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# **Simulated seawater flooding reduces oilseed rape growth, yield, and progeny performance**

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**Running Head:** *Seawater flooding reduces oilseed rape yield and seedling performance*

- 1 • **Background and Aims** Of the many threats to global food security, one of the most  
2 pressing is the increased incidence of extreme weather events. In addition to extreme  
3 rainfall, a combination of global sea level rise and storm surge is likely to result in  
4 frequent episodes of seawater flooding in arable systems along low-lying coasts. Our  
5 aim was to elucidate the effects of simulated seawater and freshwater flooding on the  
6 survival, growth, and reproductive potential of four cultivars of the important seed  
7 crop, *Brassica napus* (canola, or oilseed rape [OSR]).
- 8 • **Methods** Established plants were exposed to 24- or 96-hour freshwater or seawater  
9 root zone immersion (with a no immersion ‘control’). Initial post-treatment  
10 performance over 7-weeks was quantified using dry weight biomass. A second group  
11 of plants, cultivated until maturity, were used to quantify reproductive yield (silique  
12 and seed number, and seed size) and subsequent progeny performance (germination  
13 and seedling growth).
- 14 • **Results** OSR growth and reproductive responses were unaffected by freshwater, but  
15 seawater negatively affected growth and silique number for all cultivars, and seed mass  
16 for two (Agatha & Cubic). In addition to impacts on crop yield, the growth of seedlings  
17 cultivated from seed collected from maternal plants subjected to seawater immersion  
18 was also reduced.
- 19 • **Conclusions** Our results demonstrate the potential impact of seawater inundation on  
20 coastal cropping systems; although OSR may survive acute saline flooding, there are  
21 longer-term impacts on growth and yield for some cultivars. The threat may necessitate  
22 changes in land-use practice and/or the development of salt-tolerant cultivars to  
23 maintain economically viable yields. In addition, by evidencing a hitherto unknown  
24 effect on reproductive performance (i.e. reduced seed yield) and subsequent seedling

25 growth our study highlights an important potential impact of coastal flooding on plant  
26 community dynamics for (semi) natural habitats.

27 **Key Words** – Flooding, Food security, Osmotic Stress; Salinity, Sea-level rise; Storm  
28 surge

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## INTRODUCTION

33 Food security and climate change are two of the most pressing environmental issues facing  
34 the planet (Godfray *et al.*, 2010; IPCC, 2014). When combined however, the increased  
35 food demand imposed by a growing human population coupled with global-scale shifts in  
36 temperatures, precipitation, and sea-level rise will inevitably impose significant demands  
37 on agriculture (Parry *et al.*, 2005; Lobell *et al.*, 2008). Much of the environmental threat  
38 from climate change stems from acute extreme events rather than simply longer-term  
39 chronic change alone (Rahmstorf & Coumou, 2011; Vasseur *et al.*, 2014; Parmesan &  
40 Hanley, 2015), and of the former, flooding represents one of the greatest challenges. Future  
41 climate scenarios consistently predict major regional shifts in the intensity of extreme  
42 precipitation episodes across the globe and concomitant increases in large-scale regional  
43 flooding along river catchments (Li *et al.*, 2013; Singh *et al.*, 2013; IPCC, 2014). In  
44 addition to fluvial flooding however, a combination of changes in sea-surface  
45 temperatures, weather patterns, and sea-level rise are predicted to increase the frequency  
46 and severity of oceanic storm surges (Vousdoukas *et al.*, 2016; Vitousek *et al.*, 2017). The  
47 result will be that many low-lying coastal areas face an increased risk of seawater  
48 inundation over coming decades (Nicholls & Cazenave, 2010; Hoggart *et al.*, 2014). When  
49 taken together, extreme precipitation, storm surge, and sea-level rise are likely to cause  
50 significant flood risk to global agriculture and as a result understanding crop response to,  
51 and tolerance of, coastal flooding has become a research priority (Jackson & Ismail, 2015).

52 Although flooding can cause significant losses at any time of the crop production cycle,  
53 yields are most likely to suffer if flooding occurs during critical development stages  
54 (Rosenzweig *et al.*, 2001; Parry *et al.*, 2005). For the United Kingdom for example,

55 extreme winter and spring precipitation is projected to increase (Fowler *et al.*, 2010) at a  
56 time when many arable crops are establishing ahead of summer harvest. This is one reason  
57 why the severe, but localised, 2014 winter floods that affected only a small portion of  
58 southern England (14,000 Ha), caused economic losses estimated at £6.9 million in arable  
59 systems alone (ADAS, 2014). Globally, severe freshwater and seawater flooding has long  
60 been identified as a significant economic problem for agriculture, and one that poses an  
61 additional regional threat to human nutrition (Page & Williams, 1926; Mirza, 2002; Chau  
62 *et al.*, 2015).

63 The impact of flooding on crop plants is well known, although research has almost  
64 exclusively focussed on the effects of freshwater inundation. Flooding typically causes soil  
65 anoxia, as O<sub>2</sub> is consumed without replacement, and the accumulation of various ions  
66 (Mn<sup>2+</sup>, Fe<sup>2+</sup>, S<sup>2+</sup>) and compounds detrimental to plant growth, while submergence also  
67 denies plant tissues access to O<sub>2</sub> and CO<sub>2</sub> (Flowers & Colmer, 2008; Perata *et al.*, 2011).  
68 All of these factors can limit significantly crop growth and productivity (Malik *et al.*, 2001;  
69 Perata *et al.*, 2011; Ren *et al.*, 2014; Mutava *et al.*, 2015). Due its high salinity (typically  
70 about 35 gL<sup>-1</sup> salt of which chloride and sodium contribute 19 gL<sup>-1</sup> and 11 gL<sup>-1</sup>  
71 respectively), seawater flooding imposes additional osmotic and ionic stresses; the former  
72 limits the plant's ability to absorb water and the latter can result in toxicity through the  
73 accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in tissues (Munns & Tester, 2008). As a response, plants have  
74 adapted to avoid or lessen the impact of salt ions via the synthesis and accumulation of  
75 stress metabolites and the regulation of other tissue ions (i.e. K<sup>+</sup>) to exclude or  
76 compartmentalize Na<sup>+</sup> and Cl<sup>-</sup> and re-establish homeostatic function (Maathuis &  
77 Amtmann, 1999; Munns & Tester, 2008).

78 From the perspective of agricultural production, even if crop plants survive freshwater or  
79 seawater flooding, any impact on growth or reproductive potential is of concern. Our  
80 understanding of the effects of salinity on crop performance is, however, focussed largely  
81 on soil salinization in arid cropping systems (Pitman & Läuchli, 2002; Albacete *et al.*,  
82 2008; Flowers *et al.*, 2010), and only a handful of studies have examined how  
83 agriculturally important species respond to short-duration immersion in seawater. White *et*  
84 *al.* (2014) report how immersion in seawater for only 24-hr resulted in the accumulation  
85 of Na<sup>+</sup> and Cl<sup>-</sup> in leaves of the pasture crop white clover (*Trifolium repens*). A concomitant  
86 increase in two key organic solutes (proline and sucrose) to re-establish osmotic balance  
87 was also observed, but nonetheless flooded plants showed reduced growth and flowering  
88 potential. Interestingly White *et al.* (2014) also found variation between the responses of  
89 three different ecotypes to seawater immersion, suggesting that the relative tolerance of  
90 one ecotype could be of value in producing flood-tolerant cultivars for cultivation in low-  
91 lying coastal pastures. Sun *et al.* (2015) is the only study to look at effects of seawater  
92 immersion on arable crops, their experiment focussing on the responses of 1 month old  
93 plants of ten crop plants (six of which were Brassicaceae) to 24-hr seawater immersion.  
94 All crops were negatively affected by immersion, with two (cucumber and Chinese greens)  
95 suffering complete mortality and the remainder showing reduced growth. Since plants  
96 were harvested only 2 weeks after immersion treatments were imposed, it is unclear  
97 however, what potential any of the surviving crops had for long-term recovery or whether  
98 other key aspects of the crop production cycle (flowering & fruit/seed production) were  
99 affected by flooding.

100 The aim of this study was to determine how exposure to simulated seawater and freshwater  
101 flooding affected both immediate growth and longer-term reproductive performance of the

102 widely cultivated crop canola, or oilseed rape (OSR - *Brassica napus* L.). Beyond gaining  
103 an understanding of the short-term impact of flooding on mortality and growth of  
104 established plants, we also test the hypothesis that impacts on reproductive potential and  
105 subsequent seedling performance vary across four different cultivars. Elucidation of these  
106 responses is important for several reasons; OSR is grown primarily for seed yield and thus  
107 any impact of prolonged fluvial or seawater flooding on reproductive output has potential  
108 economic repercussions. In addition, establishing variation in growth and yield responses  
109 may help identify established genetic materials from which to develop flood/salinity  
110 tolerant cultivars. A more general understanding of plant growth and reproductive  
111 responses to acute seawater flooding is also lacking in the literature (see Hanley *et al.*,  
112 Submitted), and no study to date has tested the hypothesis that progeny performance is  
113 affected by the exposure of parent plants to short-duration seawater immersion.  
114 Consequently, our study offers an important insight into the longer-term impacts that this  
115 increasing environmental issue may have on natural, as well as agricultural, plant species  
116 and communities.

## 117 MATERIALS AND METHODS

### 118 *Study species*

119 Oilseed rape is a hybrid of *B. rapa* and *B. oleracea* (Chalhoub *et al.*, 2014), wild forms of  
120 the latter being a coastal species with some natural salt tolerance (Snogerup *et al.*, 1990).  
121 OSR is grown globally and its seeds are harvested to produce food and bioenergy oils, and  
122 for use as an animal feedstock. It accounts for 14% (by area) of agricultural land use in the  
123 UK (Garthwaite *et al.*, 2018) and major producers globally include the EU, Canada, India  
124 and China (USDA, 2015), regions likely to experience significant flooding events over

125 coming decades (IPCC, 2014). The OSR cultivars used in these experiments were Cracker  
126 (LS Plant Breeding, Impington, UK), Agatha, Astrid, and Cubic (Grainseed Ltd., Eye,  
127 Suffolk, UK). All are commonly cultivated in the UK.

### 128 *Plant cultivation*

129 One hundred seeds of each cultivar were germinated in 90 mm-diameter Petri dishes  
130 containing two layers of Whatman No. 1 filter paper moistened with 5 ml of distilled water,  
131 maintained in an incubator at 16.5 °C on a 12:12 light:dark cycle. On germination (radicle  
132 emergence), seventy seedlings per cultivar were transplanted individually into 50mm  
133 diameter, 50mm deep plastic pots containing John Innes No.2 potting compost and grown  
134 on in a naturally lit greenhouse with weekly watering with tap water, until early November  
135 2014. At this time seedlings possessing at least the first true leaf (equivalent to OSR  
136 growth-stage 1.1; Sylvester-Bradley, 1985), were re-potted into larger 110 x 110 x 120  
137 mm plastic pots containing John Innes No. 2 potting compost. Greenhouse temperatures  
138 during this cultivation period were: mean daily minimum = 10.4 °C ( $\pm 0.4$  °C SE); mean  
139 daily maximum = 25.7 °C ( $\pm 1.0$  °C).

### 140 *Effect of immersion on growth*

141 When the plants were 67-d-old (early December 2014), twelve individuals from each  
142 cultivar (OSR growth stage 1.3-1.6; i.e. emergence of third-sixth true leaf) were allocated  
143 at random to one of five treatment groups. These were 24-, or 96-hrs immersion in seawater  
144 (*Sw*) (collected from Plymouth Sound - electrical conductivity = 45.51 mS cm<sup>-1</sup> at 16.2  
145 °C); 24-, or 96-hrs immersion in deionised water (*Dw*); or a no-immersion control  
146 treatment. Although river floodplains can experience much longer periods of immersion,  
147 sometimes extending to several months (Van Eck *et al.*, 2004; Muchan *et al.*, 2015), in this

148 way we simulated the average 1-d long seawater flooding event reported for low-lying UK  
149 coastline habitats and extended the period to the maximum reported flood duration of 4-d  
150 (Environment Agency, 2014).

151 We recognise that inundation following coastal storm-surge or fluvial flooding events  
152 would likely result in full shoot submergence, but by immersing to pot-level (in large  
153 plastic tubs) our approach allowed us to separate the effect of ionic imbalance in the root-  
154 zone rather than the impact of oxygen deficiency caused by full immersion that both  
155 treatments would impose. Immediately after immersion, the pots were allowed to drain  
156 fully before being arranged randomly on a wire mesh-topped bench inside the greenhouse;  
157 the wire mesh allowed free drainage and prevented cross-contamination between treatment  
158 groups. The pots were watered to capacity (with tap water) 48-hr after seawater immersion.

159 Eight plants per treatment/cultivar combination were retained inside the greenhouse; the  
160 remainder were positioned outside, on adjacent elevated mesh. For both sets of plants, each  
161 individual was positioned at random, 20cm apart from its nearest neighbour in 1m long  
162 rows separated by 30cm to simulate the recommended field density for OSR cropping  
163 systems in the UK (HGCA, 2014).

164 Greenhouse plants were watered twice weekly for a further 56-d; temperatures during this  
165 phase of the experiment were 4.9 °C ( $\pm$  0.5) min and 18.0 °C ( $\pm$  0.6) max. Surviving plants  
166 (growth stages 2.0-2.3; i.e. plant has a rosette growth form and extended stem with  
167 internodes) from each treatment/cultivar group were harvested at 113-d-old (late January  
168 2015), cleaned of any adhering compost and oven-dried at 50 °C for 24-hr. A Levene's test  
169 for homogeneity of variance across treatment and cultivar levels was negative ( $F_{19,189} =$   
170 2.896,  $P < 0.001$ ) and biomass data were  $\log_{10}$  transformed, resulting in a positive Levene's

171 test ( $F_{19,189} = 1.335$ ,  $P = 0.165$ ). Univariate GLM analyses were used to compare the results  
172 of experimental flooding on transformed biomass. Rather than classifying plants in  
173 different groups into two separate factors (water:  $D_w$  vs.  $S_w$ , and time: 24h vs. 96h), which  
174 would decrease the power of the tests, contrasts were employed to evaluate specific  
175 differences between treatment levels (control,  $D_w24$ ,  $D_w96$ ,  $S_w24$  and  $S_w96$ ). In addition  
176 to the  $F$  statistic and its probability, we report effect sizes ( $\eta_p^2$ ) and power at  $P < 0.05$   
177 ( $\text{power}_{0.05}$ ).

### 178 *Effect of immersion on crop yield and progeny performance*

179 The four individual plants from each treatment/cultivar group positioned on elevated  
180 outdoor standing were grown to reproductive maturity in order to assess the effects of  
181 immersion on seed yield. Plants were exposed to natural weather conditions and watered  
182 (to capacity with tap water) only following prolonged dry periods. Since individuals were  
183 randomly arranged with respect to treatment and cultivar, they were able to cross-  
184 pollinate and so resulting progeny were most likely hybrids between cultivars. Three  
185 plants (Agatha 24-hr  $D_w$ ; Cracker 96-hr  $D_w$ ; Agatha 96-hr  $S_w$ ) died during this period.  
186 The main stems of plants were harvested in mid-June when most siliqua were fully  
187 ripened (growth stage 9.9). We quantified the total number of siliqua per plant, and from  
188 six (randomly selected) siliqua per plant, quantified seed number and mean individual  
189 seed mass per pod (averaging across all 6 replicate siliqua for each plant).

190 All seeds from each of the six sampled siliqua per plant were pooled. From these, 40  
191 seeds were set to germinate in 90 mm-diameter Petri dishes containing two layers of  
192 Whatman No. 1 filter paper moistened with 5 ml of deionised water, maintained in an  
193 incubator at 18°C on a 12:12 light:dark cycle. In addition to the three plants that died

194 before harvest, a further two parent plants - Cracker 96-hr  $D_w$  & Agatha 96-hr  $S_w$  –  
195 failed to yield sufficient seeds for the germination/seedling growth trials. Petri dishes  
196 were checked daily for 14 days; on germination (appearance of the radicle), seedlings  
197 were counted and removed. Six seedlings from each Petri dish were retained, and planted  
198 into a 50-mm diameter pot containing John Innes No.2 potting compost. These seedlings  
199 were grown in controlled conditions (15°C on a 12:12 light:dark cycle with daily  
200 watering to capacity) until 14-d-old when harvested and oven dried (at 50 °C for 24-hr)  
201 to determine dry weight biomass.

202 Levene's tests of homogeneity of variances were significant for siliqua number ( $F_{19,57} =$   
203 2.621,  $P = 0.003$ ), seed number ( $F_{19,56} = 2.962$ ,  $P < 0.001$ ) and seedling biomass ( $F_{19,498}$   
204 = 2.291,  $P = 0.002$ ), but did not show departure from homogeneity for seed mass ( $F_{19,56} =$   
205 1.264,  $P=0.244$ ). Logarithmic transformation of the former three homogenised the  
206 variance for siliqua number ( $F_{19,57} = 1.304$ ,  $P=0.217$ ), but not for seed number ( $F_{19,56} =$   
207 10.625,  $P < 0.001$ ) and seedling mass ( $F_{19,497} = 3.289$ ,  $P < 0.001$ ). Consequently, we  
208 present the results of GLM's hypothesis testing for; (i) seed mass where the  
209 untransformed variable did not depart from homogeneous; (ii) seed number and seedling  
210 mass where logarithmic transformation did not homogenise the variance; (iii) log siliqua  
211 number where logarithmic transformation resulted in variance homogeneity.

## 212 RESULTS

### 213 *Effect of immersion on plant growth*

214 'Treatment' had a significant effect on adult plant biomass with a moderate effect size  
215 ( $F_{4,189} = 17.71$ ,  $P < 0.001$ ;  $\eta_p^2 = 0.273$ ;  $\text{power}_{0.05} = 1$ ; Fig 1), and with the contrasts between

216 control and each of the other four treatment levels significant only for *Sw*96 (Contrast = -  
217 0.339, *SE* = 0.066, *P* < 0.001). More specific comparison showed: (i) a negative contrast  
218 between both *Sw* treatments vs. Control (Contrast = -0.219, *SE* = 0.058, *P* < 0.001); (ii) a  
219 small, but significant, positive contrast between *Dw* treatments vs. Control (Contrast =  
220 0.114, *SE* = 0.057, *P* = 0.048); and (iii) a then obvious positive contrast between *Dw* and  
221 *Sw* (Contrast = 0.333, *SE* = 0.045, *P* < 0.001), highlighting the smaller size of plants  
222 immersed in seawater. Plant biomass did not vary between cultivars but the cultivar effect  
223 size was small ( $F_{3,189} = 1.85$ , *P* = 0.140;  $\eta_p^2 = 0.029$ ;  $\text{power}_{0.05} = 0.475$ ). A significant  
224 ‘Treatment × Cultivar’ interaction with moderate effect size ( $F_{12,189} = 2.95$ , *P* = 0.001;  $\eta_p^2 =$   
225 0.158;  $\text{power}_{0.05} = 0.989$ ) emphasised several treatment- and cultivar-specific departures  
226 from the general trends described above (Astrid and Agatha for example, were both  
227 tolerant of 24-hr seawater immersion). No plants died during this first part of the  
228 experiment.

### 229 *Effect of immersion on crop yield and progeny performance*

230 The number of siliqua (Table 1) varied with ‘Treatment’ with a small effect size ( $F_{4,57} =$   
231 5.974, *P* < 0.001;  $\eta_p^2 = 0.295$ ;  $\text{power}_{0.05} = 0.978$ ) but not by ‘Cultivar’ ( $F_{3,57} = 2.004$ , *P* =  
232 0.124;  $\eta_p^2 = 0.095$ ;  $\text{power}_{0.05} = 0.489$ ) and there was no interaction ( $F_{12,57} = 1.283$ , *P* = 0.254;  
233  $\eta_p^2 = 0.213$ ;  $\text{power}_{0.05} = 0.643$ ). The contrast analyses revealed a significant negative effect  
234 of *Dw* and *Sw* treatments compared to the Control (Contrast = -0.103, *SE* = 0.050, *P* =  
235 0.046); specifically highlighting lower siliqua numbers in *Sw* vs. Control (Contrast = -  
236 0.141, *SE* = 0.055, *P* = 0.014). There was no variation between *Dw* and *Sw* (Contrast =  
237 0.76, *SE* = 0.046, *P* = 0.105) or *Dw* vs. Control (Contrast = -0.065, *SE* = 0.056, *P* = 0.250).

238 Seed number per pod (Table 1) was not influenced by ‘Treatment’ ( $F_{4,57} = 2.206$ ,  $P =$   
239  $0.080$ ;  $\eta_p^2 = 0.134$ ;  $\text{power}_{0.05} = 0.612$ ), and while varying between cultivars ( $F_{3,57} = 20.265$ ,  
240  $P < 0.001$ ;  $\eta_p^2 = 0.516$ ;  $\text{power}_{0.05} = 1$ ), there was no interaction with ‘Treatment’ ( $F_{12,57} =$   
241  $1.31$ ,  $P = 0.239$ ;  $\eta_p^2 = 0.216$ ;  $\text{power}_{0.05} = 0.655$ ). Mean individual seed mass (Table 1) did  
242 however, vary according to ‘Treatment’ ( $F_{4,57} = 5.456$ ,  $P = 0.001$ ;  $\eta_p^2 = 0.277$ ;  
243  $\text{power}_{0.05} = 0.965$ ) and ‘Cultivar’ ( $F_{3,57} = 19.658$ ,  $P < 0.001$ ;  $\eta_p^2 = 0.509$ ;  $\text{power}_{0.05} = 1$ ),  
244 although there was no significant interaction ( $F_{12,57} = 1.799$ ,  $P = 0.070$ ;  $\eta_p^2 = 0.275$ ;  
245  $\text{power}_{0.05} = 0.822$ ). While contrasts highlighted that  $D_w$  and  $S_w$  had an overall difference  
246 with Control (Contrast =  $-0.009$ ,  $SE = 0.004$ ,  $P = 0.036$ ), this was driven primarily by  
247 variation between  $S_w$  and Control (Contrast =  $-0.010$ ,  $SE = 0.004$ ,  $P = 0.023$ ), and not by  
248 the difference between  $D_w$  and Control plants (Contrast =  $-0.007$ ,  $SE = 0.005$ ,  $P = 0.122$ ).  
249 Interestingly, however, there was no variation between  $D_w$  and  $S_w$  (Contrast =  $0.003$ ,  $SE$   
250  $= 0.004$ ,  $P = 0.373$ ). These results emphasise the different intensity of effects on different  
251 cultivars; i.e., Agatha and Cubic showed the most marked negative responses in the 96-  
252 hour  $S_w$  treatment (Table 1).

253 Germination was unaffected by any of the immersion treatments imposed on parent plants  
254 (data not shown); the lowest germination for any one cultivar/treatment group being the  
255 76% recorded for seeds produced by cv Cracker 96-hr  $S_w$  (with 3 of 4 Petri dishes for this  
256 group nonetheless attaining  $>85\%$  germination).

257 Finally, seedling mass (Figure 2) differed between treatments with a small effect size  
258 ( $F_{4,498} = 6.192$   $P < 0.001$ ;  $\eta_p^2 = 0.047$ ;  $\text{power}_{0.05} = 0.988$ ), but not among cultivars ( $F_{3,498} =$   
259  $1.874$   $P = 0.133$ ;  $\eta_p^2 = 0.011$ ;  $\text{power}_{0.05} = 0.486$ ), and there was a significant ‘Treatment  $\times$   
260 Cultivar’ interaction also with a small effect size ( $F_{12,498} = 2.295$   $P = 0.008$ ;  $\eta_p^2 = 0.052$ ;

261 power<sub>0.05</sub>=0.959). Contrasts analysis revealed significant differences between Control and  
262 *Dw* & *Sw* together (Contrast = -0.004, *SE* = 0.001, *P* < 0.001), between Control and *Dw*  
263 (Contrast = -0.003, *SE* = 0.001, *P* = 0.004), and between Control and *Sw* (Contrast = -  
264 0.005, *SE* = 0.001, *P* < 0.001), but not between *Dw* and *Sw* (Contrast = 0.002, *SE* = 0.001,  
265 *P* = 0.089). The significant interaction between the two main factors highlights differences  
266 in the intensity and direction of cultivar responses. The most affected, Cubic, displayed  
267 reduced seedling growth in both the 24-hr and 96-hr treatments, while progeny collected  
268 from cv Cracker showed no response to *Sw* immersion (Fig 2). Seedlings grown from  
269 Astrid and Agatha parents exhibited reduced growth in the 96-hr *Sw* treatments only,  
270 although given the low parental replication for Agatha (only two plants survived to  
271 reproduction), the apparent negative response for seedlings collected from this cultivar  
272 should be taken with caution.

273

## DISCUSSION

274 Our results evidence substantial differences in OSR response to acute freshwater and  
275 seawater inundation; while the former exerted some impact on some of the responses  
276 examined here, the latter was more generally associated with reduced parent plant  
277 growth, seed yield, and even progeny performance. As far as we are aware this is the first  
278 time that the impacts of short-duration, acute, *Sw* flooding on several key growth and  
279 reproductive responses have been demonstrated across stages of the life-cycle for any  
280 plant species. Indeed the only previous study to examine this issue in arable crops, Sun *et*  
281 *al.*, (2015) was limited to the immediate (2-week) post-immersion response, and while  
282 White *et al.*, (2014) did look at growth and flowering in white clover over a 70-d post-  
283 immersion period, they did not consider seed yield or progeny performance. While there

284 was some variation between cultivars, this study shows that even transitory immersion in  
285 seawater has significant, consistent, and long-lasting impacts on OSR crop yield. Indeed,  
286 the fact that reduced reproductive output (most notably siliqua number) was manifest 6  
287 months after maternal plants were exposed to *Sw* immersion highlights the potential  
288 long-term impacts of seawater flooding on arable cropping systems.

289 The consequences of reduced seed yields on agricultural crops like OSR are obvious, but  
290 our results also raise the possibility that wild plant species could suffer reduced  
291 reproductive success as a result of acute seawater flooding stress on seed development. It  
292 is well established that maternal plant environment alters phenotypic expression in  
293 progeny (Herman & Sultan, 2011) and as such the reduced growth of seedlings from  
294 maternal plants subjected to *Sw* immersion reported here is unsurprising. Nonetheless,  
295 and while there is evidence that salinity stress can induce similar shifts in maternal  
296 provisioning and seedling performance (for example as shown in the grass *Cenchrus*  
297 *ciliaris*; Ruiz & Taleisnik (2013)), the fact that parent immersion in seawater for only 4  
298 days can negatively influence subsequent seedling growth, highlights more generally the  
299 impact of coastal flooding on plant community dynamics. Put simply, even if parent  
300 plants (of any native species) survive prolonged flooding, their later ability to contribute  
301 to the recovering community could be compromised. We know of no study to have  
302 demonstrated this response. For two of our maternal cultivars (Cubic and Agatha) where  
303 96-hour *Sw* immersion reduced mean seed mass by 50% or more, the most plausible  
304 explanation for the arrested growth of seedlings is that it resulted from reduced seed  
305 provisioning by the parent (Zas *et al.*, 2013). We cannot however, discount the  
306 possibility that other responses to salinity stress, including epigenetic changes, impact  
307 progeny performance and highlight this as a key area for future research.

308 From the perspective of arable plant species, progeny performance may be of minor  
309 importance however, since crops are routinely cultivated from commercially sourced  
310 seeds. Nonetheless, we demonstrated a consistent and marked reduction in siliqua number  
311 and seed mass for at least two cultivars (Cubic and Agatha) following 96-hr *Sw* immersion,  
312 highlighting the significant economic impact that seawater flooding might have on coastal  
313 OSR crop yields. While a number of studies have shown that prolonged (i.e. one to several  
314 weeks) waterlogging reduces both OSR growth and yield (Cannell & Belford, 1980; Zhou  
315 & Lin, 1995; Xu *et al.*, 2015), these studies looked at freshwater effects only. Although  
316 the relatively short-term *Dw* immersion treatments imposed here did not yield similar  
317 responses (but noting that we did not consider seed oil yield or content (Cannell & Belford,  
318 1980; Xu *et al.*, 2015)), all four cultivars exhibited much reduced plant growth, and later  
319 siliqua number in the longer duration *Sw* treatment (see also Hanley *et al.*, 2013). This  
320 highlights the challenge posed by salinity; even if a plant survives acute seawater  
321 immersion it must prevent or alleviate damage caused by the accumulation of salt ions  
322 ( $\text{Na}^+$  and  $\text{Cl}^-$ ) in tissues. It is interesting that even the most prolonged immersion times (4  
323 days) imposed here failed to induce mortality; a response that perhaps reflects an innate  
324 salinity tolerance due to OSR's heritage in *B. oleracea* and this parent species' natural  
325 affinity for maritime conditions (Snogerup *et al.*, 1990). Salt tolerance is however, often  
326 accomplished by the accumulation of stress metabolites and the regulation of tissue ions  
327 to exclude or compartmentalize the potentially damaging  $\text{Na}^+$  and  $\text{Cl}^-$ . Nonetheless even if  
328 successfully achieved, as seems to be the case here, there are costs on subsequent plant  
329 growth and reproductive performance (Munns & Tester, 2008; White *et al.*, 2014).

330 At a time of human population growth and economic development there are increasing  
331 demands on food supplies, but when coupled with unpredictable and likely ever more

332 extreme climate events, global food security is far from assured (Godfray *et al.*, 2010;  
333 IPCC, 2014). Flooding is widely recognised as one of the key threats to arable crops, but  
334 most research emphasis is placed on pluvial, freshwater flooding where the major negative  
335 impact comes from soil anoxia. In the UK the threshold for crop viability under this  
336 scenario is 15 days (ADAS, 2014), but as we show here, by virtue of the added effect of  
337 salinity, seawater flooding of only 4 days duration can impact OSR yield (while the same  
338 duration under freshwater does not). Historically much of the global agricultural  
339 salinization problem stems from poor irrigation coupled with excessive evaporation and/or  
340 deforestation in hot, dry climates (Vinod *et al.*, 2015). Nonetheless, sea-level rise and the  
341 expected increase in frequency and severity of storm surges (Vousdoukas *et al.*, 2016;  
342 Vitousek *et al.*, 2017) are likely to increase the risk of seawater inundation to temperate  
343 coastal arable systems (Nicholls & Cazenave, 2010). Under these conditions, farmers face  
344 a choice between changing land-use practise, or cultivation of flood-tolerant crops. There  
345 is a rich literature documenting salt-tolerance in crops grown in regions where the  
346 problems of soil salinization are long established; indeed for one of the most important,  
347 rice, crops grown near to coasts are frequently subjected to seawater intrusions and a  
348 genetic capacity for salt-tolerance has been identified (Ganie *et al.*, 2014). The impact of  
349 seawater immersion demonstrated here for OSR, coupled with the increasing risk of  
350 seawater flooding for coastal agriculture globally, underscores a new impetus for research  
351 into salt tolerance in a wider range of arable crop species (Jackson & Ismail, 2015).

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355

356

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496

497 Table 1: The effect of root-zone immersion in deionised (*Dw*) and seawater (*Sw*) on plant reproductive potential (crop yield) of four  
 498 oilseed rape (*Brassica napus*) cultivars (Cracker, Cubic, Agatha & Astrid) 7 months after plants (growth stage 1.3-1.6) were subject  
 499 to transient immersion (24, or 96 hours with a zero hour control). N=4 for all treatment/cultivar combinations except 'Cracker' 96h  
 500 *Dw*, and 'Agatha' 24h *Dw* & 96h *Sw* where N=3.

501

Treatment		Number of siliqua per plant				Seeds per siliqua				Seed mass (mg)			
		Cracker	Cubic	Astrid	Agatha	Cracker	Cubic	Astrid	Agatha	Cracker	Cubic	Astrid	Agatha
<b>Control</b>	Mean	35.5	29.8	22.0	35.8	13.2	22.4	22.3	20.3	0.04	0.08	0.07	0.06
	SE	8.6	2.8	2.4	4.8	2.7	1.2	1.0	1.2	0.009	0.009	0.004	0.005
<b>24h <i>Dw</i></b>	Mean	30.3	18.3	22.8	21.7	16.5	21.6	22.5	17.7	0.04	0.06	0.07	0.04
	SE	3.4	2.7	6.2	3.3	0.6	1.0	2.0	1.0	0.005	0.003	0.01	0.004
<b>96h <i>Dw</i></b>	Mean	40.7	28.0	28.8	27.5	11.4	25.0	20.9	19.9	0.03	0.07	0.06	0.05
	SE	13.2	3.6	2.7	5.0	4.9	0.5	1.1	1.4	0.02	0.004	0.005	0.004
<b>24h <i>Sw</i></b>	Mean	25.8	38.5	25.5	43.8	13.8	22.8	23.0	20.2	0.04	0.08	0.07	0.07
	SE	6.0	11.1	0.9	5.9	1.6	0.8	0.7	1.5	0.004	0.006	0.008	0.006
<b>96h <i>Sw</i></b>	Mean	24.3	11.5	17.5	14.0	13.6	19.4	22.5	9.2	0.03	0.04	0.07	0.02
	SE	6.2	3.2	3.0	5.1	3.2	2.0	1.2	4.7	0.01	0.006	0.006	0.01

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506 Figure Legends

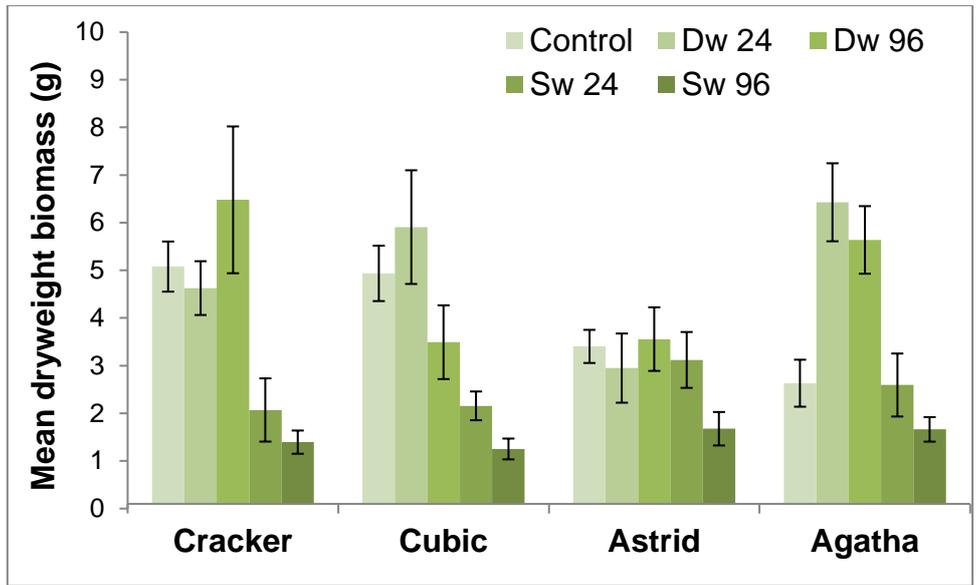
507 Figure 1: The effect of root-zone immersion in deionised (Dw) and seawater (Sw) on  
508 mean ( $\pm$  SE) total above-ground dry mass of four oilseed rape (*Brassica napus*)  
509 cultivars (Cracker, Cubic, Agatha & Astrid) 2 months after plants (67-d-old; growth  
510 stage 1.3-1.6) were subject to transient immersion (24, or 96 hours with a zero hour  
511 control). N=8 for all treatment/cultivar combinations.

512

513 Figure 2: The effect of root-zone immersion in deionised (Dw) and seawater (Sw) on  
514 mean ( $\pm$  SE) seedling dry mass of four oilseed rape (*Brassica napus*) cultivars  
515 (Cracker, Cubic, Agatha & Astrid) of plants grown from seeds collected from parent  
516 plants subject to transient immersion (24, or 96 hours with a zero hour control, at  
517 growth stage 1.3-1.6). N=4 for all treatment/cultivar combinations except 'Agatha'  
518 24h Dw where N =3 and 'Cracker' 96h Dw, & 'Agatha' 96h Sw where N=2.

519

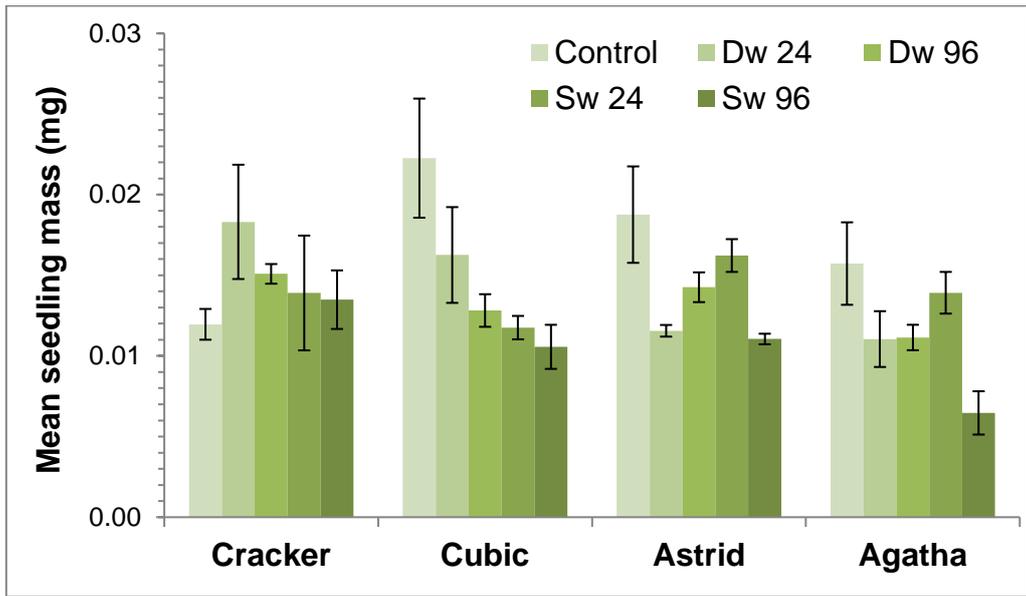
520 Fig 1



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523 Fig 2



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