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Artificial seagrass leaves shield transplanted seagrass seedlings and increase their survivorship

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ABSTRACT

Seagrass meadows provide important ecosystem functions and services. The progressive global deterioration of seagrass meadows requires management strategies to stop and recover seagrass losses. Seagrass restoration can be promoted using seagrass seedlings germinated *in vitro*, but early mortality of seedlings is a severe bottleneck for successful restoration programs. In this study, we tested a technique to promote *in situ* survivorship of *in vitro* germinated seedlings of the seagrass *Cymodocea nodosa*. In particular, we predicted that artificial seagrass leaves (plastic green raffia, i.e. 'Artificial Seagrass Shields', ASSs) surrounding seagrass seedlings would increase the long-term survivorship of seedlings by decreasing herbivory-induced mortality. After 2 years, the survivorship of seagrass seedlings in plots without the ASSs was nil, whereas one third of plots containing ASSs developed small-sized seagrass patches, including a one order of magnitude exponential increase in shoot density. Herbivory intensity on seagrass leaves significantly decreased when surrounded with ASSs. This study demonstrates how ASSs facilitate the establishment of transplanted seagrass seedlings and suggests ASSs could be a cheap, easy-to-use, restoration element to promote the survivorship of seagrass seedlings by decreasing the magnitude of herbivory.

1. Introduction

Seagrass meadows worldwide provide important ecosystem functions and services. A progressive deterioration of seagrass meadows requires management strategies to stop and recover seagrass losses. Seagrass losses are difficult to recover as a result of the slow growth of seagrasses, particularly for long-live species. Transplantation of grown seagrasses, or seagrass patches, has been suggested as a method of restoration. This has, however, been controversial because collection of material for transplantation deteriorate the donor meadows. Alternatively, seagrass restoration can be carried out using seagrass seedlings germinated *in vitro* from seeds (Renton et al., 2011). The optimization of these *in vitro* techniques allows the production of large quantities of seedlings, with up to a 70% of germination success, such as *Cymodocea nodosa* (Zarranz et al., 2010). A bottleneck, however, arises from the low survivorship of seagrass seedlings once planted to the ocean (Balestri and Lardicci, 2012). Several mechanisms can impact seedlings survivorship, including wave-induced turbulence, high sedimentation, lack of resistance to pathogens and high grazing levels (Orth et al., 2010). Consequently, it is important to develop methods to promote the *in situ* survivorship and performance of seagrass seedlings initially germinated *in vitro*.

The seagrass *C. nodosa* (Ucria) Ascherson is distributed throughout the Mediterranean Sea and the adjoining north-eastern Atlantic coasts, playing a fundamental role, as a 'habitat engineer', in the Canary Islands (Reyes et al., 1995; Tuya et al., 2014a,b), creating patchy and continuous meadows (Barberá et al., 2005; Tuya et al., 2013). This seagrass has suffered a severe regression in the last decades, mainly in the most populated islands (Fabbri et al., 2015; Tuya et al., 2014a).

In this study, we aimed to test a field technique to promote *in situ* survivorship of *C. nodosa* seagrass seedlings germinated *in vitro*. In particular, we predicted that artificial (plastic) seagrass leaves surrounding seagrass seedlings would increase seedling survivorship by decreasing herbivore-induced mortality. Two complementary

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field experiments were carried out to (i) demonstrate an increase in seagrass seedling survivorship over 2 years, and (ii) to show that a perimeter of artificial seagrass decreases the intensity of herbivory on seagrass seedlings.

2. Materials and methods

2.1. Seed collection and production of seedlings

Seagrass seeds were collected in February (2013) by SCUBA divers at at Juncalillo del Sur (Juan Grande), located on the south-east coast of Gran Canaria Island (27845945.480 N; 15833904.010 W), at 5–10 m depth. Seeds were transported to a culture facility at the University of Las Palmas, where seed germination was induced following Zarranz et al. (2010). Cultivation conditions included PAR at ca. 30 μmol photons m^{-2} s^{-1} at 20 °C; the photoperiod was 16 h light: 8 h darkness. Salinity was decreased to ca. 18 psu to stimulate germination (Caye et al., 1992). After 1 month, the culture medium was changed and salinity increased to 36 psu to facilitate seedling growth. A total of 200 seedlings were produced; all seedlings developed leaves and enough below-ground material to be transferred to the field.

2.2. Field experimentation

Fifteen seedlings were allocated to each of n = 12, 40 × 40 cm, plots. Plots consisted of a mesh frame with an interior hole, where seedlings were deployed by gently burying below-ground compartments (Fig. 1a). The corners of each plot were anchored to the bottom with metal stakes. Artificial seagrass leaves (green plastic raffia) were attached to the perimeter of 6 plots (Fig. 1, 'Artificial Seagrass Shields', ASS), whereas 6 plots lacked artificial leaves. Plots were randomly allocated on a sandy area (40 m^2) at 11 m depth on the 3rd August 2013. This is within the depth range where *C. nodosa* is normally found in the study region. Adjacent plots were, at least, ca. 3 m apart. This experimental area is *ca.* 30 m away from an adjacent seagrass meadow located at Gando Bay (27°55′37.12″ N, 15°22′40.49″ W). Plots were visited on 6 occasions through a 2-year period (1st October, 2013, 7th February, 2014, 24th April, 2014, 3rd July, 2014, 10th November, 2014 and 7th August, 2015). On each occasion, we annotated for each plot (patch) the total number of seagrass shoots and the mean leaf length. At the end of the experimental period, we quantified (for those surviving patches) patch growth (the rate of rhizome lateral spread, cm y^{-1}) by measuring the distance between the center of the plot (patch) and the edge of the major axis of the patch (Vidondo et al., 1997).

To test if a perimeter of artificial (plastic) seagrass leaves would decrease the intensity of herbivory on seagrass seedlings, we carried out a parallel experiment. We constructed square plots (50 × 50 cm) assembled using a mesh frame to which a seagrass vegetative fragment containing 3–5 shoots (between 8–12 large-sized leaves >15 cm) were attached using cable ties. Epiphytes were initially removed from each leaf using a razor blade. The perimeter of each plot was covered with 0, 1, 3 and 5 rows of either natural of artificial (plastic) seagrass leaves. A total of n = 3 plots per treatment were randomly allocated on the same sandy bottom as the previous experiment. All plots were buried with sand to simulate natural conditions. After 14 days, plots were retrieved. We re-counted the number of leaves per fragment as a way to estimate defoliation rates (i.e. the proportion of detached leaves relative to the initial number of leaves) according to the different experimental treatments. We also noted the number of leaves with at least one bite mark, and calculated the proportion of leaves with at least 1 bite, as a simple indicator of herbivory intensity.

2.3. Statistical analyses

Differences in seagrass shoot density between plots with and without ASS through times were tested by means of a Generalized Least Squares (GLS) model fitted by means of the R package 'REML'. This model was selected, as it accounted for within plot temporal autocorrelation in seagrass shoot density (de Smith, 2015). Differences in defoliation rates (the proportion of detached leaves) between plots with and without ASS, and according to the number of rows perimeter leaves were attached, were tested using a logistic regression. Similarly, differences in the proportion of leaves with at least one bite were also tested by a logistic regression. Logistic regressions were fitted by implementing Generalized Linear Models (GLM) in R (3.1.2).

3. Results

Seagrass seedlings rapidly disappeared in 10 out of the 12 plots (Fig. 2). However, two plots with ASS maintained a steady number of shoots during the first winter and experienced a progressive increase in shoot density until the end of the experimental period (Figs. 1b and 2), including a one order of magnitude increase in shoot density after two years. Statistically, this outcome was indicated by a significant interaction between tested factors (Table 1, GLS: '+/−ASS × Time', $P < 0.05$); this interaction denotes a self-acceleration of patch growth with time, i.e. an exponential increase in shoot density after the first year (Fig. 2). Patch growth rates for these two surviving patches, at the end of the study, were calculated at 55 and 63 cm y^{-1}.

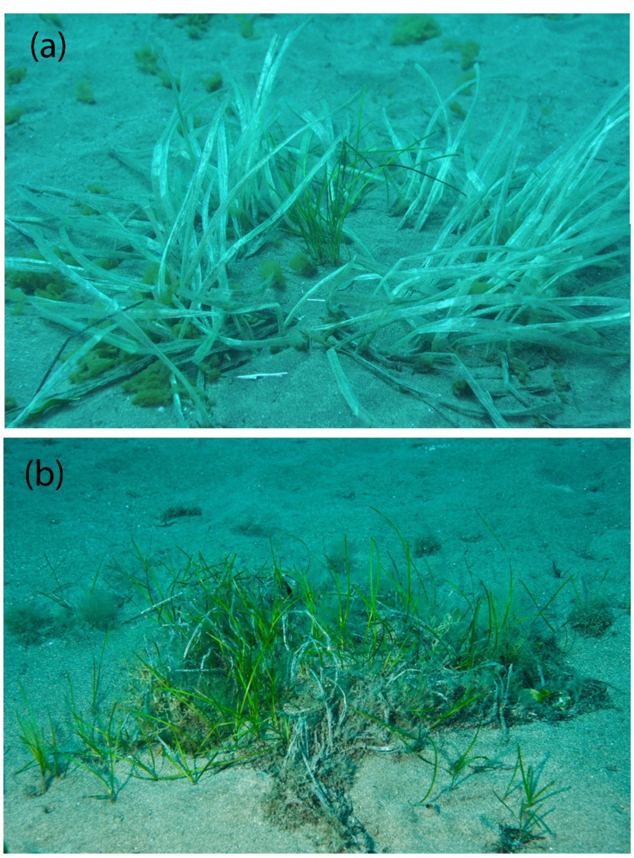

Fig. 1. (a) Transplanted seagrass seedlings in a plot with an arrangement of artificial seagrass leaves around the plot perimeter; (b) seagrass shoots in a plot after 2 years. Runners – plagiotrophic rhizomes containing seagrass shoots developing perpendicular to the plot perimeter – demonstrate the clonal expansion and the subsequent creation of a seagrass patch.