Universidade de Évora

Reproductive Ecology of *Sargassum muticum* (Yendo) Fensholt in Viana do Castelo (Northern Portugal)

Ecologia Reprodutiva do *Sargassum muticum* (Yendo) Fensholt em Viana do Castelo (Norte Portugal)

Carla Alexandra da Silva Monteiro

Tese para obtenção do grau de mestre em Biologia e Ecologia do Litoral Marinho

Orientadores
Doutor Rui Santos
Doutor Aschwin Engelen

Évora • 2007

Dissertação de Tese de Mestrado, apresentado à Universidade de Évora

Intitulada:

Reproductive Ecology of *Sargassum muticum* (Yendo) Fensholt in Viana do Castelo (Northern Portugal)

Ecologia Reprodutiva do *Sargassum muticum* (Yendo) Fensholt em Viana do Castelo (Norte Portugal)

Apresentada por:

Carla Alexandra da Silva Monteiro

para aceder ao grau de mestre em Biologia e Ecologia do Litoral Marinho

Orientadores

Doutor Rui Santos[1]
Doutor Aschwin Engelen[1]

[1]ALGAE research group, CCMAR, CIMAR-Laboratório Associado, Universidade do Algarve, FCMA, Campus de Gambelas, 8005-139 Faro, Portugal

Évora • 2007

O conteúdo desta dissertação é de exclusiva responsabilidade da autora

Dezembro de 2007

Agradecimentos

Agradeço aos meus orientadores, Doutor Rui Santos e Doutor Aschwin Engelen por terem aceite a orientação deste trabalho e pelo apoio no decorrer desta investigação.

À Doutora Ester Serrão e ao Doutor Gareth Pearson pelo apoio na parte estatística e também pelos preciosos apontamentos e criticas efectuados que beneficiaram este trabalho.

Aos Amigos do Mar que mais uma vez me receberam de uma forma calorosa e por terem sempre demonstrado disponibilidade para me acolherem no vosso laboratório.

Agradeço a hospitalidade dos meus tios, Lurdes e Fernando, que mais uma vez me acolheram como se fosse uma filha. Obrigado pelos cuidados e preocupações que tiveram nas minhas "aventuras de campo", sozinha na praia Norte.

Aos meus amigos, pela energia e apoio que sempre me concederam.

Ao Luís por tudo. Obrigado pela compreensão e cooperação nos momentos mais difíceis.

Aos meus pais e ao meu irmão que sempre me apoiaram nas minhas escolhas académicas e profissionais, mesmo quando não compreendiam os meus objectivos.

Aos meus avós, dedico este trabalho. Mesmo na ausência física a vossa presença permanecerá para sempre no meu coração.

The first manuscript (Chapter 1) of this thesis was submitted to a journal with international referees: Marine Ecology Progress Series

Contents

Ecologia Reprodutiva do *Sargassum muticum* (Yendo) Fensholt
em Viana do Castelo (Norte Portugal)

Resumo

O sucesso das espécies invasoras depende das suas características intrínsecas e das do ecossistema. Estudar estratégias reprodutivas nos estados iniciais de vida é importante na compreensão das adaptações das espécies invasoras. Esta tese pretende descrever e comparar a ecologia reprodutiva da alga *Sargassum muticum* em dois *habitats*: poças na zona intermareal média e canal na zona intermareal inferior.

O primeiro Capítulo estuda quando ocorre expulsão de gâmetas e assentamento de embriões, revelando que ambos apresentam uma periodicidade semilunar durante as marés vivas. Variações temporais no momento de expulsão dos ovos entre *habitats*, sugerem que a amplitude de maré apresenta um papel importante neste processo. O segundo Capítulo foca diferenças entre habitats no sucesso de recrutamento e de sobrevivência dos micro-recrutas. O recrutamento e a sobrevivência dos micro-recrutas foram superiores nas poças do que no canal e demonstrou-se que a meso-herbivoria é importante na sobrevivência do micro-recrutas, especialmente nas poças.

Reproductive Ecology of *Sargassum muticum* (Yendo) Fensholt
in Viana do Castelo (Northern Portugal)

Abstract

The success of invasive species is dependent on intrinsic characteristics of the species and on ecosystem characteristics. Studies of reproductive strategies in early life history are important to understand adaptations of invasive species. The aim of this thesis is to describe and compare reproductive ecology of the invasive seaweed, *Sargassum muticum* in two different habitats: mid-intertidal pools and a low-intertidal channel.

Chapter one focuses on the timing of egg expulsion and embryo settlement and reveals that both processes had a semilunar periodicity, during spring tides. Temporal variation in the timing of egg expulsion between habitats suggests that tidal amplitude cues play an important role in this process. Chapter two focuses on habitat related differences in recruitment success and micro-recruit survival. Recruitment and micro-recruit survival were higher in the pools than in the channel and showed that meso-herbivory plays an important role on micro-recruit survival, especially in the pools.

General Introduction

Introductions of species across natural barriers or biological invasions, affect the integrity of natural communities in many different ecosystems around the world (Vitsousek et al. 1997). As a consequence of these and others effects, non-indigenous species potential alter native communities and cause biodiversity loss worldwide (Wilson 1992, Villele & Verlaque 1995, Wilcove et al. 1998, Levin et al. 2002). The success of invasive species is a wide environmental tolerance, meaning a tolerance to the stresses of environmental fluctuations and extremes (Boudouresque & Verlaque 2002), ability to grow on a wide variety of substrates, from sand to artificial substrates, a great grazing resistance and finally a somewhat higher impact through a large size and their morphology (Nyberg & Wallentinus 2005). The success of reproductive features, the relative rates of settlement, survival and growth are extremely important for the persistence of any, introduced or native population (Neushul et al. 1976). Nerveless, ecological information and especially experimental data about growth strategies, life span and effects of grazers are often lacking (Nyberg & Wallentinus 2005).

Sargassum muticum: introduction and establishment

The Japanese brown seaweed *Sargassum muticum* (Yendo) Fensholt is one of the most invasive algae in Europe and North America coasts (Norton 1977b) and most probably accidentally introduced with *Crassostrea gigas* (Thunberg) imported from Japan (Druehl 1973, Farnham et al. 1973, Critchley & Dijkema 1984). Consequently this oyster constituted a primary vector for the introduction and subsequent spread of *S. muticum* within in European waters (Critcley & Dijkema 1984) and west coast of North America in the late 1940's (Ambrose & Nelson 1982). It has rapidly spread and firmly established itself as a major coloniser of lower littoral and shallow sublittoral regions (Critchley 1983a). The first record of *S. muticum* was in San Juan Island in 1948 and by 1955 was widespread and abundant throughout the area (Norton 1977b). Initially restricted to the Pacific Northwest, it spread to Mexico (Espinoza 1990). In Europe, this specie was found for the first time at Bembridge, Isle of Wight (England) in 1973 (Farham et al. 1973) and presently is distributed from Norway to Portugal (Pru'homme van Reine 1977a,b, Lluch et al. 1994).

Life history of *Sargassum muticum*

Sargassum muticum is a pseudo-perennial alga with an annual cycle of growth and reproduction in which the newly recruited individuals and the new fronds of perennial holdfast begin to grow in the early fall. The individuals grow vegetatively for a period of time and then undergo a period of reproduction. After reproduction, the fronds become senescent and die back, leaving only the perennial holdfast and basal leaves (Norton 1976).

Sargassum muticum is monoecious, which develops specialized reproductive organs called receptacles alternatively along the axes of terminal branchlets. Inside the receptacles are numerous spherical conceptacles, in separate conceptacles eggs and sperm are produced, called oogonia and antheridia, respectively (Fletcher & Fletcher 1975b). Receptacles are recognisably swollen at a fairly stage in development being lanceolate in shape and borne on a short stalk (Fletcher & Fletcher 1975a). Fertilization is external and this species has the ability for self-fertilization (Fletcher 1980, Norton 1981). Eggs are expelled from receptacles in a number of discrete pulses, but instead of being released immediately into the seawater, are fertilized and retained outside the receptacle for one to several days after fertilization and developing rhizoids (Fletcher 1980, Norton 1981, Deysher & Norton 1982, Umezaki 1984, Hales & Fletcher 1990).

The embryos of *Sargassum muticum* are able to grow and develop under a wide range of temperatures between 10-30°C, with a optimal temperature at 25°C (Deysher 1984, Hales & Fletcher 1990) and with a wide range of salinity (Norton 1977a) between 14.7 and 27.1 ‰ (Steen 2004). In optimal conditions young embryos ranges from 0.2 to 0.36 mm day^{-1} (Hales & Fletcher 1989). Although, this species has a low tolerance to desiccation (Norton 1977a, De Wreede 1983), to mechanical stress by wave-action (Viejo et al. 1995, De Wreede 1983), log abrasion and sea urchin grazing (De Wreede 1983).

The reproductive period in natural habitat is between April and May (Okuda et al. 1984) but in invader environments was very variable from region to region, and even between years (for further review see Fletcher & Fletcher 1975a, Critchley 1983a, De Wreede 1983, Deysher 1984, Espinoza 1990). In temperate regions the peak of abundance generally occurs during the colder months of the year, e.g., in north of Spain (Aramar) the population became reproductive in April-May (Fernández et al. 1990, Arenas & Fernández 1998) and the peak of proportion of mature plants was recorded in August, and September is the last fertile month (Arenas & Fernández 1998). In southwest Portugal is during April to July, but there are differences between localities (Engelen personal communication). The patterns of temperature and reproduction between regions indicate that temperature has an important role in timing of the reproductive period and growth of this species (Deysher 1984).

The preferential habitat and the success of *Sargassum muticum*

In Japan, *Sargassum muticum* often grows with *S. thunbergii,* but the upper limit is lower than the former species and is confined to sheltered localities influenced by warm (20-28 °C) waters of the Kuroshio current (Norton 1977b). In invaded coastline, this specie is able to inhabit tidal and non-tidal zones in small isolated groups, only exceptionally single specimens occur (Pru'homme van Reine & Nienhuis 1982), but normally are confined to the low water channels attached to the bedrock within harbours (Critchely 1983a), or restricted to the rockpools and stable boulders (Norton 1977b), in Northern Portugal (e.g. Viana do Castelo) the population occur in tidal pools and tidal channel, as well as in subtidal. Physical and biological barrier of expansion of this species are free space available for the recruits, wave exposure (Andrew & Viejo 1998b), turbidity and light quality/irradiance and grazing pressure (Norton 1977b, Hales & Fletcher 1989).

This invasive alga had the ability to became the dominant species at low-tide level by pushing out indigenous species, like *Cystoseira* spp., *Laminaria* spp. (Fletcher & Fletcher 1975a) and *Rhodomela larix* (De Wreede 1983) or could potentially reduce the native algae recruitment, by shading (Ambrose & Nelson 1982, Critchley 1983b, Viejo 1997), e.g. *Macrocystis pyrifera* (Ambrose & Nelson 1982) and *Bifurcaria bifurcata* (Viejo 1997); ultimately disturb the ecology of the coastline (Fletcher & Fletcher 1975a). Although, crustose and the articulated calcareous algae are not affected, probably they are more tolerant to the *Sargassum muticum* shading (Viejo 1997).

The success and spread of *Sargassum muticum* is probably due to its ability to tolerate and respond to environmental parameters like that of broad thermal tolerance (Hales & Fletcher 1989), its reproductive potential (Umezaki 1984), and strong seasonal patterns of reproduction (Kendrick 1993). Furthermore, detached branches from the parent algae are able to continue growth and form large floating mats (Fletcher & Fletcher 1975a, Norton 1977a, Critchley & Dijkema 1984, Deysher 1984) that may become fertile and produce gametes, increasing dispersal success (Norton 1977a, Deysher & Norton 1982, Critchley 1983a, Critchley & Dijkema 1984, Fernández et al. 1990, Hales & Fletcher 1990, Andrew & Viejo

1998a). Once established in an area, individuals are self-sustaining, making their removal difficult (Walker & Kendrick 1998).

Study area

The present study took place in Praia Norte, Viana do Castelo (Northern Portugal), situated at south of the Fort of Vigia. The selection of this beach was made in accordance with the presence of *Sargassum muticum* in an intertidal channel and tide pools, this provide the opportunity to test the effects of habitat on reproduction of this specie. Furthermore, this place is protected from wave action by offshore rocky bluffs that greatly reduce the predominantly NW wave action. The calm conditions of this place could permit the permanence on intertidal rock during the tidal cycle, more than is expected in many others sites.

Thesis Outline

The potential of *Sargassum muticum* disturb the intertidal communities in Portugal coastline is unknown, although and/or consequently, an understanding of the biology and ecology of this specie is necessary to provide a basis for a future management program. Furthermore, understand the life history, including strategies of reproduction, growth and the dispersive capacity of the invader, provides the limits to invasion (Elton 1958, Ehrlich 1986, Ashton & Mitchell 1989). It is during the early life history that in general an individual or a species are more sensitive to stress and grazing. Study the timing of reproduction and synchrony during the lunar and the tidal cycle is an important step to understand the initial journey and the population dynamic, as well is important study differences between habitats for understand the flexibility of the reproductive strategy of this invasive species. Various conditions are required for the successful establishment of a invasive specie but not all the process are known and guesses have to be made.

The aim of this thesis is to compare important reproductive and early life history aspects of *Sargassum muticum* in two habitats, middle intertidal pools and a lower intertidal channel. This thesis is based on two Chapters, the first focuses on the timing of egg expulsion and embryo settlement during tidal and lunar cycle between habitats. The second Chapter focuses on habitat related differences in recruitment success, micro-recruit survival and the role of meso-herbivores.

Habitat differences in the timing of reproduction of the invasive alga *Sargassum muticum* (Yendo) Fensholt over tidal and lunar cycles

Carla Monteiro[1], Aschwin Engelen[1], Ester A. Serrão[2] and Rui Santos[1]
[1]ALGAE research group, [2]MAREE research group
CCMAR, CIMAR-Laboratório Associado, Universidade do Algarve, FCMA, Campus de Gambelas, 8005-139 Faro, Portugal

ABSTRACT: *Sargassum muticum* is an invasive species that is firmly established on intertidal and subtidal rocky shores of Europe and the East Pacific coast of America. Local success and spread of *S. muticum* rely on its reproductive potential that seems dependent on exogenous factors like tidal and lunar cycles. In this study we describe and compare natural egg release and embryo settlement of *S. muticum* in two different intertidal habitats: mid-intertidal pools and low-intertidal channel. We hypothesise that 1) the reproductive periodicity of egg expulsion and embryo settlement is dependent on tidal and/or lunar cycles and 2) the periodicity of egg expulsion and settlement differs between habitats. The combination of monthly, daily and tidal samples at triplicate sites within each habitat showed a semilunar periodicity of egg expulsion and embryo settlement coincident with increasing tidal amplitude just before full and new moons. The duration of each egg expulsion event was about one week. Expulsion and settlement in tidal pools took place 1-2 days earlier than in the channel. Embryo settlement occurred during the first daily low tide and with the incoming high tide during spring tides. Our results suggest that *S. muticum* uses a combination of cyclic cues (tidal) and local factors for the exact timing of gamete expulsion and embryo release, that differ slightly between mid- and low-intertidal habitats.

KEY WORDS: Reproductive ecology · *Sargassum muticum* · egg expulsion periodicity · embryo settlement periodicity · tidal and lunar cycle

INTRODUCTION

The intertidal zone of rocky shores presents large variations in physical conditions that occur over small distances, although comparable in magnitude to those found over scales of kilometres in most terrestrial ecosystems. These extreme gradients make intertidal rocky shores a good model to study the role of environmental factors on the reproductive success of marine species. The combination of favourable pre- and post-settlement environmental conditions is crucial for the success, distribution and abundance of marine populations and has been suggested to be a major factor regulating population structure on rocky shores (Menge 1991, 2000, Roberts 1991). In marine populations settlement is highly variable at a range of temporal and spatial scales (e.g., Connel 1985, Jenkins et al. 2000). The timing of reproduction is caused by the interactions of several biological and physical factors. Physical fluctuations on intertidal shores vary along time-scales, mostly with the light-dark, tidal, lunar and seasonal cycles (Yamahira 2004). The influence of tidal and/or lunar cycles on reproductive synchrony has been reported for many marine organisms, the most famous being the coral spawning events on a few full-moon and last-quarter-moon nights per year (Harrison et al. 1984).

In several fucoid algae the reproductive periodicity is correlated with lunar or tidal cycles, as has been reported for *Silvetia compressa* (previously named *Pelvetia*, Johnson & Brawley 1998), *Fucus ceranoides* (Brawley 1992), *Fucus distichus* (Pearson & Brawley 1996), *Fucus vesiculosus* (Andersson et al. 1994, Serrão et al. 1996, Berndt et al. 2002, Pearson & Serrão 2006), *Fucus spiralis* (Monteiro et al. unpublished), *Sargassum vestitum* (May & Clayton 1991) and *Sargassum muticum* (Fletcher 1980, Norton 1981, Okuda 1981). The reproductive periodicity might however vary across the geographic range within a species if tidal cycles are involved, because these show geographic variations (for further review see Pearson & Serrão 2006). The studies conducted so far on patterns of gamete release and reproductive investment do not allow to detect small scale habitat-related variation in such patterns, because they do not include comparisons of timing of release between habitats within the same intertidal rocky shore (e.g. between middle and low intertidal). Reproductive timing and synchronized reproduction itself may increase fertilization success, and can be important for the survival of eggs and larvae and/or dispersal capacity and successful settlement, as well as predator avoidance (Yamahira 2004).

The Asian brown seaweed *Sargassum muticum* (Yendo) Fensholt is one of the most invasive algae in Europe and North America (Norton 1977), having rapidly spread and firmly established itself as a major coloniser of lower littoral and shallow sublittoral regions (Critchley 1983a,b). The success and fast spread of *S. muticum* along European coasts is at least partly attributed to its reproductive potential (Umezaki 1984), extensive fertile season and large number of reproductive structures produced (Hales & Fletcher 1989). In addition, the capacity for self-fertilization (Fletcher 1980, Norton 1981) might increase fertilization success, as it has been suggested for self-compatible hermaphroditic Fucacean algae (Brawley & Johnson 1992, Pearson & Brawley 1996). This is considered one of the main reasons for the success of introduced species (Norton 1976, Prud'homme van Reine & Nienhuis 1982). This species is an interesting system for studies of reproduction because: 1) it has shown the capacity for rapid colonization along European and North American coasts (Norton 1977, Norton 1981, Critchley 1983b); 2) it can inhabit different types of habitats and 3) gametes are expelled from receptacles in a number of discrete pulses, but instead of being released immediately into the seawater, they are fertilized and retained outside the receptacle for one to several days after fertilization (Fletcher 1980, Norton 1981, Hales & Fletcher 1990). This facilitates observations of gamete release pulses.

Seasonal patterns of growth and reproduction have been reported for *Sargassum muticum* in different latitudes (for further description see Fletcher & Fletcher 1975, Okuda 1981, Critchley 1983a, De Wreede 1983, Deysher 1984, Espinoza 1990, Fernández et al. 1990, Arenas & Fernández 1998). In their native region, Japan, the liberation of eggs takes place during spring tides, probably with a semilunar release pattern (Okuda 1981). In invaded regions like England and Southern California, embryo release is synchronized and occurs on average every 13 days, just after spring tides (Fletcher 1980, Norton 1981). Laboratory studies showed that the release is influenced by temperature and lunar phase, but in the field the lunar phase seems to have more influence than temperature (Fletcher 1980, Hales & Fletcher 1990). In Portugal, the fertile season occurs between January and September depending on the location, and varies from year to year. The expulsion and embryo settlement in tidal pools occurs during spring tides but dependent on local conditions, as differences between locations and pools have been observed (Engelen et al. in press). Other studies reveal annual differences in fertility as well as differences between invaded and native regions (Deysher 1984, Arenas & Fernández 1998).

Reproductive patterns in *Sargassum muticum* have been studied at regional scales, but never at a local scale, between different types of habitats. In this paper, we describe natural egg release and embryo settlement in two different intertidal, mid-intertidal (tidal pools) and low-intertidal (tidal channel) habitats. Our hypotheses are that 1) the reproductive periodicity of egg expulsion and embryo settlement is dependent on tidal and/or lunar cycles and 2) the periodicity of egg expulsion and embryo settlement differs between habitats. To test these hypotheses we quantified the daily variation of egg release and embryo settlement along tidal cycles in both habitats.

MATERIAL AND METHODS

Study site. The Portuguese coastline is influenced by a semidiurnal tidal regime, in which two similar tidal cycles occur per day. The study was carried out at the intertidal zone of Praia Norte (Viana do Castelo - Northern Portugal, 41°41'47 N 8°51'10 W) where the shoreline is protected from wave action by offshore rocky bluffs that greatly reduce the predominantly NW wave action. *Sargassum muticum* inhabits mid intertidal pools, lower intertidal channels and subtidal zones; the lower intertidal channels are submersed during the neap tides and form large pools during the spring tides. For further description of the area see Ladah et al. (2003).

Periodicity of eggs expulsion. The egg expulsion of *Sargassum muticum* were monitored from August to September 2005 and from May to June 2006, in three mid-intertidal pools and three sites in a tidal channel dominated by *S. muticum*. As it is not possible to distinguish by eye eggs from zygotes or early embryos, an increase in egg occurrence on the surface of the receptacles was interpreted as the expulsion of eggs from the conceptacles and a decrease represents the release of embryos from the surface of the receptacles to the water column. Eggs are fertilized on the surface of the receptacles and settlement takes place already at the embryo stage (Deysher & Norton 1982). Egg expulsion was assessed by collecting two fertile secondary laterals from five randomly selected individuals in each pool and site in the channel. Sampling was done every hour during low tide, as soon as the algae were accessible, which usually included some time before and after the area was uncovered by the tide. Ten ripe receptacles per lateral were examined for the presence or absence of eggs on their surface. During 2005, egg expulsion events were based on field observations of branches that carried receptacles with extruded eggs. The percentage of lateral branches bearing eggs at the surface of any receptacle was calculated. In 2006, fertile receptacles were carefully observed first in the field and then immediately fixed in a solution of acetic acid:ethanol (1:3) and transported to the laboratory. In the laboratory, the presence or absence of eggs on the surface of ten receptacles in each branch was observed under a dissecting microscope.

Periodicity of embryo settlement. The embryo settlement of *Sargassum muticum* were monitored daily, from August to September 2005 and from May to June 2006, in three mid-intertidal pools and three sites in a tidal channel (in same pools and sites used for the egg expulsion work). The periodicity of embryo settlement on the substratum was assessed with artificial substrates (5.96 cm^2) with rough surfaces to promote embryo adhesion (for further descriptions see Ladah et al. 2003). In each pool and site in the channel, a pvc holder containing three settlement discs was fixed at two randomly selected positions. The discs were collected daily during low tide and the number of embryos on each disc was counted in the laboratory under a dissecting microscope.

Statistical analyses. The percentage of receptacles with extruded eggs was calculated based on observations of 10 receptacles from 30 branches ($n = 300$) collected in each habitat, in each hour. In order to test whether there were significant differences ($p < 0.05$) between habitats in the percentage of branches (2005) or receptacles (2006) bearing eggs throughout the tidal cycle, a test for proportions using the normal approximation to the binomial was used for comparison between habitats and times (Zar 1999).

The rates of increase and decline of the number of branches (2005) and receptacles (2006) bearing reproductive bodies throughout the low tide was estimated in each habitat by deriving the slopes of the linear variation of their frequency through time. A positive slope indicates an increase of receptacles bearing extruded eggs and thus events of egg release to the receptacle surface, whereas a negative slope indicates the decline of fertilized eggs attached to receptacle margins, i.e. events of embryo release from the receptacle (and consequent settlement).

To detect significant differences ($p < 0.05$) between habitats in the amount of settled eggs, a t-test was used.

RESULTS

Egg expulsion

Egg expulsion in *Sargassum muticum* occurred with a periodicity of two weeks (semi-lunar) coincident with full and new moons and with spring tides in both the mid- and low-intertidal (Figs. 1 & 2). The first occurrence of egg expulsion was observed before new or full moons in mid-intertidal pools and one or two days later in the low-intertidal channel. In both habitats egg release started at the evening low tide, except in the low-intertidal on August 5 and September 2 of 2005, when release started during the morning low tide. The duration of the egg expulsion period varied between years. In 2005, when sampling took place between August and September, expulsion periods of 4-5 and 3-5 days were observed, respectively, in mid-intertidal and in low-intertidal (Fig. 1). In 2006, when sampling took place between May and June, expulsion was observed during longer periods, 7-9 days. The major events were observed from 20 to 28 June 2006 in mid-intertidal and from 21 to 29 June 2006 in the low-intertidal (Fig 2).

In 2005, the egg release at mid-intertidal increased daily until the full and new moon days, whereas in the low subtidal such an increase only took place a few days later, in general after full and new moon (Fig. 1). In 2006, there were stronger release events before the moons (full and new) and weaker events during or after the moons (Fig. 2). Field observations on marked branches showed intra-individual differences in the time of egg expulsion; in other words not all primarily laterals of a single individual expel the eggs at same time (not shown). In both habitats, egg expulsion took place either just before or after full/new moon.

In general, we found significant differences between habitats in 2005 and 2006. In 2005, there were significant differences in egg expulsion between habitats in 78 out of 92 observations (84.8 %) (dark grey boxes in Fig. 1). Non-significant differences corresponded to observations done in the first morning of an expulsion period, except in August 17 at 17:00. In 2006, 113 (57.1 %) out of a total of 198 observations showed significant differences between habitats (Fig. 2). The percentage of branches (2005) and receptacles (2006) with extruded eggs reached a maximum of 100 % in both years. The data from 2006 illustrate that in the first 2-4 days of each event, the percentages are normally near 100 %. The standard errors of the percentage of receptacles with extruded eggs per branch were small in both habitats, indicating that egg expulsion was synchronized between the three pools and between the three channel sites.

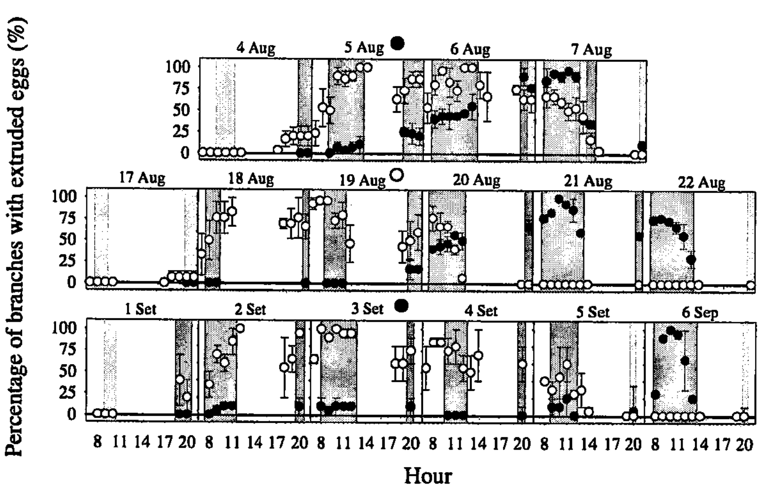

Fig. 1. Egg release by *Sargassum muticum* during diurnal low tide periods in 2005. Percentage of branches (mean ± SE) with extruded eggs in mid-intertidal pools (open symbols) and low-intertidal channel (closed symbols). Dark grey backgrounds represent periods with significant differences between habitats, as opposed to light grey backgrounds. White backgrounds represent a period of time when the two or one of the habitats was not sampled. Open and closed circles above plots indicate full and new moon, respectively.

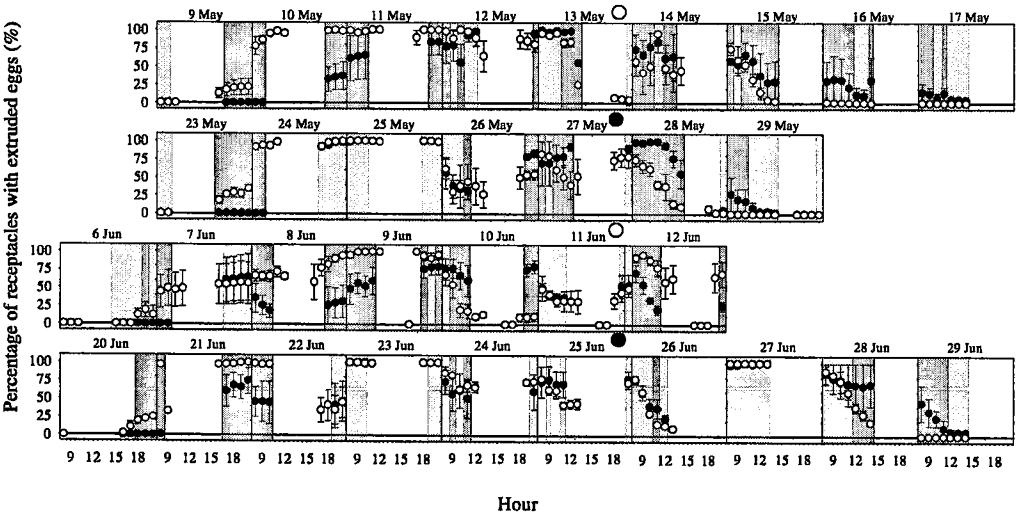

Fig. 2. Egg release by *Sargassum muticum* during diurnal low tide periods in 2006. Percentage of receptacles (mean ± SE) with extruded eggs per branch in mid-intertidal (open symbols) and low-intertidal (closed symbols). Dark grey backgrounds represent periods with significant differences between habitats as opposed to light grey backgrounds. White backgrounds represent a period of time when the two or one of the habitats was not sampled. Open and closed circles above plots indicate full and new moon, respectively.

In 2005, the onset of egg extrusion and embryo release was faster in algae located in the pools than in the channel (Fig. 3). Both the rate of increase and decline of branches with reproductive bodies (eggs and embryos, respectively) were faster in the pools both in the morning tides and in the evening tides. In 2006, the decline in receptacles bearing embryos was faster in the morning tide than in the evening tide, in both habitats (Fig. 3), which indicates better conditions in the morning tides for embryo release. No clear differences were found between habitats in the onset of both egg extrusion and embryo release in 2006.

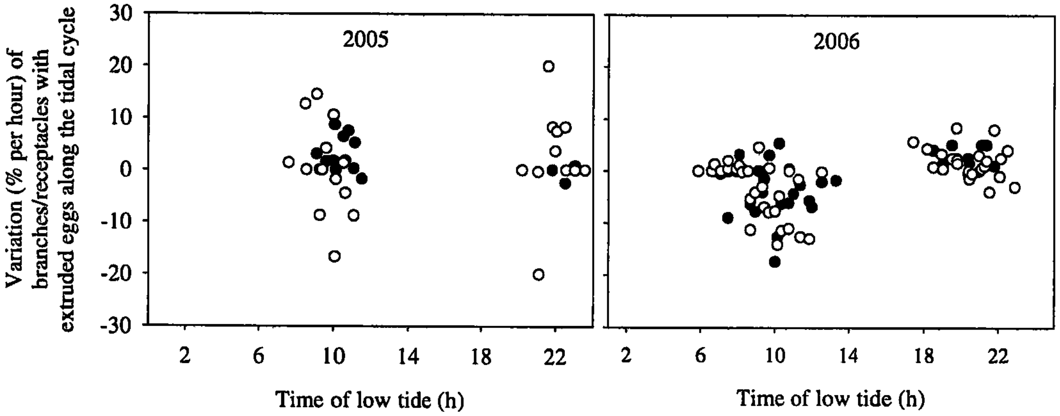

Fig. 3. Rates of increase (positive numbers, revealing ongoing egg extrusion) and decline (negative numbers, revealing embryo release from the receptacle) of *Sargassum muticum* branches (2005) and receptacles (2006) bearing reproductive bodies in mid-intertidal pools (open symbols) and in low-intertidal channel (closed symbols), estimated as the slopes of the lines in Fig 1. These are shown in relation to the time of low tide on the respective sampling day.

Embryo settlement

Embryo settlement showed a clear semi-lunar pattern, coinciding with, or just after, the full and new moons (Fig. 4). The settlement period was 3 to 5 days in the late summer of 2005 and 4 to 7 days in the early summer of 2006. In both years, egg settlement started 1 to 2 days earlier in mid-intertidal pools than in the low intertidal channel. A significant difference in the density of eggs settled between habitats was found. In 2005, the maximum egg density was 1.79 embryos.cm^{-2} in tidal pools and 0.43 embryos.cm^{-2} in the tidal channel whereas in 2006, 2.18 embryos.cm^{-2} and 1.34 embryos.cm^{-2} settled in the pools and in the channel, respectively. Within both habitats settlement was always very consistent within replicates as indicated by the low standard error.

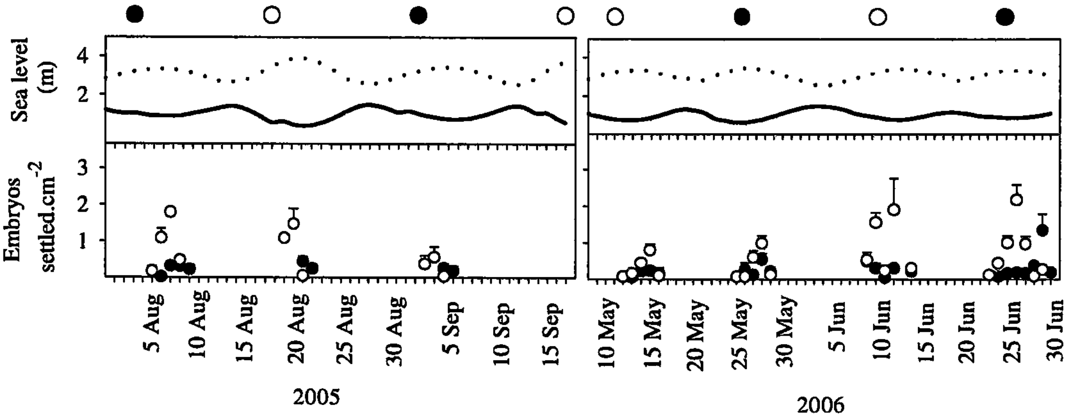

Fig. 4. Daily embryo settlement of *Sargassum muticum* in tidal pools (open symbols) and a tidal channel (closed symbols) in 2005 and 2006. Error bars show standard error (n = 3). Lunar phases (full and new moons) are shown above the graphs. The low tide (black line) and high tide (dotted line) levels are shown in the box above each plot.

DISCUSSION

Sargassum muticum showed a semi-lunar pattern of egg expulsion and embryo settlement, coincident with full and new moons (spring tides) in both habitats (mid-intertidal pools and low intertidal channel). This pattern is in agreement with natural reproduction in Japan (Okuda 1981), but not with patterns in England and Southern California where these occur after spring tides (Fletcher 1980, Norton 1981). The association of propagule release with lunar or tidal phases has been reported in many marine organisms, including fucoids, but phase shifts in the timing of release may occur across different geographic locations even within the same species (e.g., Pearson & Serrão 2006). On a biogeographic scale, the occurrence of phase shifts associated to the lunar cycle within a species, suggest that the differences in reproductive periodicity may be associated with geographic shifts in daily tidal patterns between sites. Smaller scale differences in propagule release patterns are however rarely documented and poorly understood (but see Pearson and Brawley 1996, Pearson et al. 1998).

In this study we have documented very local scale differences in the timing and rate of propagule between sites at different levels along the intertidal zone. Both egg expulsion and embryo release started a few days earlier in the mid-intertidal (pools) than in the low intertidal (channel). Both types of events were asynchronous between habitats but were synchronous within habitats suggesting that the trigger mechanism(s) may be associated to the amount of time that the habitat is disconnected from the sea, and thus to tidal amplitude. Many physical factors vary in tide pools as the tidal amplitude increases, namely faster tidal currents and, during the low tide interval absence of wave action and, during sunny days, an increase in temperature, salinity and pH and decreasing CO_2 availability for photosynthesis in the unstirred layer surrounding photosynthetic organisms in still water. The lower intertidal environment would need more time to attain a certain threshold in any of the parameters that change related to low tide exposure than the higher mid-intertidal population. Indeed in other species of fucoid algae that occur in tide pools, gamete release is triggered at low tide when the unstirred layer surrounding the algae reaches limiting concentrations of inorganic carbon for photosynthesis, which acts as a signal for gamete expulsion (Pearson et al. 1998). A similar type of mechanism could explain our observations that higher in the intertidal gamete expulsion starts a few days earlier than in the low intertidal, since a putative requirement for a threshold amount of time in still water in the light would be reached earlier in the higher intertidal.

Besides the duration of time in still water and consequent formation of boundary layers, other factors that may vary between the mid-intertidal and lower intertidal regions could potentially influence the patterns of propagule release. Fluctuations of physical factors such as photoperiod and temperature in the habitat have been shown to regulate the physiological mechanism of reproduction in this species and others (Fletcher 1980, Norton 1981, Okuda 1981, Santelices 1990), but these processes have been reported to take place along larger temporal scales than the daily scales of differences detected in this study. Most available information concerning the effects of such factors on reproductive success of *Sargassum muticum* is related to physiological limits for propagule survival rather than any potential influence on patterns of propagule release, so the information is scant and further studies are warranted. Eggs of *S. muticum* have been reported to survive in a wide range of temperatures, between 10-30° C, with an optimal temperature at 25° C (Deysher 1984, Hales & Fletcher 1990) or 7° C - 17° C (Steen 2003). Lower concentration of nutrients could decrease egg density, especially at 17°C (Steen 2003). Low salinity had been reported to be an important factor limiting initial stages of *S. muticum* and probably is a physiological barrier to expansion of this species into low salinity habitats such as estuaries or the Baltic Sea (see Norton 1977, Hales & Fletcher 1989). Low salinities decrease growth and reproduction in *S. muticum* and the optimal values range between 14.7 and 27.1 ‰ (Steen 2004).

The synchrony of egg expulsion and embryo settlement within habitats is an important factor that could contribute for the success of this species. Synchrony increases the concentration of eggs and sperm and consequently increases fertilization success and satiation for the herbivores (for further review see Reed et al. 1997), and *Sargassum* spp. are known to be susceptible to grazing by sea urchins (De Wreede 1983), fish (McCook 1996), amphipods and gastropods (Norton & Benson 1983). The influence of synchrony on fertilization success may be less important for *Sargassum muticum* because it is a monoecious seaweed capable of self-fertilization (Fletcher 1980, Norton 1981). Furthermore eggs are retained outside the receptacle for several days during which fertilization can occur (Fletcher 1980, Norton 1981, Hales & Fletcher 1990), as observed in this study (eggs were retained for 1-2 days in 2005 or 2-3 days in 2006, see below possible reasons for these differences), a process which tends to increase the success of fertilization by integrating the effects of variable sperm concentrations over a time period of a few days, as has been reported for marine invertebrates (e.g., Wahle and Gilbert 2002). This egg retention at the surface of the receptacles is also expected to increase recruitment success by facilitating fast adhesion to the substrate, because when embryos settle they already possess developing rhizoids (Deysher & Norton 1982).

Synchrony of release may however be important for allowing at least some cross-fertilizations between different individuals for avoidance of potential inbreeding depression that could result from selfing, and which could reduce competitiveness. It is not known whether any mechanism exists in *Sargassum muticum* that allows for selection of non-selfed sperm if multiple sperm are available during synchronous release periods, and this is another field of study that deserves further attention. Given the proximity of female and male conceptacles in the receptacle, a significant amount of selfing is expected, even under the synchronous release conditions detected in our study. Selfing does however provide reproductive assurance, a very important characteristic for the colonization potential and invasiveness of an introduced species, allowing single individuals to start new populations. Field observations on marked branches showed intra-individual differences in the time of egg expulsion in other words not all primarily laterals of a single individual expel the eggs at same time. The asynchrony of egg expulsion between primarily laterals branches indicates an intra-individual variance that could influence population genetic structure (Pearson & Serrão 2006). This aspect deserves more study.

The temporal variation in the duration of egg expulsion events between 2005 (3-5 days) and 2006 (7-9 days), and in embryo settlement (2-4 days in 2005 and 4-7 days in 2006), may reflect the temporal variation of the reproductive season. In 2005 the study was carried out in the last two months of the reproductive season, when lateral branches started dying. Observation in *Fucus* spp. at the same site showed that expulsion events can be longer during the peak of reproduction compared to the rest of year (Monteiro et al. in prep.). In 2006, the fieldwork was carried out in May and June, corresponding to the maximum reproductive biomass. As has been observed in Asturias, northern Spain (Arenas et al. 1995, Arenas & Fernández 1998), during this period growth stops, the maximum percentage of fertile fronds is reached and the senescent phase begins (Hales & Fletcher 1989). Furthermore, the reproductive period of egg expulsion in 2005 could have been underestimated as branches were only observed in the field as opposed to 2006 when the branches were observed both in the field and in the laboratory under a dissecting microscope. Underestimation could have been the case when expulsion rates were low as in the beginning and end of each expulsion event. Consequently, observations in 2005 might correspond only to the major peak of embryo expulsion. In addition, the population age could be responsible for some interannual variations, since these algae are able to increase the number of laterals branches each year (Arenas & Fernández 1998), which might induce variation in the reproductive patterns.

Settlement was significantly higher in the pools than in the channel. The magnitude of settled embryos was greatest in June 2006, during both moons (full and new moon), although we do not know whether this represents the yearly peak because the time of the year when maximum settlement occurs is not known. Our results revealed surprisingly low settlement densities of *Sargassum muticum*, in the order of 2.18 embryos.cm^2 (maximum average between pools), which is much less than observations in the southwest coast of Portugal, on the order of 80 embryos.cm^{-2} (Engelen et al. in press). These lower settlement densities in our study are not likely to be due to differential loss during the process of sampling in the water column due to weak attachment to the artificial disks because the embryos appeared sufficiently attached to the disks to not be dislodged by the simple lifting of the disks, and specially because the sampling procedure and disks used were the same in both studies.

Putative differences in density of reproductive branches between the two regions are another possible cause for settlement differences. A perhaps more likely hypothesis could be the occurrence of different levels of embryo dispersal between these two regions, in N Portugal (this study) and SW Portugal (Engelen et al. in press), although this appears contrary to expectation from hydrodynamic conditions, given that our site appears less exposed than that in the study of Engelen et al. (in press). It could however be explained by potentially faster attachment rate in SW Portugal due to higher temperatures being reached in the pools at low tide in the SW Portugal region than at our sites, and faster attachment might more easily avoid dispersal to unfavourable sites when the tide covers the site again. Furthermore, even if most embryos were able to be securely attached to the bottom at low tide before exposure to the incoming tide, still settlement within a smaller pool increases the probability of falling on the sampling disks, in comparison with settling inside larger pools or channels, as were those at this site, where the disks cover a much smaller proportion of the total area available for settlement. An alternative explanation for the differences in settlement between these two regions might be grazing pressure, one of the greatest sources of mortality in early life stages (Brawley & Johnson 1991). Differences in grazing assemblages between habitats could influence the early survival, although we do not know which grazers affect *Sargassum muticum* in these two areas. Studies suggest that *Sargassum* spp. is susceptible to grazing by sea urchins (De Wreede 1983) and other grazers such as *Littorina littorea* (Sjøtun et al. 2007), fish (McCook 1996), amphipods and gastropods (Norton & Benson 1983).

This study is the first to compare the reproductive patterns of the invasive species *Sargassum muticum* in two different habitats in the middle and low intertidal. Our results show a strong relationship of both egg expulsion and embryo settlement with spring tides (during full and new moons) in both habitats. However, the consistent earlier start and faster egg expulsion and embryo settlement observed in higher intertidal levels suggests that tidal amplitude and consequent small scale variations in local environmental cues play an important role in the exact timing of reproduction at different tide levels. This capacity for synchronous egg expulsion and embryo settlement during spring tides and in response to very local cues may play an important role in the high reproductive success and invasiveness of this invasive species.

Acknowledgements. We would like to thank "Associação dos Amigos do Mar" (Viana do Castelo) and the staff, for providing facilities in this laboratory work, and Gareth Pearson for help in reviewing the manuscript.

LITERATURE CITED

Andersson S, Kautsky L, Kalvas A (1994) Circadian and lunar gamete release in *Fucus vesiculosus* in the atidal Baltic Sea. Mar Ecol Prog Ser 110:195-201

Arenas F, Fernández C (1998) Ecology of *Sargassum muticum* (Phaeophyta) on the North Coast of Spain. III. Reproductive ecology. Bot Mar 41:209-216

Arenas F, Fernández C, Rico JM, Fernández E, Haya D (1995) Growth and reproductive strategies of *Sargassum muticum* (Yendo) Fensholt and *Cystoseira nodicaulis* (Whit.) Roberts. Sci Mar 59:1-8

Berndt M, Callow JA, Brawley SH (2002) Gamete concentrations and timing and success of fertilization in a rocky shore seaweed. Mar Ecol Prog Ser 226:273-285

Brawley S, Johnson L (1991) Survival of fucoid embryos in the intertidal zone depends upon developmental stage and microhabitat. J Phycol 27:179-186

Brawley SH (1992) Fertilization in natural populations of the dioecious brown alga *Fucus ceranoides* and the importance of the polyspermy block. Mar Biol 113:145-157

Brawley SH, Johnson L (1992) Gametogenesis, gametes and zygotes: an ecological perspective on sexual reproduction in the algae. Br Phycol J 27:233-252

Connel JH (1985) The consequences in initial settlement vs. post-settlement mortality in rock intertidal communities. J Exp Mar Biol Ecol 93:11-45

Critchley AT (1983a) The established and increase of *Sargassum muticum* (Yendo) Fensholt populations within the Solent area of Southern Britain. I. An Investigation of the increase in number pf population individuals. Bot Mar 26:539-545

Critchley AT (1983b) The established and increase of *Sargassum muticum* (Yendo) Fensholt populations within the Solent area of Southern Britain. II. An Investigation of the increase in canopy cover of the alga at low water. Bot Mar 26:547-552

Deysher L (1984) Reproductive phenology of newly introduced populations of brown alga, *Sargassum muticum* (Yendo) Fensholt. Hydrobiologia 116/117:403-407

Deysher L, Norton TA (1982) Dispersal and colonization in *Sargassum muticum* (Yendo) Fensholt. J Exp Mar Biol Ecol 56:179-195

De Wreede RE (1983) *Sargassum muticum* (Fucales, Phaeophyta): regrowth and interaction with *Rhodomela larix* (Ceramiales, Rhodophyta). Phycologia 22:153-160

Engelen A, Espírito-Santo C, Simões T, Monteiro C, Serrão E, Pearson G, Santos R (in press) Periodicity of propagule expulsion and settlement in the competing native and invasive brown seaweeds, *Cystoseira humilis* and *Sargassum muticum* (Phaeophyta). Eur J Phycol

Espinoza J (1990) The southern limit of *Sargassum muticum* (Yendo) Fensholt (Phaeophyta, Fucales) in the Mexican Pacific. Bot Mar 33:193-196

Fernández C, Gutiérrez LM, Rico JM (1990) Ecology of *Sargassum muticum* on the north coast of Spain. Preliminary Observations. Bot Mar 33:423-429

Fletcher RL (1980) Studies on the recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. III. Periodicity in gamete release and "incubation" of early embryo stages. Bot Mar 23:425-432

Fletcher RL, Fletcher SM (1975) Studies on recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. I. Ecology and reproduction. Bot Mar 18:149-156

Hales JM, Fletcher RL (1989) Studies on the recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. IV. The effect of temperature, irradiance and salinity on germling growth. Bot Mar 32:167-176

Hales JM, Fletcher RL (1990) Studies on the recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. V. Receptacle initiation and growth, and gamete release in laboratory culture. Bot Mar 33:241-249

Harrison PL, Babcock RC, Bull GD, Oliver JK, Wallace CC (1984) Mass spawning in tropical reef corals. Science 223:1186-1189

Jenkins SR, Åberg P, Cervin G, Coleman RA, Delaney J, Della Santina P, Hawkins SJ, LaCroix E, Myers AA, Lindergarth M, Power A-M, Hartnoll RG (2000) Spatial and temporal variation in the settlement and recruitment of the intertidal barnacle *Semibalanus balanoides* (L.) (Crustacea: Cirripedia) over a European scale. J Exp Mar Biol Ecol 243: 209-225

Johnson LE, Brawley SH (1998) Dispersal and recruitment of a canopy-forming intertidal alga: the relative roles of propagule availability and pos-settlement process. Oecologia 117:517-526

McCook LJ (1996) Effects of herbivores and water quality on *Sargassum* distribution on the Great Barrier Reef: cross-shelf transplants. Mar Ecol Prog Ser 139:179-192

Ladah L, Bermudez R, Pearson G, Serrão E (2003) Fertilization success and recruitment of dioecious and hermaphroditic fucoid seaweeds with contrasting distributions near their southern limit. Mar Ecol Prog Ser 262:163-183

May DI, Clayton MN (1991) Oogenesis, the formation of oogonial stalks and fertilization in *Sargassum vestitum* (Fucales, Phaeophyta) in southern Australia. Phycologia 30:243-256

Menge BA (1991) Relative importance of recruitment and other causes of variation in rock intertidal community structure. J Exp Mar Biol Ecol 146:69-100

Menge BA (2000) Recruitment vs. postrecruitment process as determinants of barnacle population abundance. Ecol Monogr 70:265-288

Norton TA (1976) Why is *Sargassum muticum* so invasive? Br Phycol J 11:197-198

Norton TA (1977) Ecological experiments with *Sargassum muticum*. J Mar Biol Ass UK 57:33-43

Norton TA (1981) Gamete expulsion and release in *Sargassum muticum*. Bot Mar 14:465-470

Norton TA Benson MR (1983) Ecological interactions between the brown seaweed *Sargassum muticum* and its associated fauna. Mar Biol 75:169-177

Okuda T (1981) Egg liberation in some Japanese Sargassaceae (Phaeophyceae). Xth Int Seaweed Symp, Berlin p, 197-202

Pearson GA, Brawley SH (1996) Reproductive ecology of *Fucus disthichus* (Phaeophyceae): an intertidal alga with successful external fertilization. Mar Ecol Prog Ser 143:211-223

Pearson GA, Serrão EA, Brawley SH (1998) Control of gamete release in Fucoid algae: sensing hydrodynamic conditions via carbon acquisition. Ecology 79:1725-1739

Pearson GA, Serrão EA (2006). Revisiting synchronous gamete release by fucoid algae in the intertidal zone: fertilization success and beyond? Integr Comp Biol 46:587-597

Prud'homme van Reine WF, Nienhuis PH (1982) Occurrence of the brown alga *Sargassum muticum* (Yendo) Fensholt in The Netherlands. Bot Mar 25:37-39

Reed DC, Anderson TW, Ebeling AW, Anghera M (1997) The role of reproductive synchrony in the colonization potential of kelp. Ecology 78:2443-2457

Roberts CM (1991) Larval mortality and composition of coral ref fish communities. Trends Ecol Evol 6:83-87

Santelices B (1990) Patterns of reproduction, dispersal and recruitment in seaweeds. Oceanogr Mar Biol Ann Rev 28:177-276

Serrão EA, Pearson GA, Kautsky L, Brawley SH (1996) Successful external fertilization in turbulent environments. Proc Natl Acad Sci USA 93:5286-5290

Sjøtun K, Eggereide SF, Høisæter T (2007) Grazer-controlled recruitment of the introduced *Sargassum muticum* (Phaeophyceae, Fucales) in northern Europe. Mar Ecol Prog Ser 342:127-138

Steen H (2003) Intraspecific competition in *Sargassum muticum* (Phaeophyceae) germlings under various density, nutrient and temperature regimes. Bot Mar 46:36-43

Steen H (2004) Effects of reduced salinity on reproduction and germling development in *Sargassum muticum* (Phaeophyceae, Fucales). Eur J Phycol 39:293-299

Umezaki I (1984) How many eggs will be discharged from the plant of *Sargassum horneri*? Hydrobiologia 116/117:398-402

Wahle RA, Gilbert AE (2002) Detecting and quantifying male sea urchin spawning with time-integrated fertilization assays. Mar Biol 140:375-382

Yamahira K (2004) How do multiple environmental cycles in combination determine reproductive timing in marine organisms? A model and test. Funct Ecol 18:4-15

Zar JH (1999) Biostatistical analysis. 4th edn Prentice-Hall, Upper Sladle River, N J

Recruitment and effects of mesograzers on the micro-recruits of the invasive *Sargassum muticum* (Yendo) Fensholt in two different habitats (mid- and low-intertidal)

Carla Monteiro[1], Aschwin Engelen[1] and Rui Santos[1]
[1]ALGAE research group, CCMAR, CIMAR-Laboratório Associado, Universidade do Algarve, FCMA, Campus de Gambelas, 8005-139 Faro, Portugal

ABSTRACT: The successes of invading species are dependent on the success of early life history stages. The timing of recruitment and their subsequent survival in different habitats is important for the reproductive strategies of species, in general. For algae grazing has a very strong impact on their distribution, abundance and community structure. The objective of this study was to test habitat and lunar phase related differences in recruitment and micro-recruit survival and the importance of meso-grazing. The invading brown seaweed *Sargassum muticum* was our model organism. Results revealed that recruitment and micro-recruits survival is higher in the pools than in the channel, probably due to more favourable environmental conditions. Meso-herbivory is an important mortality factor for micro-recruits, and more so in the tidal pools than in the channel. No clear lunar effects were found. In conclusion tidal pools are a more favourable environment for micro-recruits of *S. muticum*.

KEY WORDS: *Sargassum muticum* · recruitment · micro-recruit survival · mid-intertidal pools · low-intertidal channel

INTRODUCTION

Biological invasions affect the integrity of natural communities in many different ecosystems around the world (Vitousek et al. 1997) and have been increasing with the increase of international shipping, aquaculture and aquarium activity (Rueness 1989). Ecological effects or consequences of non-indigenous species in new habitats are speculative (Britton-Simmons 2004). Nevertheless, studies suggest that non-indigenous compete with native species (Mack et al. 2000), hybridize with natives (Simberloff 2001), and facilitate the establishment of subsequent invaders in a synergistic fashion (Levin et al. 2002). As a consequence, non-indigenous species have the potential to alter native communities and cause biodiversity loss worldwide (Wilson 1992, Villele & Verlaque 1995, Wilcove et al. 1998, Levin et al. 2002).

The persistence of any, introduced or native, population requires the success of reproductive features, the relative rates of settlement, survival and growth (Neushul et al. 1976). Survival of early post-settlement is a critical phase for the successful establishment of marine benthic populations (for further review see Vadas et al. 1992) and is dependent on many processes, including grazing, disturbance and physical requirements and canopy density (Kendrick 1994). In benthic marine organism it is important to understand the impact one species exerts on another, and it is imperative how these organisms adapt (Neushul et al. 1976). Timing and synchronisation of gamete release (or spawning) can be critical for recruitment assurance. In several fucoids algae the patterns of reproduction or timing of release shows a positive correlation with the lunar or tidal cycle. Studies on the recruitment and survival of micro-recruits have mainly been focussing on species with a rather restricted vertical distribution (for further review see Pearson & Serrão 2006). Little is know about species with a wide vertical distribution on intertidal rocky shore. Testing the influence of the time of release and survival in different habitats may be important to understand the reproductive strategies of species.

Grazing has a strong impact on algal distribution, abundance and community structure (Rosemond et al. 1993, Hawkins & Hartnoll 1985, Heck et al. 2000). The success and proliferation of non-indigenous species is at least partly attributed to the fact that novel areas often experience reduced predation pressure (Lawton & Brown 1986, Wilson 1989). This is also the reason why some of the most important invasion theories stress the importance of grazing or rather the lack of grazing on invasive species relative to native species (Enemy Release Hypothesis, see Mack et al. 2000, Keane & Crawley 2002, Mitchell & Power 2003). Grazing pressure is one of the greatest sources of mortality in early life stages of seaweeds in general as well as in fucoids (Vadas et al. 1992).

The effects of grazing on recruitment and early survival vary on spatial scales. Differences in grazing have been reported among different geographic zones even when they have equal species composition (e.g. Dethier & Duggins 1988, Boaventura et al. 2002). Density and composition of the grazer assemblage (Underwood & Jernakokk 1981, Underwood 1984) as well as abundance and growth rates of algae may differ among close locations (Arrontes et al. 2004) causing small scale variations in grazing effects on algae. Single herbivore species or a reduced group of herbivore species can have a disproportionate effect (for further review see Hawkins et al. 1992, Viejo et al. 1999). With this variation in mind we hypothesized differences in the importance of recruit survival and grazing pressure over small spatial scales when a strong selective gradient is present, like in the intertidal zone.

Sargassum muticum is invasive alga and is a pest species that forms extreme dense beds and replaces the native seaweeds and seagrasses (Druehl 1973). This non-indigenous species was probably accidentally introduced by juveniles of *Crassostrea gigas* (Thunberg) imported from Japan (Druehl 1973, Farnham et al. 1973, Critchley & Dijkema 1984). It rapidly spread around European coasts and firmly established itself as a major coloniser of lower littoral and shallow sublittoral regions. The patterns of reproduction are related with semilunar cycle, with periodicity of two weeks coincident with spring tides (full and new moons) (Okuda 1981) or after spring tides (Fletcher 1980, Norton 1981). Circadian rhythms are endogenously generated and synchronized to the environment by light-dark cycles (Underwood 1984). Timing of reproduction can influence lifetime reproductive success (see Pearson & Serrão 2006). This species is an attractive system for studies on recruitment and early stage survival: 1) it is one of the most invasive algae in Europe and North America coasts (Norton 1977, Norton 1981), so is important understand each life stage that contribute for the success of this invasive specie; 2) it inhabit diverse types of habitats (tide pools, tide channel) at different vertical distribution (mid and low intertidal and subtidal); and 3) few studies have been performed regarding recruitment, mostly focussing on density effects on recruitment and survival (Andrew & Viejo 1998a). As far as we are aware no study has been performed dealing with the influence of grazing on micro-recruit survival.

The aim of this study was to investigate habitat related differences in recruitment and micro-recruit survival of the invader *Sargassum muticum* and examine the role of meso-grazing by means of a grazer exclusion experiment. Since the patterns of reproduction in this species have a semilunar periodicity, which could influence micro-recruit survival, the experimental design was intended to make comparisons of the role of grazers on micro-recruit survival between different lunar phases. Since the structuring role of grazers on the composition and dynamics of species assemblages decreases from mid to lower tidal levels (Hawkins & Hartnoll 1983) our first hypothesis is that there will be habitat related differences in recruitment and micro-recruit survival between tide pools and channel. It is expected that recruitment and survival in the channel will be higher than to the pools. Considering the evolutionary importance of the timing of release for the persistence of populations, we expect to find more micro-recruit survival during moon phases in which naturally release takes place.

MATERIALS AND METHODS

Study site: The study was carried out at Praia Norte, near Forte da Vigia (Viana do Castelo - Northern Portugal, 41°41'47 N 8°51'10 W). The intertidal zone of Praia Norte is a protected shoreline from wave action by offshore rocky bluffs that greatly reduce the predominantly NW wave action (for further description of the area see Ladah et al. 2003).

Recruitment: Recruitment was determined as the total number of recruits settled on high rugosity artificial settlement discs ($5.96.cm^{-2}$, see Ladah et al. 2003). Fieldwork was performed during two different reproductive seasons, from 10 August to 23 September 2005 and from 8 May to 30 June 2006 (sampled discs). Six sampled discs were fixed at random positions under the canopy, replicated in 3 tidal pools and 3 sites in a tidal channel. The discs were collected at the end of each sampling period. The number of recruits on the sampled discs was evaluated by counting under a dissecting microscope. Recruitment data were analyzed by ANOVA with a factorial design with the following factors year (2005 and 2006 - 2 levels, random), habitat (tide pools and channel - 2 levels, fixed) and sites (3 levels, random). Recruitment was analysed for a period of two months in each year. Six replicates discs were used in each site. Variances were tested for homogeneity using Cochran's C-test, and were log-transformed. Student-Newman-Keuls (SNK) multiple comparisons were used as post-hoc tests, using $\alpha = 0.05$.

Micro-recruit survival: Micro-recruit survival was estimated from natural settlement on artificial substrates in 2005 and from settlement in the laboratory in 2006. The number of recruits on each disc was evaluated under a dissecting microscop before and after incubation in the field. The micro-recruit survival experiment followed a factorial design to test the effect of habitat (tide pools and channel - 2 levels, fixed factor), grazing (3 levels, fixed factor), and lunar phase (4 levels, fixed factor) on recruit survival. The experiments were performed for a period of three days around full, new and each half moon. Grazing was evaluated using grazer exclusion cages, built from an aluminium 5 mm mesh sewn around the sampling discs and no cage. The cage effect was evaluated using cages in which the two outer ends remained open. Three (in 2005) and four (in 2006) replicates discs were used for each treatment. Micro-recruit survival data were analyzed by ANOVA and Student-Newman-Keuls (SNK) multiple comparisons, using $\alpha = 0.05$ in both cases. The variances were homogenous and no transformation was performed.

RESULTS

Recruitment

The ANOVA showed that the number of recruits was 14 times smaller in 2005 than in 2006 (Fig. 1) and the number of recruits observed was three times more in 2005 and almost two times in 2006 in the pools than in the channel (year and habitat interaction, Table 1). Significant differences were detected between random sites in pools and random sites in channel, indicating spatial variation within each habitat on recruitment.

Table 1. ANOVA results on the recruit settlement of *Sargassum muticum* during two years in two habitats (intertidal pools and channel). Data was log (recruits+1) transformed.

Source of variation	df	MS	F	p
Year	1	28.382	576.455	0.001
Habitat	1	3.678	74.671	0.001
Site (Habitat)	4	0.140	2.839	0.032
Year x Habitat	1	0.455	9.243	0.035
Year x Site (Habitat)	4	0.194	3.938	0.066
Residual	60	0.49		

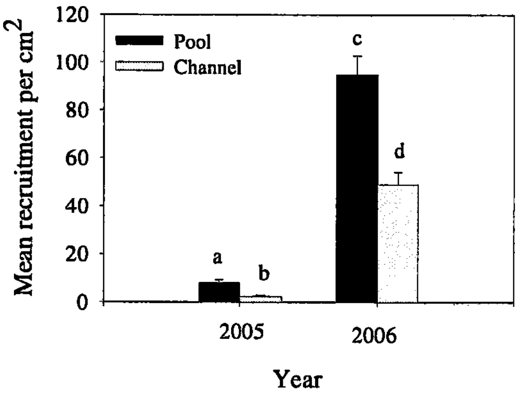

Fig. 1. The mean recruitment (cm^{-2}) of *Sargassum muticum* during two years (2005 and 2006) in two habitats: middle intertidal pools (black) and low intertidal channel (grey). Different letters above bars indicate significant differences between means based on SNK test ($p < 0.05$). Error bars show standard error ($n = 18$ discs per treatment).

Effects of grazing on micro-recruit survival

In 2005, there were significant differences in the percentage of micro-recruit survival among habitats, sites and a significant interaction between moon and cage (Table 2). The results showed that micro-recruit survival was 1.6 times higher in the pools than in the channel (Fig. 2). Significant spatial variation was detected between random sites in pools but not in the channel, indicating that micro-recruits survival is more dependent on the meso-herbivory in pools than in channel. Caging significantly increased the micro-recruits survival by 10 % relative to the natural situation in pools, but no effect was observed in channel.

In 2006, the percentage of micro-recruits survival was 1.2 times higher in the pools than in the channel (Fig. 2). Significant differences among treatments and significant interaction between habitat and cage were observed (Table 2). The percentage of micro-recruits survival protected by cages was 1.3 and 1.2 times higher than in uncaged micro-recruits in pools and channel, respectively. Moreover, a significant difference was found among all cage treatments in the pools, but not in the channel (Fig. 2). These differences indicate a bigger influence of herbivory and differences in herbivore community in pools than in the channel. In both years the success of survival is higher in the pools than in the channel and in the discs with protected cage from the herbivores, indicating the importance of habitat on the success of micro-recruits survival.

Table 2. ANOVA of *Sargassum muticum* micro-recruit survival during a grazer exclusion experiment (treatment: cage and no cage) in two habitats (tidal pools and channel), three sites per habitat and repeated four lunar phases in 2005. Data untransformed.

Source of variation	df	MS	F	p
Habitat	1	31329.000	11.639	0.0270
Site (Habitat)	4	2691.785	5.713	0.0004
Moon	3	4911.083	15.576	0.0002
Treatment	1	2916.000	15.015	0.0179
Habitat x Moon	3	520.130	1.650	0.2303
Habitat x Treatment	1	1.361	0.007	0.9373
Moon x Site (Habitat)	12	315.294	0.669	0.7768
Treatment x Site (Habitat)	4	194.201	0.412	0.7995
Moon x Treatment	3	324.204	3.893	0.0373
Habitat x Moon x Treatment	3	252.157	0.535	0.6593
Treatment x Moon x Site (Habitat)	12	83.285	0.177	0.9990
Residual	96	471.181		

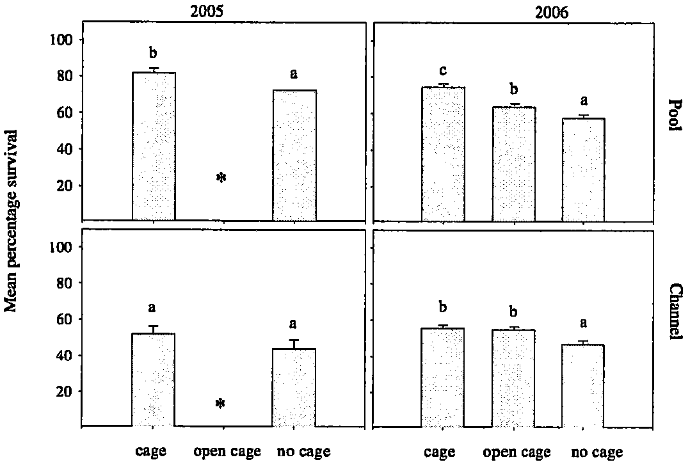

Fig. 2. Mean percentage of *Sargassum muticum* recruit survival in two years (2005 and 2006) in two habitats (intertidal pool and channel) with cage, open cage and no cage treatment. Asterisks indicate absence of open cage treatment. Different letters above bars indicate significant differences between mean percentages based on SNK test ($p < 0.05$). Error bars show standard error n = 12 and n =16 replicates per treatment in 2005 and 2006, respectively).

Table 3. ANOVA of *Sargassum muticum* micro-recruit survival during a grazer exclusion experiment (treatment: cage, open cage and no cage) in two habitats (tidal pools and channel), three sites per habitat and repeated four lunar phases in 2006. Data untransformed.

Source of variation	df	MS	*F*	*p*
Habitat	1	11742.015	50.084	0.0021
Site (Habitat)	4	234.445	1.369	0.2456
Moon	3	358.834	1.510	0.2622
Treatment	2	3978.790	46.376	0.0001
Habitat x Moon	3	822.729	3.368	0.0548
Habitat x Treatment	2	665.601	7.758	0.0134
Moon x Site (Habitat)	12	244.272	1.427	0.1552
Treatment x Site (Habitat)	8	85.795	0.501	0.8547
Moon x Treatment	6	91.273	1.846	0.1323
Habitat x Moon x Treatment	6	91.291	0.533	0.7827
Treatment x Moon x Site (Habitat)	24	49.438	0.289	0.9996
Residual	216	171.222		

DISCUSSION

In both years, recruitment in *Sargassum muticum* was dependent on habitat: higher in pools than in the channel. This difference could be caused by physical differences between these habitats, like differences in temperature, nutrients and salinity. Photoperiod and temperature control the propagules release (Santelices 1990), but eggs of *S. muticum* survive in a large range of temperature, 5° C - 30° C with a optimal temperature at 25°C (Norton 1977, Hales & Fletcher 1989) or 7° C - 17° C (Steen 2003). The temperature in tidal pools could be higher than in the channel, especially at low tide during neap tides, when the tidal channel is always in contact with the sea. Lower concentration of nutrients could decrease eggs density, especially in 17°C (Steen 2003). Another important factor for early life history stages is the salinity. Salinity probably constitutes a physiological barrier to expansion of *S. muticum* (see Norton 1977, Hales & Fletcher 1989) because at low salinities the growth and reproduction decrease (optimal values range between 14.7 and 27.1 ‰, Steen 2004). This aspect deserves more study.

Others physical factors could potentially influence the recruitment like, water motion, shadow or populations density. Physical stress could inhibit completion of life-cycle of *Sargassum muticum* at sites more exposed (Viejo et al. 1995) and in a shading sites the recruitment is lower (Deysher & Norton 1982). Survivorship of *S. muticum* juveniles is lesser at higher densities (Andrew & Viejo 1998b), but is not influenced by wave exposure in the first 4 months of macroscopic growth (Andrew & Viejo 1998a). However, wave exposure could decrease settlement and/or early post-settlement survivorship (Norton 1983, Andrew & Viejo 1998a). Both eggs settlement (Chapter 1) and recruitment are higher in pools than in the channel. The differences in recruitment between habitats could be explained by physical differences between habitats, like wave action and temperature, as well as the differences in grazer assemblages between habitats (see below the importance of grazer assemblages on micro-survival).

Differences in recruitment between years, higher in 2006 than in 2005 may reflect the temporal variation of the reproductive season (see Chapter 1). In 2005, the study was carried out in the last two months of the reproductive season, when lateral branches started dying. In 2006, the fieldwork was carried out in May and June, corresponding to the maximum reproductive biomass. Observation in *Fucus* spp. at the same site showed that recruitment is higher during the peak of reproduction compared to the rest of year (Monteiro et al. in prep.); the same pattern was found in this study.

The micro-recruit survival experiment showed significant differences between cage treatments in 2005 and a significant interaction between habitat and cage treatment, in 2006. In the pools, significant differences were observed between all treatments (cage, open cage and no cage), whereas in the channel no significant differences between cage and open cage were detected. This indicates that the natural survival was less dependent on meso-grazers smaller than the cage grid (5 mm) in the channel compared to the pools, and that the grazing community in the two habitats could be different. So meso-grazers negatively influence the micro-recruits survival, especially in pools.

Herbivores have a significant effect on algal population (Rosemond et al. 1993), they are responsible for the limits of distribution of algae (Hawkins & Hartnoll 1985) and the variability of algal density (Heck et al. 2000) furthermore, herbivory is one of the most important sources of mortality in early recruitment (Vadas et al. 1992), e.g. *Sargassum fiffifolium* (Diaz-Pulido & McCook 2003) and *Sargassum muticum* is grazed by *Littorina littorea* in laboratorial experiences (Sjøtun et al. 2007). Others studies suggest that *Sargassum* spp. are susceptible to grazing, e.g. by sea urchins (De Wreede 1983), herbivorous fish (McCook 1996), amphipods and gastropods (Norton & Benson 1983). Gastropod grazing is thought to be the most important factor determining the upper limit of lower algal beds, and only in the absence of grazers would the physical factors become important in determining the upper limit of macroalgae (Jernakoff 1983). In the eastern Atlantic, patellid gastropods are common grazers and an important source of mortality for fucoid germlings (Hawkins & Hartnoll 1983). Furthermore, herbivory also differs between early and adult seaweed stages (Diaz-Pulido & McCook 2003). For example, litorinid influence the patterns of recruit survival, especially at later stages in *Ascophyllum nodosum* (Viejo et al. 1999) and mussels exerted a positive effect on the length of fronds of *Cystoseira compressa,* but inhibited its recruitment (Benedetti-Cecchi et al. 1996).

A significant difference in micro-recruit survival was observed between habitats (higher in pools than the tidal channel), in both years. Physical differences between habitats, like temperature, nutrients or salinity could be responsible for micro-recruit survival, like was explained above for recruitment variability. The patterns of early survival are a result of the interaction between different physical and biological factors (Santelices 1990). Furthermore, based on significant differences observed in micro-recruit survival between habitats and significant interaction between habitat and cage we conclude that differences in grazer assemblages between habitats could play an important role on micro-recruit survival and probably in the recruitment to. Not all species in a grazer assemblage have the same affect on algae (Underwood & Jernakoff 1981, Underwood 1984). For example, limpets are normally the key grazers in mid- and high tidal levels (Hawkins et al. 1992), but limpet, *Cellana tramoserica* can show differences in grazing patterns at similar densities in the two habitats (rocky shores and seawalls) (Bulleri et al. 2004).

The micro-recruit survival experiments were performed during four lunar phases because the reproductive cycle of *Sargassum muticum* correlated with semilunar cycles. The influence of lunar rhythms on the activity of many marine organisms has been documented (e.g. reproductive cycles, synchronizing gamete release, synchronizing locomotion or feeding, for further review see Hawkins & Hartnoll 1983, Naylor 2001, deBruyn & Meeuwig 2001). Interactions between semilunar rhythms and locomotion activity have been reported for example in the isopod *Eurydice pulchra* (Alheit & Naylor 1976) and the amphipod *Talitrus saltator* (Williams 1979). Teleosts fishs depend on tides for feeding, exhibiting lunar and semi-lunar checks (discontinuities) in otolith growth, but others studies suggest that this theory is more complex than a simply tidal variation (for further references see Farbridge & Leatherland 1987). Based on this information, we initially hypothesized that there would be moon related differences in micro-recruit survival. Considering the evolutionary importance of the timing of release for the persistence of populations, we expected to find higher survival during moon phases in which naturally release takes place. In fact, there were significant differences in the percentage of recruit survival among lunar phases in 2005, but no effect was observed in 2006. In 2005, the percentage of micro-recruits survival was higher during full and new moon in treatments with cage, but this was not observed during full moon in treatments without cage. The significant interaction between moon and cage observed in 2005 was unexpected, and we are not able to provide a plausible explanation. The relation between moon cycles and micro-recruit survival deserves more study.

The goal of this study was to test for differences between habitats on recruitment and micro-recruit survival and to assess the importance of grazing on the micro-recruits survival in *Sargassum muticum*. Our results, revealed a great difference in recruitment and micro-recruits survival between habitats. Physical differences between both habitats could have influence on recruit success, but we hypothesize that differences in grazer assemblages could play an important role on micro-recruit survival and probably in the recruitment between habitats. Furthermore, we demonstrated a clear role of meso-herbivory on micro-recruit survival, especially in the tide pools. The invasive alga *S. muticum* occurs in different habitats ranging from the subtidal to middle intertidal, and in Europe the species is distributed from Norway to Portugal, experiencing a variety of habitat conditions and grazers assemblages. Experimental studies on interactions between grazers and *S. muticum* should be performed in different habitats along the invader coastline in order to assess the effects of grazing assemblages on the recruitment success of this species.

Acknowledgements. We are thankful to the "Associação dos Amigos do Mar" in Viana do Castelo, for supporting us during the entire sample period.

LITERATURE CITED

Alheit J, Naylor E (1976) Behavioural basis of intertidal zonation in *Eurydice pulchra* Leach. J Exp Mar Biol Ecol 23:135-144

Andrew NL, Viejo RM (1998a) Effects of wave exposure and intraspecific density on the growth and survivorship of *Sargassum muticum* (Sargassaceae: Phaeophyta). Eur J Phycol 33:251-258

Andrew NL, Viejo RM (1998b) Ecological limits to the invasion of *Sargassum muticum* in northern Spain. Aquat Bot 60:251-263

Arrontes J, Arenas F, Fernandez C, Rico JM, Oliveros J, Martinez B, Viejo RM, Alvarez D (2004) Effect of grazing by limpets on mid-shore species assemblages in northern Spain. Mar Ecol Prog Ser 277:117-133

Benedetti-Cecchi L, Nuti S, Cinelli F (1996) Analysis of spatial and temporal variability in interations among algae, limpets and mussels in low-shore habitats on the west coast of Italy. Mar Ecol Prog Ser 144:87-96

Boaventura D, Alexander M, Della Santina P, Smith ND, Ré P, Fonseca LC, Hawkins SJ (2002) The effects of grazing on the distribution and composition of low-shore algal communities on the central coast of Portugal and on the southern coast of Britain. J Exp Mar Biol Ecol 267:185-206

Bulleri F, Chapman MG, Underwood AJ (2004) Patterns of movement of the limpet *Cellana tramoserica* on rocky shores and retaining seawalls. Mar Ecol Prog Ser 281:121-129

Britton-Simmons KH (2004) Direct and indirect effects of the introduced alga *Sargassum muticum* on benthic, subtidal communities of Washington State, USA. Mar Ecol Prog Ser 277:61-78

Critchley AT, Dijkema R (1984) On the presence of the introduced brown alga *Sargassum muticum*, attached to commercially imported *Ostrea edulis* in the S.W. Netherlands. Bot Mar 27:211-216

deBruyn AMH, Meeuwig JJ (2001) Detecting lunar cycles in marine ecology: periodic regression versus categorical ANOVA. Mar Ecol Prog Ser 214:307-310

De Wreede RE (1983) *Sargassum muticum* (Fucales, Phaeophyta): regrowth and interaction with *Rhodomela larix* (Ceramiales, Rhodophyta). Phycologia 22:153-160

Dethier MN, Duggins DO (1988) Variation in strong interactions in the intertidal zone along a geographical gradient: a Washington-Alaska comparison. Mar Ecol Prog Ser 50:97-105

Druehl LD (1973) Marine transplantations. Science 179: 12

Deysher L, Norton TA (1982) Dispersal and colonization in *Sargassum muticum* (Yendo) Fensholt. J Exp Mar Biol Ecol 56:179-195

Diaz-Pulido G, Mcook LJ (2003) Relative roles of herbivory and nutrients in the recruitment of coral-reef seaweeds. Ecology 84(8):2026-2033

Farbridge KJ, Leatherland JF (1987) Lunar cycles of coho salmon, *Oncorhynchus kisutch.* J Exp Biol 129:165-178

Farnham W, Fletcher RL, Irvine LM (1973) Attached *Sargassum* found in Britain. Nature 243:231-232

Fletcher RL (1980) Studies on the recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. III. Periodicity in gamete release and "incubation" of early embryo stages. Bot Mar 23:425-432

Hales JM, Fletcher RL (1989) Studies on the recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. IV. The effect of temperature, irradiance and salinity on germling growth. Bot Mar 32:167-176

Hawkins SJ, Hartnoll RG (1983) Grazing of intertidal algae by marine invertebrates. Oceanogr Mar Biol Annu Rev 21:195-282

Hawkins SJ, Hartnoll RG (1985) Factors determining the upper limits of intertidal canopy-forming algae. Mar Ecol Prog Ser 20:265-271

Hawkins SJ, Hartnoll RG, Kain JM, Norton TA (1992) Plant–animal interactions on hard substrata in the Northeast Atlantic. In: John DM, Hawkins SJ, Price J.H (eds) Plant–animal interactions in the marine benthos. Oxford University Press, Oxford, p 1–32

Heck KL, Pennock JR, Valentine JF, Coen LD, Sklenar SA (2000) Effects of nutrient enrichment and small predator density on seagrass ecosystems: an experimental assessment. Limnol Oceanogr 45:1041-1057

Jernakoff P (1983) Factors affecting the recruitment of algae in a midshore region dominated by barnacles. J Exp Mar Biol Ecol 67:17-31

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol 17:164-170

Kendrick GA (1994) Effects of propagule settlement density and adult canopy on survival of recruits of *Sargassum* spp. (Sargassaceae: Phaeophyta). Mar Ecol Prog Ser 103:129-140

Ladah L, Bermudez R, Pearson G, Serrão E (2003) Fertilization success and recruitment of dioecious and hermaphroditic fucoid seaweeds with contrasting distributions near their southern limit. Mar Ecol Prog Ser 262:163-183

Lawton JH, Brown KC (1986) The population and community ecology of invading insects. Philos Trans R Soc Lond B 314:607-617

Levin PS, Coyer JA, Petrik R, Good TP (2002) Community-wide effects of non-indigenous species on temperate rocky reefs. Ecology 83:3182-3193

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl 10:689-710

McCook LJ (1996) Effects of herbivores and water quality on *Sargassum* distribution on the Great Barrier Reef: cross-shelf transplants. Mar Ecol Prog Ser 139:179-192

Mitchell CE, Power AG (2003) Release of invasive plants from fungal and viral pathogens. Nature 421:625-627

Naylor E (2001) Marine animal behaviour in relation to lunar phase. Earth Moon and Planets 85/86:291-302

Neushul M, Foster MS, Coon DA, Woessner JW, Harger BWW (1976) An *in situ* study of recruitment, growth and survival of subtidal marine algae: techniques and preliminary results. J Phycol 12:397–408

Norton TA (1977) Ecological experiments with *Sargassum muticum*. J Mar Biol Ass UK 57:33-43

Norton TA (1981) Gamete expulsion and release in *Sargassum muticum*. Bot Mar 14:465-470

Norton TA (1983) The resistance to dislodgement of *Sargassum muticum* germlings under defined hydrodynamic conditions. J Mar Biol Assoc UK 63:181-193

Norton TA Benson MR (1983) Ecological interactions between the brown seaweed *Sargassum muticum* and its associated fauna. Mar Biol 75:169-177

Okuda T (1981) Egg liberation in some Japanese Sargassaceae (Phaeophyceae). Xth Int Seaweed Symp, Berlin, p 197-202

Pearson GA, Serrão EA (2006). Revisiting synchronous gamete release by fucoid algae in the intertidal zone: fertilization success and beyond? Integr Comp Biol 46:587-597

Rosemond AD, Mulholland PJ, Elwood JW (1993) Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology 74:1264-1280

Rueness J (1989) *Sargassum muticum* and other introduced Japonese macroalgae: biological pollution of European coasts. Mar Poll Bull 20(4):173-176

Santelices B (1990) Patterns of reproduction, dispersal and recruitment in seaweeds. Oceanogr Mar Biol Annu Rev 28:177-276

Simberloff D (2001) Biological invasions - how are they affecting us, and what can we do about them? West N Am Naturalist 61(3):308-315

Sjøtun K, Eggereide SF, Høisæter T (2007) Grazer-controlled recruitment of the introduced *Sargassum muticum* (Phaeophyceae, Fucales) in northern Europe. Mar Ecol Prog Ser 342:127-138

Steen H (2003) Intraspecific competition in *Sargassum muticum* (Phaeophyceae) germlings under various density, nutrient and temperature regimes. Bot Mar 46:36-43

Steen H (2004) Effects of reduced salinity on reproduction and germling development in *Sargassum muticum* (Phaeophyceae, Fucales). Eur J Phycol 39:293-299

Underwood H (1984) The pineal and circadian rhythms. In: Reiter RJ (Ed) The pineal gland. Raven Press, New York, p 221-252

Underwood AJ, Jernakoff P (1981) Effects of interactions between algae and grazing gastropods on the structure of a low-shore intertidal community. Oecologia 48:221-233

Vadas RL, Johnson S, Norton TA (1992) Recruitment and mortality of early pos-settlement stages of benthic algae. Br Phycol J 27:331-351

Viejo RM, Arrontes J, Andrew NL (1995) An experimental evaluation of the effect of wave action on the distribution of *Sargassum muticum* in Northern Spain. Bot Mar 38:437-441

Viejo RM, Åberg P, Cervin G, Lindegarth M (1999) The interactive effects of adult canopy, germling density and grazing on germling survival of the rockweed *Ascophyllum nodosum*. Mar Ecol Prog Ser 187:113-120

Villele X, Verlaque M (1995) Changes and degradation in a *Posidonia oceanica* bed invaded by the introduced alga *Caulerpa taxifolia* in the North Western Mediterranean. Bot Mar 38:79-87

Vitousek PM, D'António C, Loope LL, Rejmànek M, Westbrooks M et al. (1997) Introduced species: a significant component of human-caused global change. N Z J Ecol 21:1-16

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. BioSci 48:607-615

Williams JA (1979) A semilunar rhythm of locomotor activity and moult synchrony in the sand beach amphipod *Talitrus saltator*. In: Naylor E, Hartnoll RG (eds) Cyclical phenomena in marine plants and animals. Pergamon Press, Oxford, p407-414

Wilson CG (1989) Post-dispersal seed predation of an exotic weed, *Mimosa pigra* L., in the Northern Territory. Aust J Ecol 14:235-240

Wilson EO (1992) The diversity of life. Harvard University Press, Cambridge, MA

General Conclusions

The introduction of invasive species offers a unique opportunity to study ecological adaptations, since these adaptations have been taking place over very small evolutionary time frames. Only a small number of species survive in a new region and become established and even fewer will cause disturbance. The success of invasive species is dependent on the intrinsic characteristics of the species as well as on the characteristics of the recipient ecosystem. After entering a new region the success of species is greatly dependent on its life history and reproductive strategies, as well as on levels of predation/grazing in the recipient ecosystems.

Sargassum muticum (Yendo) Fensholt is an invader species that is firmly establish on intertidal and subtidal rocky shores of Europe and North America and can be found in different habitats ranging from high intertidal flats and pools to the shallow subtidal (Norton 1977b, Ambrose & Nelson 1982, Critchley 1983a, Critcley & Dijkema 1984). The success and rapid spread of this species is probably due to the strong seasonal patterns of reproduction (Kendrick 1993) and its reproductive potential (Umezaki 1984) that seems dependent on temperature and on exogenous factors like tidal and lunar cycles.

This thesis contains the first study that describes and compares natural egg release, embryo settlement, recruitment and early-survival of *Sargassum muticum* in two different intertidal habitats, tidal pool (mid-intertidal) and tidal channel (low-intertidal). The first Chapter showed that in both habitats egg expulsion and embryo settlement had a semilunar periodicity, occurring during spring tides and coinciding with higher tidal amplitude (periods of full and new moons). However, egg expulsion and settlement occurred 1-2 days earlier in pools than in the channel. During the daily tidal cycle a significant difference in egg expulsion was found between habitats, suggesting that factors related to the tidal amplitude could be the cues for triggering the exact timing of release.

The second Chapter showed that recruitment (14 times higher in pools than in the channel) and micro-recruits survival (1.6 and 1.2 times higher in pools than in the channel in 2005 and 2006, respectively) is different between habitats and higher in pools than in channel. In addition, we demonstrated a clear role of meso-herbivory on micro-recruit survival, especially in the tide pools. Even so, *Sargassum muticum* recruitment and micro-recruits survival are more efficient in intertidal pools than in intertidal channel.

Understanding the reproductive features in early life history (e.g. settlement, recruitment and early survival), and compare the reproductive strategies between habitats is important step to understand the flexibility of the reproductive strategy of this invasive specie. In the last years, research on biological invasion has made a shift from studies on invader characteristics to ecosystem approaches in which especially the characteristics of the recipient community are receiving more attention. This thesis shows that the success of the invasive specie *Sargassum muticum* may include synchronous eggs expulsion and embryos settlement during spring tides. Furthermore, the eggs expulsion and embryos settlement was asynchronous between habitats and synchronous within habitats suggesting that tidal amplitude is a trigger factor on timing of reproduction. Moreover, synchrony of egg expulsion and embryo settlement within habitats increases the concentration of eggs and sperm and consequently increases fertilization success and satiation for the herbivores (Reed et al. 1997). This thesis provides evidence for the correctness of this new approach since it was found that for example herbivory can play an important role on the survival of invasive recruits. This knowledge could be of importance for management strategies as well as for the construction of invasion theory.

Literature Cited

Ambrose RF, Nelson BV (1982) Inhibition of giant kelp recruitment by an introduced brown alga. Bot Mar 25:265-267

Andrew NL, Viejo RM (1998a) Ecological limits to the invasion of *Sargassum muticum* in northern Spain. Aquat Bot 60:251-263

Andrew NL, Viejo RM (1998b) Effects of wave exposure and intraspecific density on the growth and survivorship of *Sargassum muticum* (Sargassaceae: Phaeophyta). Euro J Phycol 33:251-258

Arenas F, Fernández C (1998) Ecology of *Sargassum muticum* (Phaeophyta) on the North Coast of Spain. III. Reproductive ecology. Bot Mar 41:209-216

Ashton PJ, Mitchell DS (1989) Aquatic plants: pattern and modes of invasion, attributes of invaing species and assessment of control programmes. In: Drake JA, Mooney HA, di Castri F, Groves RH, Kruger FJ, Rejmanele M, Williamson M (eds) Biological invasion: a global perspective. Wiley & Sons, New York, p 111-149

Boudouresque CF, Verlaque M (2002)Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. Mar Pollut Bull 44:32-38

Critchley AT (1983a) The established and increase of *Sargassum muticum* (Yendo) Fensholt populations within the Solent area of Southern Britain. I. An Investigation of the increase in number pf population individuals. Bot Mar 26:539-545

Critchley AT (1983b) The established and increase of *Sargassum muticum* (Yendo) Fensholt populations within the Solent area of Southern Britain. II. An Investigation of the increase in canopy cover of the alga at low water. Bot Mar 26:547-552

Critchley AT, Dijkema R (1984) On the presence of the introduced brown alga *Sargassum muticum*, attached to commercially imported *Ostrea edulis* in the S. W. Netherlands. Bot Mar 27:211-216

Deysher L (1984) Reproductive phenology of newly introduced populations of brown alga, *Sargassum muticum* (Yendo) Fensholt. Hydrobiologia 116/117:403-407

Deysher L, Norton TA (1982) Dispersal and colonization in *Sargassum muticum* (Yendo) Fensholt. J Exp Mar Biol Ecol 56:179-195

De Wreede RE (1983) *Sargassum muticum* (Fucales, Phaeophyta): regrowth and interaction with *Rhodomela larix* (Ceramiales, Rhodophyta). Phycologia 22(2):153-160

Druehl, LD (1973) Marine transplantations. Science 179: 12

Elton CS (1958) The ecology of invasions by animals and plants. Methuen and Co, London, UK

Ehrlich PR (1986) Which animal will invade? In: Mooney HA, Drake JA (eds) Ecology of Biological Invasions of North America and Hawaii. Springer-Verlag, New York, p 79-95

Espinoza J (1990) The southern limit of *Sargassum muticum* (Yendo) Fensholt (Phaeophyta, Fucales) in the Mexican Pacific. Bot Mar 33:193-196

Farnham W, Fletcher RL, Irvine LM (1973) Attached *Sargassum* found in Britain. Nature 243:231-232

Fernández C, Gutiérrez LM, Rico JM (1990) Ecology of *Sargassum muticum* on the north coast of Spain. Preliminary Observations. Bot Mar 33:423-429

Fletcher RL (1980) Studies on the recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. III. Periodicity in gamete release and "incubation" of early embryo stages. Bot Mar 23:425-432

Fletcher RL, Fletcher SM (1975a) Studies on recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. I. Ecology and reproduction. Bot Mar 18:149-156

Fletcher RL, Fletcher SM (1975b) Studies on recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. II. Regenerative ability. Bot Mar 18:157-162

Hales JM, Fletcher RL (1989) Studies on the recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. IV. The effect of temperature, irradiance and salinity on germling growth. Bot Mar 32:167-176

Hales JM, Fletcher RL (1990) Studies on the recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. V. Receptacle initiation and growth, and gamete release in laboratory culture. Bot Mar 33:241-249

Hoffman AJ (1987) The arrival of seaweed propagules at the shore: a review. Bot Mar 30:151-165

Kendrick GA (1993) *Sargassum* beds at Rottnest Island: species composition and abundance. In: Wells FE, Walker DI, Kirkman H, Lethbridge R. WA (eds) Proc. 5th International Marine Biological Workshop: the flora and fauna of Rottnest Island, Western Australia, Museum Press, Perth, p 455-472

Levin PS, Coyer JA, Petrik R, Good TP (2002) Community-wide effects of non-indigenous species on temperate rocky reefs. Ecology 83:3182-3193

Lluch JR, Garreta G, Barceló MC, Ribera MA (1994) Mapas de distribucíon de algas marinas de la Península Ibérica e Islas Baleares. VII. *Cystoseira* C. Agardh (Grupo *C. bacata*) y *Sargassum* C. Agardh (*S. muticum* y *S. vulgare*). Bot Complut 19:131-138

Neushul M, Foster MS, Coon DA, Woessner JW, Harger BWW (1976) An *in situ* study of recruitment, growth and survival of subtidal marine algae: techniques and preliminary results. J Phycol 12:397–408

Norton TA (1976) Why is *Sargassum muticum* so invasive? Br Phycol J 11:197-198

Norton TA (1977a) The growth and development of *Sargassum muticum* (Yendo) Fensholt. J Exp Mar Biol Ecol 26:41-53

Norton TA (1977b) Ecological experiments with *Sargassum muticum*. J Mar Biol Ass UK 57:33-43

Norton TA (1981) Gamete expulsion and release in *Sargassum muticum*. Bot Mar 14:465-470

Nyberg CD, Wallentinus I (2005) Can species traits be used to predict marine macroalgal introductions? Bio Inv 7:265-279

Okuda T, Karei H, Yamada M (1984) Settlement of germlings in ten fucalean species. Hydrobiologia 116/117:413-418

Pru'homme van Reine WF (1977a) Zeewieren in Nederland. Natura 74:15-19

Pru'homme van Reine WF (1977b) Japans bessenwier aan onze kust. Het Zeepaard 37:58-63

Pru'homme van Reine WF, Nienhuis PH (1982) Occurrence of the brown alga *Sargassum muticum* (Yendo) Fensholt in The Netherlands. Bot Mar 25:37-39

Reed DC, Anderson TW, Ebeling AW, Anghera M (1997) The role of reproductive synchrony in the colonization potential of kelp. Ecology 78:2443-2457

Steen H (2004) Effects of reduced salinity on reproduction and germling development in *Sargassum muticum* (Phaeophyceae, Fucales). Eur J Phycol 39(3):293-299

Umezaki I (1984) How many eggs will be discharged from the plant of *Sargassum horneri*? Hydrobiologia 116/117:398-402

Viejo RM, Arrontes JM, Andrew NL (1995) An experimental evaluation of the effect of wave action on the distribution of *Sargassum muticum* in northern Spain. Bot Mar 38:437-441

Viejo RM (1997) The effects of colonization by *Sargassum muticum* on tidepool macroalgal assemblages. J Mar Biol Ass UK 77:325-340

Villele X, Verlaque M (1995) Changes and degradation in a *Posidonia oceanica* bed invaded by the introduced alga *Caulerpa taxifolia* in the North Western Mediterranean. Bot Mar 38:79-87

Vitsousek PM, D'António C, Loope LL, Rejmànek M, Westbrooks M (1997) Introduced species: a significant component of human-caused global change. N Z J Ecol 21:1-16

Walker DI, Kendrick GA (1998) Threats to macroalgal diversity: marine habitat destruction and fragmentation and introduced species. Bot Mar 41:105-112

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. BioSci 48:607-615

Wilson EO (1992) The diversity of life. Harvard University Press, Cambridge