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Assessing the suitability of twelve polymer substrates for the cultivation of macroalgae *Laminaria digitata* and *Saccharina latissima* (Laminariales)

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ABSTRACT

For the cultivation of the European phaeophyte macroalgae *Laminaria digitata* and *Saccharina latissima*, meiospores are settled onto twines within a hatchery, where they are grown for several months. The twine used is often a customarily selected synthetic polymer, polyamide or polypropylene. However, little is known about the impacts of this choice on hatchery performance. To test the effect of substrate material, we settled and cultured meiospores from both *L. digitata* and *S. latissima*, independently on twelve polymer blocks for 4 mo. They were first grown for 2 mon under laboratory conditions, then a further 2 mon in outdoor tanks. Meiospore settlement varied significantly between polymers by up to 15-fold ($p < 0.0001$) with some species-specific differences also observed ($p < 0.0001$). Tufnol was the least suitable polymer, as formaldehyde leachate reduced settlement and inhibited juvenile growth/development. After 8 wk, all polymers excluding Tufnol, were performing similarly with generally ~1 mm sporophytes present at a density of 1–2 mm^{-2}, A negative density-dependent effect of sporophyte size and density was observed in both species ($p < 0.05$). At the end of the experiment, two distinct grouping of polymers were identified regarding *S. latissima*. Those that initially had very high settlement (high density polyethylene, polymethyl methacrylate, polyoxymethylene copolymer/homopolymer and polytetrafluoroethylene) had the lowest final mean lengths, % cover and biomass (<0.2 g wet weight·block^{-1}) at the end of the experiment. Conversely many of the polymers with the lowest initial settlement (polyamide, polycarbonate, medium density polyethylene and polyvinylchloride) had the highest final mean lengths, % cover and biomass (1.7–4.9 g wet weight·block^{-1}). This reversal of fortunes is discussed regarding discriminatory meiospore settlement, differences in apparent adhesion strength of the seaweed holdfast and the transition of the growing sporophytes from a viscous force dominated boundary layer environment to a turbulent dominated environment with increasing drag as the sporophyte grows.

1. Introduction

Saccharina latissima and *Laminaria digitata* are temperate phaeophyte macroalgae of the order Laminariales which are common subtidal species, native to the European Atlantic coastline [45]. They grow to metres in length and contain a seasonally variable composition, with maximal sugar contents of 30–35% in summer and maximal protein contents of 8–11% in autumn-winter [1,27,37]. These seaweeds have economic value as fertiliser, animal feed, for human consumption and the extraction of chemicals such as alginates [4,43]. They can also be used for the bioremediation of nutrients lost from animal mariculture as part of integrated multi-trophic aquaculture (IMTA) systems [35] or for conversion into biofuel [17,20,38].

In East Asia, the related species *Saccharina japonica* is already cultivated on an industrial scale, mainly for food and chemicals [13]. Its annual production of 7.7 mt makes it the highest volume world aquaculture product in 2014 [14]. In Europe, Laminariales cultivation has been research based over the past decades, although more recently it has become commercialised at a small scale in a number of locations. For cultivation, algal meiospores are extracted from fertile sporangial tissue and settled onto twine reels within enclosed tanks. These are then cultured under controlled conditions until sporophytes of 2–10 mm are present [21]. The twines are then wound around a carrier rope at a coastal farm site and after 4–8 months, they reach their adult size. This cultivation method was first developed for *S. japonica* in 1950s China [12]. Two materials have traditionally been utilized as settlement twines: Locally abundant palm fibres which must first be conditioned through hammering and boiling [12,13], and Kuralon, a synthetic polymer manufactured in SE Asia composed of polyvinylalcohol (PVA) fibres which is woven or spun into a slightly fluffy twine [22,44].

In Europe, cultivation trials have sometimes utilized Kuralon [36,44] but more routinely have opted for cheaper alternatives. Often this is polyamide (PA) [10,15,30,40], although polypropylene (PP) has also frequently been used [5,25,28,34]. Polyvinylchloride (PVC) is not available as a twine, but has been used for cultivation experiments before,

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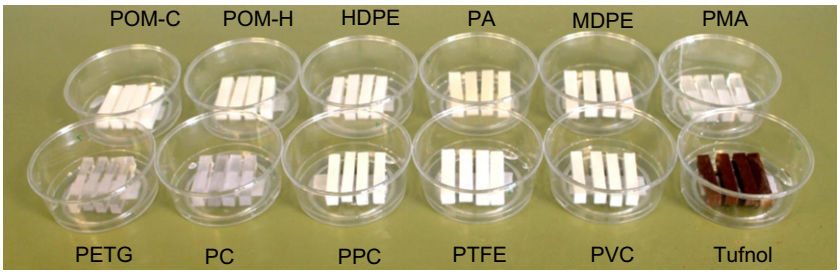

Fig. 1. The twelve polymer blocks examined. Four ~50 × 10 × 10 mm blocks were secured with hook and loop tank into replicated polystyrene basins for experimentation.

and is reported to provide a comparable attachment force to rock in *S. japonica* [19]. As far as the authors are aware, differences in substrate suitability for the settlement and growth of European Laminariales macroalgae has not been reported. This information would provide empirical rather than customary substrate selection to aid the European industry's development.

The surface chemistry is known to affect the settlement choice and adhesion strength of marine organisms including chlorophyte macroalgal zoospores, barnacles and mussels [6,23]. Settlement choice has been reported in North American Laminariales meiospores, and can vary between species [3]. It is anticipated that the same will be true in European Laminariales species. In addition, certain polymers are known to leach compounds which can have a negative effect on algal survival and growth [11]. This may influence the suitability of some polymer as substrates for Laminariales growth.

1.1. Aim and objectives

The aim of this paper is to assess the settlement of meiospores from two European Laminariales species, *S. latissima* and *L. digitata*, and their growth into juvenile sporophytes on twelve different polymers over 4 mon. The objectives are to determine 1) Which polymer/s have the maximum settlement; 2) Which polymers lead to the highest final biomass, making them suitable substrates for cultivation and; 3) Whether polymer exudates are responsible for the patterns of settlement and growth.

2. Materials and methods

2.1. Preparation of blocks and basins

The settlement preference and sporophyte development of the species *L. digitata* and *S. latissima* was evaluated on twelve polymers (Fig. 1): High density polyethylene (HDPE), PA, polycarbonate (PC), medium density polyethylene (MDPE), polyethylene terephthalate glycol (PETG), polymethyl methacrylate (PMA), polyoxymethylene copolymer (POM-C), polyoxymethylene homopolymer (POM-H), polypropylene carbonate (PPC), polytetrafluoroethylene (PTFE), PVC and phenol formaldehyde resin (Tufnol). Sheet plastic was cut into blocks with dimensions of 50 mm length, 7.5–10 mm width and 10 mm height. The top surfaces were milled so that the polymer blocks had similar surface roughness and all corner burs were removed using a razor blade.

The blocks were cleaned thoroughly using 5% Decon90 solution (Decon Laboratories Ltd., UK) and a PA bristled brush to remove dirt and residues from the manufacturing and cutting process. All of the polymers examined are highly resistant to detergent.[1] Blocks were then soaked for 24 h in frequently changed distilled water and dried at 35 °C. Following this, a patch of hooks, from hook and loop tape, was attached using additive free acetic acid cure silicone sealant. After curing for 6 h, the blocks were again soaked for 24 h in frequently changed distilled water to remove acetic acid leachate. These were then dried again at 35 °C. A section of corresponding loops, were secured to the bottom of 300 mL polystyrene basins using ethyl cyanoacrylate glue (Fig. 1). These were then washed and dried similar to the blocks. Four replicate blocks were attached in each of eight replicate basins.

[1] Based on data sheets available from www.bayplastics.co.uk, www.quadrantplastics.com, www.k-mac-plastics.com, www.theplasticshop.co.uk.

2.2. Meiospore extraction

Five fertile individuals of *L. digitata* and *S. latissima* were collected from Seil Sound, UK (56.31724, −5.58309). Meiospores were extracted using the method of Kerrison et al. [21]. The sporangial areas were cut from the thalli, rinsed with Tyndallized seawater [18] and then wiped firmly until dry using laboratory tissue (Kimtech, UK). This was repeated 4–5 times. These were cut into 1–2 cm^2 pieces and gently desiccated overnight in a 4 °C refrigerator between layers of tissue. The following morning, the pieces were placed in 8.5 °C F/2 medium without silicate (F/2-Si) enriched Tyndallized seawater (salinity 33) and incubated in the dark for 1 h [16], with agitation every 15 min to encourage meiospore release. The solution was passed through a 50 μm filter and kept in motion using a magnetic mixer while the meiospore concentration was determined using a Sedgewick Rafter counting chamber.

2.3. Laboratory incubation (wk 0–8)

Basins containing polymer blocks were filled with 300 mL of F/2-Si at 8.5 °C, with 0.125 mL L^{-1} of saturated GeO_2 solution to preclude diatom growth [21] and 100,000 meiospores (2 species × 12 polymer × 4 replicates). The basins were incubated in the dark for 48 h to optimise settlement [21], then the media was refreshed to remove unsettled meiospores and other organics. The basins were incubated at 15–25 μmol m^{-2} s^{-1} by cool white fluorescent light on a 12:12 light:dark cycle. Each wk following, the blocks were transferred into new basins of fresh F/2-Si medium, and the light increased to 30–50 μmol m^{-2} s^{-1} for a further seven wk.

At two timepoints, a randomly selected polymer block was sacrificed from each basin for examination using epifluorescent microscopy. Cells were identified through the autofluorescence of chlorophyll *a* using a Axioskop 2 microscope combined with a UV light source and filter set 09 (Zeiss, Germany). Meiospore density was determined following initial settlement. At wk 3, the density of all meiospores, gametophytes and sporophytes was determined and size measurements were taken of the largest individuals present in each field of view (predominantly sporophytes).

In February 2013, after 8 wk, all surfaces of the polymer blocks excluding the top, were wiped clean with tissue. All blocks were photographed through a stereomicroscope (Axioskop, Zeiss, Germany), using a camera (1100D, Canon, UK) and laptop running EOS Utility software (Canon, UK), capturing a 51–69 mm^2 section (dependent on block width). ImageJ v 1.45 s (National Institutes of Health, USA) was then used to determine sporophytes·mm^{-2} and measure the length of the ten largest sporophytes present. The smallest detectable sporophytes