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**1 The role of density and relatedness in wild juvenile Atlantic salmon growth**

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16

17 **ABSTRACT**

18 Growth is a key life history trait in fishes that is influenced by both abiotic factors (such

19 temperature and water chemistry) and biotic factors (such as density and food availability).

20 Investigating how growth performance is influenced by such factors in the wild is important

21 for understanding how population processes influence animals in natural environments and

22 for predicting the response to conservation and management strategies that manipulate these

23 conditions. The theory of kin selection predicts that significant growth and survival benefits

24 are conferred upon animals associating with close relatives. However, resource competition

25 may be more intense among close relatives, and little is known about the trade-off between

26 these two processes under different ecological conditions. Here we examine the correlation

27 between naturally occurring densities and kin-biased growth rate using a species where kin-  
28 recognition has a strong impact on behaviour in laboratory studies, but where, paradoxically,  
29 field investigations have failed to document predicted kin-biased growth or survival. Intra-  
30 and inter-family differences in growth rate of juvenile Atlantic salmon (*Salmo salar*) were  
31 studied to examine how relatedness (groups of full-sibling fish and groups of mixed-sibling  
32 fish) and sibling group (family/genotype) affects salmon parr growth, and the correlation of  
33 growth rate under a range of naturally-occurring densities. Parentage and relatedness of  
34 neighbouring fish were assigned using microsatellite and passive integrated transponder (PIT)  
35 tags which allowed the growth estimation of individual fish. Results show that growth rate  
36 was significantly influenced by both sibling group (family of origin) and also by an  
37 interaction between relatedness and density. The latter finding indicates that at higher  
38 densities full-sibling groups achieved higher growth rates in comparison to mixed-sibling  
39 groups. Thus, the growth benefits of associating with relatives are not conferred under all  
40 ecological conditions, but it becomes most apparent at high density when resource  
41 competition is greatest.

42

43 Key words: Atlantic salmon, family traits, relatedness, heterogeneous advantage, growth rate,  
44 density, kin selection, kin-biased behaviour

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49 INTRODUCTION

50 Growth is a key life history trait and faster growth can provide animals with a competitive  
51 advantage to access available resources (Arendt & Wilson 1997), and plays an important role

52 in survival and reproductive success in the wild (Einum, Thorstad & Næsje 2002). Growth  
53 rate has been shown to be dependent on ecological factors such as density (Grant & Imre,  
54 2005), food abundance (Imre, Grant & Keeley 2004), genetics (García de Leániz *et al.*, 2007),  
55 and relatedness of neighbouring animals (Hamilton, 1964). For example, tadpoles (*Rana*  
56 *cascadae*) reared together with siblings grow faster than when reared with non-siblings (see  
57 Hokit & Blaustein, 1994; Gramapurohit, Shanbhag & Saidapur, 2008). Moreover, this effect  
58 is mediated by resource levels; as food availability decreases, the cost of helping a relative  
59 (e.g. by sharing resources) rises and animals including amphibians (Pakkasmaa & Laurila,  
60 2004), birds (Royle *et al.*, 1999) and mammals (Nichols *et al.*, 2012) may be less willing to  
61 pay the cost of helping. Among fish, however, the concurrent effects of kin selection and  
62 resource competition are largely unknown, and evidence to date is contradictory (e.g. Brown  
63 & Brown, 1993a; Griffiths & Armstrong, 2001; Griffiths, Armstrong & Metcalfe, 2003).

64 Full-sibling groups of salmonid fish are less aggressive towards one another than non-  
65 siblings (see Brown & Brown, 1993b; Olsén & Järvi, 1997) and invest more time and energy  
66 in foraging (Brown & Brown, 1996) consequently achieving higher growth rates and densities  
67 than fish in non-sibling groups (Brown & Brown, 1993a; Olsén, Järvi & Löf, 1996). Genetic  
68 studies have failed to find evidence of sibling aggregation in the wild (see Brodeur *et al.*,  
69 2008; Fontaine & Dodson, 1999; Garant, Dodson & Bernatchez, 2000; Olsén *et al.*, 2004),  
70 despite the advantages of associating with relatives implicit from laboratory studies. Indeed,  
71 in field studies, growth rate in Atlantic salmon (*Salmo salar*) (Griffiths & Armstrong, 2001)  
72 and brown trout (*Salmo trutta*) (Greenberg *et al.*, 2002) have been higher among fish in  
73 mixed-sibling groups. One potential explanation for this may be that unrelated individuals are  
74 able to exploit a wider range of ecological niches than closely related individuals that share  
75 many genes in common and exhibit similar ecological needs (Blaustein *et al.*, 1991;  
76 Fernandes *et al.* In Press). Furthermore, kin selection advantages may be maximised, not by  
77 kin association, but rather by kin avoidance under different resource conditions. For example,

78 when food resources are unlimited, juvenile salmon increase territory- and food-sharing  
79 among closely related, but not unrelated fish (Griffiths & Armstrong, 2002). However, they  
80 avoid sharing streambed shelters during winter when resources are likely to be scarce,  
81 presumably to reduce competition among relatives (Griffiths *et al.*, 2003).

82         A further possible explanation for these contradictory outcomes may come from  
83 considering the discrepancy between laboratory studies of behaviour and genetic studies  
84 conducted in field experiments. Brodeur *et al.* (2008) point out that under laboratory  
85 conditions of low water volume and flow, highly concentrated odour cues may allow kin  
86 recognition to be achieved easily and may be misinterpreted as indicating high levels of  
87 conspecific density and competition. Perhaps also, the discrepancy between observations of  
88 kin-biased behaviour in the lab and field studies can be explained by differences in density  
89 /perceived differences in resource availability. The density of salmonid fish tested in  
90 laboratory studies of kin discrimination ranges from 1.85-50m<sup>-2</sup> (Brodeur *et al.*, 2008), while  
91 much lower densities have been documented for field studies; ranging from 0.27 m<sup>-2</sup> (Brodeur  
92 *et al.*, 2008) to <1m<sup>-2</sup> (Fontaine & Dodson, 1999; Carlsson & Carlsson, 2002). Interestingly,  
93 the only study to record kin-biased distribution in the wild was conducted at relatively high  
94 density (2.6 m<sup>-2</sup>) (Carlsson *et al.*, 2004). Kin association has been documented in shoaling fish  
95 (e.g. Evans & Kelley, 2008), however in territorial fish kin selective benefits can be accrued  
96 by reducing aggression towards related fish (Brown & Brown, 1993a) and sharing resources  
97 (Griffiths & Armstrong, 2002). It remains far from clear, however, how fish trade-off the costs  
98 and benefits of kin selection and resource competition under a range of ecologically-relevant  
99 naturally-occurring densities.

100         First, the present study will investigate the relationship between relatedness and  
101 density, and thus, the trade-off between the theories of kin-selection and resource  
102 competition. Second, since previous studies have shown that growth rate has a strong genetic  
103 basis, this study investigates the effect of sibling group (genotype) on the individual growth

104 rate in the wild. This study used an Atlantic salmon population of known parentage in a  
105 natural river habitat, which offered opportunities for genetic and environmentally mediated  
106 responses to be expressed.

107

## 108 METHODS

### 109 *Experimental Animals*

110 Full-sibling groups were created by fertilising the eggs of one female with the milt of one  
111 male (refer to supplementary materials for adult brood stock details). Twelve distinct sibling  
112 groups were made in this way (n = 6 in 2006, n = 6 in 2007). Each batch of fertilised eggs  
113 (sibling groups) was placed into a separate incubator (as per Government of Canada, 1980) (at  
114 the Watergates hatchery, Dorchester, Dorset). Each incubator was supplied from a common  
115 source of ground water through an independent siphon to ensure that sibling groups were  
116 chemically isolated from one another.

117         Within 24 hours of the fish emerging as fry from the incubator, groups of full-sibling  
118 or mixed-sibling fish were released into designated sites over a 1.5 km stretch of the River  
119 Cerne (a tributary of the River Frome, Dorset, UK, Fig 1a & b). As habitat has previously  
120 been shown to influence salmon parr growth rate (e.g. Riley et al. 2009), this particular stretch  
121 of river was chosen for its relatively homogeneous appearance and consistent stream width.  
122 Furthermore, it was not subject to management measures, allowing bankside and instream  
123 vegetation to grow freely, therefore providing an undisturbed habitat for juvenile salmon. Fish  
124 from the different sibling groups were stocked into a number of different sites on this stretch  
125 of river in both years thus allowing fish from the different sibling groups to grow in all the  
126 different available habitats. No notable changes in habitat were observed during both years of  
127 the experiments. Furthermore, owing to an impassable weir located downstream of the  
128 experimental sites, naturally occurring wild salmon were not present, therefore all juveniles  
129 caught after stocking belonged to the sample of this present study making the identification

130 and testing of the effects of relatedness easier. The weir acted as a barrier to reduce the  
131 likelihood of stocked fish moving outside the experimental stretch of river. Other fish species  
132 present in the experimental stretch of the River Cerne included trout, *Salmo trutta*, grayling  
133 *Thymallus thymallus*, pike, *Esox lucius*, minnows, *Phoxinus phoxinus*, bullhead, *Cottus gobio*,  
134 stone loach, *Barbatula barbatula*, eel, *Anguilla anguilla* and brook, *Lampetra planeri*, and  
135 river, *Lampetra fluviatilis*, lamprey (refer to Supplementary Materials for further details of  
136 Experimental Animals and Experimental Procedure).

137

138 **[FIGURE 1a & b]**

139

140 *Molecular Methods*

141 Molecular analysis of adipose tissue was carried out at Cardiff University to assign juveniles  
142 ( $n = 243$ ) to their parents and therefore determine family of origin. Genomic DNA was  
143 extracted from parental and juvenile adipose fin tissue using the Qiagen tissue DNA  
144 extraction kit (Qiagen catalogue no. 69506). DNA yield was quantified on a 1 % agarose gel  
145 and visualised on a UV transilluminator.

146         Nine loci were chosen on the basis of their reliability in the use of parentage  
147 assignment based on their use in previous salmon genetic studies and their allelic size range  
148 (see Table 1). (Refer to Supplementary Materials for further details of Molecular Methods).

149

150 **[TABLE 1]**

151

152 *Data analysis*

153 The baseline weight measurements taken from 25 emerging fry in both years were used to  
154 calculate the growth rate between stocking fish and the first sampling session. To ascertain  
155 the rate at which the fish were growing, Specific Growth Rate (SGR) (g), a measure of

156 percentage increase per day of body weight (g) per individual fish, was calculated. SGR (g) of  
157 full-sibling fish were compared to the SGR (g) of mixed-sibling fish within and between time  
158 periods. The SGR (g) of fish originating from different sibling groups (of the same parentage)  
159 were also compared.

160 For each fish ( $n = 243$ ), the increase in weight between time periods ( $t_1$  and  $t_2$ , and  $t_1$   
161 and  $t_3$ ) and was used to calculate SGR (g) using the equation (Wootton, 1990):

162

163 Specific Growth Rate (SGR) (g) =  $100 \times (\log W_2 - \log W_1) / (t_2 - t_1)$ .

164

165 Statistical analyses were based on data collected from all sampling sessions, whereas  
166 analyses between years was based on growth rate between fry stage to first sampling stage as  
167 this was the only time period when data was collected in both years at around the same time  
168 enabling comparisons between years to be made. The density (population estimate) of  
169 juvenile salmon at each site and in each sampling session was calculated using the software  
170 REMOVE (Clarke, 1996). The program uses maximum likelihood estimates of the population  
171 size in a given area ( $m^2$ ) extrapolated from the number of fish caught during each fishing  
172 attempt within that area.

173 To test the effect of sibling group on specific growth rate, a Generalised Linear Mixed  
174 Model (GLMM) was carried out in ASReml v.2.0. The dependent term in the model was  
175 specific growth rate. The main terms (F = Factor, C = Covariate) and interactions between  
176 terms in the starting model were: sibling group (F), time period (F), density (C), sibling group  
177 x time period, sibling group x density, density x time period. The identity of individual fish  
178 and the sample site were set as random effects to account for data collected repeatedly from  
179 the same individual and same area. Sampling site had no effect on specific growth rate during  
180 analysis and was therefore removed from the model. The modelling method used started from



181 the full model and achieved the minimal adequate GLMM model by sequential removal of  
182 non-significant terms.

183         While electrofishing, four fish that were stocked in 2006 were captured in 2007  
184 however these fish were not included in the 2007 analysis owing to a larger size. Genotyping  
185 results revealed that fish had dispersed from their original stocking sites into unstocked areas  
186 of the river as well as other stocked areas further down- and up-stream, therefore sites  
187 originally stocked with full-sibling fish consisted also of fish from other genotypes. In total,  
188 14% of tagged and recaptured fish within full-sibling sites were fish not originally stocked in  
189 the full-sibling sites. Despite this, all fish that had moved from their original stocking sites  
190 were returned to their site of capture for sibling group analyses. Furthermore, it is unclear  
191 exactly when altruistic benefits began to accrue between related fish, despite the genetic  
192 integrity of the sites, therefore, the sibling group analysis involved all fish caught within sites  
193 regardless of their original stocking location ( $n = 243$  fish in data set). However, fish that had  
194 moved from original stocking sites into full-sibling sites were removed from the data set prior  
195 to the relatedness analysis and coefficient of variation analysis ( $n = 208$  fish in data set. Time  
196 period 1  $n = 208$ , Time period 2  $n = 17$ , Time period 3  $n = 35$ ). An independent samples t-test  
197 (assuming unequal variances) showed no difference between the growth rate of fish between  
198 years (2006  $n = 160$ , 2007  $n = 83$ ,  $t_{1,0.409} = 0.683$ ,  $P = 0.097$ ) therefore data from both years  
199 were pooled together to form one large data set.

200         To test the effect of relatedness on growth, a Generalised Linear Mixed Model  
201 (GLMM) was carried out in ASReml v.2.0. The dependant term in the model was growth rate.  
202 The main terms (F = Factor, C = Covariate) and interactions between terms in the starting  
203 model were: relatedness (refers to whether fish were stocked in a full-sibling group or a  
204 mixed-sibling group) (F), year (refers to year of study: 2006 or 2007) (F), time period (F),  
205 density (population estimate) fish  $m^{-2}$  (C), relatedness x year, relatedness x time period,  
206 relatedness x density, density x year, density x time period. The identity of individual fish and

207 the sample site was set as random effects to account for data collected repeatedly from the  
208 same individual and same area. Residuals from all final models showed a normal distribution.  
209 Sampling site had no effect on specific growth rate during analysis and was therefore  
210 removed from the model as described above. Identity of individual fish was not statistically  
211 significant in either final model ( $P = > 0.05$ ), however this term was left in both models to  
212 allow the test to use up one degree of freedom throughout the process of making the final  
213 model, thus making the test more conservative and robust. Coefficient of variance (CV) (%)  
214 of length and weight of full-sibling and mixed-sibling fish within time periods was carried out  
215 in SPSS v.14.0. It was necessary to include fish sampled more than once in order to observe  
216 variation in all sampling sessions. The CV gives a measure of the variability in the sizes of the  
217 fish in a group and was calculated using the following method:

218

$$219 \text{ CV (\%)} = (100 \times SD) /$$

220

221 where  $SD$  = standard deviation of length or weight, and  $x$  = mean of fork length or weight).

222 CV has no units (expressed as a percentage) and is therefore a useful tool for comparing the

223 variability of samples that have widely differing means, this giving a measure of inequality

224 among individuals.

225

## 226 RESULTS

227 Growth rate varied significantly between families (GLMM  $F_{5,292} = 5.27$ ,  $P = 0.001$ ) (see

228 Figure 2a). Growth rate also differed significantly between time periods (GLMM  $F_{2,292} =$

229  $3079.36$ ,  $P = 0.001$ ) (see Figure 2b). Interestingly, the interaction between sibling group and

230 density had a significant effect on SGR ( $F_{5,292} = 4.60$ ,  $P = 0.001$ ) (Figure 3), with sibling

231 group 3 showing a positive relationship between density and growth rate, while sibling groups

232 5 and 6 show a negative relationship between density and growth rate. Residuals from the

233 final model showed a normal distribution. Identity of individual fish (random term) was not  
234 statistically significant in the final model ( $P > 0.05$ ). Despite the slight differences in  
235 methodologies between years, and small sample sizes, there was no effect of year or sampling  
236 site on specific growth rate in either model.

237

238 **[FIGURE 2a & b]**

239

240 **[FIGURE 3]**

241

242 Growth rate of juvenile salmon was not significantly affected by relatedness of  
243 neighbouring fish (GLMM  $F_{1,254} = 0.98$ ,  $P = 0.324$ ) but varied significantly between time  
244 periods (GLMM  $F_{2,254} = 2314.73$ ,  $P = 0.001$ ) (time period 1,  $n = 243$ , time period 2,  $n = 25$ ,  
245 time period 3,  $n = 38$ ; Fig. 4a). Interestingly, the significant interaction between relatedness  
246 and density (GLMM  $F_{1,254} = 8.56$ ,  $P = 0.010$ ; Fig. 4b) suggests a positive relationship  
247 between density and growth rate for fish reared among full-siblings, but a negative  
248 relationship for groups of mixed-siblings.

249

250

251 **[FIGURE 4a & b]**

252

253 There was no significant difference in mean fork length and mean wet weight between  
254 full-sibling and mixed-sibling fish within each time period (Fisher LSD  $P > 0.05$ ). However,  
255 the length ( $CV_l$ ) (Fig 5a) and weight ( $CV_w$ ) (Fig 5b) was higher in mixed-sibling fish in time  
256 period 1 (1.24 % higher  $CV_l$  and 4.73 % higher  $CV_w$ ) and higher in time period 2 (0.99 %  
257 higher  $CV_l$  and 7.25 % higher  $CV_w$ ), Fig 5a. A smaller difference was found in time period 3  
258 with mixed-sibling fish obtaining 0.14 % higher  $CV_l$  and kin fish obtaining 1.31 % higher

259  $CV_w$  than mixed-sibling fish. It seems that  $CV_l$  and  $CV_w$  of full-sibling and mixed-sibling fish  
260 was higher in mixed-sibling fish during warmer periods of the study, but much later in the  
261 study (Winter) full-sibling fish obtained higher  $CV_l$ .

262

263 **[FIGURE 5a & b]**

264

## 265 DISCUSSION

266 The results from this field study show that the effect of relatedness on growth rate is  
267 influenced by density, time period, and sibling group (family of origin). Intriguingly we found  
268 a significant interaction between relatedness and density indicating a strong relationship  
269 between density and its influence on the role of relatedness in juvenile Atlantic salmon  
270 growth. Growth rate is higher in full-sibling groups at high density, but lower growth rates are  
271 achieved at low density. Density had an opposite effect on the growth rate of mixed-sibling  
272 fish. We also show that size variation of length and weight was higher in mixed-sibling fish  
273 during Summer and Autumn, but during Winter, higher variation in length was achieved by  
274 full-sibling fish.

275 Growth rate is influenced by density (Grant & Imre, 2005), genetics (García de Leániz  
276 *et al.*, 2007) and relatedness (Hamilton, 1964). Higher growth rate is one outcome of kin  
277 selection behaviour and this is driven by cooperation (Brown & Brown, 1993a, 1993b; Olsén  
278 & Järvi, 1997) and by sharing resources (Griffiths & Armstrong, 2002) among relatives. An  
279 alternative outcome of kin biased behaviour is that groups of related fish attain higher  
280 densities and have smaller, tightly packed territories (Griffiths & Armstrong, 2002). It is  
281 known that both growth rate and aggressive behaviour cannot be maximised simultaneously  
282 (Vøllestad & Quinn, 2003) and these high metabolic demands may have resulted in decreased  
283 density within mixed-sibling groups seen in our study, since larger foraging territories are  
284 needed to gain sufficient food to offset increased energy expenditure. It seems that by

285 associating among close relatives, therefore, individuals may gain kin selection benefits  
286 (Griffiths & Armstrong, 2002), however, it remains unclear how fish trade-off the costs and  
287 benefits of kin selection and resource competition under a range of ecologically-relevant  
288 naturally-occurring densities. The interactive effects of kin selection and resource competition  
289 among fish are largely unknown, and evidence to date has been inconsistent (e.g. Brown &  
290 Brown, 1993a; Griffiths & Armstrong, 2001; Griffiths *et al.*, 2003).

291         While previous laboratory studies have found a positive effect of relatedness on  
292 growth rate (e.g. Brown & Brown, 1993a; Olsen *et al.*, 1996), field studies have failed to  
293 demonstrate a similar effect (and in some cases have shown growth rate to be higher in  
294 mixed-sibling groups) (e.g. Griffiths & Armstrong, 2001; Greenberg *et al.*, 2002). In fact,  
295 there is surprisingly little evidence for kin-biased association patterns in the wild among  
296 territorial fishes (e.g. see Brodeur *et al.*, 2008; Fontaine & Dodson, 1999; Garant *et al.*, 2000;  
297 Olsén *et al.*, 2004). A potential explanation for these outcomes is that the confinement of fish  
298 to the small, simple habitats for long periods may allow stronger associations with tankmates  
299 to be formed than would naturally occur (Griffiths & Ward, 2011) and odour cues might be  
300 highly concentrated in such low water volume that kin recognition can be easily achieved  
301 (Courtenay *et al.*, 2001). Another potential explanation is that unrelated individuals (different  
302 genotypes) are able to exploit a wider range of niches in the wild, thereby reducing intra-  
303 family competition, whereas individuals that share many genes in common; i.e. close  
304 relatives, exhibit similar ecological requirements (Blaustein *et al.*, 1991; McLaughlin,  
305 Ferguson & Noakes, 1999) and may actively avoid kin (Griffiths *et al.*, 2003). Our field study  
306 findings are consistent with Brown & Brown (1993a), Griffiths *et al.* (2003) and Toobaie &  
307 Grant, (2013) which appears to suggest that when the quality of habitat is low, for example in  
308 Winter, competition for resources increase and aggression rises in both related and unrelated  
309 groups of fish. It seems, therefore, that growth rate is driven by density and relatedness -  
310 limited food and space availability might reduce the magnitude of kin-biased behaviour (West

311 *et al.*, 2001, West, Penn & Griffin, 2002). We also found size variation (coefficient of  
312 variation) to be higher in full-sibling groups during the Winter when resources are limited  
313 (Griffiths *et al.*, 2003). An increase in CV usually indicates competition and aggressive  
314 behaviour between individuals (Jobling, 1995) and the greater variability in size among  
315 relatives that we found may suggest that subordinate fish submit to dominant siblings to  
316 increase their own chances of survival (Olsén & Järvi, 1997) in the long run, however this is  
317 at the cost of reduced foraging in the short term.

318         Higher levels of stress are experienced by fish that are held in confined areas at high  
319 densities and this may impair growth rate despite unlimited food availability (Laursen, Silva,  
320 Larsen & Höglund, 2013). It is possible that fish in the wild experience stress at lower  
321 densities, but have opportunities to escape or hide (Salonius & Iwama, 1993). In laboratory  
322 studies of kin recognition however, Brodeur *et al.*, (2008) pointed out that densities range  
323 from 1.85 to 50 fish m<sup>-2</sup> and by comparison, densities in wild studies are usually much lower,  
324 ranging from 0.27 fish m<sup>-2</sup> to <1 fish m<sup>-2</sup> (e.g. Fontaine & Dodson 1999; Carlsson & Carlsson,  
325 2002). The density over the two years in the present study only reached between 0.004 and  
326 0.15 fish m<sup>-2</sup>, similar to previous wild kinship studies e.g. 1 – 1.7 fish m<sup>-2</sup> (Griffiths &  
327 Armstrong 2001) and 0.33 fish m<sup>-2</sup> (Greenberg *et al.*, 2002). Notably, the only field studies  
328 that have found evidence of kin-biased association were conducted at high densities  
329 approaching those used in lab studies, e.g. 2.6 fish m<sup>-2</sup> (Carlsson *et al.*, 2004). Our field study  
330 has allowed kin-biased behaviour to be measured under naturally-occurring high and low  
331 densities and we show that some families achieve faster growth rates in higher densities. We  
332 also show that there is a clear effect of density in mediating the effect of kinship. It appears  
333 that in reduced habitat quality, the cost of helping relatives is outweighed by the individual's  
334 need for survival (Griffiths & Armstrong, 2001), therefore individuals may also accrue kin  
335 selection benefits by actively avoiding close relatives when resources are scarce (Griffiths *et*  
336 *al.*, 2003) and this is likely to happen in high density areas as shown by our results. Our

337 findings, therefore, suggest that the benefits of associating with relatives in the wild may only  
338 be accrued under specific ecological conditions and become most apparent at high density  
339 when resource competition is at its greatest.

340

341

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349

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- 483 **Table 1** Atlantic salmon (*Salmo salar*) microsatellite multiplexes used in the present study.
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- 485 **Figure 1 A)** Configuration in 2006 of sites stocked with six full-sibling (dark shaded) sites  
486 and six mixed-sibling (light shaded) groups of juvenile Atlantic salmon (*Salmo salar*) into on  
487 the River Cerne, (Dorset, England). **B)** Configuration in 2007 of three single-sibling (dark  
488 shaded) sites and one large mixed-sibling (light shaded) site (the size of three single-sibling  
489 sites) of Atlantic salmon.

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491 **Figure 2 A)** GLMM SGR (g) ( $\pm$  se) in juvenile Atlantic salmon (*Salmo salar*) in the River  
492 Cerne, Southern England: Family group (2006: sibling group 1  $n = 47$ , sibling group 2  $n = 39$ ,  
493 sibling group 3  $n = 55$  and 2007: sibling group 4  $n = 129$ , sibling group 5  $n = 14$ , sibling  
494 group 6  $n = 22$ ) effect on growth rate on SGR (g). **B)** GLMM SGR (g) ( $\pm$  se) in juvenile  
495 Atlantic salmon (*Salmo salar*) in the River Cerne, Southern England: time period (time period  
496 1  $n = 243$ , time period 2  $n = 25$ , time period 3  $n = 38$ ) effect on SGR (g).

497

498 **Figure 3 A) - F)** Sibling group x density interaction effect on SGR (g) in Atlantic salmon  
499 (*Salmo salar*): sibling groups 1 – 6. Solid line = mean, dotted line = standard error of the  
500 mean.

501

502 **Figure 4 A)** GLMM SGR (g) in juvenile Atlantic salmon (*Salmo salar*) ( $\pm$  se) in the River  
503 Cerne, Southern England: time period effect on SGR (g) (time period 1  $n = 208$ , time period 2  
504  $n = 17$ , time period 3  $n = 35$ ). **B)** GLMM SGR (g) in juvenile Atlantic salmon (*Salmo salar*)  
505 ( $\pm$  se) in the River Cerne, Southern England: relatedness x density effect on SGR (g).

506 **Figure 5** Coefficient of variation A) of length (cm) and B) weight (g) in juvenile Atlantic  
507 salmon (*Salmo salar*) in the River Cerne at time of sampling (time period 1: full-sibling fish  $n$   
508 = 73, mixed-sibling fish  $n = 135$ , time period 2: full-sibling fish  $n = 6$ , mixed-sibling fish  $n =$   
509 11, time period 3: full-sibling fish  $n = 14$ , mixed-sibling fish  $n = 21$ ). Open columns represent  
510 full-sibling groups, shaded columns represent mixed-sibling groups.

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*The role of density and relatedness in salmon growth*

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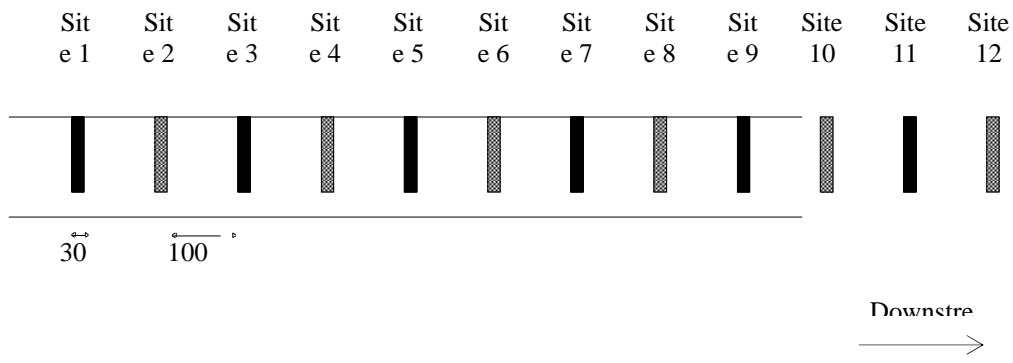
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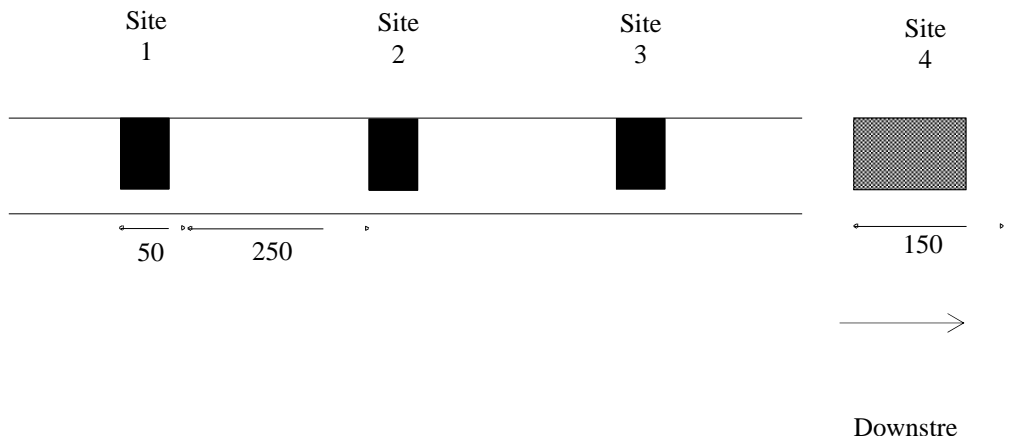
Multi-plex	Locus	Authors/Genbank no.	Primer sequence	Motif	Allele min.-max.
1	$\mu$ F43	Sánchez <i>et al.</i> (1996)	Forward: 5'-AGC GGC ATA ACG TGC TGT GT-3' Reverse: 5'-GAG TCA CTC AAA GTG AGG CC-3' (HEX)	AC/TG	103-143
	Ssa289	McConnell <i>et al.</i> (1995)	Forward: 5'-CTT TAC AAA TAG ACA GAC T-3' Reverse: 5'-TCA TAC AGT CAC TAT CAT C-3' (NED)	GT	113-125
	Ssa12	U58900	Forward: 5'-GGT TAC ACA CCA TTA GAA TGG-3' Reverse: 5'-GCT CCA TAG CTA CGA AGG CTG G-3' (NED)	GT	176-192
	Ssa132	U58901	Forward: 5'-CCG GTC ATG TCG TCA GTA GGC C-3' Reverse: 5'-GCT TGT GCT TCT AGT TCC-3' (FAM)	GT	190-210
	SSLEEN82	U86706	Forward: 5'-CAT GGA GAA TCC CAC TTT CTT A-3' (HEX) Reverse: 5'-CAG GGA GTG ATA TGG GAC ATA A-3'	CT	204-224
2	$\mu$ 20.19	Sánchez <i>et al.</i> (1996)	Forward: 5'-TCA ACC TGG TCT GCT TCG AC-3' Reverse: 5'-CTA GTT TCC CCA GCA CAG CC-3' (FAM)	AC/TG	96-102
	SSa85	O'Reilly <i>et al.</i> (1998)	Forward: 5'-AGG TGG GTC CTC CAA GCT AC-3' Reverse: 5'-ACC CGC TCC TCA CTT AAT C-3' (HEX)	GT	110-138
	SSa197	O'Reilly <i>et al.</i> (1998)	Forward: 5'-GGG TTG AGT AGG GAG GCT TG-3' Reverse: 5'-TGG CAG GGA TTT GAC ATA-3' (NED)	(GT)C(TG)TC(TG)A(GTGA)	131-203
	Ssa202	O'Reilly <i>et al.</i> (1998)	Forward: 5'-CTT GGA ATA TCT AGA ATA TGG C-3' Reverse: 5'-TTC ATG TGT TAA TGT TGC GTG-3' (HEX)	(CA)(CTCA)	268-320

539 Table 1

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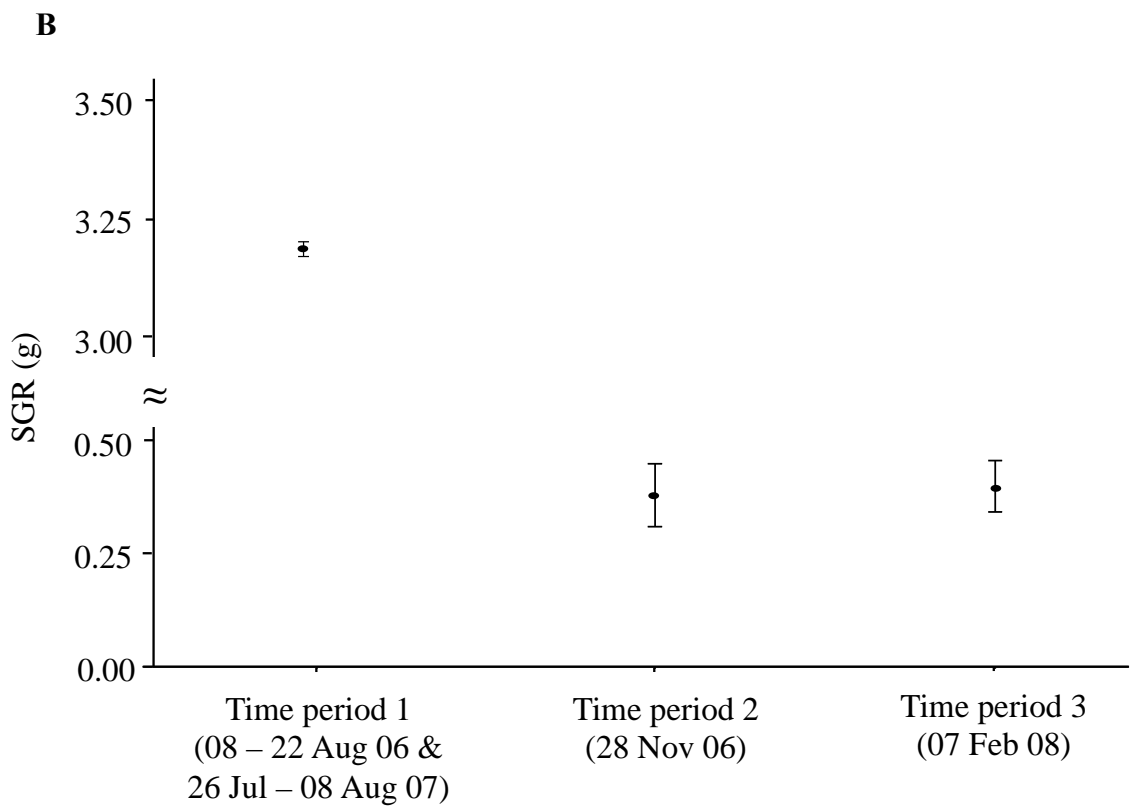
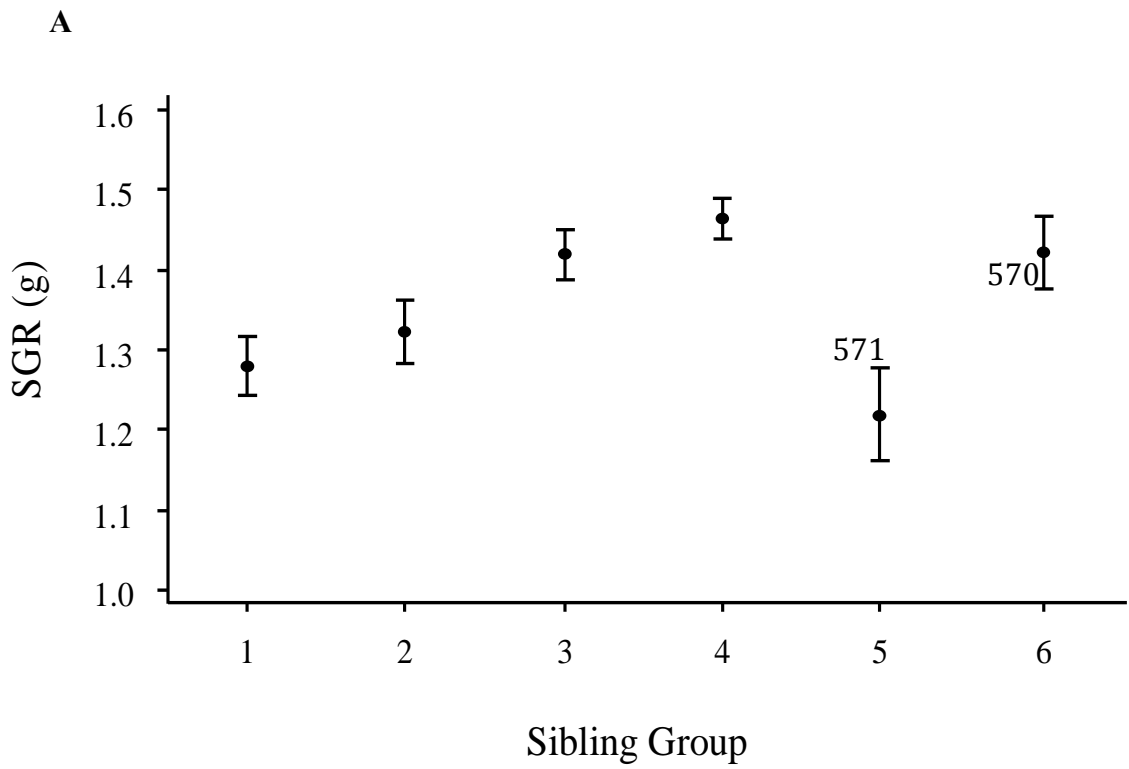


**b**





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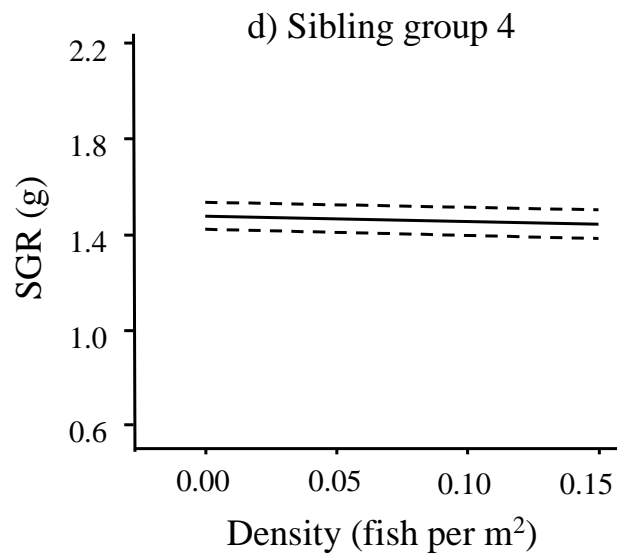
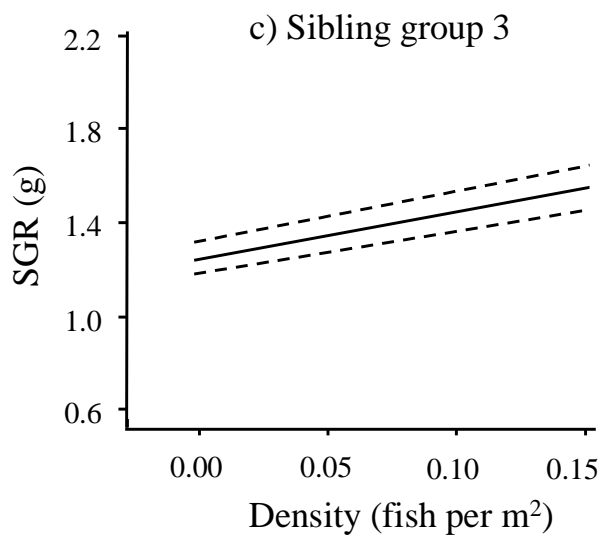
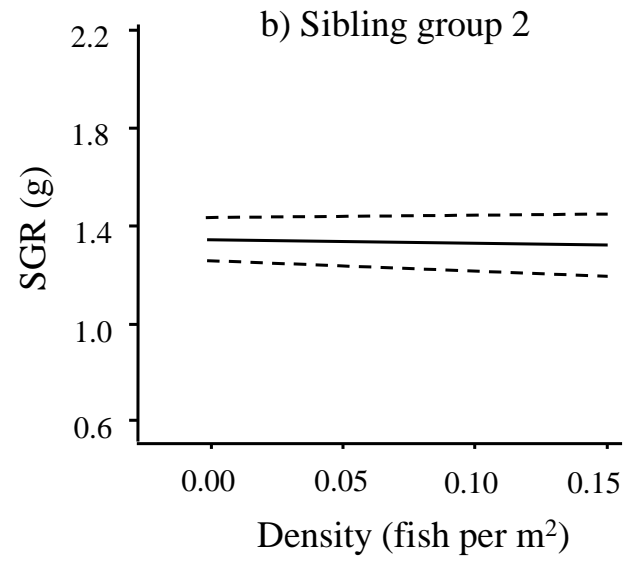
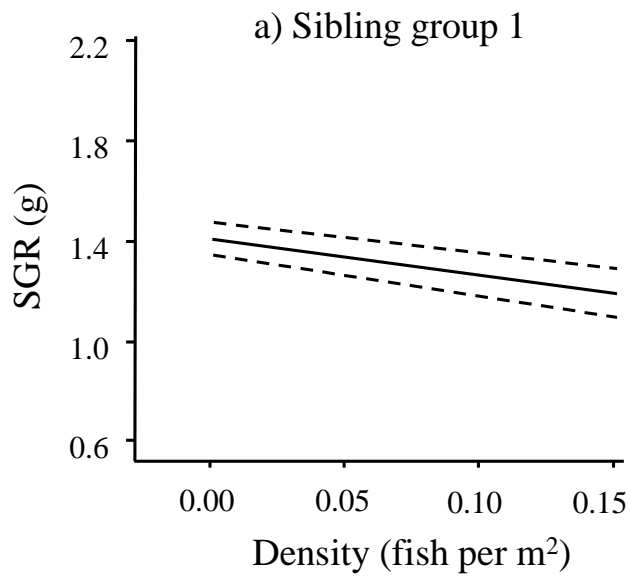
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591 Figure 2a & 2b

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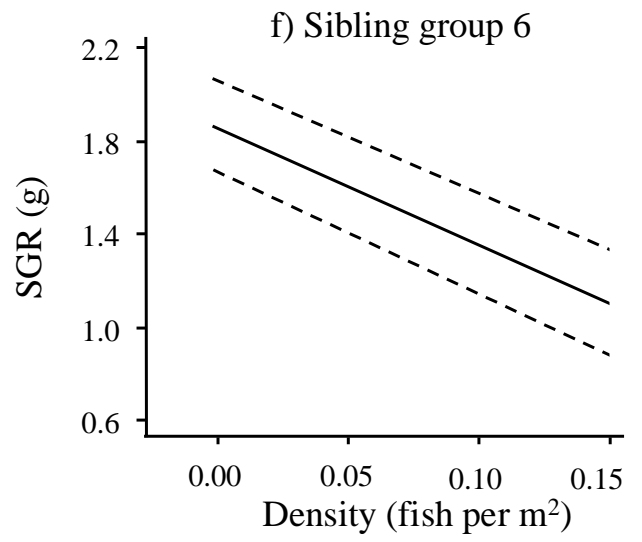
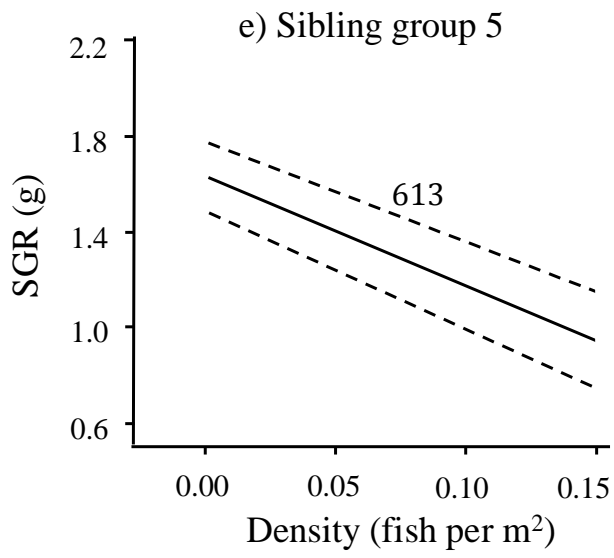
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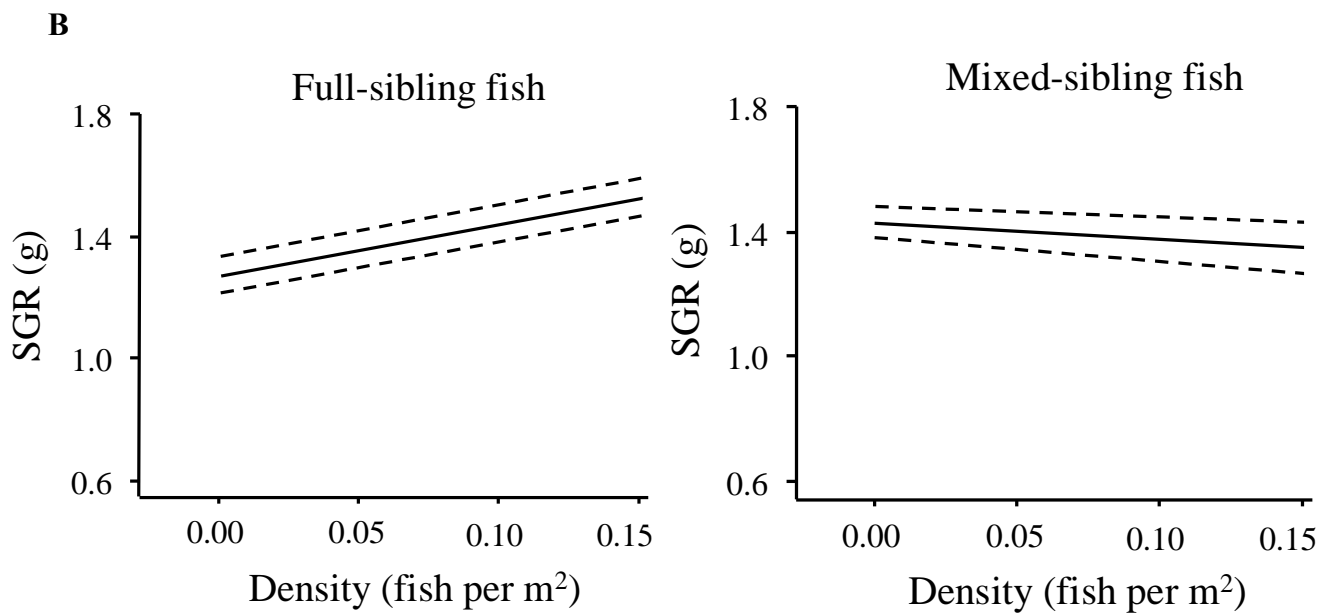
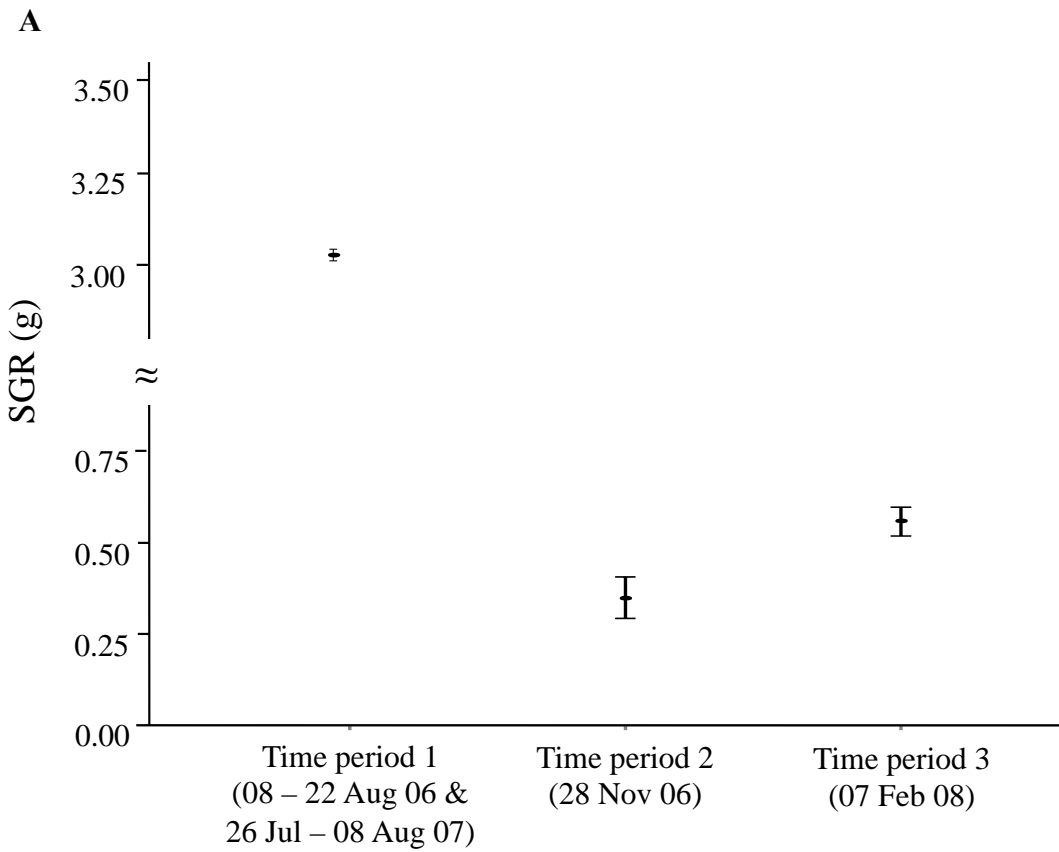
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618 Figure 3a - f



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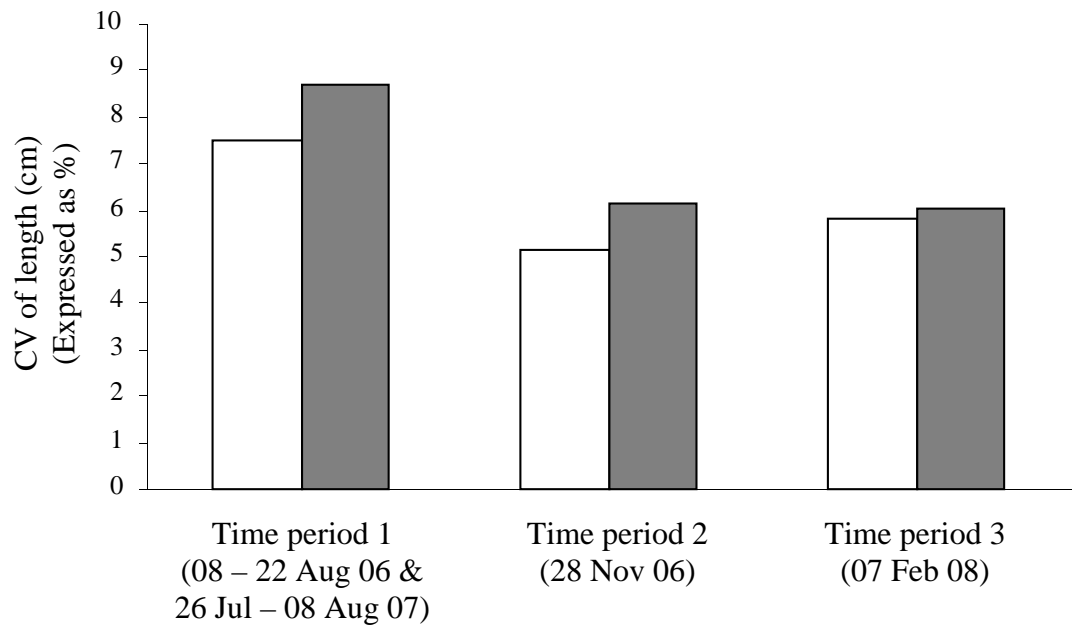
645 Figure 4a & 4b

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**A**

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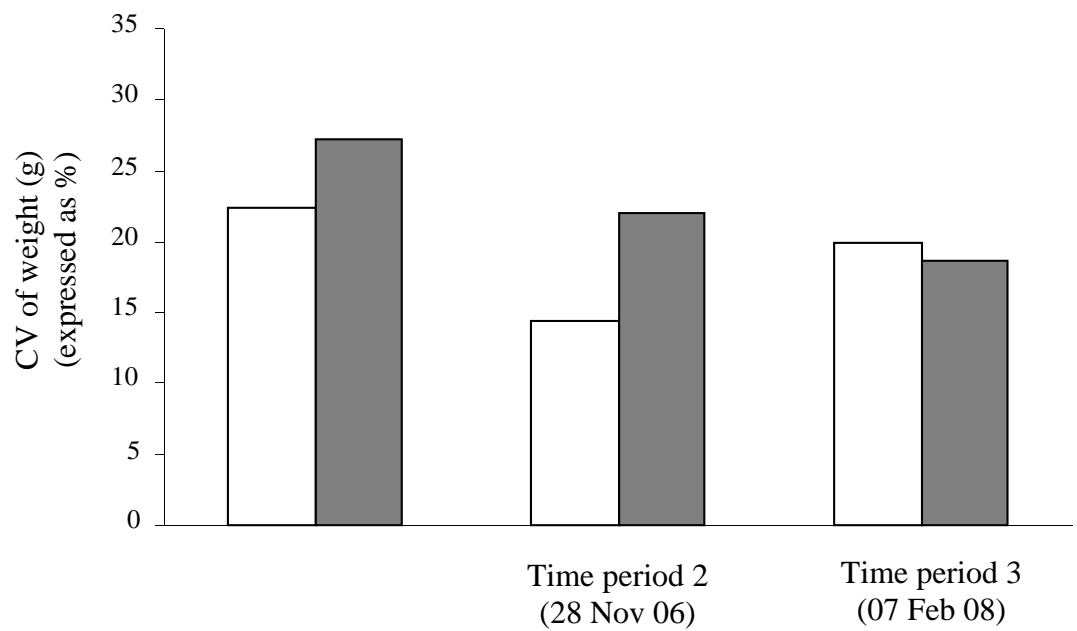
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**B**

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Time period 1  
(08 – 22 Aug 06 &  
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Figure 5a & 5b

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673 SUPPLEMENTARY MATERIALS

674

675 METHODS

676 *Experimental Animals*

677 To create groups of fish that were raised apart and were either related or unrelated, Atlantic  
678 salmon eggs and milt were obtained from wild adult specimens caught by electric fishing  
679 from the main stem of the River Frome, Dorset, UK, between Dorchester and East Stoke  
680 (SY68381 91720 – SY86479 86755). Parental fish were paired in the order in which males  
681 and females were caught. The adult fish were anaesthetised with 2-phenoxyethanol, and then  
682 eggs or milt expelled by gently squeezing the lower body of the fish (Edwards, 1978). An  
683 adipose tissue sample was taken from each adult and stored in 100% ethanol at 4 °C for  
684 genetic analysis. Once the fish were fully recovered from anaesthesia, they were returned to  
685 their site of capture. Fertilised eggs were placed into separate incubators.

686       When juveniles began to emerge from the incubators, fork length and wet weight  
687 (means to the nearest mm) of 25 individuals from each sibling group were measured. The  
688 three sibling groups most similar in size were chosen each year (n = 6 in total) for  
689 subsequent use to minimise any possible effects of inter-family variation in size. Mean ( $\pm$   
690 SE) fork length and wet weight for each sibling group in 2006 was: sibling group 1: 27.2  
691 mm  $\pm$  0.15, 0.171 g  $\pm$  0.00; sibling group 2: 27.0 mm  $\pm$  0.14, 0.157 g  $\pm$  0.00; sibling group  
692 3: 26.8 mm  $\pm$  0.11, 0.151 g  $\pm$  0.00; and in 2007: sibling group 4: 26.7 mm  $\pm$  0.12 and 0.177  
693 g  $\pm$  0.00; sibling group 5: 24.8 mm  $\pm$  0.19 and 0.127 g  $\pm$  0.00; sibling group 6: 24.9 mm  
694  $\pm$ 0.14 and 0.126 g  $\pm$ 0.00. Mixed-sibling groups were formed by combining equal numbers  
695 of fish from the three chosen sibling groups in each year, therefore ensuring identical  
696 genotype composition in full-sibling and mixed-sibling treatments within years. The average  
697 initial length and weight for all sibling groups in each year provided the baseline  
698 measurements for the mixed-sibling groups (2006: length 27.0 mm and weight 0.159 g;  
699 2007 length 25.5 mm and weight 0.143 g).

700 In April 2006, six sites on the river were designated as full-sibling sites and fry from  
701 each full- sibling group were released into two sites. An additional six sites were designated  
702 as mixed-sibling sites. The full- and mixed-sibling sites were alternated along the river to  
703 prevent stream altitude from influencing the results (Fig. 1a). Stream sites were 30 m in  
704 length, on average 4 m wide and were separated from one other by 100 m, a distance based  
705 on models of existing data (Crisp, 1995) which show that dispersal distance of most newly  
706 hatched salmon is < 20 m downstream. In 2007, to further ensure the genetic integrity of  
707 stocked areas, all full-sibling sites were situated upstream from mixed-siblings sites (Fig.  
708 1b) and the distance between stocked sites was increased to 250 m. Additionally, to utilise  
709 the river to its full capacity the length of full-sibling and mixed-sibling sites was increased to  
710 50 m and 150 m respectively.

711

#### 712 *Experimental procedure*

713 Fry release and two re-sampling events occurred each year, allowing kin-biased growth rate  
714 to be calculated over three time periods spanning a range of naturally-occurring densities  
715 across replicate seasons and years. Time period 1 extended from the date of fry release  
716 (03/04/06 – 09/04/06 and 21/03/07 – 09/04/07) to sampling event 1 (08/08/06 - 22/08/06,  
717 and 26/07/07 – 08/08/07). Time period 2: from date of fry release in 2006 to re-sampling  
718 event 2 (28/11/06). Time period 3: from date of fry release in 2007 to sampling event 3  
719 (07/02/08). To enable growth rates of individual fish to be compared between time periods,  
720 fish caught in time periods 2 or 3 were only included in the data analysis if they were also  
721 caught during time period 1.

722 All juvenile salmon caught during resampling were anaesthetised with 2-  
723 phenoxyethanol then measured (fork length and wet weight) and tagged with a Passive  
724 Integrated Transponder (PIT) tag as described by Riley *et al.* (2003) to enable repeated  
725 identification of individual fish. Also, an adipose fin clip was taken (stored in 100 %  
726 ethanol) allowing each fish to be allocated to family of origin, and for the genetic identity of

727 fish captured in full-sibling or mixed-sibling stream sites to be confirmed. In each year, two  
728 electric-fishing passes were made in each site. Where more than two fish were caught during  
729 the second pass, a further pass was made in an effort to gain a more accurate number of fish  
730 in each site.

731 Initial stocking density in 2006 and 2007 was approximately 2.7 and 4.1 fish m<sup>-2</sup>  
732 respectively. These densities were chosen to maximise the chances of measuring the (kin-  
733 biased) responses of fish under a range of densities.

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### 735 *Molecular Methods*

736 Each microsatellite locus (Table 1) was initially amplified separately, using a  
737 fluorescently labelled primer and an unlabelled primer to check the size range of PCR  
738 products. PCR products were quantified on 1 % agarose gel and visualised on a UV  
739 transilluminator. After the amplified fragments were optimised and size ranges were  
740 established, primers were clustered together into two multiplex groups according to the  
741 fragment size ranges. A Multiplex PCR Kit (QIAGEN catalogue no. 206143) was used  
742 following the manufacturer's protocol in a final reaction volume of 10 µl: 5 µl of 2 ×  
743 QIAGEN Multiplex Master Mix, 1 µl of primer mix (mix of forward and reverse primers for  
744 each locus), 2.5 µL of H<sub>2</sub>O and 1.5 µl of template DNA. PCR conditions were: 15 min of  
745 denaturation at 95 °C and 45 cycles of 30 s of initial denaturation at 94 °C, 90 s of annealing  
746 at 58 °C, 90 s of extension at 72 °C and 30 min of final elongation at 72 °C for 45 min.  
747 Amplifications were conducted in a GeneAmp 2700 Thermocycler (Applied Biosystems).

748 One microlitre of diluted (1/20) PCR product was added to 10µl Hi-di formamide  
749 and electrophoresis was performed using an ABI 3100 outsourced to KBiosciences, using  
750 0.25 µl of GS350 size standard (Applied Biosystems). Results were recovered electronically  
751 and all scoring was performed using Genemapper software (version 4) (Applied  
752 Biosystems). The program CERVUS version 3.0.0 (Marshall, 2007) was used to assign each  
753 juvenile ( $n = 243$ ) to their original parent pairs. CERVUS uses an inclusionary approach. It

754 compares the candidate parents' genotypes with the offspring's and assesses the relative  
755 likelihood (logarithm of odds) at each offspring's genotype having been inherited from all  
756 possible parents. The parent with the highest LOD score is usually assigned as the true  
757 parent if its likelihood is significantly higher than the next most likely parent. The average  
758 proportion of sampled candidate mothers and fathers was 100 % (6 mothers and 6 fathers: 3  
759 parent pairs in 2006 and 3 different parent pairs in 2007). The error rate in likelihood  
760 calculations was assumed at 1 %.