ºC during winter and 27ºC in the summer. Salinity values range from 35.5 to 36 PSU along the year except during heavy rainfalls, when this value can be as low as 15 PSU (Falcão & Vale 1990), or even lower inside the small ponds formed temporarily during the low tide (2 to 10 PSU) (personal observation). The specific study site is located at Marim (Figure 2) and was selected based on its characteristics: it is an extensive and well-developed monospecific *Z. noltii* meadow, relatively flat, so that the sampling area lies within a narrow tide height range. The meadow has limited public access because the area is a private concession where no clam harvesting occurs.

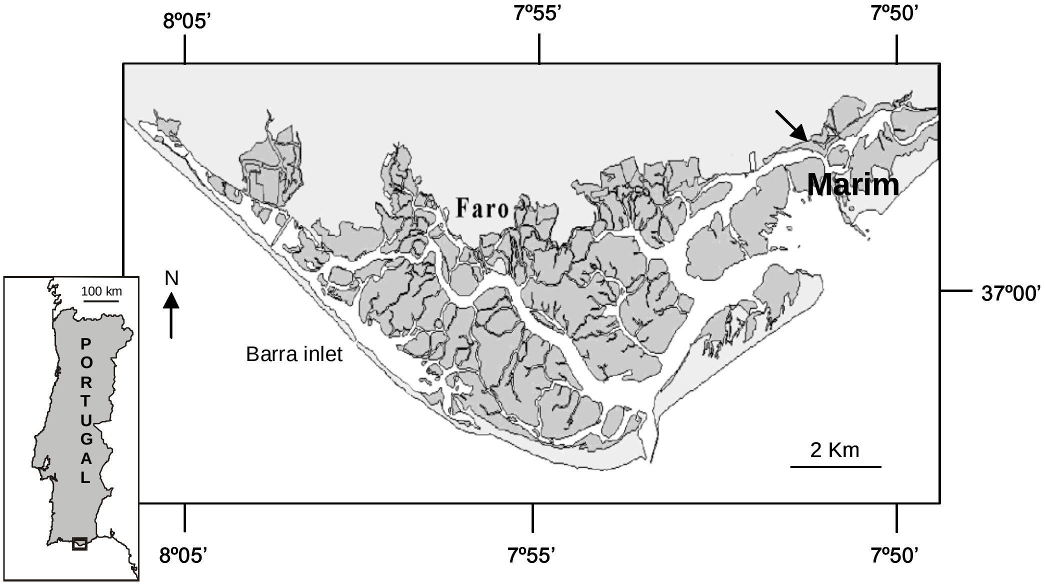

Figure 2 - Map of Ria Formosa with the location of the study site and the Ancão inlet.

3.2.2 Method

In order to identify the beginning of the flowering season and the collection of the samples, a weekly field search was carried out to detect the appearance of the first flowering shoots. At the beginning of June, samples of *Z. noltii* were collected using a 12 cm ø corer. The samples were washed up in the field to remove the sediment and transported to the laboratory in seawater. In the laboratory, the flowering shoots were carefully observed with a magnifier lens. The morphological development of the flowers between the different stages of maturation was recorded. These morphological stages were then classified into maturation stages in order to construct a maturation scale. The stage of maturity of the fruits within the inflorescence was estimated based on their size and shape inside the ovary.

3.3 Results

3.3.1 Flowering season

In Ria Formosa, the flowering season of *Z. noltii* extended from late March to early November. Although sampling was only initiated in June, the first flowers were observed in March. However, differences in the beginning and ending of the flowering season were observed in the subsequent years, with the first flowers appearing in late April and persisting until early October or even later than November. Some flowers have been observed in December in meadows located at the western sector of the Ria Formosa.

3.3.2 Description of the reproductive structures

The flowering shoots flourish from the rhizome and they are very similar to the vegetative ones except that the latter lack the reproductive structures. The full-grown flowering shoot consists of several inflorescences, each containing the flowers inside. Inside the inflorescence, the male and female flowers are arranged in two rows along a longitudinal axis. The female flowers consist of an ovary with one ovule, a style and two long and thin stigmas. The male flowers consist of two ovoid thecae arranged side by side, enclosing the filiform pollen. The flowers are wrapped and protected with an inflorescence sheath that remains closed before pollination of the female flowers and after the pollen release.

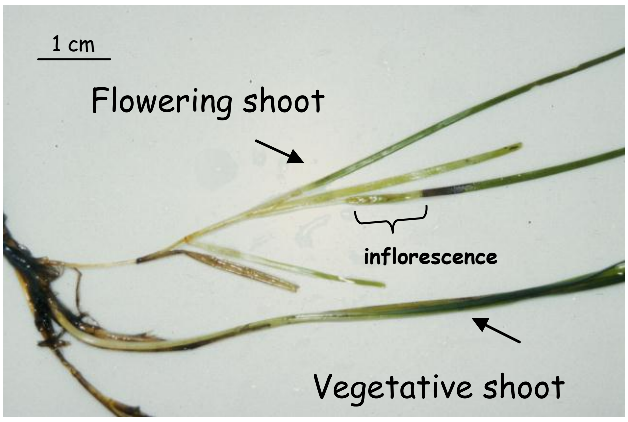

Figure 3 – Vegetative shoot and flowering shoot of *Zostera noltii*, indicating the location of the inflorescence.

3.3.3 Evolution of the maturation stages

Initially, the fully-developed male and female flowers are aligned longitudinally on a single stem, inside the inflorescence (Figure 3). At this time, the inflorescence presents a yellow-whitish colour and the flowers are totally enclosed inside the protection sheath (Stage I).

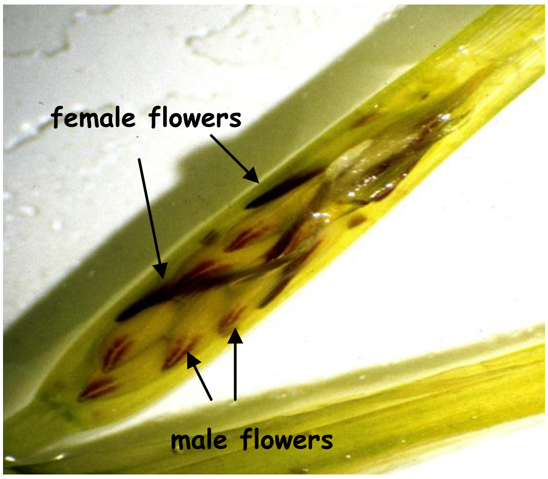

Figure 4 – Inflorescence at the initial stage of development (Stage I). Male and female flowers are indicated with arrows.

When the inflorescence is completely formed and the female flowers are mature, the style and the stigmas of the female flowers start to bend outside the sheath to be pollinated (Stage II) (Figure 4). The bending of the female flowers seems to be an acropetal movement, i.e. the female flowers start to bend in sequence from the base of the inflorescence upwards. After females have been pollinated, the stigmas fall off and the styles bend backward to the interior of the sheath. This step may be confounded with the Stage I, because the female flowers are again aligned with the male flowers inside the sheath. However, careful observations elucidate the absence of the stigmas in the female flowers (that have fallen off after pollination). Additionally, one may note that the protection sheath is not completely closed as prior to the emergence of the stigmas and that the overall inflorescence presents a dark yellow colour.

Figure 5 - Inflorescences of *Z. noltii* at stage II. The erected female flowers outside the sheath to be pollinated (indicated with the arrows).

Following the pollination of the female flowers, the male flowers bend upwards, dehisce and release the pollen to the water (Stage III) (Figure 5). After this, some of the male flowers may retract into the inflorescence instead of falling off. Empty thecae were occasionally observed inside the inflorescence.

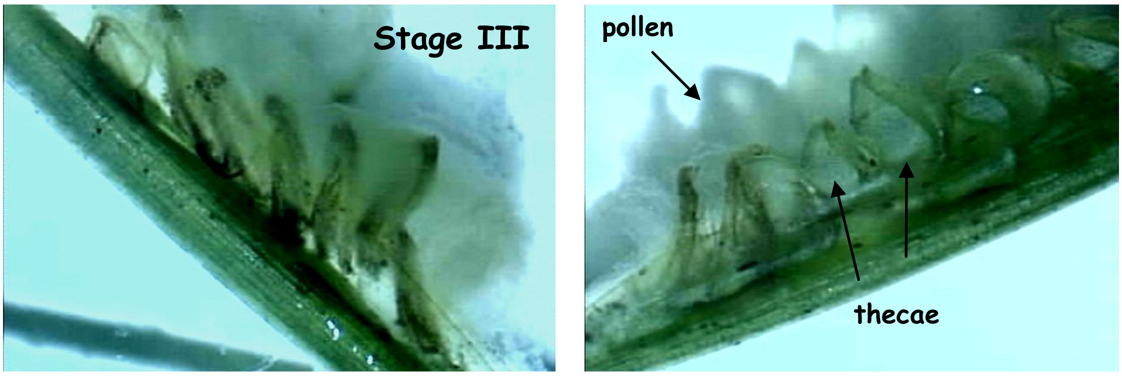

Figure 6 – Inflorescences at stage III. The male flowers release the pollen to the water (thecae and pollen are indicated with the arrows).

An inflorescence containing female flowers with no stigmas indicates that the females have been pollinated (Stage IV). The pollination process is concluded (Figure 6).

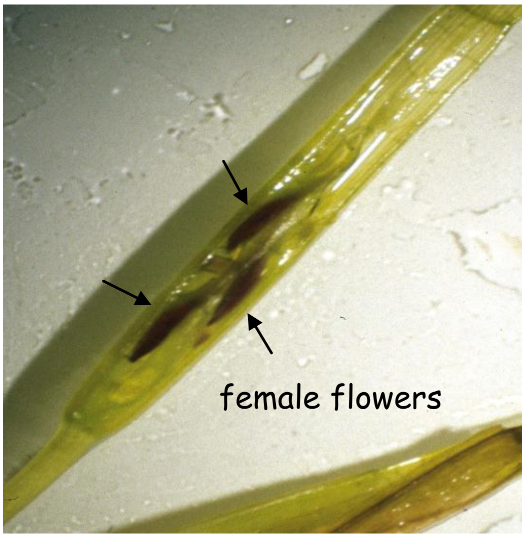

Figure 7 - Inflorescence at stage IV with three pollinated females (female flowers are indicated with arrows).

The next steps concern the development of the fruits inside the ovary of the pollinated females (Stage V) (Figure 7). The fertilized ovule starts to grow inside the female, until it fills up the ovary. In a first step, the small embryo is visible inside the ovary (Stage Va). Later, the ovary is completely filled up with the fruit (Stage Vb). After this, a thin coat starts to cover the fruit, which presents a white colour inside (Stage Vc). The thin coat becomes thicker and dark brown, and the fruit becomes greenish-blue inside, forming the seed (Stage Vd). It may happen that, inside the same inflorescence, there are, simultaneously, fruits in the early stages and seeds fully developed. When this was the case, the most advanced stage of maturity was considered.

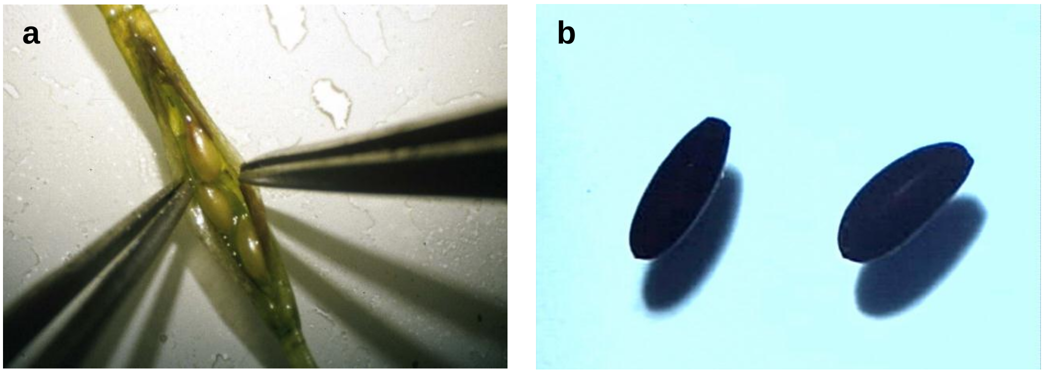

Figure 8 - Inflorescence at stage V. a) three fruits are forming inside the ovary (Stage Vc); b) two seeds completely formed, with a hard dark brown coat (Stage Vd).

3.3.4 Maturation scale

After the observation of the development of the flowering process, a maturation scale was created, pointing out the main characteristics observed in the reproductive structures (Table I).

Table I – Maturation scale showing the main characteristics of the reproductive structures in each stage of maturity.

Maturation stage	**Main characteristics**
I	Flowers aligned on a single stem; yellow-whitish inflorescence; sheath closed
II	Mature females with styles and stigmas erect outside sheath for fertilization
III	Mature males releasing filiform pollen (anther dehiscing)
IV	Inflorescence with fertilized females, styles shrink; stigmas absent; thecae empty (if still present)
V	Presence of fruits
Va	White young fruit inside ovary
Vb	Ovary filled up with fruit
Vc	White fruit presents a thin coat
Vd - Seed	Greenish-blue fruit presents a hard brownish-black coat

3.3.5 Male to female flowers ratio

The number of male and female flowers was counted only in inflorescences on stages I and II because the following stage III is related to pollen release and may therefore lack some male flowers; in Stage IV only fertilized females are present inside the inflorescence; and stage V is only related to fruit formation, and therefore males are no longer present in the inflorescence. No seasonal pattern was found in the ratio of male to female flowers along the flowering event. The mean male to female ratio obtained for *Zostera noltii* was 1:1 (n=30). In each inflorescence there was a mean of 4±0.14 S.E. male flowers to 4±0.13 S.E. female flowers.

3.4 Discussion

The flowering event of *Zostera noltii* is poorly documented, being detailed only in the studies of Loques *et al.* (1988) and more recently in Curiel *et al.* (1996). In *Z. noltii*, the length of the flowering season varies from place to place. In Ria Formosa, the first flowers appeared at the beginning of March and lasted until November, while in the Netherlands they were first observed in late June, lasting until October (Hootsmans *et al.* 1987); in the French Mediterranean the flowering season is shorter and takes place from May until late August (Loques *et al.* 1988), which was very similar to that observed for *Zostera noltii* from the Adriatic Sea (Curiel *et al.* 1996). The water temperature, the daylength and fluctuating salinity regimes have been pointed out as the main factors controlling the flowering of seagrasses in general (McMillan 1982; Loques *et al.* 1988; Phillips & Meñez 1988; Buia & Mazzella 1991; Ramage & Schiel 1998). Tidal amplitude is also referred as responsible for the greater nutrient transport which seems to be related to flowering events during spring tides (Pettit, 1984). In Ria Formosa, flowering is probably triggered by the increase in water temperature and irradiance verified at the onset of spring. The end of the flowering season, which happens suddenly, is probably related to the first heavy rainfalls that occur in autumn, which reduce water salinity. At this time, the flowering shoots acquire a rotten appearance and quickly fall off.

Flowering shoots have been observed to persist until December in meadows near the Ancão inlet (Figure 2). In this area, the *Z. noltii* meadows are composed of sandy sediment, which differ from the muddy sediment meadows common in the Ria. *Z. noltii* flowering season was observed to last longer in recently formed meadows and newly colonized beds composed of sandy sediments (Auby & Labourg 1996; Curiel *et al.* 1996). Thus, flowering may be controlled by other factors, like sediment type, than temperature and daylenght.

The reproductive structures described in this study for *Z. noltii* are identical to those referred in Loques *et al.* (1988) for the same species in the Mediterranean, and for *Z. marina* (De Cock 1980). The main difference among these two species is related to the size of the structures, which are about twice in *Z. marina*.

The development of the maturation stages described here for *Z. noltii* is also very similar to the observations made by Loques *et al.* (1988) and De Cock (1980) for *Z. marina*. De Cock (1980) have also constructed a maturation scale for *Z. marina* based on extensive laboratory observations on the time elapsed between successive stages in the flowering process and seed development. In the present study, there are no data on the time elapsed between successive maturation stages, so no comparisons can be made on this subject.

Within the same inflorescence, the female flowers are pollinated before the male flowers release their pollen (Loques *et al.* 1988), which is considered a mechanism to avoid self pollination. Simultaneous flowering of male and female flowers is considered to be exceptional and occurs only if pollination is prevented by exposing the plants to extended periods of abnormal conditions, mainly high water temperatures (up to 25ºC) (De Cock 1980). Despite this fact, and because the inflorescences of a same flowering shoot are at different maturation stages at the same time, female flowers may be pollinated with pollen released by the male flowers of any other inflorescence.

The male to female ratio may provide an index of pollination potential, i.e. the higher the ratio of male flowers to female flowers the higher is the probability of the female flowers to be pollinated. Highest pollination rates were recorded within sites presenting the highest ratio of male to female flowers (Conacher *et al*. 1994). The ratio observed in the present study (1:1) is similar to that recorded by Loques *et al.* (1988) for *Z. noltii* but is lower than that reported in Conacher *et al*. (1994) for *Z. capricorni*, in which the mean ratio of male to female flowers was 2.2:1.

Seagrass seeds are typically negatively buoyant (Pettit 1984). In *Z. noltii* it was noted that the seeds always sink to the bottom of an aquarium after being released from the ovary, which was also observed by De Cock (1980) for *Zostera marina* and Loques *et al.* (1988) for *Z. noltii*. In the field, seeds have never been found in the sediment. This suggests that seed dispersal may occur immediately after the seeds are released, instead of standing in the sediment. Seed dispersal is probably made by water currents that transport detached flowering shoots containing seeds over long distances (Phillips & Meñez, 1988; Loques *et al.*, 1988; Curiel *et al.*, 1996). In fact, after the flowering event is completed, signs of necrosis were observed in the flowering shoots. Their green colour changed to dark brown and finally they detached from the rhizome. Large fishes that feed on the flowering shoots or animals that ingest large quantities of sediment in which *Z. noltii* seeds may have accumulated may also contribute to the seed dispersal (Loques *et al.*, 1988).

4. Descriptive study

4.1 Introduction

Clams have been grown in the Ria Formosa for a long time. Commercial clam harvesting is a very traditional and significant economic activity in the lagoon, representing up to 90% of the national clam production (Morais & Carvalho 1992). During low tide, a large number of fishermen harvest the clams not only in the bare sand areas but particularly within the intertidal vegetated meadows of *Zostera noltii*. These meadows may provide suitable conditions for the existence of a variety of clam species, since a higher number of clams is collected there compared to bare sand zones (Irlandi 1997). Although fishermen state that is harder to search the clams inside these meadows because of the leaves and rhizomes, they know from experience that these areas provide a larger number of clam seeds and so they frequently clam harvest on *Z. noltii* beds.

This fishing practice produces local reductions in the seagrass density because the shoots are cut and removed from the sediment with a clam harvesting knife. Moreover, during the summer, tourists intensively harvest the clams for self consumption, increasing the impact of the activity on *Z. noltii* meadows. The time required to recover from disturbance depends on the frequency and intensity of the impact on the meadows (Boese 2000). Areas with an easier public access are regularly more visited by fishermen than restricted areas (for example, private concessions).

Z. noltii has the capacity to develop through vegetative growth of the rhizomes and the apical shoots. Under an intense and persistent disturbance (natural or anthropogenic) vegetative development may be insufficient to sustain the meadows. In this case, the ability to reproduce sexually plays a crucial role in the maintenance of the species or in the recolonization processes from seeds into new areas (Duarte & San-Jensen 1990). There are studies that relate different types of disturbance, namely burial derived from hurricanes and subaqueous dune migration) with the enhancement of seagrass flowering (Gallegos *et al.* 1992; Marbà & Duarte 1995). This suggests that there is probably a coupling between vegetative growth and sexual reproduction patterns controlling the continuity of disturbed meadows.

The objective of the present chapter is to describe the sexual reproduction effort of the seagrass *Zostera noltii* under disturbance by clam harvesting in two sectors of the Ria Formosa. The development of the maturation stages of the inflorescences along the flowering season was also observed in both sectors.

4.2 Methodology

4.2.1 Study site

Ria Formosa extends along the south coast of the Algarve, Portugal, from Praia do Ancão (near Vilamoura) to Cacela, in a total of 55 km. Two sectors may be considered in the lagoon: a western sector including the areas of Vilamoura and Praia de Faro and an eastern sector, enclosing the region of Olhão to Cacela (Figure 8).

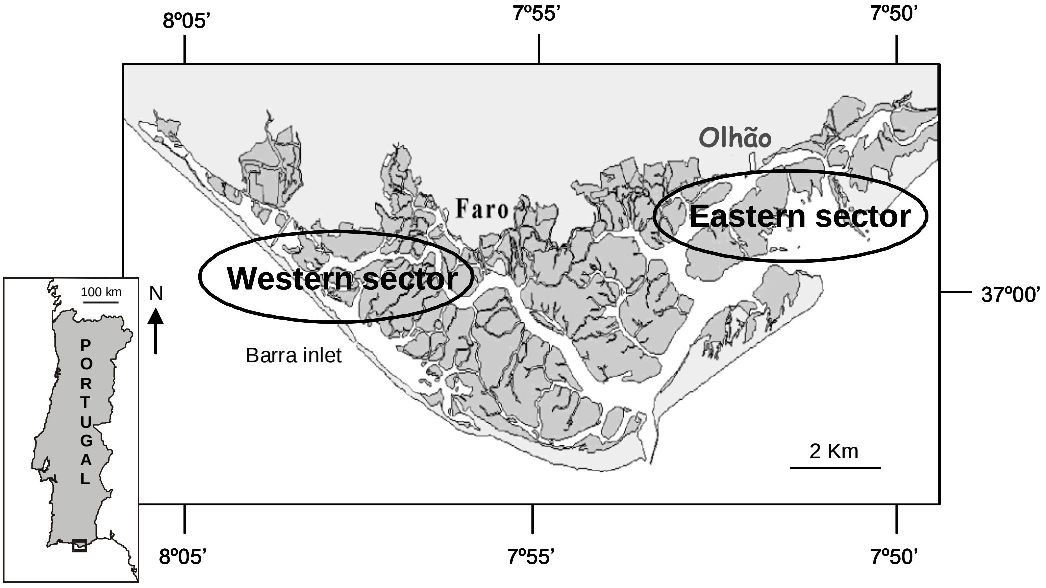

Figure 9 – Map of Ria Formosa with location of the two sectors studied and the Ancão inlet.

In the western sector, most of the intertidal meadows are easily accessed by people in general. In here, a large number of fishermen daily harvest the clams. Recreational clam harvesting by summer tourists also contribute to the increase of disturbance in these areas. Therefore, these meadows are constantly disturbed and a low plant density is visible. On the contrary, in the eastern sector, there are a great number of private concessions in which only the owner is allowed to clam harvest, and most of the meadows can only be accessed by boat. Thus, in these meadows, the disturbance is supposed to be lesser than in the western sector meadows, as suggested by the higher plant density.

4.2.2 Method

In each sector, sampling sites were chosen according to different levels of disturbance by clam harvesting: a disturbed meadow, where a daily clam harvesting activity occurs, and an undisturbed meadow (control), with no clam harvesting impact. The four sites were sampled on a fortnightly basis, from June until the end of the flowering season in early November, when no more flowering shoots were observed. In each site, replicate samples of *Zostera noltii* were randomly collected using a 12 cm ø corer. In the laboratory, each replicate sample was analyzed in order to obtain the density of both vegetative and flowering shoots. These shoots were dried (48h at 60ºC) and weighed separately for above-ground biomass determinations.

The reproductive effort of *Z. noltii* was determined as the ratio of the dry weight of reproductive shoots to total above-ground plant dry weight x 100, i.e.

the proportion of total shoot biomass (vegetative and flowering shoots) allocated to sexual reproduction (flowering shoots) (Auby & Labourg 1996; Kaldy & Dunton 2000).

A two-way ANOVA was used to test significant differences between disturbance levels, between sectors and interactions between both factors. In order to avoid the huge variability within samples, created by a high number of zero values in the flowering shoot density, the total number of flowering shoots per m^2 was used in Chi-square calculations to test significant differences between disturbance levels.

4.3 Results

In both sectors, vegetative shoot density of *Z. noltii* in the disturbed sites was significantly lower than in the control sites ($p<0.001$) (Figure 9). The eastern sector presented a significantly higher vegetative shoot density than the western sector, in both disturbed and control sites ($p<0.001$). There was no significant interaction between sectors and disturbance levels ($p=0.779$).

In the western sector, vegetative shoot density in the disturbed site ranged from 796 to 7343 shoots m^{-2} whereas in the control site it ranged from 3539 to 13366 shoots m^{-2}. In the eastern sector, vegetative shoot density in the disturbed site ranged from 978 to 10106 m^{-2} whereas in the control sites it ranged from 4600 to 13773 shoots m^{-2}.

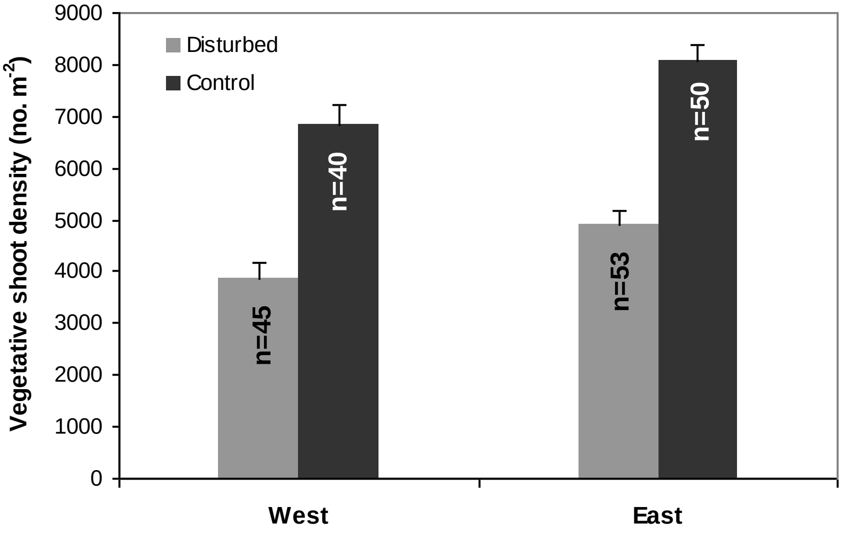

Figure 10 - Vegetative shoot density of *Zostera noltii* in the control meadows and in the meadows disturbed by clam harvesting activity at the western and eastern sectors of the Ria Formosa (Mean ± S.E.); n= number of corers analysed.

In the eastern sector, the total number of flowering shoots per m^2 was significantly higher in the disturbed site than in the control ($p<0.01$) (Figure 10). In contrast, in the western sector, the total number of flowering shoots per m^2 was significantly lower in the disturbed site than in the control ($p<0.01$).

In the western sector, flowering shoot density in the disturbed site ranged from 0 to 978 shoots m^{-2} whereas in the control site it ranged from 0 to 1504 shoots m^{-2}. In the eastern sector, flowering shoot density in the disturbed site ranged from 0 to 2200 shoots m^{-2} whereas in the control sites it ranged from 0 to 1062 shoots m^{-2}.

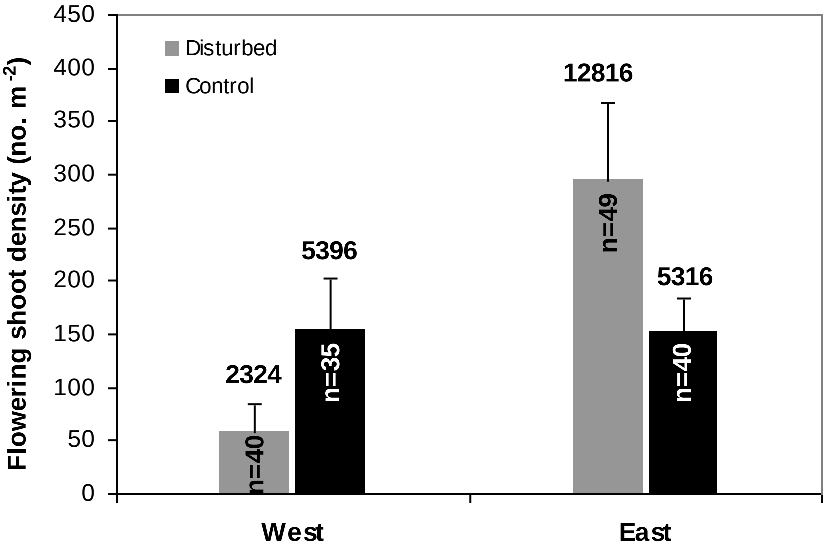

Figure 11 - Flowering shoot density of *Zostera noltii* in the control meadows and in the meadows disturbed by clam harvesting activity at the western and eastern sectors of the Ria Formosa (Mean ± S.E.); n= number of corers analysed; Numbers above bars refer to the total number of flowering shoots per m^2.

Zostera noltii reached a maximum reproductive effort of 6.38 ± 3.91% in the eastern disturbed site in August (Table II). A minimum value of 1.31 ± 0.50% was recorded in the western control site in June.

Table II – Maximum sexual reproductive effort (RE) of *Zostera noltii* in the control meadows and in the meadows disturbed by clam harvesting activity in the western and eastern sectors of the Ria Formosa (Total shoot biomass = vegetative + flowering shoot biomass; n=5; Mean ± SE).

Western sector	**Disturbed**	**Control**
Flowering shoot biomass (g DW m^{-2})	2.27 ± 2.27	3.60 ± 0.98
Total shoot biomass (g DW m^{-2})	75.35 ± 6.02	202.75 ± 38.77
RE (%)	**2.70 ± 2.70 (Jul)**	**1.31 ± 0.50 (Jun)**
Eastern sector		
Flowering shoot biomass (g DW m^{-2})	2.39 ± 1.77	3.04 ± 1.75
Total shoot biomass (g DW m^{-2})	26.91± 8.87	194.49 ± 44.45
RE (%)	**6.38 ± 3.91 (Aug)**	**1.85 ± 1.14 (Aug)**

The observations of the development of maturation stages showed that earlier stages shift to later stages as the flowering season advances (Figures 11 and 12). At the beginning of the sampling, earlier stages were presented in a higher number of inflorescences; as the season advanced, the later stages began to appear and rapidly became the most represented; by the end of the flowering season, only these stages were present. In the western sector, the earlier maturation stages are less represented in the disturbed site than in the control site, in the overall sampling event (Figure 11).

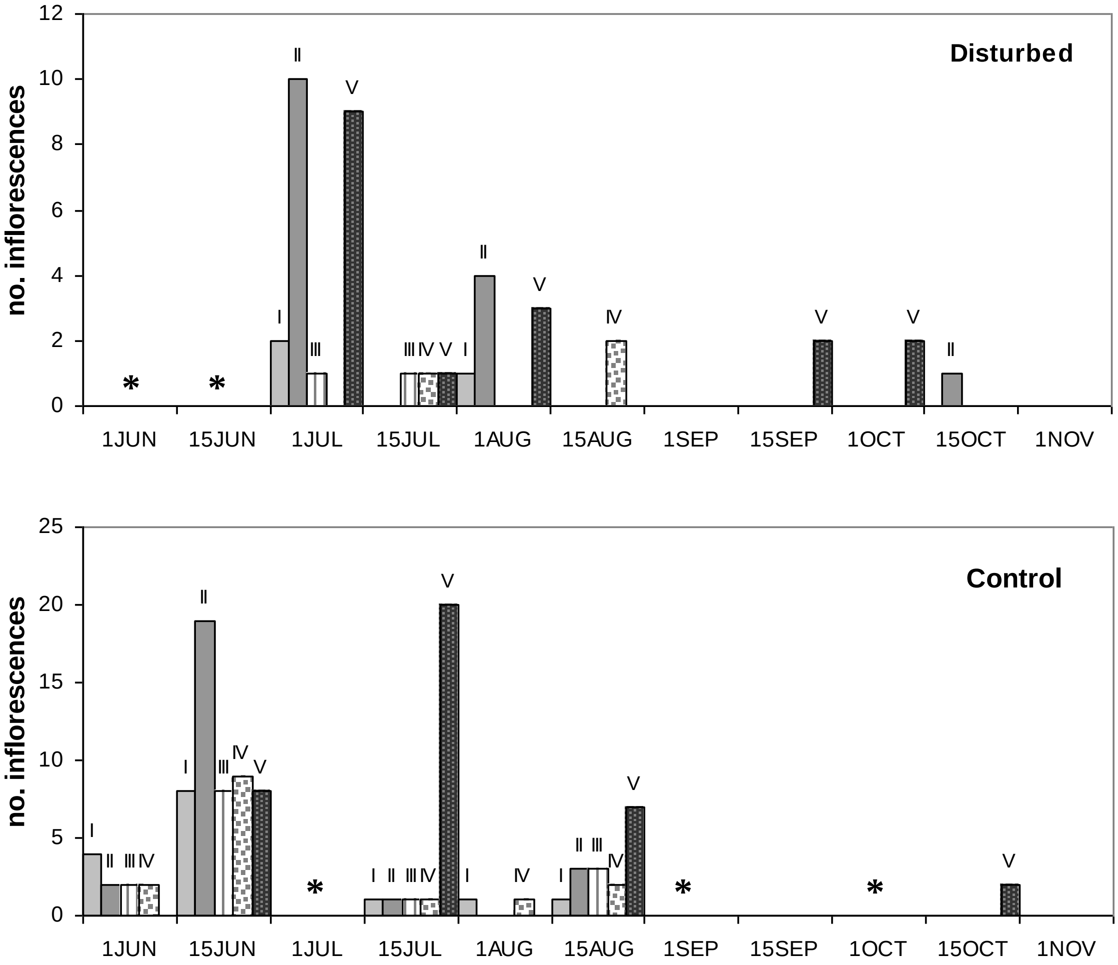

Figure 12 – Evolution of maturation stages along the sampling period, in the control meadows and in the disturbed meadows by clam harvesting activity at the western sector of the Ria Formosa; (*) not sampled.

Concerning the eastern sector, it was observed that in the control site the earlier stages disappeared from September onwards, whereas in the disturbed site all the stages remained present until the end of the flowering season. Moreover, inflorescences at stage V are in much greater number in the disturbed site than in the control (Figure 12).

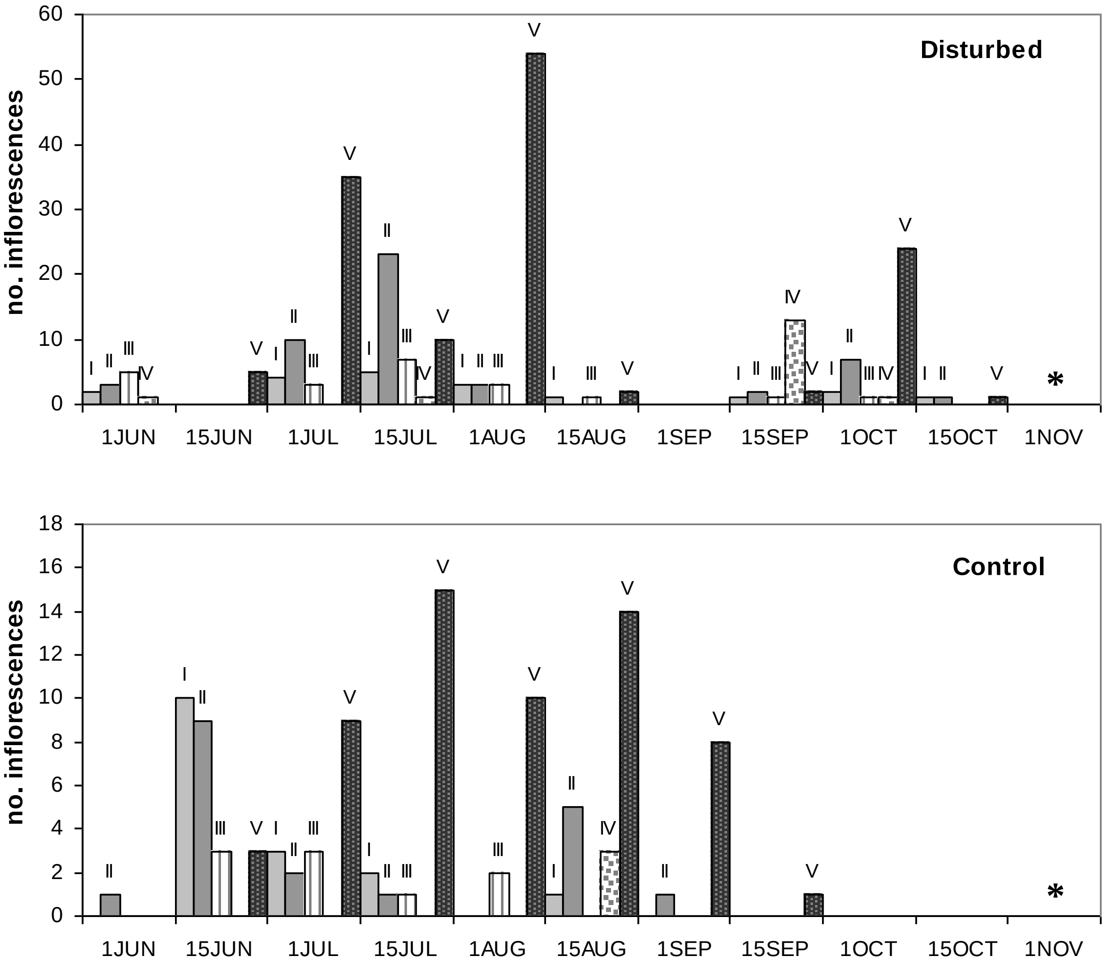

Figure 13 – Evolution of maturation stages along the sampling period, in the control meadows and in the disturbed meadows by clam harvesting activity at the eastern sector of the Ria Formosa; (*) not sampled.

4.4 Discussion

The results presented in this descriptive study indicate an adverse effect of the clam harvesting activity on the *Zostera noltii* abundance, as shown by the lower vegetative shoot density observed in the disturbed sites, in both sectors. The visual impact of disturbance was evident in the field; the disturbed meadows were highly fragmented, not only because of the cut and the removal of the plants but also because of the regular trampling of the fishermen. The negative effects of human trampling on seagrass cover by both reducing shoot density and rhizome biomass were confirmed by Eckrich & Holmquist (2000) for the seagrass *Thalassia testudinum*. Since these disturbances (clam harvesting and trampling) are frequent and persistent in the Ria Formosa lagoon, the disturbed meadows are not able to recover and sustain the high vegetative shoot densities characteristic of the undisturbed meadows. However, some results obtained for *Z. marina* suggest that, in the absence of clam harvesting activity, disturbed meadows have a great capacity to rapidly recover from this type of disturbance (Boese 2002).

In the western studied site, the disturbance by clam harvesting activity and trampling is more intense than in the eastern site. In the western site, *Z. noltii* meadows are easily accessed by people in general and thus the daily number of fishermen and visitors is probably higher. This is confirmed by the lower vegetative shoot density observed in the western disturbed site compared to the eastern one. In the eastern site, which is a private concession, the disturbance intensity must be lower than in the western site because only the owner of the concession clam harvests the meadow. In here, *Z. noltii* vegetative shoot density was higher than in the western disturbed site. However, the reproductive effort was lower in the western disturbed site, where disturbance is more intense. It may be hypothesised that the disturbance intensity here is too high for vegetative and sexual growth to maintain the meadow in the same density levels verified in the eastern disturbed site.

The results obtained showed that the reproductive effort was higher in clam harvested meadows that in undisturbed meadows. The quantification of the sexual reproductive effort provides information about the allocation of biomass to sexual reproduction in the plant and is, therefore, a useful measure of the energy investment of the species in that process (Kaldy & Dunton 2000). The higher reproductive effort observed in the present study in the disturbed sites reflects the higher investment of the species in sexual reproduction as response to the disturbance by clam harvesting. The disturbance by clam harvesting produced the fragmentation of the meadows. Auby & Labourg (1996) have also found higher values of maximal reproductive effort of *Z. noltii* in colonizing beds (27%), which are fragmented, in comparison to stabilized areas (5%). On the contrary, in Ria Formosa, continuous meadows near the Barra inlet showed higher values of reproductive effort (7%) in comparison to fragmented meadows (3%) (Alexandre *et al.* unpublished data). These higher values of reproductive effort found in the continuous meadows near the Barra may be related to the Barra opening. These meadows may be apparently stabilized and they are probably still recovering from the disturbance produced by the sediment dynamics after the Barra opening.

The flowering shoot density varies according to different types of disturbance. In Ria Formosa, the maximum density of 2200 flowering shoots m^{-2} recorded in *Z. noltii* meadows disturbed by clam harvesting activity is very similar to that obtained in australian *Z. capricorni* beds recovering from dugong grazing (2169 flowering shoots m^{-2}) (Peterken & Conacher 1997). *Z. noltii* meadows recently formed by dredging materials in the lagoon of Venice presented a much smaller mean flowering shoot density (111 ± 31.4 flowering shoots m^{-2}) (Curiel *et al.* 1996). This variability on flowering shoot density suggests that recovery depends not only on the species but also on the intensity and frequency of disturbance. The high flowering density found in this study evidences the potential capacity of *Z. noltii* in the Ria Formosa to make use of the sexual reproduction in response to the disturbance.

In the disturbed sites of the Ria Formosa, not only the earlier stages remained present until the end of the flowering season as there were also a higher number of inflorescences producing fruits at this time. These observations evidence the higher sexual reproductive investment of these plants in face of disturbance, in a continued effort to produce new flowering shoots and, consequently, more fruits and seeds. To our knowledge, there are no previous studies on the analysis of the maturation stages along the flowering season in *Z. noltii* with which this study might be compared.

The large variability in the observations revealed a clumped distribution pattern of the flowering shoots in the meadow. There were corers with a high number of shoots, while there were others collected at the same time with no shoots at all. In future work, the sampling effort must be increased to reduce this variability.

As pointed out by Hemminga & Duarte (2000), the most critical factors in the sexual reproduction success of seagrass populations seem to be flowering and seedling survival. In this study, a higher flowering shoot density and sexual reproductive effort were observed in *Z. noltii* meadows under disturbance. However, the real contribution of sexual reproduction in the maintenance of disturbed meadows can only be achieved when considering the overall reproduction cycle and the reproductive success. Determinations of seed production, the seed germination rate and seedling survival are imperative in assessing the effective contribution and the success of the sexual reproduction of *Z. noltii* in maintaining the species. These studies are currently being developed for *Z. noltii* in Ria Formosa.

5. Experimental study

5.1 Introduction

As mentioned in the previous chapter, the Ria Formosa lagoon is traditionally clam harvested every day by a considerable number of fishermen. The clams collected here represent the largest amount at a national level. As indicated by the results obtained in the descriptive study, the fishing practices employed by the local fishermen generate severe local reductions in the density of *Zostera noltii* meadows, being a source of habitat fragmentation. However, a great capacity to recover from different types of disturbance has been demonstrated by several species at different localities (Rasheed 1999; Boese 2002; Plus *et al.* 2003). In most cases, the recolonization and the re-establishment process is attributed both to the sexual reproduction success, by means of a high seedling survival rate, and to a rapid vegetative recruitment (Plus *et al.* 2003). The coupling of sexual reproduction and vegetative growth appears to be essential in the re-establishment of certain disturbed seagrass beds.

The increase in the production of flowering shoots as an enhancement of the sexual reproduction investment have been suggested as a species response to a disturbance, not only by results obtained in the descriptive study presented but also by other authors (Gallegos *et al.* 1992; Marbà & Duarte

1995). In the previous chapter it was observed that disturbed meadows showed a higher reproductive effort and that, in the eastern sector, the disturbed meadow presented a higher flowering shoot density as well. Once the descriptive study has revealed that the clam harvesting activity increases the flower density of *Z. noltii*, a controlled experiment was carried out to validate this observation.

The objective of the present chapter is to quantify experimentally the reproductive effort of *Zostera noltii* after an induced clam harvesting treatment. The capacity of the species to recover from the experimental disturbance was also investigated.

5.2 Method

The experimental design consisted of twelve 1 m^2 quadrats (6 treatments and 6 controls) randomly placed in an undisturbed Z*ostera noltii* meadow. The treatment was applied to 6 quadrats, which consisted in disturbing the meadow inside the quadrats using the same technique employed by the local harvesters to remove the clams. This technique consists of furrowing and revolving the sediment about 10 cm deep with a clam digging knife. The sediment and the seagrass are then pushed backward.

Each quadrat was sub-divided into 25 sub-quadrats. In each sampling event, five sub-quadrats (replicate samples) were collected from each $1m^2$ quadrat, using a 12 cm ø corer. The sub-quadrats were randomly collected by a sampling without replacement so that replicate corers could not be taken twice in the same sub-quadrat. The treatment was applied one month before the beginning of the sampling. All quadrats were collected on a fortnightly basis from August to October. Sampling ended in October when no more flowers were observed in the corers. Each replicate sample was analyzed in order to assess both vegetative and flowering shoot density, as well as their above-ground biomass oven-dried during 48h at 60ºC to calculate the reproductive effort. The reproductive effort was determined as the proportion of total shoot biomass allocated to sexual reproduction (flowering shoots) (Kaldy & Dunton 2000).

Significant differences in vegetative shoot density between control and treatment plots were tested using a Student's t-test. In order to avoid the huge variability within samples, created by a high number of zero values in the flowering shoot density, the total number of flowering shoots per m^2 was used in Chi-square calculations to test significant differences between control and treatment plots. Significant differences in the reproductive effort between control and treatment plots were tested using a Z-test for proportions (Zar 1999). The proportion values were obtained by dividing the total flowering shoot biomass to the total shoot biomass (vegetative + flowering shoot biomass) present in all corers within each date.

5.3 Results

The clam harvesting treatment had a negative impact on the vegetative shoot density of *Zostera noltii*. Even though there were no significant differences between the treatment quadrats and the control (p>0.05) one month after the experiment (August 02), in the two subsequent months (September and October 02) the shoot density in the clam harvested plots was significantly lower (p<0.05) (Figure 13). One year after the experiment (August 03), no significant differences were detected in shoot density between control and treatment quadrats (p>0.05).

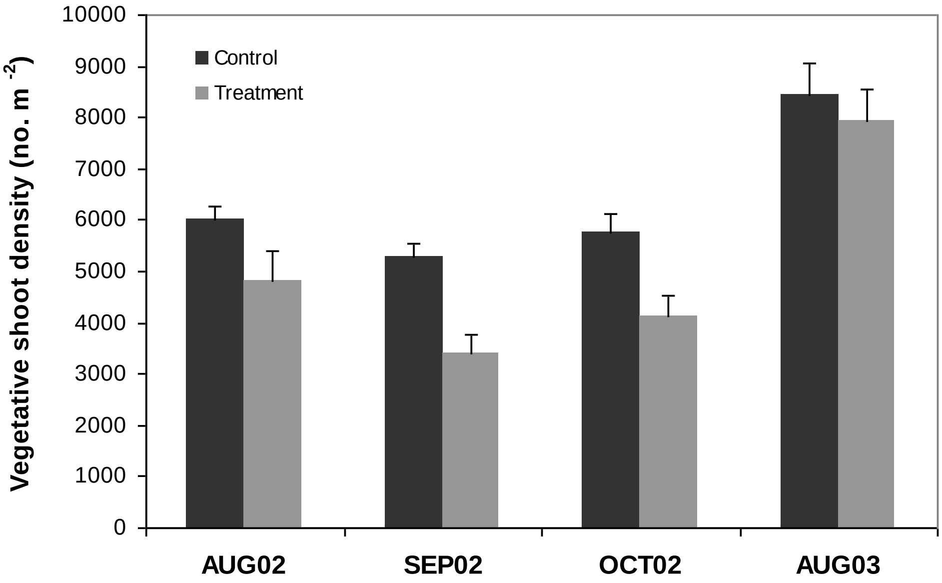

Figure 14 – Vegetative shoot density of *Zostera noltii* in the control and in the treatment (Mean ± S.E.); Control n=30, Treatment n=30.

In August 02, one month after the treatment, the control plots exhibited a significantly higher number of total flowering shoots per m^2 than the treatment plots ($p<0.01$) (Figure 14). However, one month later, in September 02, the total number of flowering shoots per m^2 was significantly higher in the treatment plots than in the control ($p<0.01$). In October 02, no flowering shoots were recorded in the samples, which may be related to the end of the flowering season. One year after the beginning of the experiment, the number of flowering shoots per m^2 in the control was about three-fold higher (1238 to 4246 shoots) while in the treatment it was almost nine-fold higher (973 to 8493 shoots).

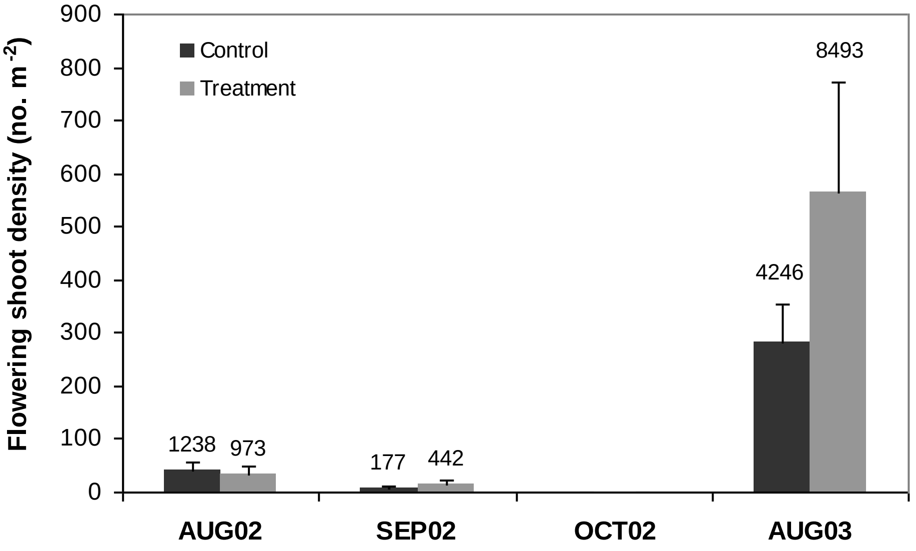

Figure 15 – Flowering shoot density of *Zostera noltii* in the control and in the treatment (Mean ± S.E.); Control n=30, Treatment n=30; Numbers above bars refer to the total number of flowering shoots per m^2.

No significant differences in the reproductive effort of *Z. noltii* between the control and treatment plots were observed in August 02, one month after the treatment. In all the subsequent sampling dates, the reproductive effort was significantly higher (p>0.05) (Figure 15).

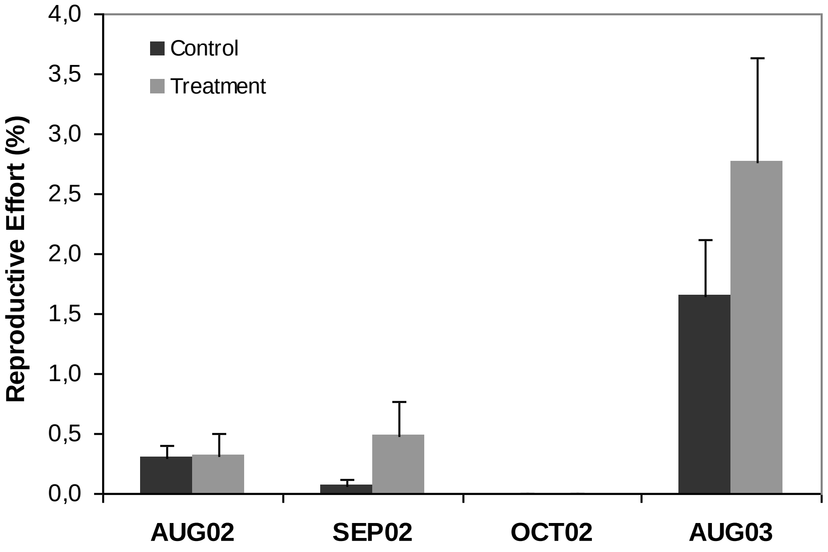

Figure 16 – Reproductive effort (%) of *Zostera noltii* in the control and in the treatment (Mean ± S.E.); Control n=30, Treatment n=30.

5.4 Discussion

The results obtained in this experimental study confirmed the negative impact of the clam harvesting activity on Zostera *noltii* shoot density observed previously in the descriptive study. In spite of the immediate negative visual impact produced by the disturbance, the effect of shoot decrease was only observed two months after the treatment. The clam harvesting technique consisted of cutting the shoots and rhizomes, pushing both the seagrass and the sediment backward. After this procedure, the damaged shoots may remain half-buried and stuck in the sediment for many tidal cycles until they are finally released by the tidal currents. Consequently, there is a probability for some of these shoots to be included in the samples collected one month after the clam harvesting treatment, thus overestimating the total shoot density alive within each sample.

Vegetative shoot densities returned to pre-treatment levels after one year, which reveals the great capacity of this species to recover from the clam harvesting treatment through vegetative development. *Z. noltii* has been described as a fast growing species with a rapid vegetative recruitment and, consequently, with a high recovery rate (Vermaat *et al.* 1987; Laugier 1999; Peralta 2000). Despite this, recovery depends not only on the species but also on the intensity and frequency of disturbance (Curiel *et al*. 1996; Peterken & Conacher 1997). The recovery of *Z. marina* stands became endangered when the disturbance intensity was such that neither rhizomes nor roots were left in the sediment (De Jonge & De Jong, 1992). The frequency of the disturbance employed in this experimental study was lower than that of the daily clam harvesting meadows because the treatment was applied only once at the beginning of the experiment. Moreover, the shoots were not totally removed from the sediment by the clam harvesting treatment. Thus, such recoveries are not expected in daily clam harvested meadows or in meadows in which the plants are completely removed from the sediment, has happen often in determined areas of the Ria Formosa.

The enhancement of flowering shoots induced by the clam harvesting treatment was evident, as shown by the higher number of shoots found in the clam harvested quadrates two months and one year after the treatment. In September, the number of flowering shoots remarkably decreased in the control plots, probably due to the ending of the flowering season. Even so, the shoot decrease verified in the treatment plots was less than in the control. In spite of the flowering shoot decrease due to the end of the flowering season, there was a higher investment in new shoot production induced by the disturbance. The production of new flowering shoots must be a rapid and continuous process during the reproductive season, in these disturbed meadows. The analysis of the development of the maturation stages in the second chapter of this study supports this hypothesis, as in the disturbed sites a higher number of flowering shoots were present in the first stages of maturation at the end of the flowering season. These results indicate that *Z. noltii* is able to increase the production of flowering shoots and the sexual reproductive effort as a response to the disturbance.

The rapid recovery of seagrass meadows after being disturbed is frequently attributed to the fast clonal growth of the species (Rasheed 1999) but re-establishment can also be made by seedling recruitment (Peterken & Conacher 1997). In some cases, even after flowering and fruiting events, no significant recovery by sexual means was observed and the seeds stored in the sediment played no role in recovery (Rasheed 1999). The entire recovery of the *Z. noltii* clam harvested plots in the year subsequent to the treatment is probably related to the species high clonal growth, once it is a typical colonizing species. Vegetative recolonization probably occurs through vegetative growth of the plants at the edges of the disturbed area (Preen 1995). Nevertheless, the higher investment in sexual reproduction observed in the clam harvested plots suggests that sexual recruitment must also play a significant role in the maintenance of this species, although it might not be the main one. The seeds released into the sediment at the end of the flowering season may be stored there throughout the winter, forming a seed bank, and possibly germinate in the next spring, producing new shoots (seedlings). In the course of this study, neither seeds nor seedlings were found in the sediment, even after sieving, as it was also reported by Loques *et al.* (1988) for *Z. noltii*. This may be related to the low seed viability of the species. Even if seeds are viable in the sediment and germinate, seedling survival is characterized by a strong interaction between temperature and salinity. The highest *Z. noltii* seed germination rates required salinities of 1‰ and water temperatures of 10ºC, and the maximal potential recruitment by seeds is at 10‰ and 30ºC. It was also estimated that less than 5% of *Z. noltii* plants originated from seeds (Hootsmans *et al.* 1987). In Ria Formosa, such low salinities occurred infrequently during low tide after a winter heavy rainfall (personal observation). The coupling of 30ºC and 10‰ in water temperature and salinity is highly improbable in the Ria, except after summer heavy rainfalls. Beside these crucial factors to seed germination, seedling development may be inhibited or the seedlings may not survive due to the strong shading effect of older shoots within a dense meadow (Harrison 1993; Olesen & Sand-Jensen 1994). Thus, given the fast clonal growth character of *Z. noltii* and the low probability in coupling the salinities and the temperatures required to seed germination and development, the recovery of disturbed meadows must occur mainly by vegetative development than by sexual reproduction.

All these constraints to seed development suggest that *Zostera noltii* sexual reproduction appears to play a secondary role in the species maintenance and/or in the re-establishment from a disturbance in Ria Formosa, nonetheless the high investment in the production of flowering shoots and seeds. Although sexual reproduction may not be considered the main via by which *Z. noltii* recovers from an intense disturbance, the advantage properties of seed dispersion appears to be essential in the colonization of new areas (Buia & Mazzella 1991; Curiel *et al.* 1996) improving genetic diversity, or in the recolonization of areas where disturbance intensity have produced total losses over particular areas of the meadows (Duarte & San-Jensen 1990).

6. General Discussion

In Ria Formosa, the flowering season of *Zostera noltii* goes from March until November, but little variations may happen from year to year or even from place to place (Hootsmans *et al*. 1987; Loques et al 1988; Curiel *et al*. 1996). Several factors are responsible for the triggering the flowering event, namely the water temperature, the daylength, fluctuating salinity regimes and tidal amplitude (McMillan 1982; Pettit 1984; Loques *et al*. 1988; Phillips & Meñez 1988; Buia & Mazzella 1991; Ramage & Schiel 1998). In Ria Formosa, the flowering season is probably related with the increasing water temperature and irradiance at the spring time, and the end of the flowering event may be related with the heavy rainfalls in the autumn, which reduce the water salinity.

The male to female flower ratio found in this study (1:1) was similar to that observed by Loques *et al*. (1988) for *Zostera noltii*, but is lower than that reported in Conacher *et al*. (1994) for *Z. capricorni* (2.2:1). The male to female ratio may provide an index of pollination potential because as higher is the male to female ratio the higher is also the probability of the female flowers to be pollinated (Conacher *et al*. 1994).

Both in the descriptive and in the experimental study, the results obtained showed that the clam harvesting activity is responsible for the lower vegetative shoot density observed in the disturbed meadows, when compared to the non-clam harvested ones. This negative effect, together with the trampling, is visible in the field and produces the fragmentation of the meadows. The clam harvesting activity also induces the production of flowering shoots. Higher values of reproductive effort were also observed in the disturbed fragmented meadows, which reflect the higher investment of *Z. noltii* in the sexual reproduction as a response to the disturbance by clam harvesting. Higher values of reproductive effort were also observed in fragmented *Z. noltii* meadows in France (Auby & Labourg 1996). In Ria Formosa, non-fragmented meadows near the Barra inlet presented values of reproductive effort similar to that found in the meadows disturbed by clam harvesting activity (Alexandre *et al*. unpublished data). These non-fragmented meadows may be apparently stabilized and are probably still recovering from the disturbance produced by the sediment dynamics after the Barra opening.

Z. noltii is described as a fast growing species with a rapid vegetative development (Vermaat *et al.* 1987; Laugier 1999; Peralta 2000). Despite this characteristic to rapid recover from disturbance (Rasheed 1999), the re-establishment of *Z. noltii* the meadows can also be made by seedling recruitment through sexual reproduction (Peterken & Conacher 1997). Recolonization by sexual reproduction requires the successful completion of the entire plant life cycle. In this study, a higher flowering shoot density and sexual reproductive effort were observed in *Z. noltii* meadows under disturbance. However, neither seeds nor seedling were found in the sediment. In spite of the high viability of *Z. noltii* seeds (70%) (Alexandre & Cabaço unpublished data), the low salinity and high temperature conditions required for seed germination and seedling development are possibly the major constraints to the success of the sexual reproduction pattern in recovering from disturbance by clam harvesting. Nonetheless the high investment in the production of flowering shoots and seeds, sexual reproduction in Z. noltii appears to play a secondary role in the recovery from disturbance by clam harvesting. However, the advantage properties of seed dispersion appears to be essential in the colonization of new areas (Buia & Mazzella 1991; Curiel *et al.* 1996) improving genetic diversity, or in the recolonization of areas where disturbance intensity have produced total losses over particular areas of the meadows (Duarte & San-Jensen 1990). Experiments to analyse the rate of success of seedling establishment and development into new bare areas would provide invaluable information on the real contribution of the sexual reproduction in maintaining the existence of the species or in the species propagation over long distances.

7. Bibliographic References

Adam, P. (1990) Saltmarsh ecology. Cambridge University Press, New York.

Alongi, D. (1998) Coastal ecosystem processes. CRC Press, Boca Raton.

Auby, I., Labourg, P. (1996) Seasonal dynamics of *Zostera noltii* Hornem. in the bay of Arcachon (France). Journal of Sea Research, 35 (4): 269-277.

Boese, B. (2002) Effects of recreational clam harvesting on eelgrass (*Zostera marina*) and associated infaunal invertebrates: in situ manipulative experiments. Aquatic Botany, 73: 63-74.

Buia, M., Mazzella, L., Pire, H., Russo, G. (1985) Fioritura di *Zostera noltii* Hornem a Ischia (Golfo di Napoli) Oebalia, XI, 3: N.S.

Buia, M., Mazzella, L. (1991) Reproductive phenology of the Mediterranean seagrasses *Posidonia oceanica* (L.) Delile, *Cymodocea nodosa* (Ucria) Aschers., and *Zostera noltii* Hornem. Aquatic Botany, 40: 343-362.

Constanza, R., Arge, R., Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K. Naeem, S., O'Neill, R., Paruelo, J. Raskin, R., Sutton, P., Belt, M. (1997) The value of the world's ecosystem services and natural capital. Nature, 387: 253-260.

Conacher, C., Poiner, I., O'Donohue, M. (1994) Morphology, flowering and seed production of *Zostera capricorni* Aschers. in subtropical Australia. Aquatic Botany, 49: 33-46.

Curiel, D., Bellato, A., Rismondo, A., Marzocchi, M. (1996) Sexual reproduction of *Zostera noltii* Hornemann in the lagoon of Venice (Italy, north Adriatic). Aquatic Botany, 52: 313-318.

Dawes, C. (1981) Marine botany. John Wiley and Sons, New York.

De Cock, A. (1980) Flowering, pollination and fruiting in *Zostera marina* L. Aquatic Botany, 9: 201-220.

De Jonge, V., De Jong, D. (1992) Role of tide, light and fisheries in the decline of *Zostera marina* L. in the Dutch Wadden Sea. Netherlands Institute for Sea Research Publication Series, 20: 161-76.

Den Hartog, C. (1970) The seagrasses of the world. North-Holland, Amsterdam.

Duarte, C., Chiscano, C. (1999) Seagrass biomass and production: a reassessment. Aquatic Botany, 65: 159-174.

Duarte, C., Sand-Jensen, K. (1990) Seagrass colonization: patch formation and patch growth in Cymodocea nodosa. Marine Ecology Progress Series, 65: 193-200.

Eckrich, C., Holmquist, J. (2000) Trampling in a seagrass assemblage: direct effects, response of associated fauna, and the role of substrate characteristics. Marine Ecology Progress Series, 201: 199-209.

Everett, R., Ruiz, G., Carlton, J. (1995) Effect of oyster mariculture on submerged aquatic vegetation: an experimental test in a Pacific NorthWestern estuary. Marine Ecology Progress Series: 125: 205-217.

Falcão, M. & Vale, C. (1990) The Ria Formosa ecosystem study: benthic nutrient remineralization and tidal variability of nutrients in the water. Hydrobiology, 207: 137-146.

Gallegos, M., Merino, M., Marbà, N., Duarte, C. (1992) Flowering of *Thalassia testudinum* Banks ex König in the Mexican Caribbean: age-dependence and interannual variability. Aquatic Botany, 43: 249-255.

Goubin, C., Loques, F. (1991) Germinating *Zostera noltii* Hornemann found in the Etang de Diana, Corsica. Aquatic Botany, 42: 75-79.

Harrison, P. (1993) Variations in demography of *Zostera marina* and *Z. noltii* on an intertidal gradient. Aquatic Botany, 45: 63-77.

Hemminga, M., Duarte, C. (2000) Seagrass Ecology. Cambridge University Press.

Hootsmans, M., Vermaat, J., 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: 275-285.

Irlandi, E. (1997) Seagrass patch size and survivorship of an infaunal bivalve. Oikos, 78: 511-518.

Kaldy, J., Dunton, K. (2000) Above- and below-ground production, biomass and reproductive ecology of *Thalassia testudinum* (turtle grass) in a subtropical coastal lagoon. Marine Ecology Progress Series, 193: 271-283.

Keddy, C. (1987) Reproduction of annual eelgrass: variation among habitats and comparison with perennial eelgrass (*Zostera marina* L.). Aquatic Botany, 27: 243-256.

Loques, F., Caye, G., Meinesz, A. (1988) Flowering and fruiting of *Zostera noltii* in Golf Juan (French Mediterranean). Aquatic Botany, 32: 341-352.

Loques, F., Caye, G., Meinesz, A. (1990) Germination in the marine phanerogam *Zostera noltii* Hornemann at Golf Juan, French Mediterranean. Aquatic Botany, 38: 249-260.

Marbà, N., Duarte, C.M. (1995) Coupling of seagrass (*Cymodocea nodosa*) patch dynamics to subaqueous dune migration. Journal of Ecology, 83 (3): 381-389.

Marbà, N. Walker, D. (1999) Growth, flowering and population dynamics of temperate Westernern Australian seagrasses. Marine Ecology Progress Series, 184: 105-118.

McMillan, C. (1982) Reproductive physiology of tropical seagrasses. Aquatic Botany, 14: 245-258.

Morais, A. & Carvalho, C. (1992) A pesca no Algarve: principais números. 7º Congresso do Algarve. Edições Racal Clube, Silves. 1: 419-430.

Olesen, B., Sand-Jensen, K. (1994) Demography of shallow eelgrass (*Zostera marina*) populations: shoots dynamics and biomass development. Journal of Ecology, 82: 379-390.

Orth, R., Luckenbach, M., Moore, K. (1994) Seed dispersal in a marine macrophyte: implications for colonization and restoration. Ecology, 75: 1927-1939.

Orth, R., Moore, K. (1983) Seed germination and seedling growth of *Zostera marina* L. (eelgrass) in the Chesapeake Bay. Aquatic Botany, 15: 117-131.

Peterken, C., Conacher, C. (1997) Seed germination and recolonization of *Zostera capricorni* after grazing by dugongs. Aquatic Botany, 59: 333-340.

Peralta, G. (2000) Estudios sobre el crecimiento en *Zostera noltii* Hornem.: Dinámica estacional y aspectos ecofisiológicos. Tese de Doutoramento. Universidade de Cádiz.

Pettit, J. (1984) Aspects of flowering and pollination in marine angiosperms. Oceanography and Marine Biology Annual Reviews, 22: 315-342.

Phillips, R., Meñez, E. (1988) Seagrasses. Smithsonian Contribution to the Marine Sciences. 34. Washington D.C. Smithsonian Institution.

Pirc, H., Buia, M., Mazzella, L. (1986) Germination and seedling development of *Cymodocea nodosa* (Ucria) Ascherson under laboratory conditions and “in situ”. Aquatic Botany, 26: 181-188.

Plus, M., Deslous-Paoli, J., Dagault, F. (2003) Seagrass (*Zostera marina* L.) bed recolonisation after anoxia-induced full mortality. Aquatic Botany, 77(2): 121-134.

Preen A. (1995) Impacts of dugong foraging on seagrass habitats: Observational and experimental evidence for cultivation grazing. Marine Ecology Progress Series, 124: 201-213.

Ramage D., Schiel D. (1998) Reproduction of the seagrass *Zostera novazelandica* on intertidal platforms in southern New Zealand. Marine Biology, 130: 479- 489.

Rasheed, M. (1999) Recovery of experimentally created gaps within a tropical *Zostera capricorni* (Aschers.) seagrass meadow, Queensland Australia. Journal of Experimental Marine Biology and Ecology, 235: 183-200.

Short, F. (1987) Effects of sediment nutrients on seagrasses: literature review and mesocosm experiment. Aquatic Botany, 27: 41-57.

Short, F., Coles, R., Pergent-Martini, C. (2001) Global seagrass distribution. pp. 5-30. *In*: F. Short, R. Coles (Eds). Global Seagrass Research Methods. Elsevier, The Netherlands.

Vermaat JE, Hootsmans MJM, Nienhuis PH (1987) Seasonal dynamics and leaf growth of *Zostera noltii* Hornem., a perennial intertidal seagrass. Aquatic Botany 28: 287-299

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, Upper Saddle River, NJ