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

Kerrison, Philip D.; Stanley, Michele S.; Black, Kenneth D.; Hughes, Adam D.

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

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7 **Kerrison, Philip D.^{1,2}**

8
9 **Stanley, Michele S.¹**

10
11 **Black, Kenneth D.¹**

12
13 **Hughes, Adam D.¹**

14
15
16 ¹ SAMS, Scottish Marine Institute, Oban, Argyll, UK, PA37 1QA.

17 ² Corresponding author: Email: Philip.Kerrison@sams.ac.uk, Telephone: +44 (0)1631 559309,

18 Fax: +44 (0)1631 559309

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21
22 For the cultivation of the European phaeophyte macroalgae *Laminaria digitata* and *Saccharina*
23 *latissima*, meiospores are settled onto twines within a hatchery, where they are grown for several
24 months. The twine used is often a customarily selected synthetic polymer, polyamide or

25 polypropylene. However, little is known about the impacts of this choice on hatchery performance.
26 To test the effect of substrate material, we settled and cultured meiospores from both *L. digitata*
27 and *S. latissima*, independently on twelve polymer blocks for 4 mo. They were first grown for 2 mo
28 under laboratory conditions, then a further 2 mo in outdoor tanks. Meiospore settlement varied
29 significantly between polymers by up to 15-fold ($p < 0.0001$) with some species-specific differences
30 also observed ($p < 0.0001$). Tufnol was the least suitable polymer, as formaldehyde leachate reduced
31 settlement and inhibited juvenile growth/development. After 8 wk, all polymers excluding Tufnol,
32 were performing similarly with generally ~ 1 mm sporophytes present at a density of $1\text{-}2\cdot\text{mm}^{-2}$. A
33 negative density-dependent effect of sporophyte size and density was observed in both species
34 ($p < 0.05$). At the end of the experiment, two distinct grouping of polymers were identified regarding
35 *S. latissima*. Those that initially had very high settlement (high density polyethylene, polymethyl
36 methacrylate, polyoxymethylene copolymer/homopolymer and polytetrafluoroethylene) had the
37 lowest final mean lengths, % cover and biomass ($< 0.2\text{g wet weight}\cdot\text{block}^{-1}$) at the end of the
38 experiment. Conversely many of the polymers with the lowest initial settlement (polyamide,
39 polycarbonate, medium density polyethylene and polyvinylchloride) had the highest final mean
40 lengths, % cover and biomass ($1.7\text{-}4.9\text{ g wet weight}\cdot\text{block}^{-1}$). This reversal of fortunes is discussed
41 regarding discriminatory meiospore settlement, differences in apparent adhesion strength of the
42 seaweed holdfast and the transition of the growing sporophytes from a viscous force dominated
43 boundary layer environment to a turbulent dominated environment with increasing drag as the
44 sporophyte grows.

45

46

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

48 1. Introduction

49 *Saccharina latissima* and *Laminaria digitata* are temperate phaeophyte macroalgae of the order
50 Laminariales which are common subtidal species, native to the European Atlantic coastline (Yesson
51 et al. 2015). They grow to metres in length and contain a seasonally variable composition, with
52 maximal sugar contents of 30-35% in summer and maximal protein contents of 8-11% in autumn-
53 winter (Marinho et al. 2015a; Schiener et al. 2015; Adams et al. 2011). These seaweeds have
54 economic value as fertiliser, animal feed, for human consumption and the extraction of chemicals
55 such as alginates (Bixler and Porse 2011; Wei et al. 2013). They can also be used for the
56 bioremediation of nutrients lost from animal mariculture as part of integrated multi-trophic
57 aquaculture (IMTA) systems (Sanderson 2009) or for conversion into biofuel (Kerrison et al. 2015a;
58 Hughes et al. 2012; Schiener et al. 2016).

59
60 In east Asia, the related species *Saccharina japonica* is already cultivated on an industrial scale,
61 mainly for food and chemicals (FAO 2004). Its annual production of 7.7 mt makes it the highest
62 volume world aquaculture product in 2014 (FAO Accessed June 2015). In Europe, Laminariales
63 cultivation has been research based over the past decades, although more recently it has become
64 commercialised at a small scale in a number of locations. For cultivation, algal meiospores are
65 extracted from fertile sporangial tissue and settled onto twine reels within enclosed tanks. These are
66 then cultured under controlled conditions until sporophytes of 2-10 mm are present (Kerrison et al.
67 2015b). The twines are then wound around a carrier rope at a coastal farm site and after 4-8 months,
68 they reach their adult size. This cultivation method was first developed for *S. japonica* in 1950s China
69 (FAO 1998). Two materials have traditionally been utilized as settlement twines: Locally abundant
70 palm fibres which must first be conditioned through hammering and boiling (FAO 1998, 2004), and
71 Kuralon, a synthetic polymer manufactured in SE Asia composed of polyvinylalcohol (PVA) fibres
72 which is woven or spun into a slightly fluffy twine (Kuraway Accessed 15Jan2015; Werner and Dring
73 2011).

74

75 In Europe, cultivation trials have sometimes utilised Kuralon (Sanderson et al. 2012; Werner and
76 Dring 2011) but more routinely have opted for cheaper alternatives. Often this is polyamide (PA)
77 (Shea and Chopin 2007; Peteiro et al. 2014; Flavin et al. 2013; Druehl et al. 1988), although
78 polypropylene (PP) has also frequently been used (Rößner et al. 2014; Buck and Buchholz 2004;
79 Macchiavello et al. 2010; Marinho et al. 2015b). Polyvinylchloride (PVC) is not available as a twine,
80 but has been used for cultivation experiments before, and is reported to provide a comparable
81 attachment force to rock in *S. japonica* (Kawamata 2001). As far as the authors are aware,
82 differences in substrate suitability for the settlement and growth of European Laminariales
83 macroalgae has not been reported. This information would provide empirical rather than customary
84 substrate selection to aid the European industry's development.

85

86 The surface chemistry is known to affect the settlement choice and adhesion strength of marine
87 organisms including chlorophyte macroalgal zoospores, barnacles and mussels (Lejars et al. 2012;
88 Callow et al. 2005). Settlement choice has been reported in North American Laminariales
89 meiospores, and can vary between species (Amsler and Neushul 1990). It is anticipated that the
90 same will be true in European Laminariales species. In addition, certain polymers are known to leach
91 compounds which can have a negative effect on algal survival and growth (Dyer and Richardson
92 1966). This may influence the suitability of some polymer as substrates for Laminariales growth.

93

94 **1.1 Aim and objectives**

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

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

101

102 **2. Materials and Methods**

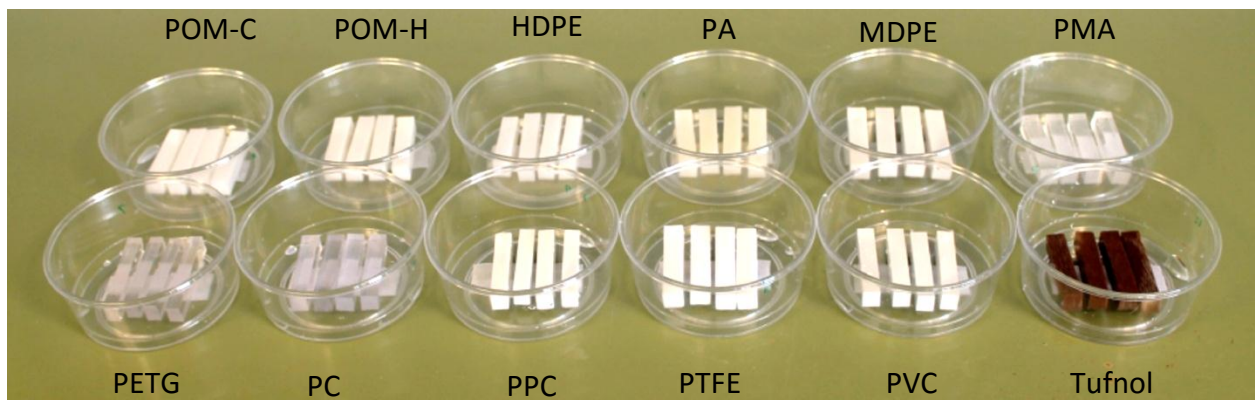
103 **2.1 Preparation of blocks and basins**

104 The settlement preference and sporophyte development of the species *L. digitata* and *S. latissima*
105 was evaluated on twelve polymers (Fig 1): High density polyethylene (HDPE), PA, polycarbonate (PC),
106 medium density polyethylene (MDPE), polyethylene terephthalate glycol (PETG), polymethyl
107 methacrylate (PMA), polyoxymethylene copolymer (POM-C), polyoxymethylene homopolymer
108 (POM-H), polypropylene carbonate (PPC), polytetrafluoroethylene (PTFE), PVC and phenol
109 formaldehyde resin (Tufnol). Sheet plastic was cut into blocks with dimensions of 50 mm length, 7.5-
110 10 mm width and 10 mm height. The top surfaces were milled so that the polymer blocks had similar
111 surface roughness and all corner burs were removed using a razor blade.

112

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

115



116

117

118 The blocks were cleaned thoroughly using 5% Decon90 solution (Decon Laboratories Ltd, UK) and a
119 PA bristled brush to remove dirt and residues from the manufacturing and cutting process. All of the
120 polymers examined are highly resistant to detergent¹. Blocks were then soaked for 24 hr in
121 frequently changed distilled water and dried at 35°C. Following this, a patch of hooks, from hook and
122 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 cm² 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·L⁻¹ of
143 saturated GeO₂ 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 µmol·m⁻²·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 µmol·m⁻²·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 mm² 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 (μm) 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 ($\text{g w wt}\cdot\text{block}^{-1}$)

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 ($10\text{-}15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 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 $10\text{-}15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 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-hF*) 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

203 **4. Results**

204 **4.1 Meiospore settlement (wk 0)**

205 Significant differences were seen in the meiospore settlement between polymers in both *S. latissima*
206 (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
207 significantly different between the species (2wAN: $p < 0.0001$, $F_{11,1,83} = 23.7$), although many
208 similarities are observed. In *S. latissima* (Fig 2), maximum settlement of meiospores was observed on
209 PTFE ($34.1 \pm 0.7 \cdot \text{mm}^{-2}$) and POM-C ($30.6 \pm 3.7 \cdot \text{mm}^{-2}$). Settlement of between 24.0 - $16.5 \cdot \text{mm}^{-2}$ was seen
210 on PMA, HDPE, PC, PA and POM-H. The lowest settlement was observed on Tufnol ($3.6 \pm 1.3 \cdot \text{mm}^{-2}$)
211 and MDPE ($7.4 \pm 0.4 \cdot \text{mm}^{-2}$). In *L. digitata* (Fig 2), maximum settlement density of meiospores was also
212 observed on PTFE ($26.4 \pm 1.1 \cdot \text{mm}^{-2}$), PMA ($25.2 \pm 1.2 \cdot \text{mm}^{-2}$) and HDPE ($24.8 \pm 1.3 \cdot \text{mm}^{-2}$). Settlement of
213 between 25.2 - $12.8 \cdot \text{mm}^{-2}$ was seen on PMA, HDPE, PC, PA and POM-H. The lowest settlement was
214 observed on Tufnol ($1.1 \pm 0.2 \cdot \text{mm}^{-2}$) and MDPE ($2.2 \pm 0.2 \cdot \text{mm}^{-2}$). Settlement on many polymers was
215 significantly higher in *S. latissima* than *L. digitata*: 30% in PTFE, 82 % higher on POM-C, 3-fold in PC,
216 MDPE and Tufnol and 10-13-fold in PA and PVC (all MW or AN: $p < 0.05$).

217

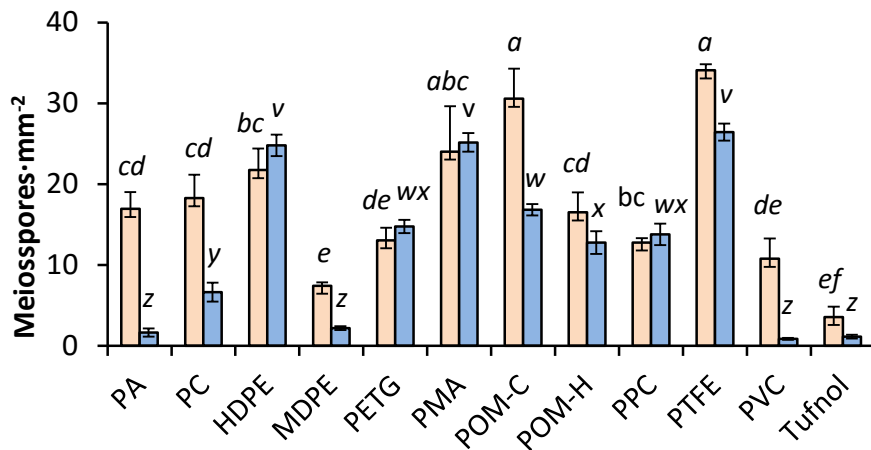


Figure 2. Meiospore settlement on twelve polymer blocks by either *Saccharina latissima* (light orange) or *Laminaria digitata* (dark blue). Mean \pm standard error. Letters show significance groupings within each species.

218

219 4.2 Microscopic development (wk 5)

220 After 5 wk, significant differences were seen in the mean maximum length of *S. latissima* growing on
221 different polymer blocks (nAN: $p < 0.0001$, $F_{11,36,96} = 19.2$). Tufnol had the smallest individuals ($38 \pm 3 \mu\text{m}$)
222 and was significantly different from all other polymers (p -*hF* $p < 0.05$), with the exception of POM-C
223 which had a length of $104 \pm 26 \mu\text{m}$. Mean maximum length on all other polymers was between 210-
224 310 μm (Table 2). Significant differences were also seen in the mean maximum length of *L. digitata*
225 (nAN: $p < 0.0001$, $F_{11,24,72} = 11.9$). *Post-hoc* Fisher's ($p < 0.05$) showed that the maximum length was
226 found on HDPE ($176 \pm 61 \mu\text{m}$), PA, PC, PPC and PVC (131-139 μm). The shortest lengths were on PETG,
227 POM-C, POM-H and Tufnol (Table 3; mean 79–94 μm).

228

229 The sporophyte %, was significantly different between polymers in *S. latissima* (AN: $p < 0.0001$,
230 $F_{11,36} = 5.9$) but not in *L. digitata* ($p > 0.05$), revealing a significant interactive effect between the
231 variables (2wAN: $p < 0.005$, $F_{11,1,11,83} = 2.9$). In *S. latissima*, the highest sporophyte % was 73 ± 12 in PETG
232 and the lowest was $3 \pm 2\%$ in Tufnol (p -*hF*: $p < 0.05$). All other polymers were 44–64% sporophyte. In *L.*
233 *digitata*, the sporophyte % was always between 34-54 % (Table 2).

234

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

239

240 4.3 Juvenile sporophytes (8 wk)

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

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

248

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

254

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

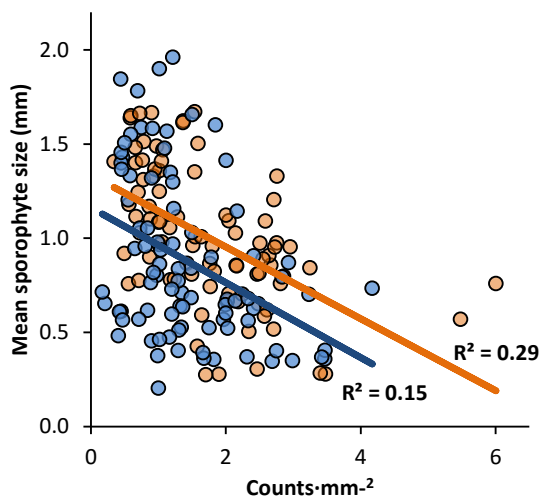
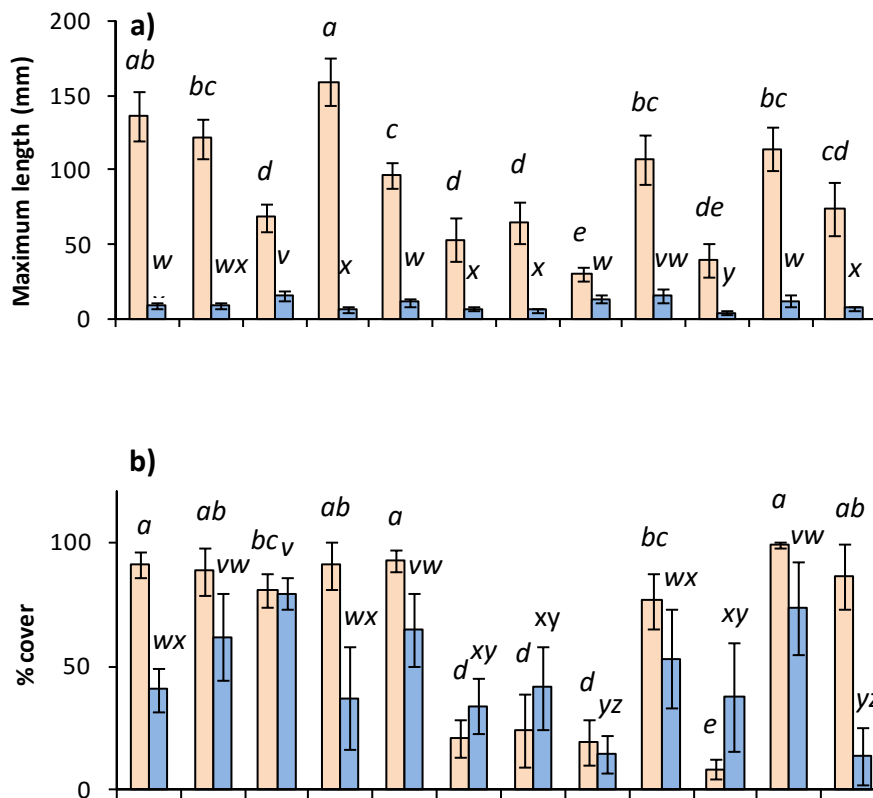


Figure 3. Density dependence of sporophyte size and density in *Saccharina latissima* (light orange) and *L. digitata* (dark blue) after 8 weeks of growth on polymer blocks.

258

259 4.4 Outdoor tank growth (wk 12)

260 The size categorized lengths were significantly different in *S. latissima* (KW: $p < 0.0001$,
 261 $H_{10,10} = 45.2$). Lengths were greatest on MDPE and PETG (mean rank 4.9) and POM-H (3.9 ± 0.4). The
 262 lowest ranking was found on PTFE and PVC (1.7 ± 0.3), while all others were between 2.9-3.5. A
 263 significant difference was also found in *L. digitata* (KW: $p < 0.0005$, $H_{10,10} = 26.4$). The highest ranking
 264 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
 265 (1.6 ± 0.3). Percentage cover also varied significantly in *S. latissima* (AN: $p < 0.0001$, $F_{10,77} = 6.0$), with
 266 maximum cover (95-99%) on POM-C, POM-H, MDPE, PVC, HDPE and PETG. The minimum cover of
 267 62-63% was observed on PA and PTFE. In *L. digitata* no significant effect was seen ($p > 0.05$).
 268



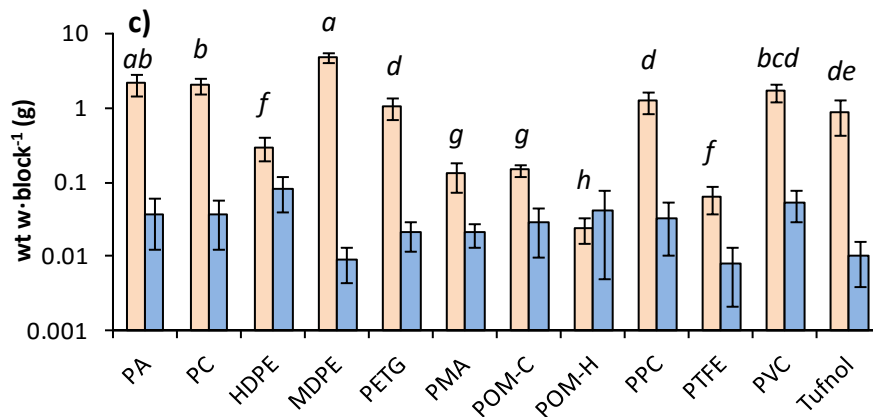


Figure 4. a) % cover and b) mean maximum length and c) the fresh weight·block⁻¹ of either *S. latissima* or *L. digitata* on twelve different polymer blocks following four months of cultivation. Shown is mean ± standard deviation. Letters denote separate significance groups for either species.

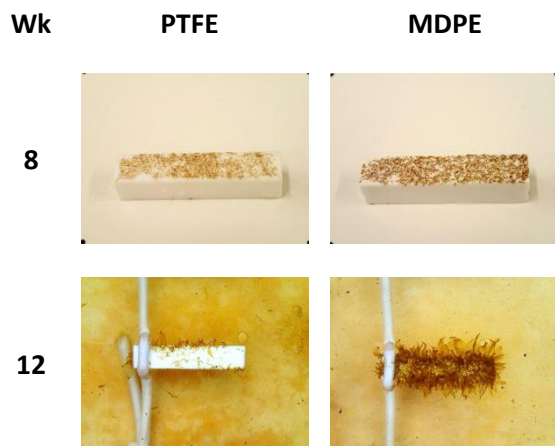
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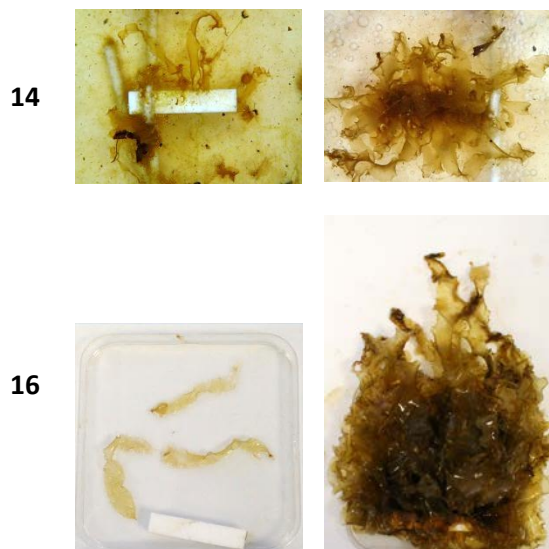
270 **4.5 End of experiment (wk 16)**

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: $p < 0.0001$, $F_{1,11,11,72} = 8.5$), 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 ± 4 mm.

277

278





279 **Figure 5.** *Saccharina latissima* sporophyte growth on 50x10x10 mm PTFE and MDPE blocks between
 280 8-16 wk. Sporophytes on PTFE after 16 wk detached easily from block when disturbed.

281
 282

283 The % cover on the *S. latissima* polymer blocks was also significantly different (Fig 4b; AN: $p < 0.0001$,
 284 $F_{11,36,47} = 16.1$). *P-h*Fs revealed that the maximum cover of 76-99% was found on PPC, HDPE, Tufnol,
 285 PC, PE, PA, PETG and PVC respectively. The lowest cover of 8-24% was found on PTFE, POM-H, PMA
 286 and POM-C. Variation in % cover of *L. digitata* was also significant (AN: $p < 0.001$, $F_{11,36,47} = 3.9$). The
 287 maximum cover was $79 \pm 6\%$ on HDPE and between 62-73% on PC, PETG and PVC. Lowest cover was
 288 $14 \pm 11\%$ on Tufnol and $15 \pm 8\%$ in POM-H. A 2wAN confirmed that the pattern was different the two
 289 species ($p < 0.0001$, $F_{1,11,11,72} = 59.8$) and polymers ($p < 0.0001$, $F_{1,11,11,72} = 11.6$), resulting in a significant
 290 interaction between the two factors ($p < 0.0001$, $F_{1,11,11,72} = 5.2$).

291

292 The $FW \cdot block^{-1}$ was significantly different between polymers in *S. latissima* (Fig 4c; AN: $p < 0.0001$,
 293 $F_{11,82} = 11.1$). MDPE was clearly the best polymer with mean 4.87 ± 2.38 g $FW \cdot block^{-1}$. The next best
 294 were PA, PC and PVC with means of 1.69–1.98 g $FW \cdot block^{-1}$. The lowest fresh weight was found on
 295 POM-H, PTFE, PMA, POM-C with means of 0.02–0.15 g $FW \cdot block^{-1}$. No difference in $FW \cdot block^{-1}$ was
 296 seen in *L. digitata* ($p > 0.05$): 0.03 ± 0.02 g. This was due to low growth and high variability between
 297 replicates.

298

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

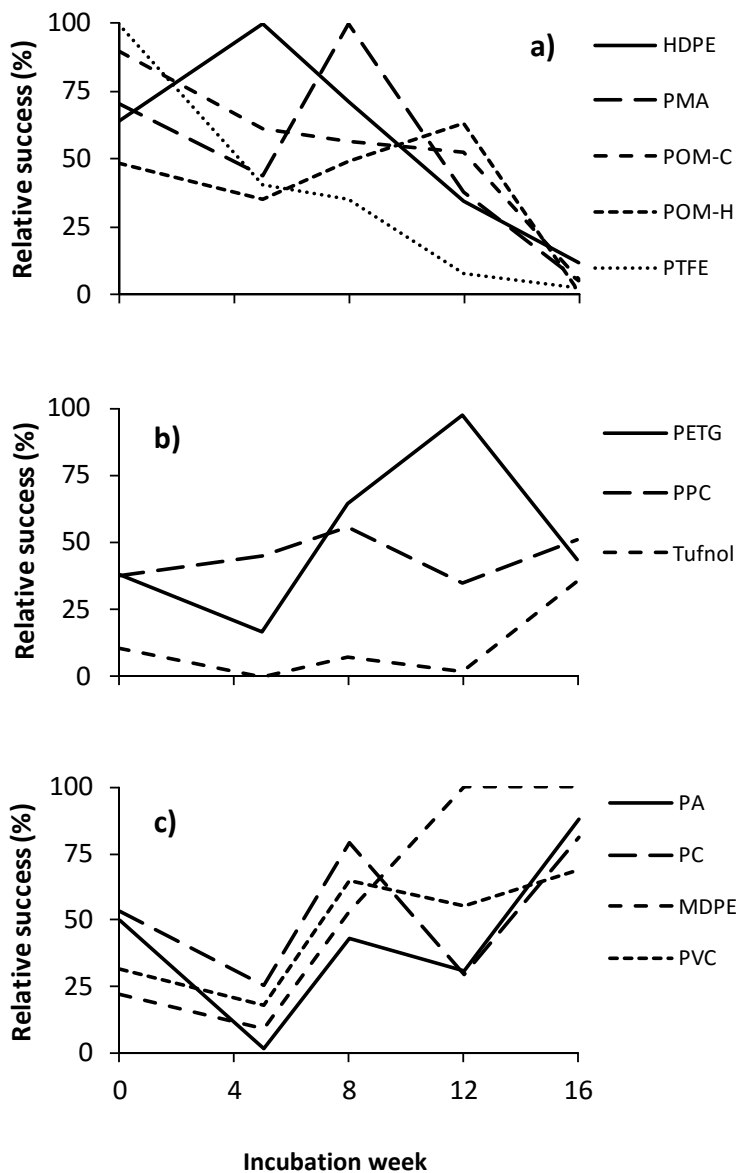


Figure 6. Relative success metric of *S. latissima* on twelve different polymer blocks over 16 wk of cultivation. Three responses were seen **a)** polymers that showed highest success during settlement and early development (0-5 weeks) and lowest final biomass (week 16). **b)** polymers that had moderate 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 leachate 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 ($19.8 \pm 22.8\%$) of the control ($51.3 \pm 9.5\%$) and was significantly different, unlike all others
320 polymers (48.1-60.4%). In *L. digitata*, Tufnol had the lowest value ($0 \pm 0\%$), while all others (28.7-
321 45.6 %) were not significantly different from the control ($36.7 \pm 5.3\%$).

322

323

324 **5. DISCUSSION**

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

331

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

339

340 **5.1 Meiospore settlement**

341 Highest meiospore settlement was observed on PTFE, PMA and HDPE while lowest settlement was
342 on Tufnol, MDPE and PVC. Flagellated Laminariales meiospores are motile, and so can exhibit taxis
343 responses to many stimuli such as light and nutrient concentrations (Amsler and Neushul 1989;
344 Kerrison et al. 2015b). This is thought to allow discrimination between suitable and unsuitable
345 settlement locations (Amsler and Neushul 1989; Maggs and Callow 2002). In *Ulva* spp. zoospores, it
346 is documented that they undergo a surface selection process: This involves 'sensing' the surface
347 using a rotating apical papilla and possibly also temporary attachment. If the substrate is suitable, a
348 golgi body derived adhesive is discharged and the flagellum is retracted (Maggs and Callow 2002). If
349 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 microtopography (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 of distinct 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

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

405

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

411

412 **5.3 Density dependent effects**

413 Density dependent effects on recruitment and growth have been well studied in the Laminariales
414 (Reed et al. 1991; Reed 1990). Dioecious gametophytes must settle within 1 mm for fertilisation to
415 occur (Reed 1990). Yet, the rapid growth of sporophytes from microscopic to macroscopic organisms
416 (reaching ~1 mm in 8 weeks in this study), requires that there exists strong intra-specific
417 competition as sporophytes grow, leading to strong negative density-dependent mortality (Reed
418 1990). This was observed in the present study, as initial settlement was 3-31 meiospore·mm⁻², but by
419 wk 5, counts had declined 3 to 20-fold. After 8 wk, the counts of visible macroscopic sporophytes
420 was similar on all polymers and in both species at around 1-2·mm⁻²; with the exception of Tufnol, the
421 mean size was also consistently 0.7-1.2 mm. The convergence of the size and sporophyte count, is
422 an indication that density-dependent self-thinning is occurring, with the largest sporophytes
423 suppressing the growth of the smallest (Steen and Scrosati 2004) and leading to convergence of the
424 relative success metric. In both species, the largest sporophytes were present when density was
425 <1·mm⁻².

426

427 For the aquaculture of kelp, the aim of the hatchery is generally to produce a dense coverage of
428 sporophyte, between 1-10 mm⁻² (Kerrison et al. 2015b). In this study, a density of 1-2
429 sporophyte·mm⁻² appeared to be the ideal, producing a monoculture with no clear space for other
430 organisms to become established. If 90% meiospore germination is presumed for both species,
431 50:50 male:female division and only one sporophyte produced per female (Kerrison et al. 2015b),
432 then the ideal settlement density is in the region of ~1.8-3.6 meiospore·mm⁻² of twine surface. This
433 density should lead to good coverage of juvenile sporophytes within the hatchery, and reveals that
434 the settlement density in this study was up to 10-fold higher than necessary.

435

436

437 **5.4 Relative success metric changes week 8-16**

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

445

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

452

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-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

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

485

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491

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