ARTICLE IN PRESS

Marine Pollution Bulletin xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Field transplantation of seagrass (*Posidonia oceanica)* seedlings: Effects of invasive algae and nutrients

Pereda-Briones L.[a], Tomas F.[a,b,*], Terrados J.[a]

[a] *Instituto Mediterráneo de Estudios Avanzados, IMEDEA (CSIC-UIB), Miquel Marques 21, 07190 Esporles, Illes Balears, Spain*
[b] *Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, OR 97331, USA*

ARTICLE INFO

Keywords:
Restoration
Facilitation
Alien species
Caulerpa
Eutrophication
Early life stage

ABSTRACT

Seedlings are a key life stage in seagrasses, providing genetic diversity and being a useful tool for restoration. We examined the influence of increased sediment nutrients and the presence of the invasive macroalga *Caulerpa cylindracea* on the success of in situ transplanting *Posidonia oceanica* seedlings in a six-month experiment. Our results indicate that one-year old seedlings successfully survive in the field and their survival and growth are positively affected by the presence of *C. cylindracea.* Furthermore, nutrient addition in the sediment had positive effects on both *C. cylindracea* (increasing its cover) and seedlings (increasing leaf development), and the increased *C. cylindracea* cover did not result in detrimental effects on seedlings. Therefore, biological invasions and nutrient addition do not reinforce each other in the short term to negatively impact transplanted seedlings, which highlights facilitative interactions between invasive algae and native seagrass and provides useful information for successful strategies of seagrass restoration.

1. Introduction

Seagrass beds are recognized as key ecosystems in shallow coastal waters because they provide an ensemble of ecosystem services (coastal protection, water quality, CO_2 sequestration, habitat for fishery species, etc.) that contribute to environmental, economic and social wellbeing (Duarte and Chiscano, 1999; Orth et al., 2006a). As with other coastal ecosystems, seagrass beds are susceptible to suffering multiple perturbations associated to human activities, both local and global (e.g. trawling, eutrophication, boat anchoring, climate change, invasive species etc.). Such disturbances threaten these important ecosystems and have caused worldwide declines of seagrass meadows during the last century (Orth et al., 2006a; Boudouresque et al., 2009; Waycott et al., 2009). Recent literature has emphasized the importance of restoration as an effective instrument to assist recovery of a disturbed or degraded ecosystem (Ganassin and Gibbs, 2008; Reynolds et al., 2016).

The process of seagrass restoration is recognized as being complex and having produced relatively few successes (Paling et al., 2003; Seddon, 2004; Ganassin and Gibbs, 2008). Over the years, numerous techniques have been developed for different species and environments, including the transplantation of adult seagrasses as well as the use of seeds and seedlings in restoration (Renton et al., 2011; Statton et al., 2012). Transplanting adult plants involves their collection from healthy beds, which may damage or degrade the donor beds. Furthermore, in many cases, transplant efforts have resulted in limited survival and spatial expansion (West et al., 1990; Paling et al., 2003). Conversely, planting seeds and seedlings is a non-destructive technique, since it may lower the damage of donor beds (Seddon, 2004; Orth et al., 2006b). On the other hand, seedlings are a critical life stage that contributes to the maintenance of existing meadows (Zipperle et al., 2009; Jarvis et al., 2014) as well as the formation of new ones via dispersal (Orth et al., 1994). In addition, seedling production represents a critical genetic diversity source that provides adaptive capacity, essential for the long term persistence of the species and the ecosystems they form. The ecological importance of seagrass seedlings is thus highlighted in the current scenario of seagrass threats and losses (Waycott et al., 2009).

Posidonia oceanica is a critical foundation species endemic to the Mediterranean Sea, and its meadows have decreased between 5% and 20% since the beginning of the twentieth century (Boudouresque et al., 2009). One-year survival represents a bottleneck in the life cycle of this species (Balestri et al., 1998; Piazzi et al., 1999). Nutrient reserves appear to only be enough to sustain the plant during the first 8 months of seedling development (Balestri et al., 2009), which could contribute to reduced development and higher mortality in seedlings established on poor-nutrient areas. Indeed, seedling abundance decreases to about half in the course of the first year of establishment and development (Piazzi et al., 1999; Balestri and Lardicci, 2008). Hence, to avoid the high mortality associated to the first year of life, it is of special interest

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[*] Corresponding author at: Instituto Mediterráneo de Estudios Avanzados, IMEDEA (CSIC-UIB), Miquel Marques 21, 07190 Esporles, Illes Balears, Spain.
E-mail address: fiona@imedea.uib-csic.es (F. Tomas).

http://dx.doi.org/10.1016/j.marpolbul.2017.09.034
Received 15 March 2017; Received in revised form 13 September 2017; Accepted 14 September 2017

Please cite this article as: Pereda-Briones, L., Marine Pollution Bulletin (2017), http://dx.doi.org/10.1016/j.marpolbul.2017.09.034

to evaluate the use of one-year-old seedlings as a source of transplanting material in seagrass restoration projects.

P. oceanica seedlings commonly settle in "dead matte" (i.e. a matrix of dead seagrass rhizomes) areas surrounding the meadow as well as on rocky bottoms (Balestri et al., 1998; Piazzi et al., 1999; Balestri and Lardicci, 2008; Alagna et al., 2013), which are areas that can be easily colonized by invasive algae (Infantes et al., 2011a; Marbà et al., 2014). Biological invasions are a major and growing concern of global biodiversity change (Kappel, 2005; Wonham and Pachepsky, 2006). The invasions of *Caulerpa* species have received much scientific and public attention (Klein and Verlaque, 2008). In particular, *Caulerpa taxifolia* (Vahl) C. Agardh and *Caulerpa cylindracea [*(Sonder) Belton et al., 2014; formerly *Caulerpa racemosa* (Forsskal) J. Agardh var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque] have rapidly colonized wide areas of the Mediterranean and Australia, forming permanent populations and interfering with the native assemblages, influencing the decrease of native algal species (Piazzi et al., 2005), and impacting macrophyte assemblages such as seagrasses (Klein and Verlaque, 2008), amongst other impacts.

Nutrients are an essential resource for primary producers, particularly in oligotrophic environments, and could be an important factor influencing competitive interactions between invasive algae and seagrass seedlings. Previous studies have demonstrated that the penetration of *C. cylindracea* into native assemblages is enhanced with increased nutrients, suggesting that the invasive capacity of this species may be facilitated under higher nutrient regimes (Gennaro and Piazzi, 2011; Gennaro et al., 2015). Therefore, increased nutrient availability could enhance negative effects of invasive algae on native seagrass seedlings. However, since the Mediterranean Sea is an oligotrophic region where nutrient limitation drives *P. oceanica* development (Romero et al., 1998; Alcoverro et al., 2001), and nutrient reserves are constrained in seedlings (Balestri et al., 2009), an increase of nutrient availability could also favor seedling competitive capacity by promoting seedling development, potentially modifying the direction and strength of interactions between the invasive alga and the native seagrass (Gennaro and Piazzi, 2011; Klein and Verlaque, 2008).

The objective of this study was to assess the success of transplanting one-year old *P. oceanica* seedlings on dead matte substrate, and how this process is influenced by increased nutrient availability in the sediment and the presence of the invasive macroalga *C. cylindracea*. To achieve this goal, an in situ experiment where *C. cylindracea* presence and nutrient availability were manipulated in plots with transplanted *P. oceanica* seedlings was performed to test the following hypothesis: (1) the presence of *C. cylindracea* increases the mortality of *P. oceanica* seedlings and reduces their vegetative development, (2) an increase of nutrient availability in the sediment promotes the abundance of *C. cylindracea* and the development of *P. oceanica* seedlings.

2. Materials and methods

2.1. Study site

The study was carried out in Alcanada (39°60′N, 3°9′E), Alcudia Bay, Mallorca, in the Balearic Islands archipelago (Western Mediterranean). The site is shallow (max. depth of 5 m) and exposed to south winds which blow most often during winter. Patches of *P. oceanica* meadows alternate with sand gaps and dead *P. oceanica* patches where the substratum is "dead matte", the mixture of rhizomes, roots and sediment that this species forms (Mateo et al., 1997). We chose dead matte as the substratum for our experiment since it is colonized by *C. cylindracea* (Infantes et al., 2011b) and it is the most successful transplant area for *P. oceanica* (Molenaar and Meinesz, 1995; Balestri et al., 1998, Piazzi et al., 1999, Terrados et al., 2013).

2.2. *Posidonia oceanica* seedlings for transplanting

The seedlings of *P. oceanica* were obtained from fruits collected on the sea shore during June 2013 along the Mallorca coast. Once in the laboratory, fruits were opened and their seed removed and placed in 60 L aquariums with seawater (salinity, 37), at a temperature of 19 °C and illuminated by white fluorescent lamps that provided 57 ± 2 $\mu mol \cdot m^{-2} s^{-1}$ of photosynthetically active radiation in a 14:10 h light:dark photoperiod. The aquariums were also equipped with an air pump and a water filter, providing a flow rate of 200 L per hour. Seeds successfully germinate under these conditions and first leaves and roots are produced within two weeks (Terrados et al., 2013). These seedlings were maintained without any substrate for the following eleven months under the described conditions, with monthly replacement of seawater and weekly manual elimination of epiphytic algae growing on seedling leaves. Seedlings were examined for proper vegetative development (presence of seed, leaves and roots) and 108 seedlings with homogeneous morphological characteristics (in terms of number and size of leaves and roots) were chosen for transplanting. These seedlings consisted of one shoot, a short rhizome (1 to 3.5 cm in length) with 2 to 5 adventitious roots and 4 to 5 leaves amounting a total leaf surface of (23.7 + 1.16 cm^2; mean + SE).

2.3. Experimental design

A factorial experiment was designed considering two factors, each with two levels: nutrient availability in the sediment (control versus fertilized) and presence of *Caulerpa cylindracea* (present versus removed), with three replicates per treatment. Twelve 30 × 30 cm^2 plots were established on dead matte areas within a depth range of 2.5–3 m. The plots were distributed in three groups (ca. 50 m apart) that contained one replicate plot of each treatment. Distance between plots within each group was 2 m. Treatments were randomly assigned and applied to each plot.

OSMOCOTE *Exact* fertilizer, NPK (15 + 9 + 9, % dry weight) was used as a source of nutrients. Fertilizer (4.31 g) was deployed inside a small hole dug in each plot cell in which seedlings were posteriorly transplanted. Nutrient loading was 64.6 $g\,N\,m^{-2}$ and 38.8 $g\,P\,m^{-2}$, respectively, and similar to a nitrogen loading rate that promotes *P. oceanica* growth (Alcoverro et al., 1997). *C. cylindracea* was manually and carefully removed without disturbing the sediment from the corresponding plots and from a perimeter of 20 cm around them to maintain a buffer zone around the plots. Removal was done before seedling transplanting and was repeated every 2 weeks throughout the experimental period.

Immediately after nutrient and *C. cylindracea* treatment application (May 2014), 9 seedlings were transplanted in each plot, following a gridded pattern of 3 × 3 cells. The experiment ran for 6 months and foliar vegetative development and seedling survival were monitored in situ four times, every 6 weeks approximately. The number of seedlings present in each plot was counted to assess seedling survival (percentage of present seedlings relative to the initial number of seedlings transplanted in each plot). Additionally, the number of leaves and the number of fish bites in the leaves were counted, and the length of all the leaves as well as the width of the second youngest leaf were measured for each seedling.

The cover of *C. cylindracea* in the plots was monitored after treatment application and every month afterwards to evaluate the effectiveness of the repeated eliminations, and to evaluate its response to nutrient addition. *C. cylindracea* cover was estimated by photographical analysis (on 15 random points) of every cell (10 cm^2) of each plot using the software CPCe-Program "Coral Point with Excel extensions" (Kohler and Gill, 2006).

At the end of the experiment, seedlings that had survived were collected to quantify their biomass and nitrogen and carbon content. Seedlings were dried at 60 °C during 48 h, divided into leaf blades,