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A life-stage conflict of interest in kelp: Higher meiospore settlement where sporophyte attachment is weak

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ABSTRACT

Meiospores of Laminariales macroalgae must select a benthic substratum suitable for their attachment and survival, but also suitable for the development of the sessile sporophyte stage which can grow metres in length. In a controlled four month experiment, meiospores of *Saccharina latissima* were allowed to settle and develop on twelve different polymer surfaces. Highest meiospore settlement was seen where the attachment force of the developing macroscopic sporophytes was weak (< 0.3 N), leading to the eventual detachment of the juveniles before they can grow 100 mm. The sporophyte holdfast cover (%) was strongly related to the biomass achieved ($R^2 = 0.68$) and negatively correlated to the water contact angle (θ_w) of the polymer ($R^2 = 0.45$). Yet, meiospore settlement was positively correlated to θ_w ($R^2 = 0.24$). The study shows that the selective settlement of the meiospore conflicts with the requirements of the macroscopic sporophyte to attach firmly. It is hypothesised that higher θ_w is used by kelp meiospores as a cue for recently disturbed environments, allowing gregarious settlement in areas with reduced interspecific competition.

1. Introduction

The subtidal zone of temperate rocky shores is typically colonized by large leathery macroalgae of the order Laminariales [1]. These, have to survive in a highly turbulent environment where water velocities can often exceed 2 $m \cdot s^{-2}$ [2], comparable to a hurricane force wind of 130 $miles \cdot h^{-1}$ [3]. The motile single-celled meiospores locate and settle on a suitable substratum, then germinate into microscopic dioecious gametophytes which inter-fertilise to produce a juvenile sporophyte attached to the substratum. The developing holdfast attaches using both a chemical adhesive [4,5] and mechanical locking with the surface topography [6] to prevent their detachment and mortality. The selection of a substratum suitable for both the microscopic and macroscopic phases is therefore essential for the successful completion of the life-cycle.

The water contact angle (θ_w) is a measure of the wettability of a surface, mainly dictated by the surface free energy [7]. High free energy surfaces have an abundance of molecules, groups or atoms available to interact with another substance in contact. When a water droplet is placed on a high free energy surface, hydrogen bonds interact strongly with the surface molecules causing the droplet to flatten, wetting the surface. This hydrophilic interaction leads to a low θ_w. On surfaces with low free energy, little interaction occurs, and so the droplet surface tension is dominant, leading to a more spherical droplet, a high contact angle and a hydrophobic interaction. While wettability and surface free energy are generally closely related, the two terms are not strictly interchangeable [7] as surface free energy is calculated based on the interaction of a surface with a number of solvents, rather than just water.

The motile phase of many marine species are known to undergo a selection process when they encounter a surface, allowing them to discriminate and show preferences based on the chemistry, roughness and biology of the surface [8]. Typically, such as with the model biofouling species of green macroalgae *Ulva* spp. Linnaeus, 1753, hydrophobic surfaces receive far higher settlement [9,10]; possibly because it allows effective exclusion of water from the interface between the algal adhesive and the substratum [11]. Yet, despite hydrophobic surfaces receiving higher settlement, they are associated with weaker adhesion due to a low chemical interaction [10,12]. Consequently, such hydrophobic surfaces with low free energy are often used to reduce marine biofouling [7,8]. This pattern of settlement is not always seen, as some organisms settle preferentially on hydrophilic, show no preference or adhere most strongly to hydrophobic surfaces [10,13–15].

Surface roughness/topography can also influence the settlement of marine species [16]. The settlement of *Ulva* spp. zoospores can be either

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increased or reduced through the creation of different microenvironments using specific engineered microtopographies [17,18]. The presence of other macroorganisms or a biofilm will also assist or hinder settlement [19].

Once juveniles grow to a macroscopic size, their surface requirements change. Macroscopic roughness allows thigmotactic attachment [20] of the holdfast, which could potentially supersede the need for chemical compatibility between the bioadhesive and substratum through mechanical interlocking with surface features. The integrity of the substratum itself can also be important [21]. Attachment to calcareous surfaces rather than rock has been shown to lead to weaker attachment in the Fucoids [22,23], possibly due it being weakened by fracturing or partially dissolution. Similarly, macroalgal communities can be structured by the underlying rock type. *Fucus vesiculosus* Linnaeus, 1753, is more abundant on Baltic sandstone and limestone than crystalline bedrock [24,25]. In New Zealand, *Ulva lactuca* Linnaeus, 1753, is found to favour sandstone over shale [26], while *Ecklonia radiata* (C. Agardh) J. Agardh 1848, from SW Australia attaches more strongly to granite and sandstone than structurally weaker limestone [27].

Saccharina latissima (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, 2006, also known as sugar kelp, is a fast-growing North Atlantic Laminariales species, with economic value as an aquaculture crop. Development of cultivation for *S. latissima* have been underway for a number of years, based on methods for the related species *Saccharina japonica* in China [28]. Sugar kelp is now cultured commercially for food on both sides of the Atlantic. Large-scale cultivation for biofuel or chemical extraction through a biorefinery approach is a future possibility [29], however, the cultivation methods still need to be optimised, including the growth substratum [30].

It is known that the choice of substratum influences the settlement and development of many marine organisms [31]. Many different substrata are used for the cultivation of seaweeds around the world. Polypropylene (PP) and polyamide (PA) are customarily favoured for *S. latissima* [32–34] despite no published evidence that they are the most suitable. It has been recently shown that large differences exist in the suitability of polymers for cultivation with medium density polyethylene (MDPE), polyvinyl chloride (PVC), polycarbonate (PC) and PA recommended as substrata, despite a low initial settlement density of meiospores compared to other materials like PP [35].

1.1. Aim and hypotheses

We will examine the physical characteristics of twelve different polymer blocks (water contact angle and roughness) in an attempt to explain the pattern of *S. latissima* meiospore settlement and sporophyte growth reported by Kerrison et al. [35]. We will also determine the % cover of holdfast bioadhesive on the blocks and the growth characteristics and attachment strength of individual sporophytes after 4 months. We hypothesise that similar to *Ulva* spp. zoospores, *S. latissima* meiospores will have high settlement on hydrophobic surfaces. We also hypothesise that the bioadhesive attachment of the developing sporophytes will be weaker on hydrophobic surfaces. If validated, it will represent a conflict of interest between the microscopic meiospore and macroscopic sporophyte life-stages. The potential use of the different polymers as cultivation substrates for *S. latissima* is beyond the scope of the present study and has already been discussed [35].

2. Materials and methods

2.1. Polymer block preparation

Twelve polymers were examined: high density polyethylene (HDPE), polyamide (PA), polycarbonate (PC), polyethylene (MDPE), polyethylene terephthalate glycol (PETG), polymethyl methacrylate (PMA), polyoxymethylene co-polymer (POM-C), polyoxymethylene homopolymer (POM-H), polypropylene carbonate (PPC), polytetrafluoroethylene (PTFE), polyvinylchloride (PVC) and a phenol formaldehyde resin (Tufnol®). Sheet plastic of each polymer was first cut into similar sized blocks (10 × 50 × 7.5–10 mm). One of the cut surfaces was then milled using a vertical face mill, to try to achieve a similar mean surface roughness on all blocks (μm scale) to allow comparison of meiospores settlement and sporophyte attachment force. Three polymers (PETG, PC and Tufnol®) could not be milled successfully as they became chipped due to their high rigidity. These were instead ground using a static belt sander down to 3000 grit (6 μm mean particle size). A razor blade was used to remove any corner burs. The blocks were cleaned thorough with 5% Decon90 detergent (Decon Laboratories Ltd., UK) and a soft PA bristled brush. These were then soaked for 24 h in frequently changed distilled water and dried at 35 °C.

2.2. Polymer block characterisation

The static water contact angle (θ_w) was measured on the machined surface of cleaned blocks using the method of Callow et al. [9]. Duplicate 20 μL standing droplets of ultra-high purity water were immediately photographed on triplicate blocks (n = 3). These were analysed using ImageJ v 1.45s (National Institutes of Health, USA) and the DropSnake plugin [36]. Another set of cleaned blocks, were gold-splutter coated with a Polaron SC7620 (Quorum Technologies Ltd., UK) fitted with a gold/palladium disc, before measurement of θ_w. The gold-coated blocks will have identical surface chemistry and so any variation in contact angle will be due to the surface roughness of the polymer blocks only. The difference between the contact angle of the gold-coated block contact angle and pure gold (70°) was then calculated ($\Delta\theta_w$).

The surface profile of each gold-coated polymer block type was measured using an optical surface profiler (NT1100, Wyko, Vecco, USA) operating in vertical scanning interferometry mode. Triplicate 600 × 450 μm areas were examined at 10× magnification on one block of each polymer except Tufnol®, where triplicate 90 × 121 μm areas were examined at 50×, due to unacceptably high granularity at 10×. For each area, the mean roughness (R_a), the root mean squares roughness (R_t) and peak-to-valley distance (R_q) was calculated. To remove noise, pixels > 5σ from R_q were excluded.

2.3. Saccharina latissima settlement and growth

A separate set of polymer blocks were used to examine the settlement and growth characteristics of *S. latissima* [35]. Briefly, meiospores of *S. latissima* were extracted from fertile sporangial tissue from five individuals collected from Seil Sound, UK (56.31724°N, −5.58309°W), using the method of Kerrison et al. [37]. Meiospores were released through immersion in 8.5 °C F/2 medium without silicate (F/2-Si), in the dark for 1 h with agitation every 15 min. The resultant suspension was then passed through a 50 μm filter. 100,000 meiospores were settled into 48 basins, each containing four identical polymer blocks, 300 mL F/2-Si and 0.125 mL·L^{-1} germanium dioxide at 8.5 °C in the dark for 48 h (12 polymers × 4 basins × 4 pseudoreplicate blocks) [37]. After this settlement period, meiospore settlement density was determined by destructively sampling one block from each basin (n = 4) using epifluorescent microscopy. Meiospores were identified through chlorophyll *a* autofluorescence with a Axioskop 2 microscope combined with a UV light source and filter set 9 (Zeiss, Germany). The media was refreshed and the basins were transferred to a 12:12 light/dark cycle at 15–25 μmol·m^{-2}·s^{-1} from a cool white fluorescent bulb for a week. For seven further weeks the blocks were moved to new basins containing fresh F/2-Si, without germanium dioxide and with light increased to 30–50 μmol·m^{-2}·s^{-1}.

After 5 weeks, a single block from each basin was destructively sampled as described in a separate study [35]. After eight weeks, all surfaces of the polymer blocks, excluding the top, were wiped clean