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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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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 mo under laboratory conditions, then a further 2 mo 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 (<0.2g 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.

**KEYWORDS: polymer; settlement; kelp; cultivation; macroalga; contact angle**

## 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 (Yesson et al. 2015). 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 (Marinho et al. 2015a; Schiener et al. 2015; Adams et al. 2011). These seaweeds have economic value as fertiliser, animal feed, for human consumption and the extraction of chemicals such as alginates (Bixler and Porse 2011; Wei et al. 2013). They can also be used for the bioremediation of nutrients lost from animal mariculture as part of integrated multi-trophic aquaculture (IMTA) systems (Sanderson 2009) or for conversion into biofuel (Kerrison et al. 2015a; Hughes et al. 2012; Schiener et al. 2016).

In east Asia, the related species *Saccharina japonica* is already cultivated on an industrial scale, mainly for food and chemicals (FAO 2004). Its annual production of 7.7 mt makes it the highest volume world aquaculture product in 2014 (FAO Accessed June 2015). 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 (Kerrison et al. 2015b). 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 (FAO 1998). Two materials have traditionally been utilized as settlement twines: Locally abundant palm fibres which must first be conditioned through hammering and boiling (FAO 1998, 2004), and Kuralon, a synthetic polymer manufactured in SE Asia composed of polyvinylalcohol (PVA) fibres which is woven or spun into a slightly fluffy twine (Kuraway Accessed 15Jan2015; Werner and Dring 2011).

In Europe, cultivation trials have sometimes utilised Kuralon (Sanderson et al. 2012; Werner and Dring 2011) but more routinely have opted for cheaper alternatives. Often this is polyamide (PA) (Shea and Chopin 2007; Peteiro et al. 2014; Flavin et al. 2013; Druehl et al. 1988), although polypropylene (PP) has also frequently been used (Rößner et al. 2014; Buck and Buchholz 2004; Macchiavello et al. 2010; Marinho et al. 2015b). Polyvinylchloride (PVC) is not available as a twine, but has been used for cultivation experiments before, and is reported to provide a comparable attachment force to rock in *S. japonica* (Kawamata 2001). 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 (Lejars et al. 2012; Callow et al. 2005). Settlement choice has been reported in North American Laminariales meiospores, and can vary between species (Amsler and Neushul 1990). 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 (Dyer and Richardson 1966). 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 mo. 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

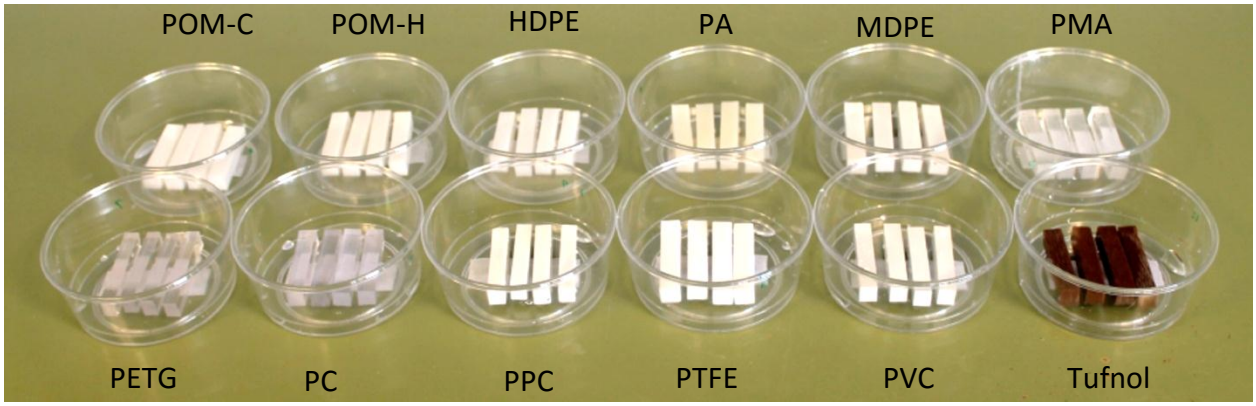
for cultivation and; 3) Whether polymer exudates are responsible for the patterns of settlement and growth.

## 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.

**Figure 1.** The twelve polymer blocks examined. Four ~50x10x10 mm blocks were secured with hook and loop tank into replicated polystyrene basins for experimentation.




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 hr 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 hr, the

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

123 blocks were again soaked for 24 hr in frequently changed distilled water to remove acetic acid
124 leachate. These were then dried again at 35°C. A section of corresponding loops, were secured to
125 the bottom of 300 mL polystyrene basins using ethyl cyanoacrylate glue (Fig 1). These were then
126 washed and dried similar to the blocks. Four replicate blocks were attached in each of eight replicate
127 basins.
128
129 **2.2 Meiospore extraction**
130 Five fertile individuals of *L. digitata* and *S. latissima* were collected from Seil Sound, UK (56.31724, -
131 5.58309). Meiospores were extracted using the method of Kerrison et al. (2015b). The sporangial
132 areas were cut from the thalli, rinsed with Tyndallized seawater (Kawai et al. 2007) and then wiped
133 firmly until dry using laboratory tissue (Kimtech, UK). This was repeated 4-5 times. These were cut
134 into 1-2 $cm^2$ pieces and gently desiccated overnight in a 4°C refrigerator between layers of tissue.
135 The following morning, the pieces were placed in 8.5°C F/2 medium without silicate (F/2-Si) enriched
136 Tyndallized seawater (salinity 33) and incubated in the dark for one hour (Guillard 1975), with
137 agitation every 15 min to encourage meiospore release. The solution was passed through a 50 µm
138 filter and kept in motion using a magnetic mixer while the meiospore concentration was determined
139 using a Sedgewick Rafter counting chamber.
140
141 **2.3 Laboratory incubation (wk 0-8)**
142 Basins containing polymer blocks were filled with 300 mL of F/2-Si at 8.5°C, with 0.125 $mL \cdot L^{-1}$ of
143 saturated $GeO_2$ solution to preclude diatom growth (Kerrison et al. 2015b) and 100,000 meiospores
144 (2 species x 12 polymer x 4 replicates). The basins were incubated in the dark for 48 hr to optimise
145 settlement (Kerrison et al. 2015b), then the media was refreshed to remove unsettled meiospores
146 and other organics. The basins were incubated at 15-25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ by cool white fluorescent light
147 on a 12:12 light:dark cycle. Each wk following, the blocks were transferred into new basins of fresh
148 F/2-Si medium, and the light increased to 30-50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for a further seven wk.

149

150 At two timepoints, a randomly selected polymer block was sacrificed from each basin for
151 examination using epifluorescent microscopy. Cells were identified through the autofluorescence of
152 chlorophyll *a* using a Axioskop 2 microscope combined with a UV light source and filter set 09 (Zeiss,
153 Germany). Meiospore density was determined following initial settlement. At wk 3, the density of all
154 meiospores, gametophytes and sporophytes was determined and size measurements were taken of
155 the largest individuals present in each field of view (predominantly sporophytes).
156 In February 2013, after 8 wk, all surfaces of the polymer blocks excluding the top, were wiped clean
157 with tissue. All blocks were photographed through a stereomicroscope (Axioskop, Zeiss, Germany),
158 using a camera (1100D, Canon, UK) and laptop running EOS Utility software (Canon, UK), capturing a
159 51-69 $mm^2$ section (dependent on block width). ImageJ v 1.45s (National Institutes of Health, USA)
160 was then used to determine sporophytes·$mm^{-2}$ and measure the length of the ten largest
161 sporophytes present. The smallest detectable sporophytes were ~100 µm. A second photograph
162 captured the entire top surface, but was not used for image analysis.
163

164 **2.3 Outdoor tank incubation (wk 8-16)**

165 All blocks were then strapped onto 0.4x1.2 m PC sheets using 5 mm elastic. Each PC sheet was
166 placed into an outdoor tank (2.6x0.4x0.5 m) receiving a constant flow of sand-filtered seawater.
167 After four weeks, the entire top surface of each block was re-photographed from above. This was
168 used to estimate % surface coverage and then categorise the sporophyte growth into six categories
169 ranging from very small (<5mm) to very large (>40mm).
170

171 After a further 8 wk (wk 16), the experiment was ended. % cover was estimated and the length of
172 the five largest sporophytes (where present) was determined. All sporophytes were cut from each
173 block, blotted dry using tissue (Kimtech, UK) and weighed to determine the total fresh weight·$block^{-1}$.
174

175 **2.4 Relative success metric**

176 Due to the differing measurement techniques used at each stage of growth, direct comparison of a
177 single measurement was not possible. Therefore, a metric of success was calculated at each growth
178 stage to integrate all measurements (Table 1). These were then converted to a % of the maximum
179 value encountered, showing a comparative metric of relative success at each stage (% rs).

180

181 **Table 1.** Measured parameters used to calculate the relative success (rs) metric at each growth stage.
182

| Week | Growth stage | Success metric calculation |
|---|---|---|
| 0 | Settlement | Meiospores ($mm^{-2}$) |
| 5 | Microscopic development | Mean length $(\mu m)^2$ * counts ($mm^{-2}$)* % sporophytes |
| 8 | Outplanted (<1-2mm) | Mean length $(mm)^2$ * counts ($mm^{-2}$) * block surface area ($mm^{-2}$) |
| 12 | Outdoor tank growth | Mean size class * % cover |
| 16 | End of Experiment | Final biomass (g w wt·$block^{-1}$) |

183

184 **2.5 Toxicity testing**

185 A separate experiment determined whether leachates from each polymer affected the survival and
186 development of either species. A separate set of polymer blocks were cleaned, soaked and dried, as
187 described previously. These were incubated in 20 mL of F/2-Si for one week under fluorescent
188 lighting (10-15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 12:12 L:D). Medium incubated without a polymer block and fresh
189 medium were used as controls. Incubated media was passed through a GF/F filter (Whatman, UK)
190 and then added to individual Petri dishes containing a cleaned microscopic slide (Kerrison et al.
191 2015b). 100,000 meiospores of either *S. latissima* or *L. digitata* were then added to each dish.
192 Similar to the main experiment, the dishes were incubated for 2 d in the dark, then transferred to
193 10-15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 12:12 L:D for 6 wk. Every 7-10 d, the media was refreshed with fresh block
194 incubated medium.

195

196 **2.6 Statistical analyses**

197 Data which satisfied the Anderson-Darling test for normality and Levene's test for homoscedasticity
198 were analysed using single way, two way or nested analysis of variance (AN, 2wAN and nAN
199 respectively). *Post-hoc* Fishers (*p-h*F) or ANs were conducted where significance was found. Non-
200 parametric data was examined using a Kruskal-Wallis test (KW) followed by Mann Whitney-U tests
201 (MW). Minitab v.15 (Minitab Inc) and Excel 2010 (Microsoft) were used for statistical testing.

202

# 4. Results

## 4.1 Meiospore settlement (wk 0)

Significant differences were seen in the meiospore settlement between polymers in both *S. latissima* (AN: $p<0.0001$, $F_{11,36}=9.4$) and *L. digitata* (AN: $p<0.0001$, $F_{11,36}=85.4$; Fig 2). The settlement was also significantly different between the species (2wAN: $p<0.0001$, $F_{11,1,83}=23.7$), although many similarities are observed. In *S. latissima* (Fig 2), maximum settlement of meiospores was observed on PTFE (34.1±0.7·mm$^{-2}$) and POM-C (30.6±3.7·mm$^{-2}$). Settlement of between 24.0-16.5·mm$^{-2}$ was seen on PMA, HDPE, PC, PA and POM-H. The lowest settlement was observed on Tufnol (3.6±1.3·mm$^{-2}$) and MDPE (7.4±0.4·mm$^{-2}$). In *L. digitata* (Fig 2), maximum settlement density of meiospores was also observed on PTFE (26.4±1.1·mm$^{-2}$), PMA (25.2±1.2·mm$^{-2}$) and HDPE (24.8±1.3·mm$^{-2}$). Settlement of between 25.2-12.8·mm$^{-2}$ was seen on PMA, HDPE, PC, PA and POM-H. The lowest settlement was observed on Tufnol (1.1±0.2·mm$^{-2}$) and MDPE (2.2±0.2·mm$^{-2}$). Settlement on many polymers was significantly higher in *S. latissima* than *L. digitata*: 30% in PTFE, 82 % higher on POM-C, 3-fold in PC, MDPE and Tufnol and 10-13-fold in PA and PVC (all MW or AN: $p<0.05$).

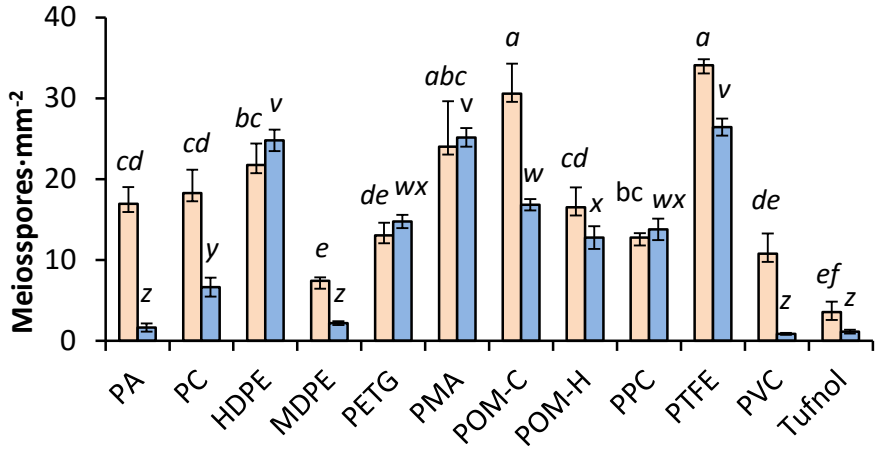



**Figure 2.** Meiospore settlement on twelve polymer blocks by either *Saccharina latissima* (light orange) or *Laminaria digitata* (dark blue). Mean ± standard error. Letters show significance groupings within each species.

**4.2 Microscopic development (wk 5)**

After 5 wk, significant differences were seen in the mean maximum length of *S. latissima* growing on different polymer blocks (nAN: $p<0.0001$, $F_{11,36,96}=19.2$). Tufnol had the smallest individuals (38±3 µm) and was significantly different from all other polymers (*p-h*F $p<0.05$), with the exception of POM-C which had a length of 104±26 µm. Mean maximum length on all other polymers was between 210-310 µm (Table 2). Significant differences were also seen in the mean maximum length of *L. digitata* (nAN: $p<0.0001$, $F_{11,24,72}=11.9$). *Post-hoc* Fisher's ($p<0.05$) showed that the maximum length was found on HDPE (176±61 µm), PA, PC, PPC and PVC (131-139 µm). The shortest lengths were on PETG, POM-C, POM-H and Tufnol (Table 3; mean 79–94 µm).

The sporophyte %, was significantly different between polymers in *S. latissima* (AN: $p<0.0001$, $F_{11,36}=5.9$) but not in *L. digitata* ($p>0.05$), revealing a significant interactive effect between the variables (2wAN: $p<0.005$, $F_{11,1,\mathbf{11},83}=2.9$). In *S. latissima*, the highest sporophyte % was 73±12 in PETG and the lowest was 3±2% in Tufnol (*p-h*F: $p<0.05$). All other polymers were 44–64% sporophyte. In *L. digitata*, the sporophyte % was always between 34-54 % (Table 2).

Individuals·mm$^{-2}$ were also significantly different in *S. latissima* (AN: $p<0.0001$, $F_{11,36}=5.1$), with the highest density of 8.9±1.5·mm$^{-2}$ on HDPE followed by 6.8-7.6·mm$^{-2}$ on POM-H, PMA, PPC and PTFE. The lowest density of 1.6-1.9·mm$^{-2}$ on MDPE, POM-C and PA. No significant different was found in *L. digitata* ($p>0.05$). No interaction was found between variables in either species ($p>0.05$).

**4.3 Juvenile sporophytes (8 wk)**

After 8 wk in the laboratory, significant differences were still seen in the mean maximum length of *S. latissima* (AN: $p<0.0001$, $F_{11,83}=5.2$). Tufnol had the smallest sporophytes of 0.35±0.09 mm while a mean of 0.75-0.93 mm was found on PTFE, POM-C, PETG, PVC, PPC and HDPE. The largest sporophytes were found on PC, POM-C, PA, PMA and MDPE (1.18–1.28 mm). *L. digitata* also had

significant differences (AN: $p<0.005$, $F_{11,81}=3.0$). Most had sporophytes between 0.83–1.23 mm, with PA and MDPE slightly less (mean 0.66-0.69 mm). The smallest sporophytes were again found on Tufnol (0.09±0.02 mm).

The sporophyte·mm$^{-2}$ were significantly different in *S. latissima* (AN: $p<0.001$, $F_{11,93}=3.4$). Highest counts were found on PVC and PPC (mean 2.4-2.7·mm$^{-2}$) while lowest was found on PA and MDPE (mean 0.7-0.8·mm$^{-2}$). The others had between 1.3-2.2·mm$^{-2}$. In *L. digitata*, the sporophyte·mm$^{-2}$ were also significantly different (AN: $p<0.0001$, $F_{11,81}=3.6$) with highest counts on PTFE, POM-C and MDPE (mean 1.7-2.1·mm$^{-2}$ and lowest on PMA, POM-H and Tufnol (mean 0.7-0.8·mm$^{-2}$).

When the mean maximum length and sporophyte counts were examined together, a negative correlation was found in both species (*S. latissima*: $R^2=0.29$; *L. digitata*: $R^2=0.16$) indicating a density dependent effect (Fig 3). Data from Tufnol was not included from this analysis (see section 5.2).

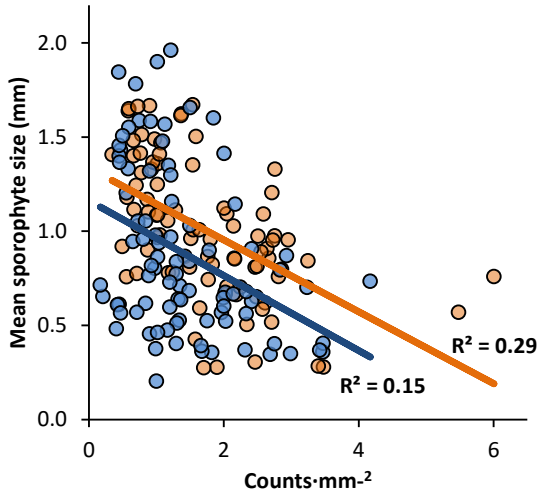
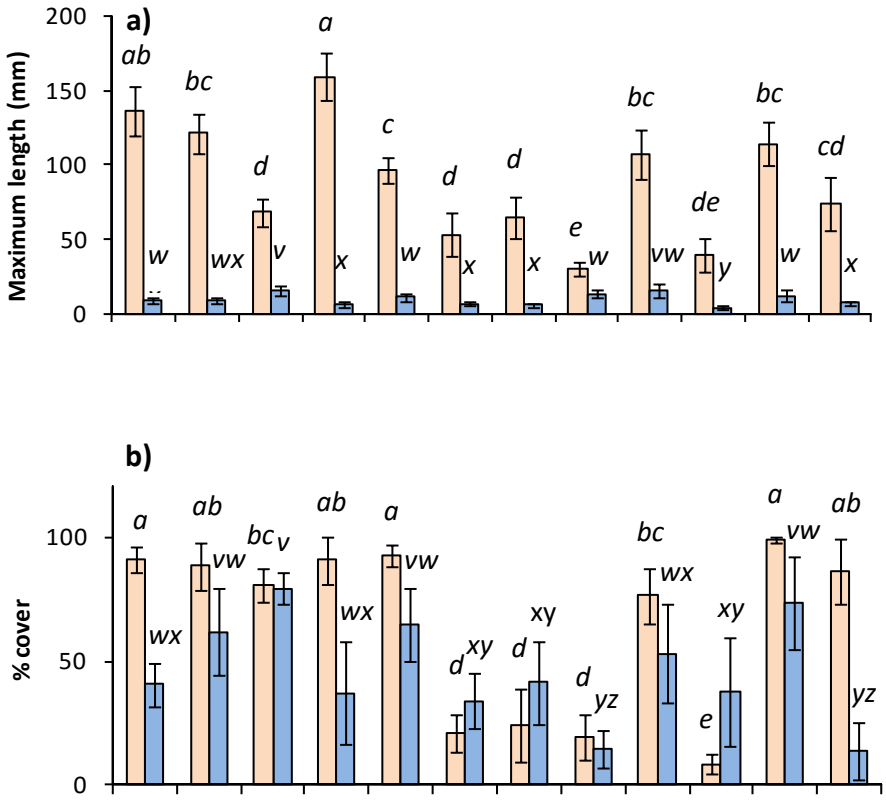



**Figure 3.** Density dependence of sporophyte size and density in *Saccharina latissima* (light orange) and *L. digitata* (dark blue) after 8 weeks of growth on polymer blocks.

### 4.4 Outdoor tank growth (wk 12)

The size categorized lengths were significantly different in *S. latissima* (KW: $p<0.0001$, $H_{10,10}=45.2$).Lengths were greatest on MDPE and PETG (mean rank 4.9) and POM-H (3.9±0.4). The lowest ranking was found on PTFE and PVC (1.7±0.3), while all others were between 2.9-3.5. A significant difference was also found in *L. digitata* (KW: $p<0.0005$, $H_{10,10}=26.4$). The highest ranking was seen on PVC (3.8±0.3), HDPE (3.3±0.5) and PMA (3.0±0.5), while the lowest ranking was on PTFE (1.6±0.3). Percentage cover also varied significantly in *S. latissima* (AN: $p<0.0001$, $F_{10,77}=6.0$), with maximum cover (95-99%) on POM-C, POM-H, MDPE, PVC, HDPE and PETG. The minimum cover of 62-63% was observed on PA and PTFE. In *L. digitata* no significant effect was seen ($p>0.05$).



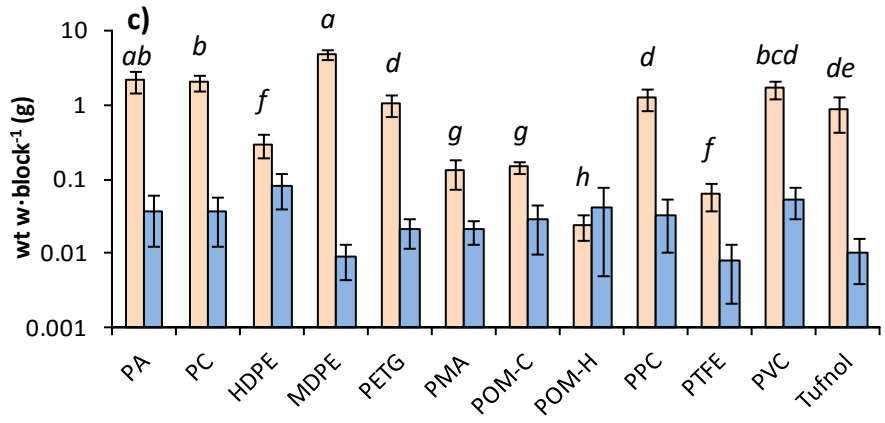


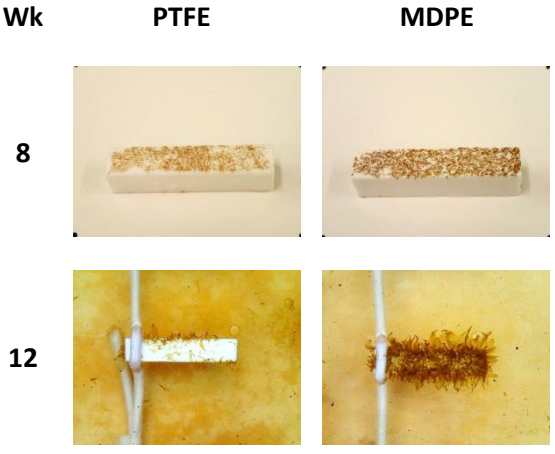

**Figure 4. a)** % cover and **b)** mean maximum length and **c)** the fresh weight·block$^{-1}$ of either *S. latissima* or *L. digitata* on twelve different polymer blocks following four months of cultivation. Shown is mean ± standard deviation. Letters denote separate significance groups for either species.

269

270 **4.5 End of experiment (wk 16)**

271 The mean maximum length was significantly different between the two species (Fig 4a; 2wAN:
272 $p<0.0001$, $F_{1,11,11,72}=328$), between polymers (2wAN: $p<0.0001$, $F_{1,\mathbf{11},11,72}=8.5$), and with a significant
273 interaction (2wAN: $p<0.0001$, $F_{1,11,11,72}=7.4$). In *S. latissima*, the largest sporophytes were found on
274 MDPE (159±32 mm; Fig 5) and PA (137±33 mm). Lengths of 97-121 mm were found on PETG, PPC,
275 PVC and PC, while the shortest were seen on PTFE (39±11 mm) and POM-H (30±5 mm). In *L. digitata*,
276 no difference was found in the mean maximum length on the different polymers ($p>0.05$): 10±4mm.

277
278



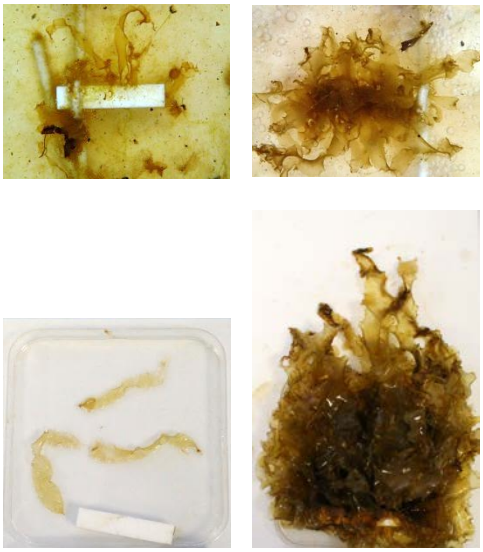



**Figure 5.** *Saccharina latissima* sporophyte growth on 50x10x10 mm PTFE and MDPE blocks between 8-16 wk. Sporophytes on PTFE after 16 wk detached easily from block when disturbed.

The % cover on the *S. latissima* polymer blocks was also significantly different (Fig 4b; AN: $p<0.0001$, $F_{11,36,47}=16.1$). *P-h*Fs revealed that the maximum cover of 76-99% was found on PPC, HDPE, Tufnol, PC, PE, PA, PETG and PVC respectively. The lowest cover of 8-24% was found on PTFE, POM-H, PMA and POM-C. Variation in % cover of *L. digitata* was also significant (AN: $p<0.001$, $F_{11,36,47}=3.9$). The maximum cover was 79±6% on HDPE and between 62-73% on PC, PETG and PVC. Lowest cover was 14±11% on Tufnol and 15±8% in POM-H. A 2wAN confirmed that the pattern was different the two species ($p<0.0001$, $F_{\mathbf{1},11,11,72}=59.8$) and polymers ($p<0.0001$, $F_{1,\mathbf{11},11,72}=11.6$), resulting in a significant interaction between the two factors ($p<0.0001$, $F_{1,11,\mathbf{11},72}=5.2$).

The FW·block$^{-1}$ was significantly different between polymers in *S. latissima* (Fig 4c; AN: $p<0.0001$, $F_{11,82}=11.1$). MDPE was clearly the best polymer with mean 4.87±2.38 g FW·block$^{-1}$. The next best were PA, PC and PVC with means of 1.69–1.98 g FW·block$^{-1}$. The lowest fresh weight was found on POM-H, PTFE, PMA, POM-C with means of 0.02–0.15 g FW·block$^{-1}$. No difference in FW·block$^{-1}$ was seen in *L. digitata* ($p>0.05$): 0.03±0.02 g. This was due to low growth and high variability between replicates.

299 **4.6 Relative success metric trajectories**

300 Three success trajectories were identified in *S. latissima* (Fig 6). The most successful group included
301 HDPE, PMA, POM-C/H and PTFE. These polymers had the highest initial settlement (50-100% rs) and
302 lowest final biomass (1-12% rs). The second group included PETG, PPC and Tufnol. These had low-
303 moderate settlement (11-38% rs) and moderate final biomass (35-51% rs). The third group was
304 composed of PA, PC, MDPE and PVC. These had low-moderate settlement (22-54% rs) and high final
305 biomass (69-100 % rs). No clear trajectories were identified in *L. digitata*, due to the non-significant
306 difference in final biomass.

307

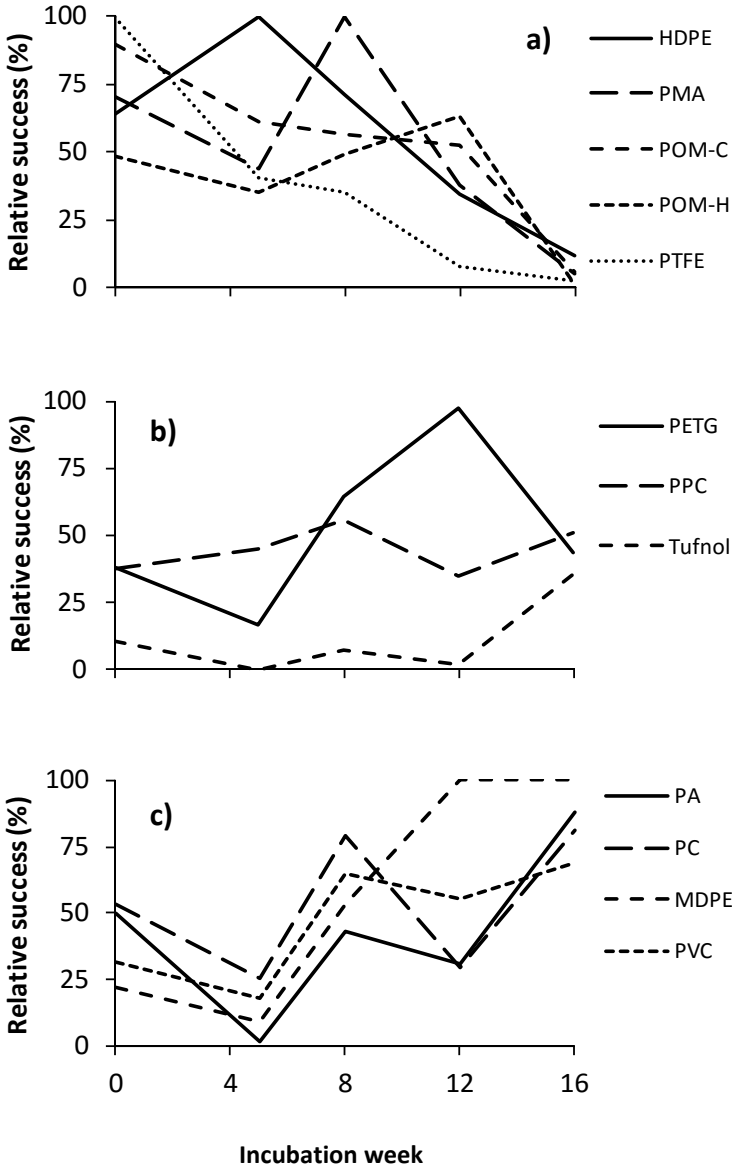


**Figure 6.** Relative success metric of *S. latissima* on twelve different polymer blocks over 16 wk of cultivation. Three responses were seen **a)** polymers that showed highest success during settlement and early development (0-5 weeks) and lowest final biomass (week 16). **b)** polymers that had moderate to low settlement success and moderate final biomass, and **c)** polymers with moderate to low settlement success and highest final biomass. Note: for better visualisation, the biomass of MDPE was divided by two before % success was calculated.

308

309

310 **4.7 Toxicity experiment**

311 After 6 wk of incubation in weekly refreshed block leachage medium, no difference was found in the
312 total number of structures on *S. latissima* ($p>0.05$), while in *L. digitata* significant differences were
313 found (AN: $p<0.0001$, $F_{12,29}=4.9$); both Tufnol (1.9±0.5) and POM-H (1.9±1.4), were significantly lower
314 than the control (2.5±1.0), while in PMA (3.2±1.0) and PVC (3.3±0.6) they were significantly higher. It
315 is unclear whether these effects are due to changes in settlement or survival.

316

317 Significant differences were present in sporophyte % for both *S. latissima* (ANOVA: $p<0.001$,
318 $F_{12,29}=4.2$) and *L. digitata* (ANOVA: $p< 0.0001$, $F_{12,29}=4.5$). In *S. latissima*, Tufnol had about half the %
319 sporophytes (19.8±22.8%) of the control (51.3±9.5%) and was significantly different, unlike all others
320 polymers (48.1-60.4%). In *L. digitata*, Tufnol had the lowest value (0±0%), while all others (28.7-
321 45.6 %) were not significantly different from the control (36.7±5.3 %).

322

323

## 5. DISCUSSION

This study examined the growth of two species of Laminariales macroalgae, of commercial importance, on twelve different polymers: from meiospore settlement until four months old and up to 150 mm in length. Large differences were observed in the suitability of the polymers, confirming that selection of an appropriate growth substrate is a very important initial step to allow the successful cultivation of the European Laminariales, allowing successful settlement and development of macroscopic sporophytes in the hatchery and their growth into adults.

Various substrates have been previously used for the cultivation of Heterokontaphyte macroalgae e.g. PA, PP and PVC (Peteiro et al. 2014; Druehl et al. 1988; Marinho et al. 2015b; Kawamata 2001), however, no published study has before systematically assessed how settlement and growth varies growth substrates. A 10x10x50 mm polymer block was chosen for this experiment as these could be cut from sheet plastic, and easily transferred between laboratory and outdoor tank cultivation. Unfortunately, this limited the selection of test materials, preventing comparison with two traditionally used materials: palm fibre and Kuralon (FAO 1998; Werner and Dring 2011).

### 5.1 Meiospore settlement

Highest meiospore settlement was observed on PTFE, PMA and HDPE while lowest settlement was on Tufnol, MDPE and PVC. Flagellated Laminariales meiospores are motile, and so can exhibit taxic responses to many stimuli such as light and nutrient concentrations (Amsler and Neushul 1989; Kerrison et al. 2015b). This is thought to allow discrimination between suitable and unsuitable settlement locations (Amsler and Neushul 1989; Maggs and Callow 2002). In *Ulva* spp. zoospores, it is documented that they undergo a surface selection process: This involves 'sensing' the surface using a rotating apical papilla and possibly also temporary attachment. If the substrate is suitable, a golgi body derived adhesive is discharged and the flagellum is retracted (Maggs and Callow 2002). If the substrate is not suitable, the zoospore can lift off and resume swimming. This process allows

350 *Ulva* spp. to discriminate surfaces with particular chemical characteristics such as contact angle and
351 surface microtopgraphy (Callow et al. 2002; Schumacher et al. 2007; Long et al. 2010).
352
353 A species-specific difference in the settlement pattern was also evident, with *S. latissima* displaying
354 higher settlement than *L. digitata* on many substrates: PA, PC, MDPE, POM-C, PTFE, PVC and Tufnol.
355 We have identified four possible explanations: Firstly, meiospores of *S. latissima* may have a higher
356 settlement competency than *L. digitata* meiospores. Given the non-significant difference in
357 settlement between the two species on half of the polymers examined, this would suggest a similar
358 level of meiospore competency. Therefore, although this may contribute, it is not likely to have a
359 controlling influence on the results. Secondly, the chemical adhesive secreted by the two species
360 may be different, resulting in differential settlement between the species. This is a possibility,
361 however, there is currently no evidence ofdistinct adhesives occurring between species of the
362 Laminariales, although differences in adhesion strength has been observed between strains of the
363 diatom *Phaeodactylum tricornutum*. Thirdly, the species may have differential tolerance to
364 chemicals leaching from the polymers. A toxic effect was observed due to Tufnol, and *L. digitata* did
365 appear to be more severely effected in comparison to *S. latissima* (see section 5.2). This can explain
366 the settlement pattern on this substrate only, and so does not explain differential settlement seen
367 on other polymers.
368
369 Finally, *L. digitata* may be more discriminatory in its settlement, while *S. latissima* is less selective.
370 This is suggested to be the main factor explaining the differential settlement between the species.
371 *Saccharina latissima* is a fast growing species, which capitalises on openings in the subtidal seaweed
372 canopy created by disturbance. This opportunistic nature, appears to make it less discriminatory,
373 settling on any available surface. *L. digitata* is a slower growing species (this study), which forms
374 long-lived subtidal beds. The more selective settlement in *L. digitata* may be to ensure it only settles
375 in the most suitable locations.

376

377 **5.2 Toxicity testing**

378 It has been long known that certain structural polymers can leach toxic or inhibitory compounds that
379 negatively affect algal growth (Dyer and Richardson 1966) and invertebrate settlement (Dan et al.
380 2002). Studies have shown that PC, transparent PE, PMA and PTFE are generally considered safe to
381 use for the cultivation of algae (Dyer and Richardson 1966). Conversely, varied results have been
382 found concerning PA, PP, black PE and PVC, which are sometimes benign, while at other times inhibit
383 algal growth (Stein 1980 and refs therein). In the present study, the toxicity test was carried out on
384 all 12 polymers to determine whether waterborne leachates influenced the settlement and growth
385 of the two species. Tufnol is a phenol-formaldehyde resin, which is known to leach toxic
386 formaldehyde. This chemical would be most concentrated within the surface boundary layer and so
387 is likely to act as a settlement deterrent for meiospores which are able to select based on the
388 chemical environment and lighting regime (Amsler and Neushul 1989; Kerrison et al. 2015b).

389

390 After 6 wk of exposure to Tufnol leachate, *S. latissima* sporophyte development was reduced by 66%
391 and growth reduced 10-fold. In *L. digitata* the effect was even more severe, with meiospore counts
392 reduced 120-fold and sporophyte development completely inhibited suggesting this species is
393 particularly susceptible to the toxic agent. This delayed/inhibited development was also seen in the
394 main experiment (0-8 wk), causing a severe developmental lag in both species in comparison to the
395 other polymers. Because of this, Tufnol was excluded from the comparative analysis of growth at 8
396 and 12 wk.

397

398 Both POM-C and POM-H are also known to leach formaldehyde under certain conditions (Rittschof
399 and Costlow 1989), however, the toxicity tests did not indicate that this is a major factor controlling
400 settlement and growth. Whilst, a negative effect was seen on POM-H leachate individuals·mm$^{-2}$ in *L.*
401 *digitata* and final size in *S. latissima*, POM-C leachate also led to increase in size in *L. digitata*. These

results do not agree with the main experiment, where POM-C/H displayed relatively high settlement. These results suggesting that formaldehyde leaching from POM-C/H is low, and so this is not the major factor determining the settlement and growth response of these species on the polymers.

All other polymers showed no indication of toxic leachates, and in fact, *L. digitata* showed significantly higher individuals·mm$^{-2}$ due to PMA or PVC leachate, and higher final size due to PPC. No similar effects were seen in the main experiment settlement results and the cause of these effects are uncertain. A toxicity effect is only truly seen in Tufnol, and so is not is responsible for the settlement pattern seen on the other polymers.

**5.3 Density dependent effects**

Density dependent effects on recruitment and growth have been well studied in the Laminariales (Reed et al. 1991; Reed 1990). Dioecious gametophytes must settle within 1 mm for fertilisation to occur (Reed 1990). Yet, the rapid growth of sporophytes from microscopic to macroscopic organisms (reaching ~1 mm in 8 weeks in this study), requires that there exists strong intra-specific competition as sporophytes grow, leading to strong negative density-dependent mortality (Reed 1990). This was observed in the present study, as initial settlement was 3-31 meiospore·mm$^{-2}$, but by wk 5, counts had declined 3 to 20-fold. After 8 wk, the counts of visible macroscopic sporophytes was similar on all polymers and in both species at around 1-2·mm$^{-2}$; with the exception of Tufnol, the mean size was also consistently 0.7-1.2 mm. The convergence of the size and sporophyte count, is an indication that density-dependent self-thinning is occurring, with the largest sporophytes suppressing the growth of the smallest (Steen and Scrosati 2004) and leading to convergence of the relative success metric. In both species, the largest sporophytes were present when density was <1·mm$^{-2}$.

For the aquaculture of kelp, the aim of the hatchery is generally to produce a dense coverage of sporophyte, between 1-10 $mm^{-2}$ (Kerrison et al. 2015b). In this study, a density of 1-2 sporophyte·$mm^{-2}$ appeared to be the ideal, producing a monoculture with no clear space for other organisms to become established. If 90% meiospore germination is presumed for both species, 50:50 male:female division and only one sporophyte produced per female (Kerrison et al. 2015b), then the ideal settlement density is in the region of ~1.8-3.6 meiospore·$mm^{-2}$ of twine surface. This density should lead to good coverage of juvenile sporophytes within the hatchery, and reveals that the settlement density in this study was up to 10-fold higher than necessary.

**5.4 Relative success metric changes week 8-16**

While settlement varied up to 15-fold between polymers, as the kelps developed from microscopic to macroscopic sporophytes of 1-2 mm over the first 8 wk, the % rs on each block tended to converge, due to similar density and sporophyte size as just described. This indicates that while there did appear to be some effect of substrate chemistry on the development rate at wk 5 – faster growth on HDPE in both species and slower growth on POM-H/C, MDPE and PETG in only one of the species – these effects were lost after 8 wk, with density becoming the controlling influence on growth.

After 12 wk, *S. latissima* began to reaching >20 mm and substrate chemistry began to again control the relative success of each polymer. MDPE and PETG became the most successful whilst the success of PTFE, HDPE and PMA declined substantially. By the end of the experiment in week 16, a clear pattern had emerged for *S. latissima*: the polymers with the highest initial settlement success (PTFE, POM-C/H, PMA and HDPE), ended with the lowest biomass, while those with lower initial settlement (MDPE, PVC, PA and PC) ended with the highest final biomass.

453 This reversal of fortune appears to occur during the transition from the low Reynolds number,
454 viscous force dominated, environment within the surface boundary layer to the higher Reynold's
455 number, turbulent flow dominated environment inhabited by macroscopic organisms (Vogel 1994).
456 Whilst PTFE, POM-C/H, PMA and HDPE all had excellent initial settlement, it was observed that at wk
457 5-8 in both species, the developing sporophytes were easily detached by any accidental physical
458 contact. This must reflect that the adhesive secreted by the Laminariales holdfast is incompatible
459 with these surfaces, which are typified by high contact angles and low surface energies (Callow and
460 Fletcher 1994). This conforms to the typical pattern, that higher contact angle surfaces are adhered
461 to more weakly (Lejars et al 2012). As the macroscopic sporophytes on these polymers grew in size,
462 they would experience increasing drag force due to turbulent flow (Vogel 1994). This drag will have
463 exceeded the adhesive attachment force leading to their detachment and loss, and corresponding
464 patchy coverage on these polymers with their near complete removal by the end of the experiment.
465 These polymers may therefore be suitable for marine applications in which kelp biofouling is best
466 avoided.
467
468 In contrast, *L. digitata* only reached final sizes of 4-16 mm on all blocks, and achieved a low biomass
469 of only 0.01-0.08 $g \cdot block^{-1}$. Since sporophytes of this species were also observed to be easily
470 removed by physical contact on PTFE, POM-C/H, PMA and HDPE, these sporophytes were not large
471 enough to have yet experienced sufficient drag for their weak attachment to be exceeded. It is
472 expected that similar detachment would be observed if the sporophytes had been allowed to
473 grow >20mm.
474
475 **5.5 CONCLUSIONS**
476 We have demonstrated that settlement and growth on MDPE, PVC, PA and PC polymers, produced
477 the highest final biomass, sporophyte size in *S. latissima* and were firmly attached to the blocks.
478 Therefore, of the polymers examined, these appear highly suitable polymers for European

Laminariales cultivation, despite poor initial settlement. Both PA and PVC have been used before as a settlement substrate. PP has also regularly been used, likely due to its low cost and wide availability; however this polymer is best avoided as it gave only mediocre performance. This study highlights that substrate selection experiments need to consider that observed settlement patterns may not reflect the best interest of the adult organism if it traverses from a low to high Reynold's number environment.

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