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

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ABSTRACT

Growth is a key life history trait in fishes that is influenced by both abiotic factors (such temperature and water chemistry) and biotic factors (such as density and food availability). Investigating how growth performance is influenced by such factors in the wild is important for understanding how population processes influence animals in natural environments and for predicting the response to conservation and management strategies that manipulate these conditions. The theory of kin selection predicts that significant growth and survival benefits are conferred upon animals associating with close relatives. However, resource competition may be more intense among close relatives, and little is known about the trade-off between these two processes under different ecological conditions. Here we examine the correlation

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

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

INTRODUCTION

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

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

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

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

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

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

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

METHODS

Experimental Animals

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

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

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

[FIGURE 1a & b]

Molecular Methods

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

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

[TABLE 1]

Data analysis

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

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

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

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

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

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

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

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

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

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

CV (%) = (100 x SD) /

where SD = standard deviation of length or weight, and x = mean of fork length or weight). CV has no units (expressed as a percentage) and is therefore a useful tool for comparing the variability of samples that have widely differing means, this giving a measure of inequality among individuals.

RESULTS

Growth rate varied significantly between families (GLMM $F_{5,292} = 5.27$, $P = 0.001$) (see Figure 2a). Growth rate also differed significantly between time periods (GLMM $F_{2,292} = 3079.36$, $P = 0.001$) (see Figure 2b). Interestingly, the interaction between sibling group and density had a significant effect on SGR ($F_{5,292} = 4.60$, $P = 0.001$) (Figure 3), with sibling group 3 showing a positive relationship between density and growth rate, while sibling groups 5 and 6 show a negative relationship between density and growth rate. Residuals from the

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

[FIGURE 2a & b]

[FIGURE 3]

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

[FIGURE 4a & b]

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

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

[FIGURE 5a & b]

DISCUSSION

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

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

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

While previous laboratory studies have found a positive effect of relatedness on growth rate (e.g. Brown & Brown, 1993a; Olsen *et al*., 1996), field studies have failed to demonstrate a similar effect (and in some cases have shown growth rate to be higher in mixed-sibling groups) (e.g. Griffiths & Armstrong, 2001; Greenberg *et al*., 2002). In fact, there is surprisingly little evidence for kin-biased association patterns in the wild among territorial fishes (e.g. see Brodeur *et al*., 2008; Fontaine & Dodson, 1999; Garant *et al*., 2000; Olsén *et al*., 2004). A potential explanation for these outcomes is that the confinement of fish to the small, simple habitats for long periods may allow stronger associations with tankmates to be formed than would naturally occur (Griffiths & Ward, 2011) and odour cues might be highly concentrated in such low water volume that kin recognition can be easily achieved (Courtenay *et al*., 2001). Another potential explanation is that unrelated individuals (different genotypes) are able to exploit a wider range of niches in the wild, thereby reducing intra-family competition, whereas individuals that share many genes in common; i.e. close relatives, exhibit similar ecological requirements (Blaustein *et al*., 1991; McLaughlin, Ferguson & Noakes, 1999) and may actively avoid kin (Griffiths *et al*., 2003). Our field study findings are consistent with Brown & Brown (1993a), Griffiths *et al*. (2003) and Toobaie & Grant, (2013) which appears to suggest that when the quality of habitat is low, for example in Winter, competition for resources increase and aggression rises in both related and unrelated groups of fish. It seems, therefore, that growth rate is driven by density and relatedness - 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^{-2} and by comparison, densities in wild studies are usually much lower,
324 ranging from 0.27 fish m^{-2} to <1 fish m^{-2} (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^{-2}, similar to previous wild kinship studies e.g. 1 – 1.7 fish m^{-2} (Griffiths &
327 Armstrong 2001) and 0.33 fish m^{-2} (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^{-2} (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

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

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Table 1 Atlantic salmon (*Salmo salar*) microsatellite multiplexes used in the present study.

Figure 1 A) Configuration in 2006 of sites stocked with six full-sibling (dark shaded) sites and six mixed-sibling (light shaded) groups of juvenile Atlantic salmon (*Salmo salar*) into on the River Cerne, (Dorset, England). **B)** Configuration in 2007 of three single-sibling (dark shaded) sites and one large mixed-sibling (light shaded) site (the size of three single-sibling sites) of Atlantic salmon.

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

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

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

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

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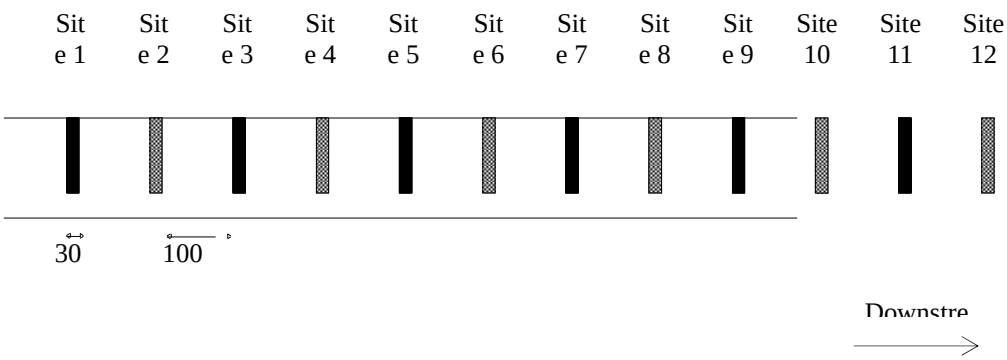
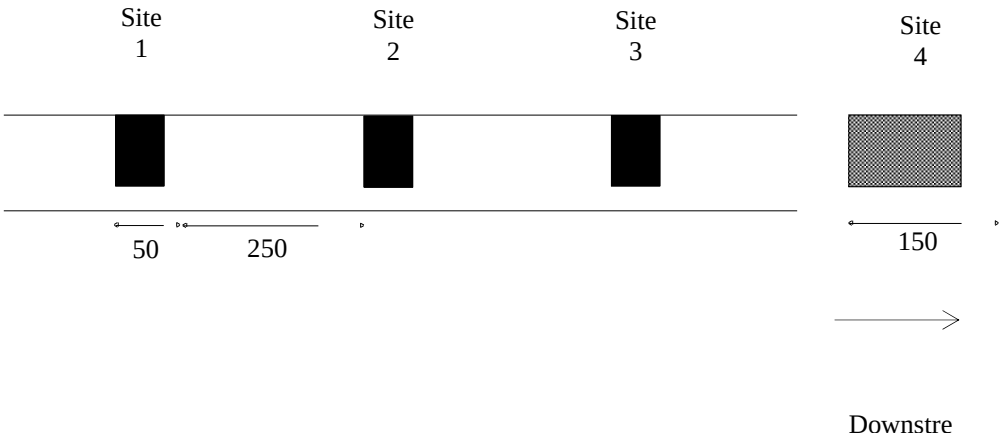
Multi-plex	Locus	Authors/Genbank no.	Primer sequence	Motif	Allele min.–max.
1	µF43	Sánchez *et al.* (1996)	Forward: 5'-AGC GGC ATA ACG TGC TGT GT-3'		
			Reverse : 5'-GAG TCA CTC AAA GTG AGG CC-3' (HEX)	AC/TG	103–143
	Ssa289	McConnell *et al.* (1995)	Forward: 5'-CTT TAC AAA TAG ACA GAC T-3'		
			Reverse : 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	µ20.19	Sánchez *et al.* (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 *et al.* (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 *et al.* (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 *et al.* (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

Table 1

a


Sit e 1
Sit e 2
Sit e 3
Sit e 4
Sit e 5
Sit e 6
Sit e 7
Sit e 8
Sit e 9
Site 10
Site 11
Site 12
30
100
Downstre

b


Site 1
Site 2
Site 3
Site 4
50
250
150
Downstre

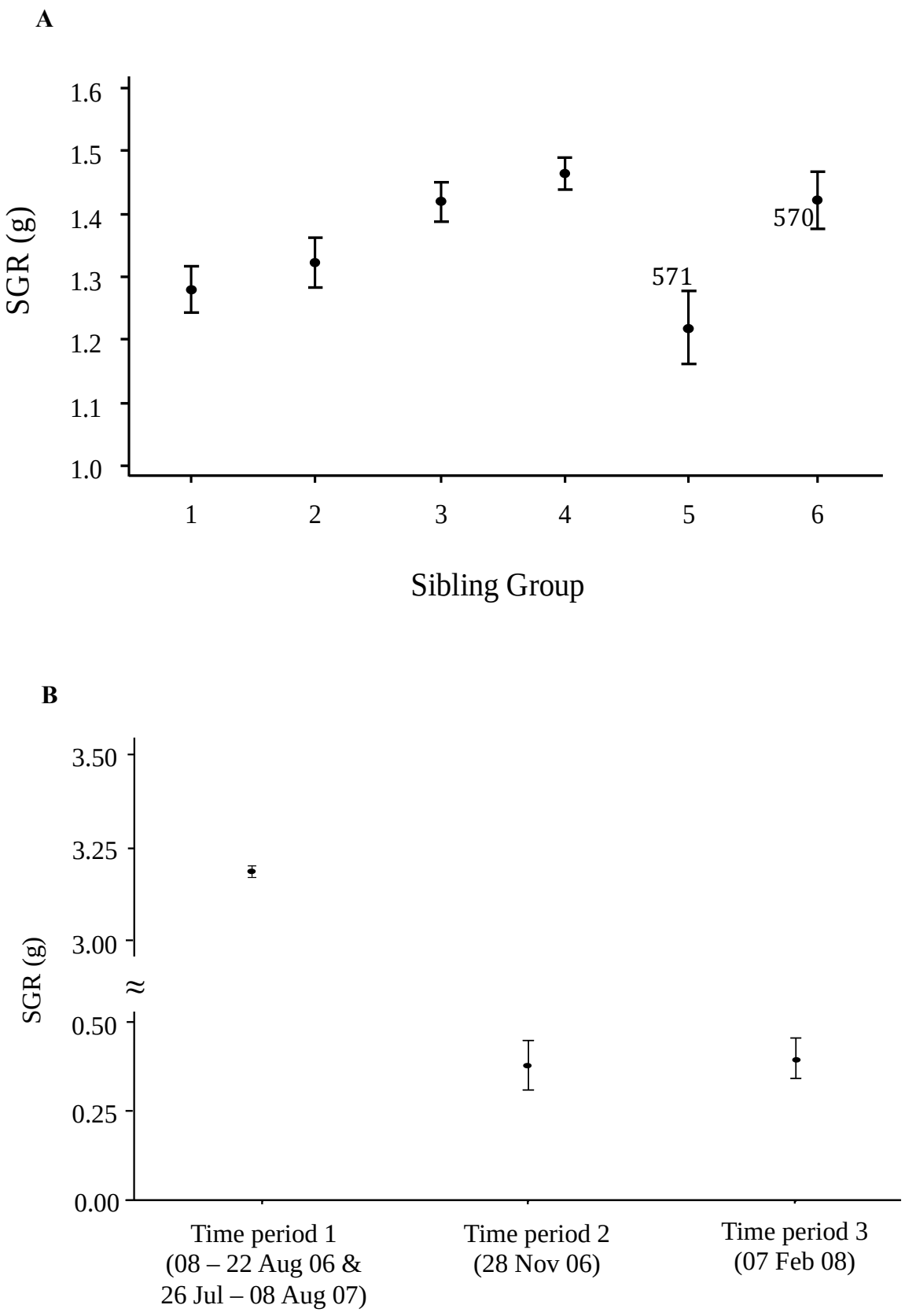
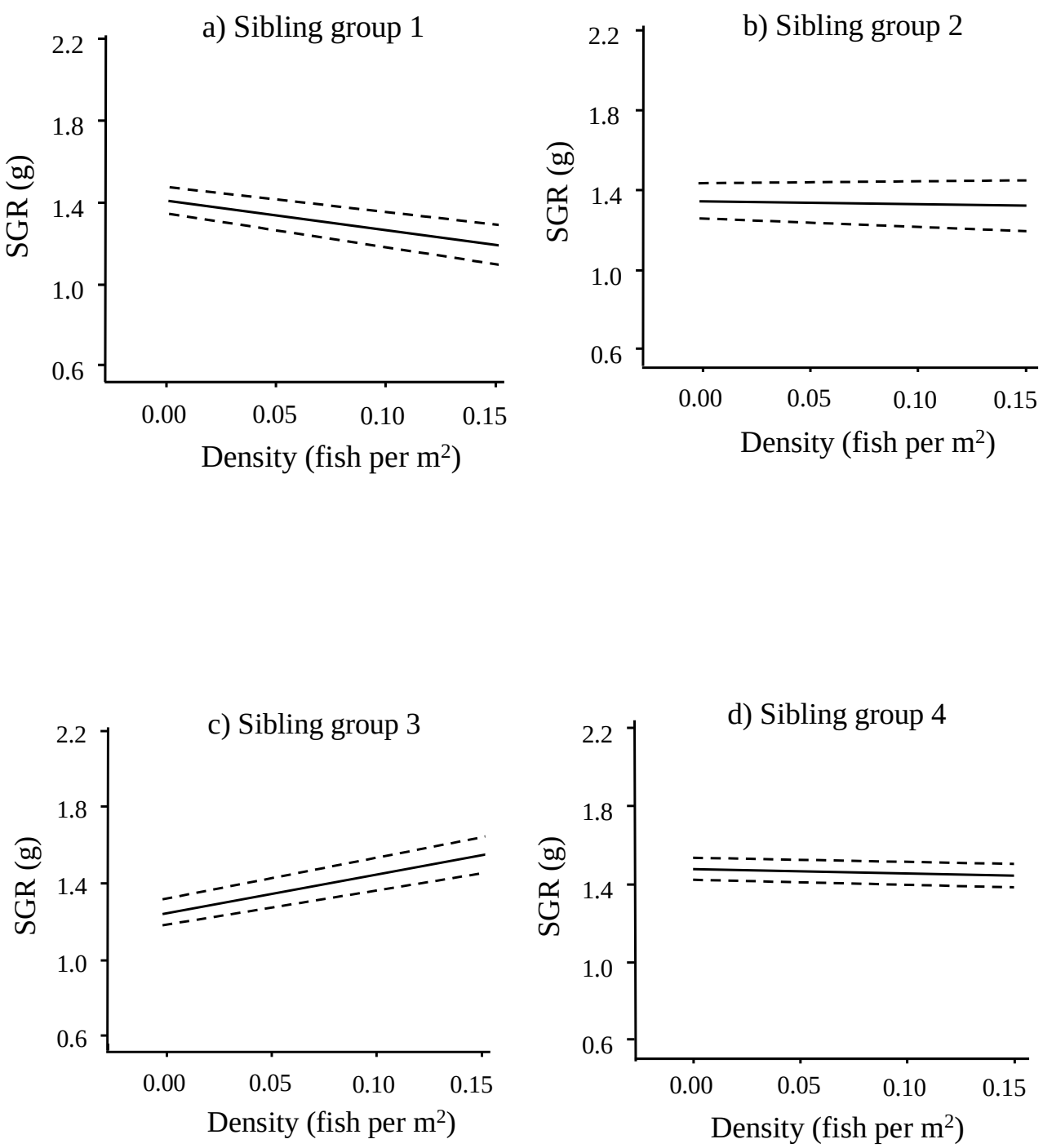

A
SGR (g)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
570
571
1
2
3
4
5
6
Sibling Group
B
SGR (g)
3.50
3.25
3.00
0.50
0.25
0.00
Time period 1
(08 – 22 Aug 06 &
26 Jul – 08 Aug 07)
Time period 2
(28 Nov 06)
Time period 3
(07 Feb 08)

Figure 2a & 2b


a) Sibling group 1
b) Sibling group 2
c) Sibling group 3
d) Sibling group 4
SGR (g)
2.2
1.8
1.4
1.0
0.6
0.00
0.05
0.10
0.15
Density (fish per m2)

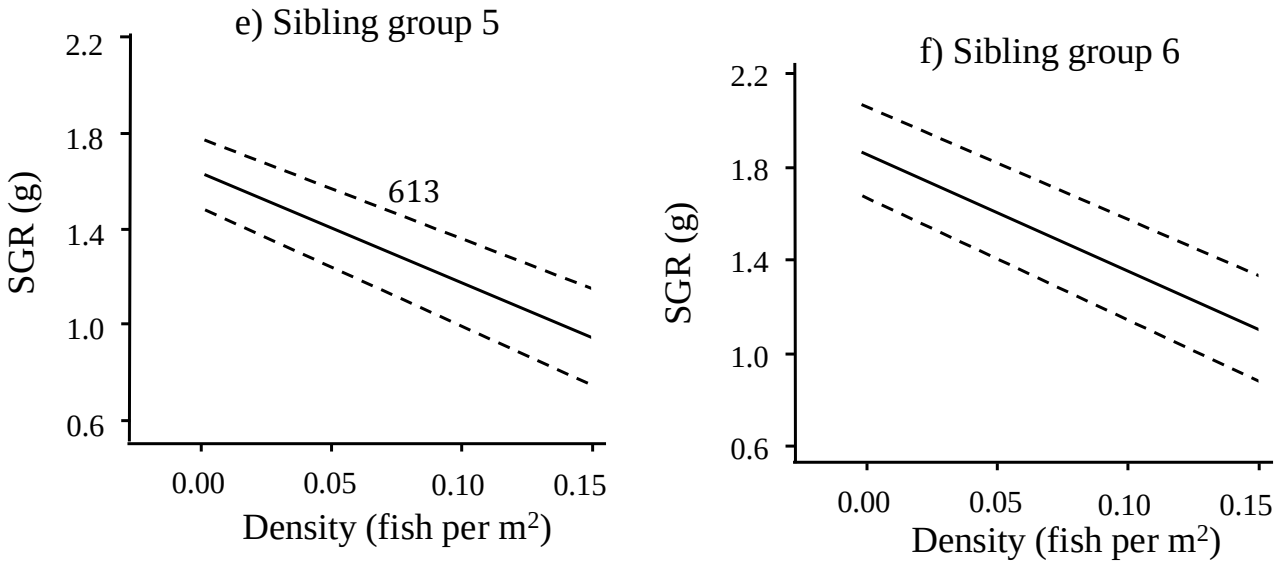
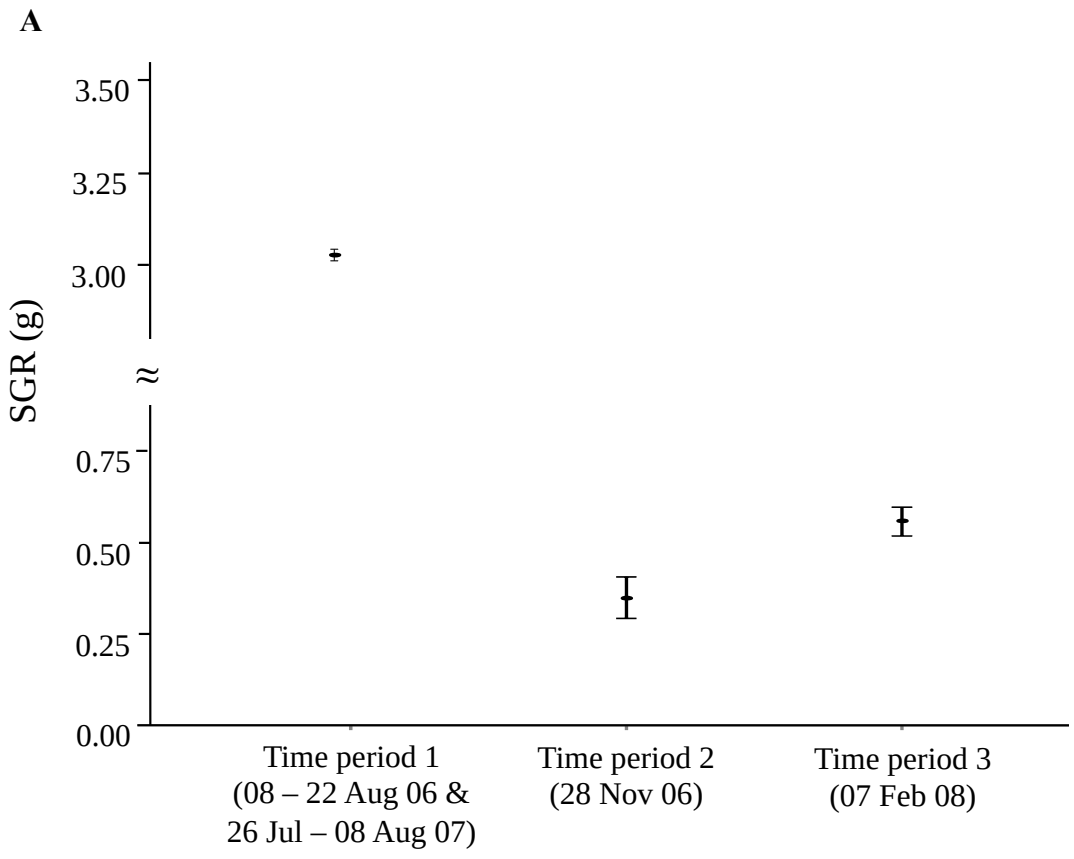
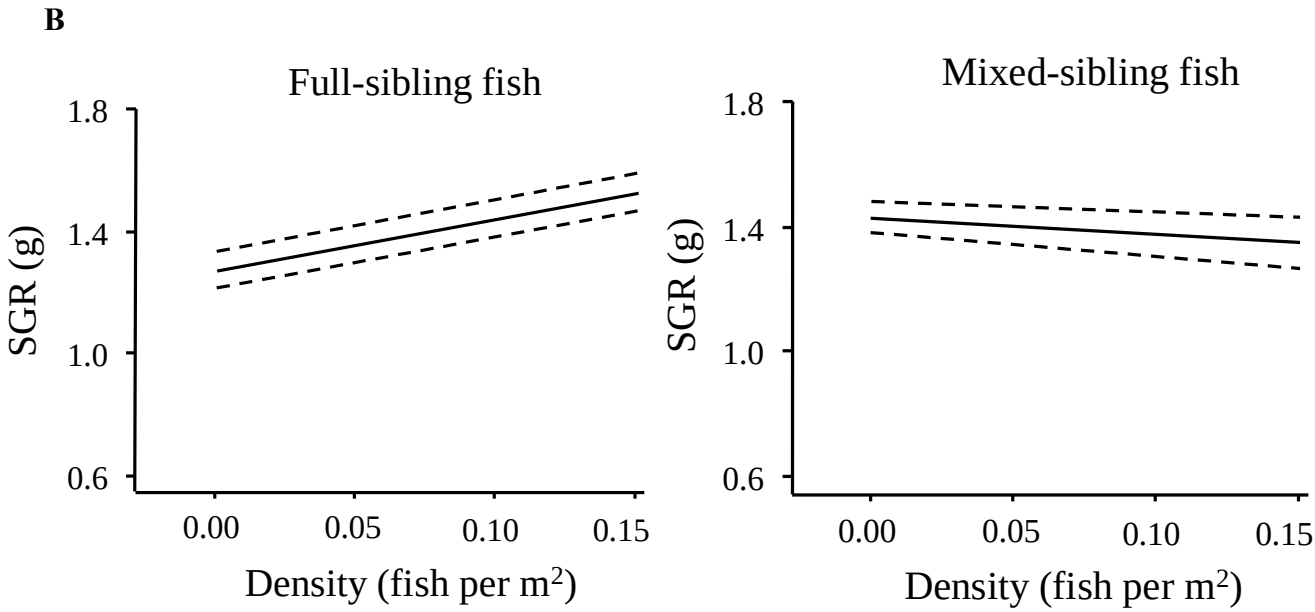

e) Sibling group 5
SGR (g)
2.2
1.8
1.4
1.0
0.6
613
0.00
0.05
0.10
0.15
Density (fish per m2)
f) Sibling group 6
SGR (g)
2.2
1.8
1.4
1.0
0.6
0.00
0.05
0.10
0.15
Density (fish per m2)

Figure 3a - f


A
3.50
3.25
3.00
SGR (g)
0.75
0.50
0.25
0.00
Time period 1
(08 – 22 Aug 06 &
26 Jul – 08 Aug 07)
Time period 2
(28 Nov 06)
Time period 3
(07 Feb 08)

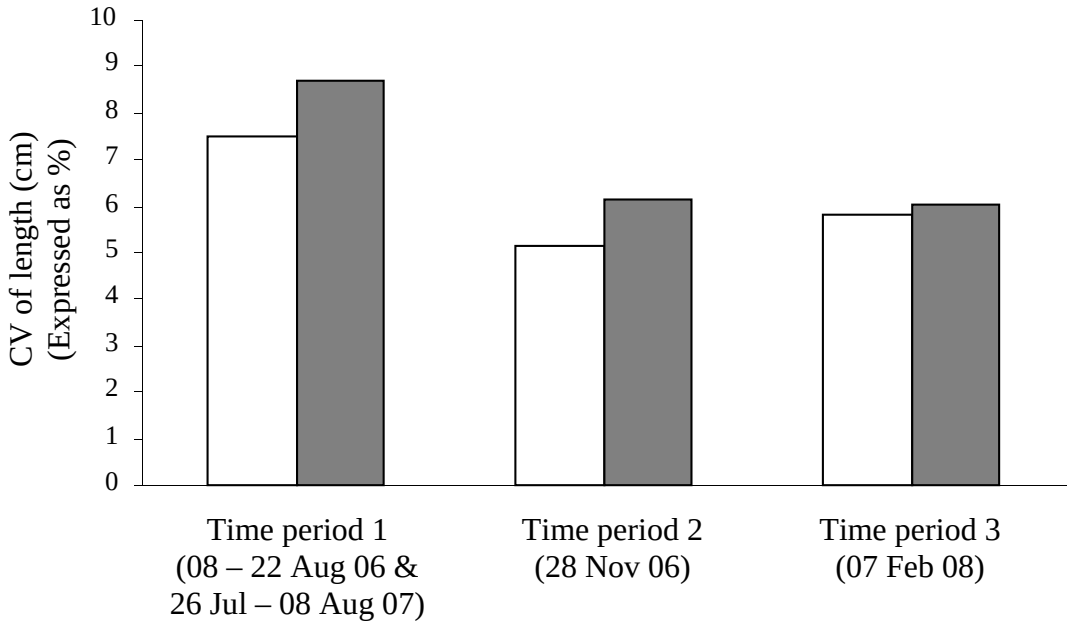

B
Full-sibling fish
Mixed-sibling fish
1.8
1.4
1.0
0.6
SGR (g)
0.00
0.05
0.10
0.15
Density (fish per m2)

644

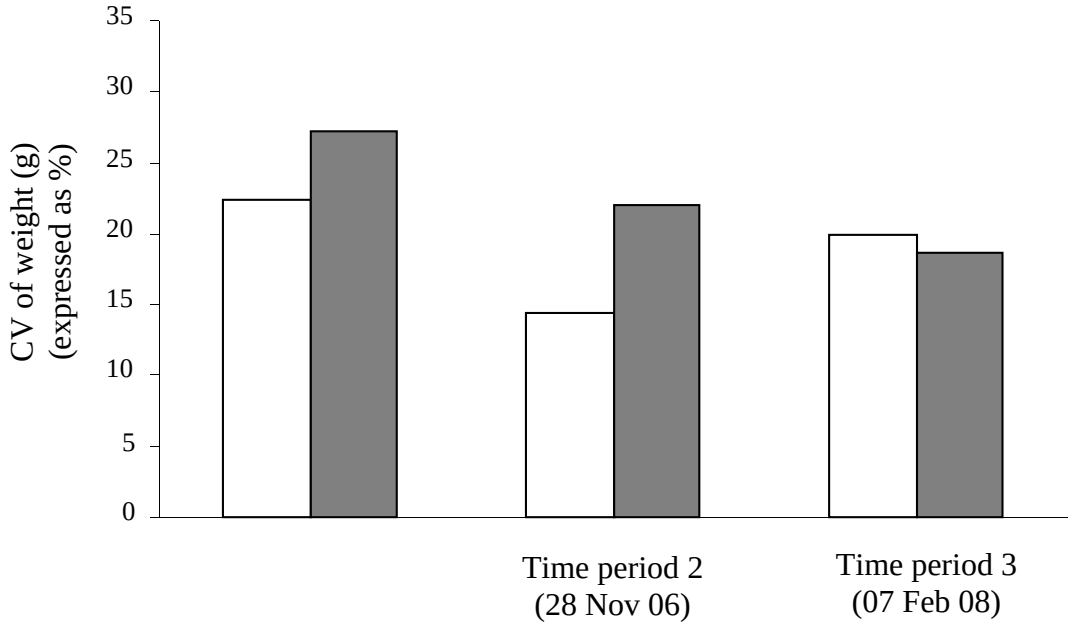
645 Figure 4a & 4b

646

A



B



Time period 1
(08 – 22 Aug 06 &
26 Jul – 08 Aug 07)

Figure 5a & 5b

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 (±
690 SE) fork length and wet weight for each sibling group in 2006 was: sibling group 1: 27.2
691 mm ± 0.15, 0.171 g ± 0.00; sibling group 2: 27.0 mm ± 0.14, 0.157 g ± 0.00; sibling group
692 3: 26.8 mm ± 0.11, 0.151 g ± 0.00; and in 2007: sibling group 4: 26.7 mm ± 0.12 and 0.177
693 g ± 0.00; sibling group 5: 24.8 mm ± 0.19 and 0.127 g ± 0.00; sibling group 6: 24.9 mm
694 ±0.14 and 0.126 g ±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).

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

Experimental procedure

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

All juvenile salmon caught during resampling were anaesthetised with 2-phenoxyethanol then measured (fork length and wet weight) and tagged with a Passive Integrated Transponder (PIT) tag as described by Riley *et al*. (2003) to enable repeated identification of individual fish. Also, an adipose fin clip was taken (stored in 100 % 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^{-2}
732 respectively. These densities were chosen to maximise the chances of measuring the (kin-
733 biased) responses of fish under a range of densities.
734
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_2O 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 %.