

1 **Title:** Differential responses of trailing-edge populations of a foundation alga to thermal stress.

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9

10 **Abstract**

11 Populations within the same species range edge may experience contrasting local conditions
12 and exhibit diverse levels of environmental tolerance. This heterogeneity within a range boundary has
13 seldom been considered in studies forecasting the impact of anthropogenic habitat alteration and
14 climate change on species distributions. Moreover, any ecological prediction under changing
15 environmental conditions requires a good understanding of the combined responses of organisms to
16 multiple stressors, in particular the effects on key life cycle stages of species. The intertidal seaweed
17 *Fucus serratus* is a dominant species on northern Atlantic shores and whose southern limit is in the
18 NW Iberian Peninsula. We examined how early developmental stages of southern-edge populations of
19 this foundation alga responded to the combined effects of environmental stressors, including salinity
20 and aerial and seawater temperature. Four populations from two different areas of the NW Iberian
21 coast were considered: two populations from open shores on the northern coast and two populations
22 from the western rias on the Atlantic coast. The study findings revealed inter-population variability in
23 the response to heat stress, with greater survival of germlings from the northern populations than those
24 from western rias. Environmental conditions are still more benign within western rias, under the
25 influence of strong summer upwelling events. The results also suggest the key role of aerial thermal
26 stress in determining the southern limit of distribution of the target species. The future of these
27 heterogeneous edge populations from NW Iberian Peninsula depends on trends in climate change and
28 the ability of populations to cope with these. Environmental changes may already be occurring at rates
29 that exceed the plastic and adaptive potential of edge populations in N Spain, while the future of
30 western rias as climate refugia for this and other foundation alga is also uncertain.

31 **Keywords:** climate change; climatic refugia; early developmental stages; foundation species;
32 interactive effects; intertidal; phenotypic differentiation; rocky shores.

33 **Introduction**

34 It has long been recognized that species are not homogeneous entities, as conspecific populations
35 exposed to different environmental conditions are often phenotypically and/or genetically distinct
36 (Linhart & Grant, 1996). However, the inter-population variability in environmental affinities and
37 tolerances has only been recently considered in studies forecasting the impact of anthropogenic habitat
38 alteration and climate change on species distributions. Thus, these projections often considered the
39 whole-species level, assumed to be a single entity with ecologically similar members (but see for
40 instance Banta et al., 2012; Nicastro et al. 2013; Valladares et al., 2014; Bennett et al., 2015; Saada et
41 al. 2016).

42 In this context, geographically peripheral populations have frequently been considered to live
43 at the margin of their environmental tolerances and to be more susceptible to extinction than central
44 populations, with which they share the same range of environmental tolerance (Sagarin & Gaines,
45 2002 and references therein). However, edge populations, in particular those at the low-latitude
46 boundary of species distributions (rear-edge *sensu* Hampe & Petit, 2005) may have unique phenotypic
47 and genetic traits and may be acclimatized or adapted to these stressful marginal environments
48 (Kawecki, 2008; Bennett et al., 2015; Rehm et al., 2015). In addition, geographically peripheral
49 populations are not always located in unfavourable, ecologically marginal sites (Pironon et al., 2017)
50 as they can occupy “core-like” locations, i.e. contemporary climatic refugia (Ashcroft, 2010; Keppel et
51 al., 2012). Different populations within the same species range edge may experience contrasting local
52 conditions and exhibit diverse levels of environmental tolerance. This edge-edge variability has
53 seldom been considered, despite its importance in range limit dynamics and species responses to
54 climate change.

55 A pre-requisite to constructing any ecological prediction under changing environmental
56 conditions is a good understanding of how organisms respond to interacting environmental factors
57 (Gunderson et al., 2016). The reaction to combined stressors, which may be additive, synergistic or
58 antagonistic, will in turn determine the impact on the performance of individuals and populations

59 (Crain et al., 2008). The effects of different environmental components on key life cycle stages of
60 species and how such effects vary among populations is a current priority in ecological research.

61 Marine organisms living in intertidal habitats are exposed to multiple environmental stresses,
62 linked to both aquatic and aerial regimes, and they may thus be considered early tracers of the effects
63 of changing climate conditions (Helmuth et al., 2006). Canopy-forming macroalgae play a key role in
64 temperate coastal ecosystems by providing food and shelter for diverse species of fauna and flora, as
65 well as sustaining complex food webs (Chapman, 1995; Schiel & Foster, 2006). Retraction of the low-
66 latitude, rear edge of these dominant habitat-forming seaweeds is predicted in northern Atlantic rocky
67 shores (Jueterbock et al., 2013) and many of these species are already in decline in this region (e.g.
68 Lima et al., 2007; Díez et al., 2012; Nicastro et al., 2013; Fernández, 2016; Assis et al., 2017).
69 However, the inter-population variability in environmental affinities and specifically the potential of
70 southern edge populations to withstand environmental changes remain poorly investigated (Pearson et
71 al., 2009; Ferreira et al., 2014; Jueterbock et al., 2014; Bennett et al., 2015). This particularly applies
72 to how early developmental stages, which are usually bottlenecks in seaweed populations (Santelices,
73 1990; Vadas et al., 1992), respond to multiple stressors, as most of these inter-population studies focus
74 on adult thalli.

75 The seaweed *Fucus serratus* L is a dominant canopy-forming species on northern Atlantic
76 intertidal shores and whose southern limit is in the NW Iberian Peninsula (Fischer-Piette, 1955;
77 Lüning, 1990). The species has two range edges in this area, one in N Spain and the other in N
78 Portugal (Lüning, 1990; Arrontes, 1993). In the last few decades, the distribution of *F. serratus* in N
79 Spain has shifted towards the west, and its presence is now almost entirely limited to scattered
80 populations in wave-sheltered Atlantic rias, and a few semi-exposed populations in N Spain, mostly on
81 the coast of the province of Lugo (Duarte et al., 2013; Araújo et al., 2014). Marginal populations from
82 western Atlantic rias and those of N Spain are exposed to contrasting environmental conditions. In
83 addition to differences in wave exposure, cooler water and greater nutrient supply in summer, and
84 higher salinity variability over the year were detected inside western rias than in northern semi-

85 exposed shores, due to the stronger influence of upwelling events and river flows (Martínez et al.,
86 2012; Abrantes et al., 2017; Duarte & Viejo, 2018 and references therein). Furthermore,
87 environmental conditions are changing rapidly, particularly in northern coastal areas, where seawater
88 and air temperatures are increasing (Gómez-Gesteira et al., 2008; Meinshausen et al., 2009; Abrantes
89 et al., 2017) and the surface salinity is decreasing (González-Pola et al., 2005; Llope et al., 2006). The
90 interactive effects of physical factors may affect these marginal populations in different ways and the
91 ability of the populations to persist will also vary. Only one previous study has investigated the
92 combined effects of several physical factors in two edge populations of *F. serratus* in experiments
93 with vegetative fronds of the species (Martínez et al., 2012b).

94 We evaluated how early developmental stages of *F. serratus* in populations in two marginal
95 geographical areas, i.e. western Atlantic rias and northern coast of the NW Iberian Peninsula, respond
96 to the combined effects of salinity, seawater and air temperature. We hypothesized that tolerance to
97 thermal stress would be higher in populations of N Spain, presently exposed to harsh conditions
98 (Duarte and Viejo 2018), while populations from rias, under the influence of freshwater inflow, would
99 be more tolerant to low salinity levels. We also expected strong effects of air temperature in the
100 performance of this intertidal species. In order to determine stage-specific variations in the
101 vulnerability of the alga to environmental factors, the vital rates of two different developmental stages
102 (germlings, juveniles) were estimated in mesocosm experiments under controlled conditions. Fine-
103 scale empirical studies like this are necessary for more accurate prediction of species responses to
104 climate change than obtained by broad-scale modelling approaches.

105

106 **Material and methods**

107 *Collection of germlings and juvenile fronds*

108 Germlings and juvenile stages of *Fucus serratus* were collected from four locations on the coast of
109 Galicia, NW Iberian Peninsula: two locations in the province of Lugo, on the open northern semi-
110 exposed Cantabrian coast (Peizás: 43°35'N, 7°16'W and San Pedro: 43°37'N, 7°20'W) and two

111 locations within large embayments or rias on the western Atlantic coast (O Freixo, in *Ría de Muros*:
112 42°47'N, 8°56'W and Isla de Arousa, in *Ría de Arousa*: 42°33'N, 8°51'W; Fig. 1). Discs made from
113 epoxy resin (4.5 cm diameter, Fetadit 55/63; Fetasa, Madrid, Spain), each with a rough surface and a
114 central hole (see Johnson, 1994), were attached to polycarbonate plates (14 x 14 cm), which were then
115 anchored to rocks with stainless steel bolts and placed under canopies of reproductive individuals of *F.*
116 *serratus*. The discs were placed in the four sites between June and September 2016 to allow algal
117 settlement and subsequent quantification and collection of germlings. A total of 80 discs on 20 plates
118 were placed within each population in the rias, and 40 discs on 10 plates as well as 26 independent
119 discs were placed under canopies at Peizás and San Pedro, respectively. Fewer discs were placed in
120 the sites in the province of Lugo because of lower abundance of reproductive individuals of *F.*
121 *serratus* at these sites on northern shores (Duarte & Viejo 2018). The discs were exposed for three
122 months in the field and were then recovered during the spring tides in September 2016. The number of
123 germlings on each disc was counted under a stereomicroscope. In addition, 96 juvenile individuals of
124 *F. serratus* were collected from each site (mean \pm SE= 0.40 \pm 0.005 g fresh weight, 4.37 \pm 0.04 cm
125 length, n = 384) and transported to the laboratory in cool boxes.

126 *Mesocosm experiments*

127 The germlings that settled on the artificial discs and the juvenile fronds collected were used in
128 mesocosm experiments, conducted at the *Estación de Ciencias Mariñas de Toralla* (ECIMAT, Vigo,
129 Spain, <https://www.ecimat.uvigo.es>). Prior to the experiments, discs with germlings and the juvenile
130 fronds were immersed in seawater of salinity \sim 35 ‰ and temperature \sim 16 °C for 9 days with a water
131 surface irradiance of \sim 450 $\mu\text{mol m}^{-2}\text{s}^{-1}$. The experiments with germlings and juveniles were then
132 started and lasted for 28 and 24 days, respectively (during September-October 2016).

133 The experimental set-up included Population (4 levels: Isla de Arousa, O Freixo, Peizás and
134 San Pedro, hereinafter IA, OF, Pz and SP, respectively), Salinity (2 levels: low and high with mean
135 values \sim 22 and 35 ‰), Seawater temperature (2 levels: cold and warm, with mean values \sim 16 and 20
136 °C), and Air temperature during emersion periods, hereafter Emersion (2 levels: low and high, with

137 maximum temperatures of about 19 and 26 °C, respectively) as fixed and orthogonal factors and Tank
138 and/or Disc as random factors (see details of the experiments and the statistical analyses below). Given
139 the smaller number of recruitment discs placed in the Lugo populations (Pz and SP), the complete
140 orthogonal design in the case of germlings was only established for the ria populations (IA and OF).
141 Germlings from Lugo were only exposed to different temperatures during emersion (at fixed
142 conditions of 35 ‰ of salinity and 20 °C of seawater temperature).

143 The salinity, seawater temperature and emersion levels were chosen to represent the different
144 environmental conditions experienced by *F. serratus* individuals in the study areas (Duarte & Viejo,
145 2018). Salinity fluctuates within the rias and decreases from 35 to ~ 22-29 ‰ due to the river supply,
146 while the values are less variable in the semi-exposed Lugo locations, ranging between 32 and 36.5 ‰
147 throughout the year (Intecmar, Xunta de Galicia, Spain, <http://www.intecmar.gal/>). The mean seawater
148 temperature is lower within the rias than on the Lugo coast during summer (Duarte & Viejo, 2018).
149 Finally, the air temperatures selected corresponded to daily mean values recorded by data loggers
150 located underneath a canopy of *F. serratus* at O Freixo, during diurnal emersion periods, both under
151 heatwave and non-heatwave conditions during summer of three consecutive years (2011-2013, authors
152 unpublished data).

153 The experiments were set up in an isothermal walk-in chamber. The mesocosm system
154 consisted of 32 transparent plastic tanks (3 L) supplied with 50 µm sand-filtered seawater from the *Ría*
155 *de Vigo* (42°12'N, 8°48'W) adjusted to the two experimental salinities with freshwater and renewed
156 every two days (mean values \pm SE = 22 \pm 0.04 ‰ and 35 \pm 0.01 ‰, n=16). Four tanks and 3 juveniles
157 per tank from each population (i.e. 12 fronds per tank) were used for each combination of Salinity x
158 Seawater temperature x Emersion (i.e. a total of 32 tanks and 384 juvenile individuals). In the case of
159 germlings, 3 tanks and 2 discs per tank and population were used for each combination of
160 environmental conditions (in populations from rias, but only for emersion treatments at the fixed
161 values of 35 ‰ of salinity and 20 °C of seawater temperature for Lugo populations). A total of 120
162 discs (48 for each population from rias and 12 for each population from Lugo) were used. The

163 experiments with germlings (settled on discs) and juveniles were conducted simultaneously in the
164 same tanks (see Supplementary Fig. 1 for an outline of the experimental design). The reverse side of
165 each disc was numbered, and juveniles (hanging from ropes that ran through the tank) were also
166 tagged with numbered plastic strips for subsequent identification.

167 Tanks were continuously aerated, and light was supplied from above by cool white fluorescent
168 lamps (F18 W/840; mean water surface irradiance: $451.87 \mu\text{mol m}^{-2}\text{s}^{-1}$ (± 6.39 SE, $n = 8$) with a 12:12
169 h light: dark photoperiod. Tanks were randomly allocated to the salinity and seawater temperature
170 treatments to minimize the effect of their position. The cold seawater treatment was determined by the
171 chamber temperature (16°C mean value), while for the warm seawater treatment the water was heated
172 using titanium aquarium heaters (100 W) regulated by a set of 3 AT Controller Twin (Aqua Medic®,
173 Germany). Seawater temperature was recorded every 5 minutes by data loggers (IButton® Data
174 Loggers, Germany). Mean daily values ranged between $15.67 (\pm 0.05$ SE) and $16.84 (\pm 0.01)^\circ\text{C}$ in the
175 cold seawater temperature treatment, and between $19.82 (\pm 0.03)$ and $21.74 (\pm 0.02)^\circ\text{C}$ in the warm
176 treatment, $n= 288$.

177 To simulate emersion conditions during low tide, recruitment discs and juveniles were
178 manually transferred once a day from the tanks to shelves located inside the same chamber and
179 covered with a canopy of *Fucus* spp. (non-reproductive fronds). Juveniles and recruitment discs from
180 the same tank were grouped on the shelves. The emersion time varied weekly throughout the
181 experiment, alternating between 4 and 6 h, in order to simulate the monthly variations in the tidal
182 range. Shelves for emersion periods were provided with light from above with cool light fluorescent
183 lamps, at the same light intensity and photoperiod as in the tanks. The irradiance values under canopy
184 were $328.50 \pm 6.77 \mu\text{mol m}^{-2}\text{s}^{-1}$ ($n = 6$). To produce temperature profiles similar to those experienced
185 by macroalgae during emersion in the field, the air temperature was increased gradually by using
186 ceramic infrared heaters (150 W, Exo Terra, Mansfield, USA) positioned over the shelves. The air
187 temperature was regulated with digital temperature controllers (AT Control System controllers, Aqua
188 Medic®, Germany) and recorded via individual temperature sensors inserted underneath the canopy.

189 This system enabled continuous control and recording of air temperature with an error of 0.2 °C. In the
190 4-hour emersion cycle, air temperature increased gradually, reaching maximal values of 18.77 °C and
191 22.50 °C in the low and high emersion treatments (Fig. 2a), while in the 6-hour cycle the maximal
192 values were 18.71 °C and 25.86 °C, respectively (Fig. 2b). Care was then taken to simulate the
193 emersion conditions experienced in the field by early developmental stages of intertidal algae, i.e.
194 cover by adult canopies, the gradual increase in aerial temperatures during each emersion period, and
195 the weekly variability in the length of the emersion periods.

196 *Post-stress conditions of recruits*

197 After the experiment, discs with surviving recruits (i.e. individuals visible to the naked eye) were
198 maintained submerged in tanks under common ambient conditions of high salinity ($35.2\text{‰} \pm 0.08$, $n =$
199 12) and low seawater temperature ($16\text{ °C} \pm 0.11$, $n = 12$) for 32 days in the isothermal walk-in
200 chamber. Only discs from the ria populations (IA and OF) previously exposed to low emersion
201 conditions, were used at this stage, due to the elevated mortality experienced by germlings in some of
202 the population and treatment combinations in the previous experiment (see results).

203 *Response variables*

204 *Survival of germlings, recruits and juveniles*

205 The number of germlings/recruits per disc was calculated under a binocular microscope as the mean
206 value from 3 random 1 cm²-subsamples per disc. The proportion of live germlings/recruits was
207 calculated at the end of the mesocosm experiment/start of the post-stress phase (after 28 days) and at
208 the end of the post-stress phase (i.e. 60 days). The state (dead or alive) of each juvenile was
209 determined 10 and 17 days after the start of mesocosm experiment (22 and 29 September 2016,
210 respectively). Dead individuals were identified by the presence of significant tissue depigmentation,
211 broken apices and necrosis.

212 *Growth of germlings, recruits and juveniles*

213 Due to the small size of germlings at the beginning of the experiment, the first measurements, i.e.
214 length measured from top of embryo body to tip of rhizoid, were taken one week after the experiment
215 started ($t = 7$ days). Mean size per disc was calculated from 10 germlings, which were randomly
216 selected and measured under a binocular microscope connected to an image analysis system (Nikon-
217 SMZ1500, NIS Elements Basic Research 4.0). The mean size of germlings/recruits per disc was also
218 estimated at the end of the mesocosm experiment and at the start/end of the post-stress phase.

219 In the case of juveniles, the fresh weight (± 0.001 g) of each individual was estimated weekly,
220 after the specimens had been dried by gently blotting them with paper towels. The growth of each
221 juvenile was calculated as the difference in the initial weight and the weight after 10 and 17 days.
222 Final weights (24 days after the start of the experiment) were not considered for analysis due to the
223 high mortality of juveniles at that time (see results).

224 *Data analyses*

225 Potential differences between populations in the mean number of germlings/recruits and the size of
226 juveniles/recruits were evaluated at the start of the mesocosm/post-stress experiments by using
227 Generalized Mixed Models (GLMMs; Zuur et al., 2007) and Linear Mixed Models (LMMs)
228 respectively. Population (fixed) and Tank (random) were included in the analyses as orthogonal
229 factors. The initial size of germlings was not known, as the first measurements were taken one week
230 after the start of the mesocosm experiment (see above).

231 GLMMs for count data (initial number of germlings/recruits) were fitted with a Poisson
232 distribution of error terms and a log-link function. Data overdispersion was detected in the germling
233 analysis (dispersion parameter, $\Phi = 7.42$; Zuur et al., 2009) and was corrected by adding observation-
234 level random effects, i.e. the disc was added as a random factor (OLRE; Harrison, 2014). As
235 differences in the initial number of germlings between populations were detected in the mesocosm
236 experiment (see results), the possible influence of these initial densities on survival was subsequently
237 determined by using 70th and 90th quantile regressions (Scharf et al., 1998; Cade & Noon, 2003).

238 Goodness of fit (R^1) was calculated for each specific quantile regression according to Koenker &
239 Machado (1999).

240 The effects of the physical factors and population of origin on the survival rate of germlings
241 was analysed at the end of the mesocosm experiment by using GLMMs fitted with a binomial
242 distribution of error terms and a logit link function. The average size of germlings per disc was
243 analysed one week after the start and also at the end of the mesocosm experiment by using Linear
244 Mixed Models (LMMs). The full or “beyond optimal” models (*sensu* Zuur et al., 2009) included four
245 orthogonal fixed factors (Population, Salinity, Seawater temperature and Emersion) and the Tank x
246 Population interaction. In each case, the explanatory variable (either survival rate or size) was
247 analysed separately for populations from rias (including all the treatment combinations) and for all
248 populations (testing only the effects of Emersion, see the mesocosm experimental set up). In the
249 analysis of the survival of germlings for all populations, the random Tank x Population structure was
250 not considered in the full model, due to the lack of a sufficient number of replicates to ensure adequate
251 statistical power. In addition, data overdispersion was also observed in this analysis (dispersion
252 parameter, $\Phi = 10.76$) and was corrected by adding Disc as a random factor.

253 Survival of juveniles 10 and 17 days after the start of the mesocosm experiment were likewise
254 analysed by GLMMs with binomial distribution and a logit link function. The response variable was in
255 this case State (dead *vs* alive; 0 *vs* 1), and the full model included the four previously mentioned
256 orthogonal fixed factors, their interactions and the random factor Tank. Growth of juveniles 10 days
257 after the start of the experiment was analysed using an LMM of the same design. Three outliers were
258 removed to achieve the data normality assumption. At $t = 17$ days, only juveniles exposed to the low
259 emersion temperature treatment were considered for modelling, due to the high mortalities detected at
260 the high emersion treatment.

261 At the end of the post-stress phase, the survival rate of recruits was analysed using a
262 Generalized Linear Model (GLM), again fitted with a binomial distribution of error terms and a logit
263 link function. As explained above, the analyses were only carried out at this phase with recruitment

264 discs from rias populations previously exposed to the low emersion temperature. Three orthogonal
265 fixed factors were considered (Population, Salinity, and Seawater temperature in the previous
266 mesocosm experiment). Tank was not included as a random factor in the full model because of the
267 small number of tanks at this stage. Data overdispersion was detected (dispersion parameter, $\Phi =$
268 10.30) and was corrected by adding observation-level random effects. The final mean size of recruits
269 per disc was tested with a Linear Model (LM) using the same design as for survival in this post-stress
270 phase. Prior to the analysis, data were log-transformed to comply with the assumption of data
271 normality.

272 Model selection in mixed models, either GLMMs or LMMs, was performed in two steps,
273 starting with the full (or beyond optimal) model. First, the optimal random structure was selected from
274 nested models fitted with restricted maximum likelihood estimation by using the hypothesis testing
275 approach. The p-values were corrected due to the problems of testing on the boundary (Zuur et al.,
276 2009). The optimal fixed structure was then selected by comparing models fitted with maximum
277 likelihood estimations. Given a selected random structure, models with different combination of fixed
278 components were compared using Akaike Information Criteria for small samples (AICc; Burnham &
279 Anderson, 2002). The differences in AICc values between each model and the model with the
280 minimum value ($\Delta_i = \text{AICc}_i - \text{AICc}_{\min}$) were also calculated. The subset of candidate models was those
281 with the lowest AICc values and $\Delta_i > 2$ with the other models (Burnham and Anderson 2002). The
282 Akaike weights (w_i) of the subset of candidate models was also determined, as was the relative
283 importance of the factor in the candidate subset of models (Burnham, 2015). Akaike weights are
284 equivalent to the probability of a given model being the best in the candidate subset of models
285 (Burnham & Anderson, 2002). The relative importance of the factor is the sum of all the Akaike
286 weights of the candidate subset of models containing each explanatory variable.

287 Data normality, homogeneity of variance and data dispersion assumptions were tested and
288 where necessary were corrected in the selected model. Once the final model was selected, a Tukey's

289 post hoc test was applied to examine pairwise differences for significant interactions terms or main
290 effects.

291 The complexity of the experimental design and the limited number of available populations
292 per area (two within rias and two on northern shores), precluded the inclusion of Area as an additional
293 main fixed factor and Population as a nested random factor in mixed models. A higher number of
294 populations should be available (> 4 but preferable > 10; Zuur et al 2007). We used a parsimonious
295 approach by considering Population as a main fixed factor, determining the existence of divergent
296 patterns between ria and northern populations by significant differences in pairwise comparisons.

297 Quantile regressions were conducted using the R package *quantreg* (Koenker et al., 2019),
298 while LMs, LMMs GLMs and GLMMs were fitted using the *lme4* package (Bates et al., 2015) and
299 model selection with information criteria was conducted using the *dredge* function of MuMIn package
300 (Barton, 2019) in the R 3.0.3 R software (R Development Core Team 2011).

301

302 **Results**

303 *Mesocosm experiment*

304 *Survival and size of germlings*

305 Germling survival and growth showed inter-population variability in response to thermal conditions,
306 with a relevant role of emersion temperature, either alone or in combination with seawater
307 temperature. When all the treatment combinations were analysed for germlings from the ria
308 populations, survival was only affected by the population of origin (Table 1a; relative importance of
309 Population factor = 1). Many more germlings from OF than from IA survived (mean percentage of
310 survival \pm SE = 48.78 ± 4 % and 5.70 ± 0.06 % for OF and IA, respectively, n = 48). However,
311 analysis of all populations (at fixed values of 35 ‰ salinity and 20 °C seawater temperature, see
312 methods) revealed that the effect of emersion temperature depended on the population of origin (see

313 Table 1b, Population x Emersion interaction). At the high emersion temperature, more germlings from
314 the Lugo populations than from ria populations survived (Fig. 3a).
315 Initial differences between populations were found in the number of germlings that settled on each
316 disc, with significantly higher densities in the rias than in Lugo (initial number of germlings per disc:
317 IA = 88.40 ± 7.27 ; OF = 59.16 ± 4.12 ; Pz = 15.69 ± 2.53 ; SP = 13.08 ± 2.0 , n = 48 and 12 for rias and
318 Lugo populations respectively; Tukey post-hoc test, IA > OF > Pz = SP, for analysis see
319 Supplementary Table 2). Survival patterns did not seem to be significantly affected by these initial
320 differences in density. Quantile regressions relating survival proportions to the initial density of
321 germlings showed negative relationships between these variables, but with low goodness of fit (R^1)
322 values and/or non-significant slopes (90th quantile regression: slope = -0.073, p = 0.025, $R^1 = 0.041$;
323 70th quantile regression: slope = -0.050, p = 0.156, $R^1 = 0.016$).

324 The combined effect of seawater and emersion temperature affected the final size of germlings
325 from the ria populations (Table 2a; relative importance of the Seawater temperature x Emersion
326 interaction, based on Akaike weights = 0.42). Germlings exposed to cold seawater and low emersion
327 temperatures reached the largest sizes (Fig. 3b). Salinity or population of origin, on the other hand,
328 appeared in a smaller proportion of the subset of candidate models, suggesting limited effects of these
329 factors on the final size of germlings (Table 2a; relative importance of Salinity and Population, 0.31
330 and 0.18, respectively). One week after the start of the experiment, salinity affected the size of
331 germlings from rias, which were smaller in the high salinity (35‰) treatment (size of germlings =
332 $647.42 \pm 35.79 \mu\text{m}$ and $444.70 \pm 18.11 \mu\text{m}$ for low and high salinity treatments respectively, n=33; for
333 analysis see Supplementary Table 2). Analysis of all four populations under fixed conditions of
334 seawater temperature and salinity revealed that temperature during emersion had a negative effect on
335 the final size of survivors (Table 2b, final size of germlings= $385.69 \pm 35.40 \mu\text{m}$ and 276.74 ± 28.27
336 μm for low and high emersion temperatures respectively, n = 21). No differences in responses were
337 detected between populations. However, one week after the start of the experiment, germlings from
338 the Lugo populations (Pz and SP) growing under the high emersion temperature were smaller than

339 those from the ria populations (IA and OF; the best model included the interaction term Population x
340 Emersion, see Supplementary Table 3 and Fig. 2).

341 *Survival and growth of juveniles*

342 Temperature during emersion was relevant in determining juvenile performance, sometimes
343 interacting with seawater temperature. Thus, juvenile survival decreased in the second week after the
344 start of the experiment, especially for the high emersion temperature; this pattern became more evident
345 over time (Fig. 4a). Ten days after the start of the experiment (22 September), the effect of emersion
346 was dependent on seawater temperature (Fig. 4b and Table 3a; relative importance of Seawater
347 temperature x Emersion interaction = 0.43). At this time, survival of juveniles was lower for warm
348 seawater and high emersion temperatures, regardless of the population of origin (Fig 4b). However, 17
349 days after the start of the experiment (29 September), the effect of emersion appeared to depend on the
350 population of origin (Table 3b; relative importance of Population x Emersion = 0.21). At the high
351 emersion temperature, juvenile survival was much lower, irrespective of the population origin. By
352 contrast, at low emersion temperature, survival of the juveniles from OF (ria population) was highest,
353 and survival of the juveniles from Pz (northern population) was lowest. Differences in survival
354 between emersion treatments were also more evident in individuals from OF and less obvious in those
355 from Pz (Fig. 4c).

356 The growth of juveniles 10 days after the start of the experiment (22 September) was
357 influenced by additive effects of population of origin and emersion temperature (Table 4a; relative
358 importance of Population=1, Emersion=1). The weight of all juvenile survivors (particularly those
359 from IA) decreased, with the exception of individuals from OF, which increased slightly in weight
360 (Fig. 5a). Likewise, at high emersion temperature the weight of the juveniles decreased, while in the
361 low emersion temperature the weight increased (Fig. 5b). The initial weight of juveniles differed
362 among populations, with juveniles from IA being heavier than those from the other populations (initial
363 weight, IA = 0.51 ± 0.010 g; OF = 0.35 ± 0.006 g; Pz = 0.39 ± 0.008 g; SP = 0.36 ± 0.008 g, n = 96,
364 Tukey post-hoc test, IA > OF = Pz = SP; see Supplementary Table 4 for model selection). Similar

365 results were obtained by considering relative growth rates rather than absolute growth values (results
366 not shown) despite the differences in initial sizes. The growth of juveniles 17 days after the start of the
367 mesocosm experiment (29 September) was only analysed for low emersion conditions (see methods).
368 At that time, juvenile growth depended on the additive effects of population of origin and seawater
369 temperature (Table 4b; relative importance of Population: 1, Temperature: 0.61), and growth tended to
370 increase in juveniles from rias (particularly those from OF) and in juveniles exposed to the seawater
371 temperature (Figs. 5c-d).

372 *Post-stress conditions*

373 The population of origin and the effect of previous treatments influenced the performance of recruits
374 maintained under common conditions during the post-stress phase. Thus, survival of recruits from OF
375 was much higher than that of those from IA, and of those previously exposed to the cold seawater
376 temperature, i.e. 16 °C, that also was the set temperature in the post-stress phase (Table 5a, Population
377 effect = 39.16 ± 8.74 % and 63.02 ± 8.13 % percentages of survival for IA and OF, n=16 and 15;
378 Seawater temperature effect = 63.16 ± 7.32 % and 33.46 ± 9.32 %, at cold and warm seawater
379 temperatures, n=18 and 13). Discs from OF presented higher initial recruit densities than those from
380 IA (the selected model was the full model which included Population, df=4, AICc=505.95; mean
381 initial number of recruits per disc \pm SE, IA = 33.04 ± 8.92 ; OF = 40.8 ± 13.05 ; n = 16 - 15).

382 At the start of the post-stress experiment, the recruits from ria populations previously exposed
383 to cold seawater temperature were larger than those exposed to warm seawater (Table 5b, initial size =
384 588.17 ± 57.39 μ m and 512.33 ± 84.11 μ m at cold and warm seawater temperatures respectively, n=18
385 and 13). However, at the end of the experiment, the size of recruits was not affected by prior exposure
386 to different seawater temperature or salinity or by the population of origin (Table 5c).

387

388 **Discussion**

389 The study findings revealed inter-population variability in the resistance to heat stress at the southern
390 rear-edge of the distribution range of *Fucus serratus*. A growing body of literature shows variability in

391 thermal tolerance between edge and central populations of geographic species distributions,
392 particularly in several habitat-forming algae including fucoids and kelps (e.g. Pearson et al., 2009;
393 Ferreira et al., 2014; Bennett et al., 2015; Saada et al., 2016; King et al., 2018). However, edge-edge
394 comparisons as shown in this study are scarce, despite their importance in unravelling range dynamics
395 under present and future climate change scenarios. Our results also highlighted the strong influence of
396 aerial thermal stress, either alone or in synergy with seawater temperature, on the performance of early
397 stages of *F. serratus*, and thus its potential role in establishing the southern boundary of the
398 distribution. Finally, the effect of salinity was subtle, only affecting the size of germlings in the ria
399 populations.

400 Germlings from the northern semi-exposed populations on the Lugo coast were more resistant
401 to thermal stress during emersion (greater survival) than those from western rias on the Atlantic coast.
402 In juvenile stages the effect of emersion temperature was predominant, without any evident
403 differences between the northern and the ria populations in vital rates. However, the greater resistance
404 of Lugo populations to heat stress was also detected in experimental studies with adult plants. Thus,
405 the survival threshold of adult plants to seawater temperature was 3°C higher in one population from
406 Lugo coast than the thresholds of other populations along the species distributional range, including
407 populations of western rias of Iberian Peninsula (A. García, unpublished data).

408 The higher thermal tolerance of germling and adult *F. serratus* occurs under current harsh
409 conditions on open northern shores. Habitat quality recently declined in the western part of northern
410 Spanish coasts, as shown by inhibition of the growth of juvenile *F. serratus* transplanted from rias to
411 the Lugo coast, and the decrease in plant size, reproduction and recruitment of the extant populations
412 (Duarte & Viejo, 2018). Significant warming and limited nutrient supply in summer due to increased
413 water stratification, have been detected on the northern coast of Spain in the last few decades (Llope et
414 al. 2006, 2007; Gómez-Gesteria et al., 2008).

415 Smaller germlings and reduced growth of juveniles from northern populations than those from
416 rias were also recorded in our mesocosm experiments. This may reflect physiological costs associated
417 with stress tolerance. Metabolic costs are involved in the synthesis of antioxidants, phenolic

418 compounds or heat shock proteins, as part of the ubiquitous cellular responses to heat stress (Feder &
419 Hofmann, 1999; Contreras-Porcia et al., 2017). High levels of expression of constitutive heat shock
420 proteins have been detected in Spanish populations of *F. serratus* in relation to core populations of N
421 Europe (Jueterbock et al., 2014).

422 The underlying mechanisms of the greater thermal tolerance in northern Spanish populations
423 than in western rias may be due to local adaptation and/or acclimatization (Kawecki, 2008).
424 Experiments often do not enable genetic responses to be disentangled from plastic responses, which
425 may also be transgenerational (Munday et al., 2013). Our findings indicated that germlings/recruits
426 may rapidly acclimate to specific environmental conditions, as survival was higher during the post-
427 stress phase in those specimens previously exposed in the mesocosm experiment to the same
428 temperature. Southern populations of *F. serratus* are genetically unique and exhibit pronounced inter-
429 population differences relative to core populations in central and northern Europe, although with lower
430 genetic diversity (Coyer et al., 2003). The limited dispersal capacity of the species and the high
431 genetic differentiation at a local scale also favour ecotypic differentiation at the southern edge of
432 *F.serratus* distribution (Coyer et al., 2003).

433 Whether adapted or acclimatized to the present conditions, the greater resistance of germlings
434 and adult plants to thermal stress, may offset poor recruitment, favouring persistence of the Lugo
435 populations. Nevertheless, the long-term viability of these peripheral northern populations is uncertain
436 and will depend on the rate of future climate change and the adaptive potential of these populations to
437 face the forthcoming conditions at a fast enough speed to avoid local extinction. Selection may be
438 strong under increasing environmental stress, as observed at small scales on the steep intertidal
439 gradient (e.g. Hays, 2007). Spanish populations of *F. serratus* exhibit signs of increased selection
440 pressure for heat tolerance over time (Jueterbock et al., 2018). However, the lower genetic diversity
441 detected in the southern populations of this species, in relation to core locations in northern European
442 shores, may limit their evolvability (Coyer et al. 2003; Pearson et al., 2009). Future climate change
443 scenarios may exceed the potential for edge populations from northern Spain to persist. In fact, north-
444 eastern populations located in the province of Asturias, which were abundant in the 1990s and

445 constituted by then the boundary of *F. serratus* distribution, are currently almost extinct (Arrontes,
446 2002; Duarte et al., 2013).

447 While conditions are becoming harsher for *F. serratus* and other habitat-forming algae in N
448 Spain such as *F. vesiculosus*, *Himanthalia elongata* or kelps (Nicastro et al., 2013; Casado-Amezúa et
449 al., 2019 and references therein), the western rias still provide benign conditions for several
450 macroalgae at the southern edge of their distributions (Duarte & Viejo, 2018). Rias can act as
451 contemporary refugia, providing favourable environmental conditions related to the presence of
452 intense summer upwelling events, shelter from wave-action and inputs from rivers (Fraga, 1981;
453 Álvarez et al., 2005). The role of upwelling areas as refugia for ecosystem engineers, including
454 canopy-former fucoids, has previously been highlighted (Chollett et al., 2010; Lourenço et al., 2016).

455 Higher heterogeneity in the performance of early stages between ria populations than between
456 northern populations was also detected in the mesocosm experiments, with more survivors and faster
457 growth rates in the O Freixo population than in the Isla de Arousa population. These sites are located
458 in the inner part of two different rias characterized by different flow rates and morphological and
459 topographical conditions (Rosón et al., 1995; Carballo et al., 2009). Cooler waters in summer and
460 higher inorganic nutrient supply were recorded in O Freixo than in the Isla de Arousa (Duarte & Viejo,
461 2018). The geographic isolation and higher environmental heterogeneity may promote larger
462 phenotypic and genetic divergence between these two populations than between populations from
463 northern open shores.

464 The study findings also revealed the important role played by thermal stress during emersion,
465 either alone or in synergy with seawater temperature, on the vital rates of early stages of *F. serratus*
466 from edge populations. Although variable, the responses of several species of genus *Fucus* to
467 combined abiotic stresses were mostly additive (Wahl et al., 2011). In particular, *F. serratus* adults
468 were affected additively by solar radiation, seawater and air temperature (Martínez et al. 2012b;
469 Fernández et al., 2015). Nevertheless, our contrasting results are not surprising, as responses often
470 differ among life stages, with early stages being more vulnerable to stressors than adults, particularly
471 to aerial exposure (Vadas et al., 1992; Davison & Pearson, 1996; Nielsen et al., 2014). Germling size

472 and juvenile survival were both synergistically affected by air and seawater thermal stress. When
473 multiple stressors occur simultaneously or in rapid succession, synergistic effects are likely to occur,
474 because the second stressor will increase the intensity or duration of the first stressor (Gunderson et
475 al., 2016). Repeated exposure to high air and water temperatures may lead to higher physiological
476 costs.

477 In our experiments, the synergies occurred in moist conditions during emersion, under adult
478 canopies, simulating field conditions. A previous study with adult fronds of *F. serratus* indicated that
479 humidity ameliorated aerial thermal stress (Martínez et al., 2012b; Fernández et al., 2015). However,
480 desiccation may confer protection during aerial exposure and increase thermo-tolerance in intertidal
481 algae by maintaining fronds in an inactive state (Davison & Pearson, 1996; Mota et al., 2015). Further
482 work is necessary to elucidate whether moisture exacerbates or alleviates thermal stress during aerial
483 exposure of early developmental stages.

484 The southern limits of the geographical distribution of various species, particularly of
485 intertidal seaweeds, have frequently been attributed to the major influence of seawater temperature (
486 e.g. Lüning, 1990; Eggert, 2012; Jueterbock et al., 2013; Saada et al., 2016). Our results indicate that
487 thermal stress during aerial exposure, and the synergistic effects of air/water warming probably play a
488 key role in determining the southern limits of distributional of *F. serratus*. Previous field observations
489 also pointed out the importance of stress at low tide in the decline and subsequent extinction of eastern
490 populations of *F. serratus* in northern Spain (Viejo et al., 2011; Martínez et al., 2012b). Indeed,
491 molecular thermal damage of intertidal organisms in temperate regions occurs at temperatures almost
492 exclusively experienced during emersion periods (Helmuth et al., 2002 and references therein).

493 In summary, populations from rias and northern shores in NW Iberian Peninsula exhibited
494 distinct resistance to heat stress and experienced contrasting local conditions. The future of these
495 marginal populations remains an open question and depends on trends in climate change and the
496 ability of populations to cope with these. Environmental changes may already be occurring at rates
497 that exceed the plastic and adaptive potential of edge populations in northern Spain, despite the
498 detected greater resistance to heat stress of these populations than those from rias. Global warming and

499 specifically heat waves are increasing in frequency and intensity (Smale et al., 2019). Warming is
500 highly heterogeneous in coastal areas and is much less pronounced in upwelling regions (Varela et al.,
501 2018). However, several studies have highlighted the weak upwelling events both on N and W Iberia
502 during the last few decades (Llope et al., 2006; Pérez et al., 2010). Furthermore, a recent study using
503 high-spatial resolution climate models predicts the future weakening of the NW Iberia upwelling
504 (Sousa et al., 2019) under emissions scenario RCP 8.5, which is characterized by large increases in
505 greenhouse gas emissions (Riahi et al., 2011). The future of western rias as climate refugia for *F.*
506 *serratus* and several foundation species will thus be threatened, and the stressful conditions on the
507 northern coast will also be exacerbated. Whether these heterogeneous rear-edge NW Iberian
508 populations persist or become extinct is a key question with important ecological and evolutionary
509 implications. The disappearance of habitat-forming species would probably cause bottom-up
510 cascading effects, leading to functional simplification and a decrease in the productivity potential of
511 coastal systems (Duarte et al., 2015; Filbee-Dexter & Wernberg, 2018). Furthermore, *F. serratus* and
512 other fucoids and kelps harbour unique genetic and phenotypic variation at the warmer rear edge, in
513 NW Iberia (Coyer et al., 2003; Provan, 2013; Neiva et al., 2015 and references therein). The loss of
514 this unique and heterogeneous phenotypic and genetic component at the rear edge of the distribution
515 may compromise the adaptive potential of these key species as a whole to increasing warming
516 conditions. Global actions that slow the future rate of greenhouse gas emissions could reduce the
517 impacts of climate change, by favouring the potential of marine species to acclimate or adapt to the
518 new conditions.

519

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527 **Author contributions**

528 All authors participated in the fieldwork, experimental set-up and execution of the experiment. RMV
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531

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780 **Tables**

781 **Table 1.** Selection of the random and fixed structures of GLMMs on survival of germlings at the end of the
 782 first mesocosm experiment from a) rias populations and b) all populations. For the random structure, table
 783 shows likelihood ratio tests comparing different nested models. For the fixed structure, the LogLikelihood
 784 (logLik), AICc and Δ_i are shown for both, the subset of candidate models and the full model, and Weights
 785 (ω_i) only for the candidate models. Selected models are marked in bold. P: Population, S: Salinity, T:
 786 Seawater temperature, E: Emersion. In the fixed component, the multiplications signs (x) indicate the
 787 inclusion of all the interactions of lower order and the implicated main factors.

788

a) Ria populations					
Random structure	df	Model comparison	logLik	L. Ratio test	p-value
MR1: P x S x T x E	16		-306.02		
MR2: P x S x T x E + (1 Tank)	17	1 vs. 2	-305.49	1.0683	< 0.001
MR3: P x S x T x E + (Population Tank)	19	2 vs. 3	-301.86	7.2617	< 0.001
Fixed structure	df	logLik	AICc	Δ_i	ω_i
MF1: P	6	-306.804	626.552	0	0.390
MF2: P + S	7	-305.993	627.258	0.705	0.274
MF3: P + E	7	-306.474	628.220	1.668	0.169
MF4: P + T	7	-306.488	628.248	1.696	0.167
Full model: P x S x T x E	19	-301.855	651.711	25.158	
b) All populations					
Random structure	df	Model comparison	logLik	L. Ratio test	p-value
MR1: P x E	8		-386.8		
MR2: P x E + (1 Tank)	9	1 vs. 2	-282.7	208.19	< 0.001
Fixed structure	df	logLik	AICc	Δ_i	ω_i
MF1: P x E	9	-282.703	588.1	0	1

789 **Table 2.** Model selection of the random and fixed structures of LMs models on size of germlings at the end
790 of the first mesocosm experiment from a) rias populations and b) all populations. For the random structure,
791 table shows likelihood ratio test comparing different nested models. For the fixed structure, the
792 LogLikelihood (logLik), AICc and Δ_i are shown for both, the subset of candidate models and the full model,
793 and weights (ω_i), only for the candidate models. Selected models are marked in bold. Abbreviations as in
794 Table 1. Null= model with no fixed structure. In the fixed component, the multiplications signs (x) indicate
795 the inclusion of all the interactions of lower order and the implicated main factors.

a) Ria populations					
Random structure	df	Model comparison	logLik	L. Ratio test	p-value
MR1: P x S x T x E	17		-88.064		
MR2: P x S x T x E + (1 Tank)	18	1 vs. 2	-88.063	0.003	0.481
MR3: P x S x T x E + (Population Tank)	20	2 vs. 3	-88.009	0.107	0.496
Fixed structure	df	logLik	AICc	Δ_i	ω_i
MF1: T x E	5	-60.141	131.031	0	0.172
MF2: E	3	-62.412	131.117	0.085	0.165
MF3: P + E	4	-61.913	132.319	1.287	0.090
MF4: S + T x E	6	-59.633	132.330	1.299	0.090
MF5: P + T x E	6	-59.649	132.361	1.330	0.089
MF6: E + T	4	-61.940	132.373	1.342	0.088
MF7: Null	2	-64.128	132.401	1.369	0.087
MF8: S + E	4	-62.023	132.539	1.508	0.081
MF9: S x E	5	-60.989	132.729	1.697	0.074
MF10: S x E + T x E	7	-58.791	133.000	1.990	0.064
Full model: P x S x T x E	17	-55.805	154.611	23.580	
b) All populations					
Random structure	df	Model comparison	logLik	L. Ratio test	p-value
MR1: P x E	9		-29.237		
MR2: P x E + (1 Tank)	10	1 vs. 2	-29.226	0.022	0.439
MR3: P x E + (Population Tank)	19	2 vs. 3	-28.451	1.549	0.604
Fixed structure	df	logLik	AICc	Δ_i	ω_i
MF1: E	3	-18.936	44.503	0	1
Full model: P x E	9	-16.832	57.289	12.786	

796

797

798 **Table 3.** Selection of the random and fixed structures of GLMMs on survival of juveniles: a) 10 days (22
799 September) and b) 17 days (29 September) after the start of the mesocosm experiment. For the random
800 structure table shows likelihood ratio tests comparing different nested models. For the fixed structure, the
801 LogLikelihood (logLik), AICc and Δ_i are shown for both, the subset of candidate models and the full model,
802 and weights (ω_i) only for the subset of candidate models. Null= model with no fixed structure. Selected
803 models are marked in bold. Abbreviations as in Table 1. In the fixed component, the multiplications signs (x)
804 indicate the inclusion of all the interactions of lower order and the implicated main factors.

805

a) 10 days					
Random structure	df	Model comparison	logLik	L. Ratio test	p-value
MR1: P x S x T x E	32		-165.16		
MR2: P x S x T x E + (1 Tank)	33	1 vs. 2	-146.43	37.459	< 0.001
Fixed structure	df	logLik	AICc	Δ_i	ω_i
MF1: T x E	6	-51.986	133.165	0	0.301
MF2: T + E	5	-49.513	133.311	0.146	0.280
MF3: Null	2	-53.834	134.381	1.216	0.164
MF4: S + T	5	-51.558	134.833	1.668	0.131
MF5: S + T x E	7	-49.018	134.936	1.771	0.124
Full model: P x S x T x E	33	-38.693	208.434	75.269	
b) 17 days					
Random structure	df	Model comparison	logLik	L. Ratio test	p-value
MR1: P x S x T x E	32		-199.10		
MR2: P x S x T x E + (1 Tank)	33	1 vs 2	-189.97	18.269	<0.001
Fixed structure	df	logLik	AICc	Δ_i	ω_i
MF1: P x E	7	-62.327	139.587	0	0.213
MF2: P + T + E	8	-61.504	140.217	0.630	0.156
MF3: E	4	-66.021	140.368	0.780	0.144
MF4: T + E	5	-65.233	140.958	1.371	0.107
MF5: P + T x E	9	-60.730	140.985	1.397	0.106
MF6: P + S x E + T x E	13	-55.967	141.128	1.540	0.099
MF7: P + S + E	8	-61.996	141.203	1.615	0.095
MF8: T x E	6	-64.434	141.562	1.975	0.079
Full model: P x S x T x E	33	-49.628	192.848	53.261	

806 **Table 4.** Selection of the random and the fixed structures of LMMs on growth of juveniles: a) 10 days (22
807 September) and b) 17 days (29 September) after the start of the mesocosm experiment. For the random
808 structure table shows likelihood ratios test comparing different nested models. For the fixed structure, the
809 LogLikelihood (logLik), AICc and Δ_i are shown for both, the subset of candidate models and the full model,
810 and weights (ω_i) only for the candidate models. Selected models are marked in bold. Abbreviations as in
811 Table 1. In the fixed component, the multiplications signs (x) indicate the inclusion of all the interactions of
812 lower order and the implicated main factors.

a) 10 days					
Random structure	df	Model comparison	logLik	L. Ratio test	p-value
MR1: P x S x T x E	33		450.06		
MR2: P x S x T x E + (1 Tank)	34	1 vs. 2	457.99	15.876	<0.001
MR3: P x S x T x E + (Population Tank)	43	2 vs. 3	466.36	16.736	0.003
Fixed structure	df	logLik	AICc	Δ_i	ω_i
MF1: P + E	19	613.933	-1187.123	0	0.367
MF2: S + P x E	20	615.001	-1186.959	0.163	0.339
MF3: T + P x E	20	614.233	-1185.422	1.701	0.157
MF4: S + T + P x E	21	615.255	-1185.150	1.973	0.137
Full model: P x S x T x E	43	623.584	-1146.211	40.911	
b) 17 days					
Random structure	df	Model comparison	logLik	L. Ratio test	p-value
MR1: P x S x T	17		147.30		
MR2: P x S x T + (1 Tank)	18	1 vs. 2	146.18	2.244	0.226
MR3: P x S x T + (Population Tank)	27	2 vs. 3	143.18	5.986	0.524
Fixed structure	df	logLik	AICc	Δ_i	ω_i
MF1: P + T	9	210.042	-400.547	0	0.392
MF2: P	8	208.879	-400.538	0.008	0.390
MF3: P x T	12	213.059	-399.382	1.165	0.218
Full model: P x S x T	20	215.000	-382.074	18.473	

813

814 **Table 5.** Selection of the best linear model on survival and growth of recruits from rias populations in the
815 post-stress experiment for: a) survival of recruits, b) initial size of recruits and c) final size of recruits. Table
816 shows the LogLikelihood (logLik), AICc, Δi and AICc weights (ω_i). Selected models are marked in bold.
817 Abbreviations as in Table 1.

a) Survival of recruits					
	df	logLik	AICc	Δi	ω_i
MF1: P + T	4	-95.156	199.852	0	0.715
MF2: P x T	5	-94.648	201.695	1.844	0.285
Full model: P x S x T	9	-93.793	214.157	14.305	
b) Initial size of recruits					
	df	logLik	AICc	Δi	ω_i
MF1: T	3	-110.524	227.936	0	0.318
MF2: Null	2	-111.908	228.245	0.309	0.273
MF3: P + T	4	-109.474	228.486	0.550	0.242
MF4: P	3	-111.164	229.218	1.281	0.167
Full model: P x T x S	9	-108.283	243.137	15.201	
c) Final size of recruits					
	df	logLik	AICc	Δi	ω_i
MF1: Null	2	-111.908	228.245	0	0.520
MF2: T	3	-111.338	229.566	1.320	0.269
MF3: P	3	-111.582	230.053	1.808	0.211
Full model: P x S x T	9	-108.430	243.432	15.186	

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820

1 **Figures**

2 **Figure 1.** Study locations on the NW Iberian Peninsula. Pz, Peizás; SP, San Pedro; OF, O Freixo (Ría
3 de Muros); IA, Isla de Arousa (Ría de Arousa). Black arrows show the current position of the two range
4 limits of the species on NW Iberian Peninsula.

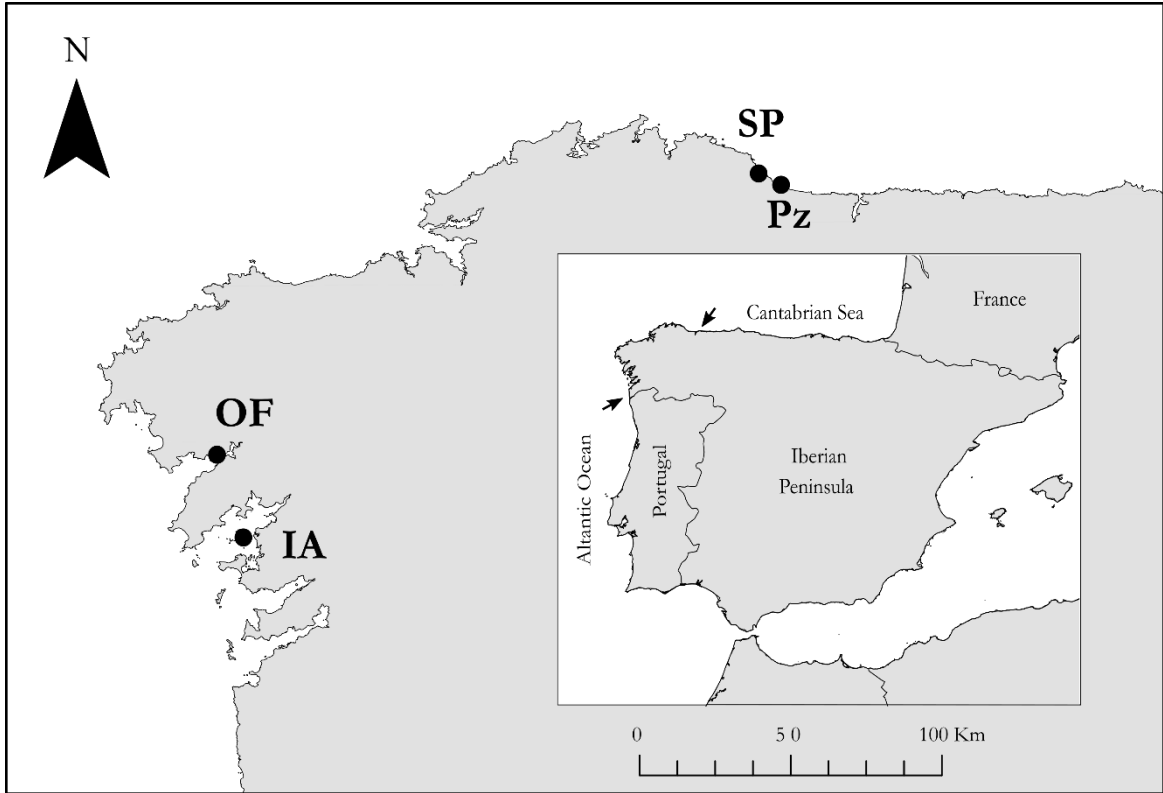
5 **Figure 2.** Air temperatures in the two emersion treatments (high and low) in the first mesocosm
6 experiment, a) 4-hour cycle; b) 6-hour cycle. Note the different scales on the axes. Average values \pm SE
7 are shown, n= 6.

8 **Figure 3.** Survival of germlings and final size (average values per disc) in the first mesocosm
9 experiment. a) Survival of germlings for each combination of population and emersion (n = 6); b) final
10 average size of germlings from the ria populations, for each combination of seawater and emersion
11 temperature (n = 20-23). Mean values \pm SE are shown. Different lower-case letters above bars indicate
12 significant differences between means based on *a posteriori* Tuckey test. Population abbreviations as in
13 Fig. 1.

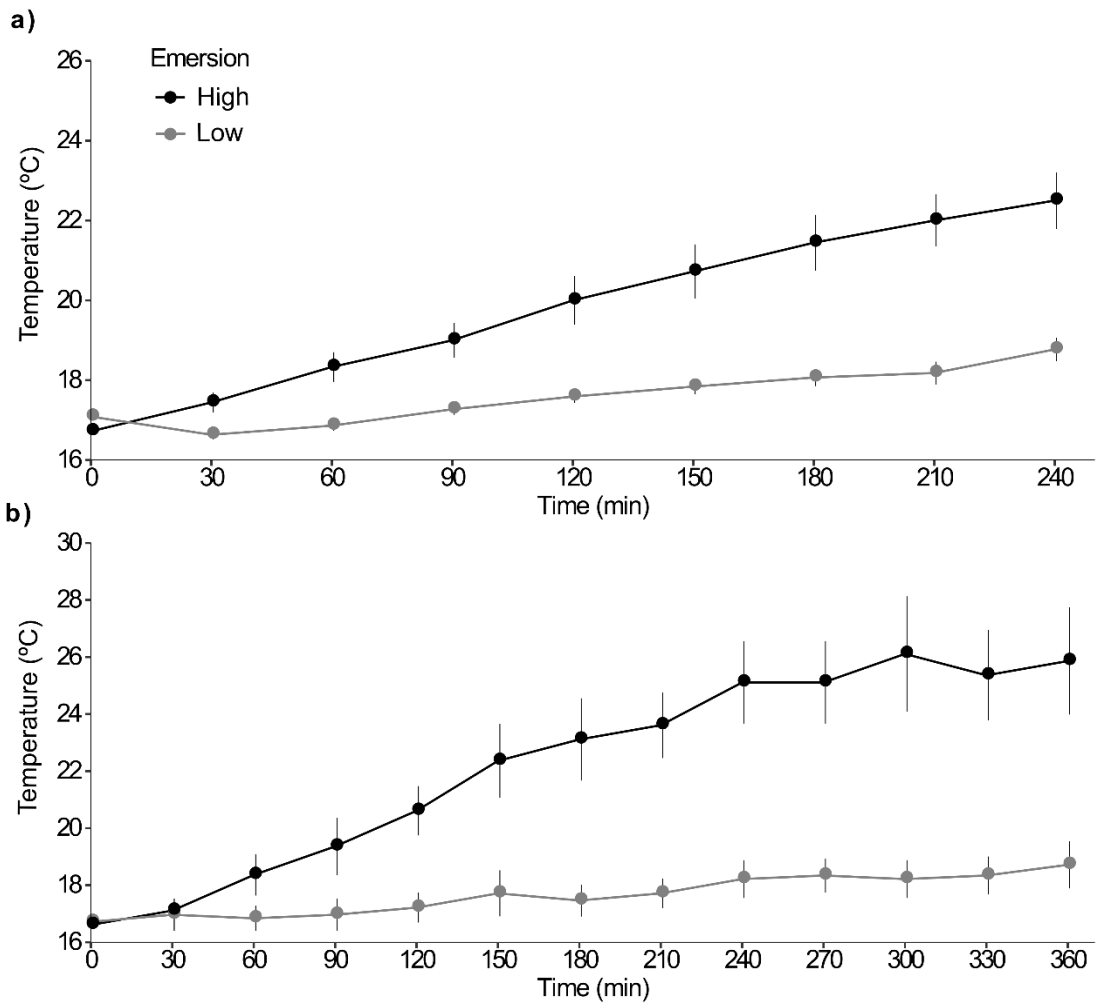
14 **Figure 4.** Survival of juveniles in the first mesocosm experiment. a) Survival over time for each
15 combination of population and emersion treatment (n = 16); b) survival of juveniles 10 days after the
16 start of the experiment for each combination of seawater and emersion temperature (n = 16); c) survival
17 of juveniles 17 days after the start of the experiment for each combination of population and emersion
18 treatment (n = 4). In a) mean values and the smallest and largest SE bars are shown; in b) and c) mean
19 values \pm SE are shown. Different lower-case letters above bars indicate significant differences between
20 means based on Tukey post hoc test. Population abbreviations as in Fig. 1

21 **Figure 5.** Growth of juveniles in the first mesocosm experiment. a) Growth (difference in final and
22 initial fresh weights) 10 days after the start of the experiment for each population (n = 67-79); b) growth
23 10 days after the start of the experiment for high and low emersion treatments (n= 140 - 157); c) growth

24 17 days after the start of the experiment for each population (n = 37-23); d) growth 17 days after the
25 start of the experiment for cold and warm seawater temperatures (n = 63-64). Mean values \pm SE are
26 shown. Different lower-case letters above bars indicate significant differences between means based on
27 a Tukey post hoc test. Population abbreviations as in Fig. 1.



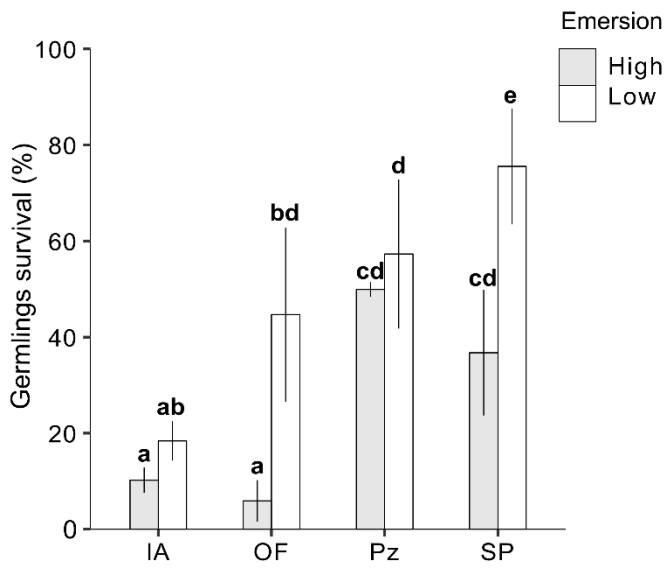
28 Figure 1



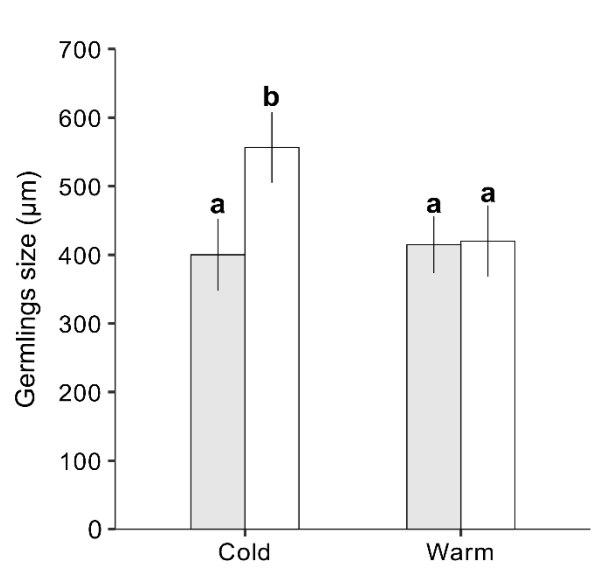
29

30 Figure 2

a) All populations, Population x Emersion

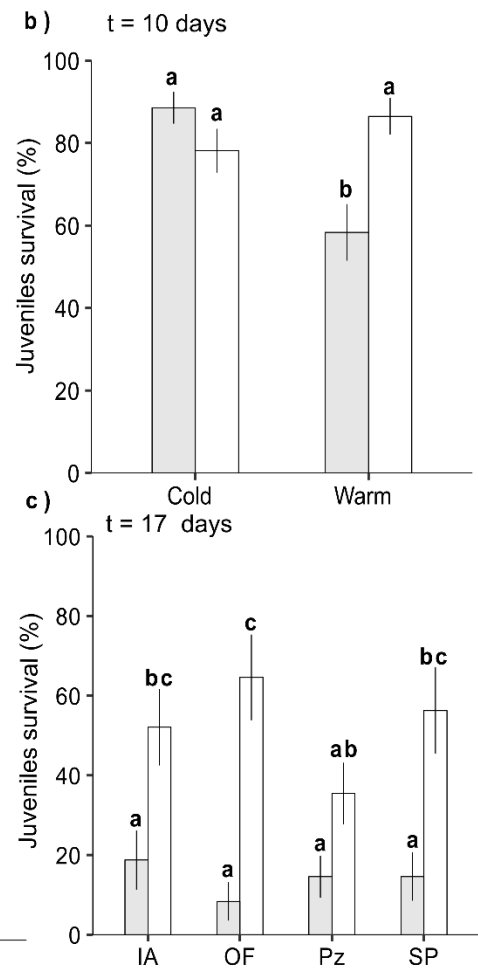
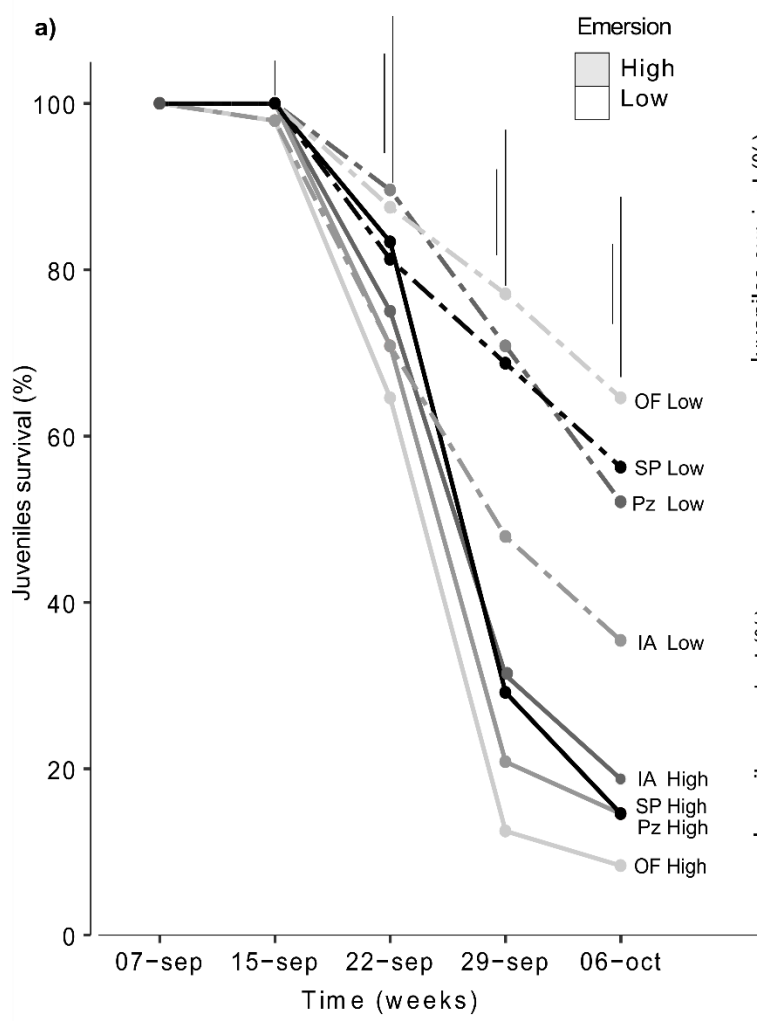


b) Ria populations, Temperature x Emersion



31

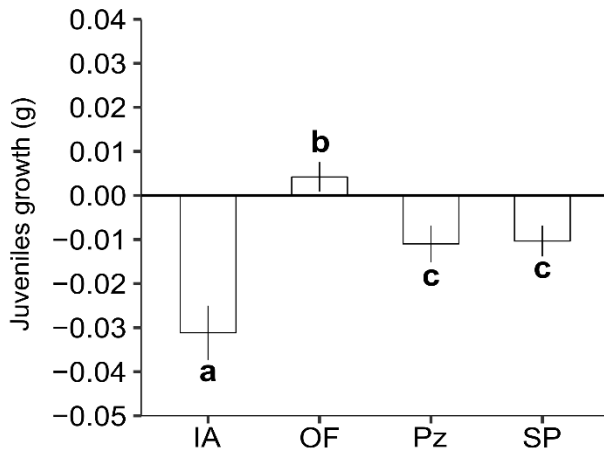
32 Figure 3



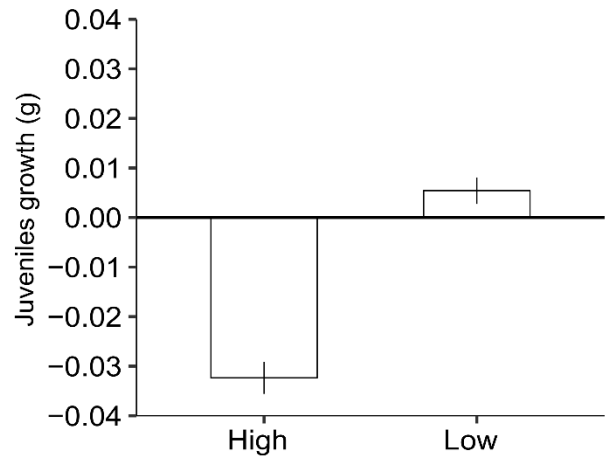
33

34 Figure 4

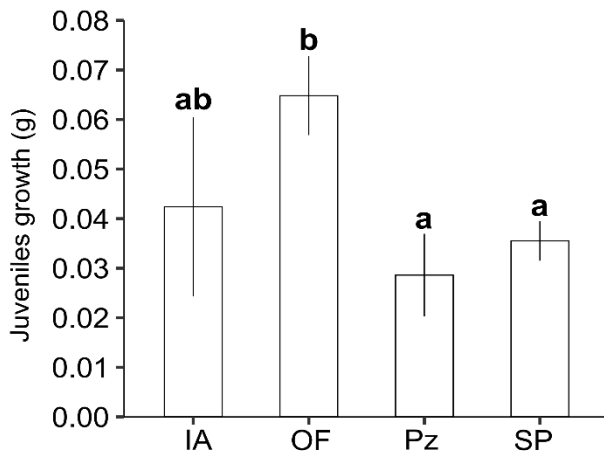
a) t = 10 days, Population



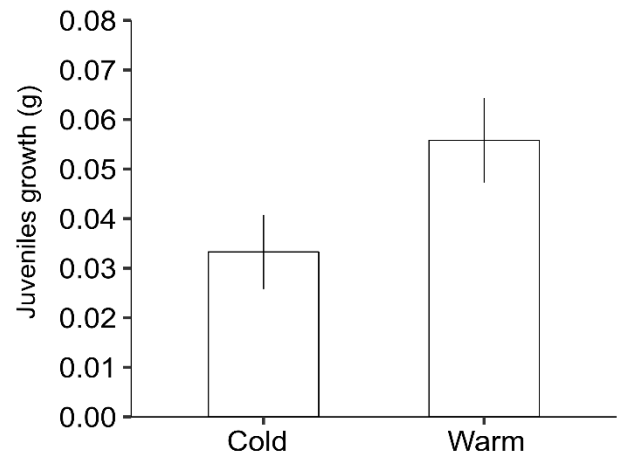
b) t = 10 days, Emersion



c) t = 17 days, Population



d) t = 17 days, Seawater temperature



35

36 Figure 5