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1 **Simulating nutrient release from parental carcasses increases the growth,**

2 **biomass and genetic diversity of juvenile Atlantic salmon**

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20

ABSTRACT

21

22 1. The net transport of nutrients by migratory fish from oceans to inland spawning areas has

23 decreased due to population declines and migration barriers. Restoration of nutrients to

24 increasingly oligotrophic upland streams (that were historically salmon spawning areas) have

25 shown short-term benefits for juvenile salmon, but the longer-term consequences are little known.

26 2. Here we simulated the deposition of a small number of adult Atlantic salmon *Salmo salar*

27 carcasses at the end of the spawning period in five Scottish upland streams ('high parental nutrient'

28 treatment), while leaving five reference streams without carcasses ('low parental nutrient'

29 treatment). All streams received exactly the same number of salmon eggs (n = 3,000) drawn in

30 equal number from the same 30 wild-origin families, thereby controlling for initial egg density and

31 genetic composition. We then monitored the resulting juvenile salmon and their macroinvertebrate

32 prey, repeating the carcass addition treatment in the next spawning season.

33 3. Macroinvertebrate biomass and abundance were five times higher in the high parental nutrient

34 streams, even one year after the carcass addition, and led to faster growth of juvenile salmon over

35 the next 2 years (but with no change in population density). This faster growth led to more fish

36 exceeding the size threshold that would trigger emigration to sea at 2 rather than 3 years of age.

37 There was also higher genetic diversity among surviving salmon in high parental nutrient streams;

38 genotyping showed that these effects were not due to immigration but to differential survival.

39 4. *Synthesis and applications:* This field experiment shows that adding nutrients that simulate the

40 presence of small numbers of adult salmon carcasses can have long-term effects on the growth rate

41 of juvenile salmon, likely increasing the number that will migrate to sea early and also increasing

42 their genetic diversity. However, the feasibility of adding nutrients to spawning streams as a

43 management tool to boost salmon populations will depend on whether the benefits at this stage are

44 maintained over the entire life cycle.

45

46 **KEYWORDS**: marine derived nutrients, oligotrophic, phosphorus, smolt

1 | INTRODUCTION

Anadromous species are born and reproduce in fresh water but achieve most of their growth in the ocean. This life cycle has consequences for freshwater ecosystems since migratory fishes can act as vectors for marine nutrients (Naiman *et al.* 2002), subsidized mostly via the production of gametes, waste products and the decomposition of adult carcasses arising from post-spawning mortality (Willson & Halupka 1995). While emigrating juveniles also transport nutrients in the opposite direction, the relative scale of the nutrient flows is such that the majority of anadromous populations generate a net import of marine-derived nutrients to freshwater communities (Naiman *et al.* 2002; Walters, Barnes & Post 2009; Flecker *et al.* 2010; Childress, Allan & McIntyre 2014). The effect - generally in the form of increased productivity and/or biomass - is detectable in freshwater food webs, especially when ecosystems are otherwise oligotrophic (Claeson *et al.* 2006; Nislow *et al.* 2010; Guyette *et al.* 2014).

Recent declines in adult populations may result in the export of nutrients from fresh waters (by emigrating juveniles) being greater than the import by the breeding adults (Moore & Schindler 2004; Scheuerell *et al.* 2005; Moore *et al.* 2011). This consequent steady decline in nutrient inputs ('oligotrophication') may significantly alter the architecture of the food webs that exist within these freshwater ecosystems (Doughty *et al.* 2016; Gerwing & Plate 2019). This process is exacerbated by a rise in the number of artificial barriers to riverine migration, such as weirs or dams constructed for the purpose of hydropower generation or water storage. These have undoubtedly contributed to observed declines in anadromous fish populations (Limburg & Waldman 2009; Lenders *et al.* 2016), which in turn have reduced the level of nutrient subsidies in ecosystems upriver of the barriers (Williams *et al.* 2009).

69   Restoring nutrient levels to some presumed previous level is one mitigation measure, but this needs

70   to be carefully managed and evidence-based to avoid causing eutrophication (Stockner, Rydin &

71   Hyenstrand 2000). Nutrients can be administered to freshwater ecosystems via the addition of

72   fertilisers (Griswold, Taki & Stockner 2003; Ward, McCubbing & Slaney 2003), fish carcasses

73   (Bilby *et al.* 1998; Williams *et al.* 2009), or fish carcass 'analogues' (Kohler *et al.* 2012; Guyette,

74   Loftin & Zydlewski 2013), the latter usually being in the form of dried pellets made from marine

75   fish (Pearsons, Roley & Johnson 2007). The addition of carcasses or their analogues has been

76   found to be more effective than adding liquid fertilisers (Kiernan, Harvey & Johnson 2010; Wipfli

77   *et al.* 2010), probably because the nutrient pulse lasts longer and also allows organisms to feed

78   directly on the added biomass in addition to creating bottom-up effects (Bilby *et al.* 1998; Wipfli,

79   Hudson & Caouette 1998). Carcass analogues have similar effects on freshwater productivity to

80   real carcasses (Wipfli, Hudson & Caouette 2004), but have the advantages that they are lighter

81   (being dried) and are more easily stored and transported (Pearsons, Roley & Johnson 2007).

82   To date, most studies on the impact of nutrient restoration in freshwater streams find that the

83   addition of carcasses or carcass analogues increases invertebrate abundance and biomass (Wipfli,

84   Hudson & Caouette 1998; Claeson *et al.* 2006; Nislow *et al.* 2010) and generally benefits fish

85   growth and body condition (Wipfli *et al.* 2003; Williams *et al.* 2009; Guyette, Loftin & Zydlewski

86   2013). However, effects on fish density and biomass are unclear, in part due to the limitations of

87   field studies in controlling for the immigration of non-experimental fish into restored areas, as

88   shown by Bilby *et al.* (1998). There is also little knowledge of the longer-term consequences of

89   nutrient manipulations, since most studies have only lasted for a few months after supplementation

90   (e.g. Wipfli *et al.* 2003; Williams *et al.* 2009). It is therefore unclear whether observed increases

91   in growth rate are sustained in the long term and/or influence subsequent life histories. For

example, the timing of emigration to sea in many anadromous fish species is size-dependent; therefore, it is possible that the age structure of migrant fish could be altered by nutritional subsidies from their parents (Nelson & Reynolds 2015). Finally, to our knowledge no previous studies have considered how the addition of marine-derived nutrients may affect the longer term genetic diversity of freshwater fish populations, which is becoming increasingly recognised as an important aspect of conservation management (Garcia de Leaniz *et al.* 2007; Kahilainen, Puurtinen & Kotiaho 2014).

Here we examine the effects of marine-derived nutrients on juvenile Atlantic salmon and their invertebrate prey. While Pacific salmon are semelparous and experience mass mortality after spawning, post-spawning mortality rates in Atlantic salmon are lower and vary on both a temporal and spatial scale (Fleming 1998; Jonsson & Jonsson 2003). Nevertheless, even relatively small influxes of marine nutrients have the potential to alter the highly oligotrophic upland streams in which these salmon typically breed (Jonsson & Jonsson 2003; Nislow, Armstrong & McKelvey 2004), and there is evidence that this species used to spawn at much higher densities than is currently the case (Lenders et al, 2016). Using a study system that allows us to exclude the potential effects of immigrant fish on calculations of fish biomass and density, we recently demonstrated experimentally that marine-derived nutrients from salmon carcass analogues can have a positive effect on juvenile Atlantic salmon genetic diversity, growth, and biomass over the first 3 months of life (Auer *et al.* 2018). Here we extend our work in this same study system to consider whether these effects persist across the freshwater stage. We also evaluate if such simulations of nutrient release may influence the age (and hence size) at which juveniles undertake the spring seaward migration, with potential implications for the subsequent marine phase of the Atlantic salmon's life history.

2 | METHODS

2.1 | Study sites and general experimental protocol

We selected 10 small headwater streams that were surrounded primarily by open moorland and drained into the Rivers Blackwater, Bran, and Meig of the River Conon catchment in northern Scotland (Fig. 1, Table S1; Auer *et al.* 2018). Hydropower dams along each of the rivers prevent the passage of most returning adult salmon (Gowans *et al.* 2003; Williams 2007). Atlantic salmon (stocked as eggs or juveniles) and resident brown trout (*Salmo trutta*) are the dominant fish species in the system. Five of the study streams were randomly assigned to the 'high parental nutrient' treatment (addition of analogue carcasses), while the other five study streams were assigned to the 'low parental nutrient' treatment (without carcasses). We then planted out eggs from genotyped salmon families in each of the streams and subsequently monitored prey availability and fish growth, biomass, density and genetic diversity of this focal cohort over the next 2 years (Fig S1). We also monitored these same fish variables in a second cohort of eggs planted out the second year to evaluate repeatability of parental nutrient effects during the first summer of growth (see Supplementary Information (SI) for details).

2.2 | Experimental families and planting out eggs and carcass analogues

Over a three-day period in December 2015, fifty-four full sibling families were created using *in vitro* fertilization of fish caught in a fish trap at a dam on the River Blackwater (Fig. 1). Parental fish were those previously stocked in headwater streams above the dam that were returning from the sea to spawn. Of the 54 families, we selected a subset of 30 families that were chosen at random with respect to paternal age but controlled for maternal life history; only families from females that had spent a single winter at sea, confirmed by scalimetry, were selected since that was the

137 dominant life history of captured adults. A small section of adipose fin was clipped from each

138 parent and preserved in 100% ethanol for later DNA analysis (see SI). Fertilized eggs were then

139 reared overwinter in family-specific trays under identical water and temperature conditions at a

140 nearby hatchery.

141 In late February to early March 2016, when eggs had reached the eyed stage of embryonic

142 development, 100 eggs from each of the 30 families were collected from the hatchery, mixed

143 together, and then planted out in a 300m^2 experimental reach (75-100m in length depending on

144 stream wet width; Table S1) in each of the study streams (Table S1). Eggs were buried beneath the

145 gravel in two Vibert boxes at the lower and upper limit of each experimental reach (n = 100 eggs

146 per box) and the rest of the eggs were planted out in 8 artificial nests (n = 350 eggs per nest;

147 McLennan *et al.* 2016) at equidistant points between the upper and lower Vibert boxes. In total,

148 each experimental 300 m^2 reach received 3,000 eggs, equating to a density of 10 eggs m^{-2} that falls

149 within the range of observed spawning densities for Atlantic salmon (Fleming 1996). The Vibert

150 boxes were found to be empty of eggs when recovered in late May/early June 2016, indicating

151 successful hatching in all streams. Similar methods were used to create the second cohort of eggs

152 that were planted out in each of the study streams the following year (see SI).

153 At the time of egg planting and again the following winter (Table S1), analogue carcasses,

154 composed of dried hatchery salmon pellets (Coral 2000+40PAX B12, made of 60% marine-

155 derived fish-based nutrients, Skretting, Invergordon UK) and similar to salmon carcasses in their

156 nutritional content and decay rate (Pearsons, Roley & Johnson 2007), were distributed in high

157 parental nutrient streams. Each experimental reach in the high parental nutrient streams received 5

158 mesh bags of pellets, each weighing 3kg, and equivalent to 25 adult salmon carcasses, an amount

159 similar to or less than that used in other nutrient supplementation experiments in Atlantic salmon

160 streams (Williams *et al.* 2009; Guyette, Loftin & Zydlewski 2013; Guyette *et al.* 2014). The bags

161 were evenly spaced along the length of each experimental reach and anchored to the substrate of

162 the stream by stones to prevent removal by scavengers. HOBO temperature data loggers (Onset

163 Computer Corporation, Bourne MA, USA) were also placed in each stream at the time of egg

164 deposition and programmed to record data every 4h (Fig S2).

165 **2.3 | Macroinvertebrate prey abundance and biomass**

166 Macroinvertebrates were sampled in each experimental reach during late May to early June 2016

167 and then again in late February to early March 2017, when the fish were in their first and about to

168 enter their second year of life (age 0+ and 1+ respectively). The sampling in March 2017 took

169 place immediately prior to the addition of the second set of carcass analogues. Invertebrates were

170 collected using the electrobugging technique (Taylor, McIntosh & Peckarsky 2001), described in

171 detail in the SI. Specimens were then later identified to the family level and their length and

172 biomass was calculated. Only those macroinvertebrates equal to or smaller than 1 mm and 2.5 mm

173 in width were included in estimates of prey abundance and biomass (for age 0+ and 1+ fish

174 respectively), since these are the maximum prey sizes that gape-limited juvenile salmon can

175 consume at their respective body sizes (Wankowski 1979). These smaller macroinvertebrates were

176 primarily from the Orders Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, and Diptera, all of

177 which are known to be in the diet of juvenile salmon in the Conon and other Scottish river

178 catchments (Table S2; Mills 1964; Maitland 1965).

179 **2.4 | Recapture of juvenile salmon**

180 Surviving juveniles were captured by triple-pass electro-fishing in July 2016 (when fish were age

181 0+, approximately 3 months old), in July 2017 (when fish were aged 1+, approximately 15 months

182 old, Table S1), and finally in March 2018 (when the fish were 22 months old, which we refer to as

183 age 2; Table S1). A total of 1272 fish were captured at age 0+ within the experimental reaches of

184 the streams, 458 at age 1+ (plus 292 caught within 50m of the experimental reaches) and 306 at

185 age 2, this time within 100m of the experimental reaches. Captured fish were anaesthetized,

186 weighed ($\pm$ 1.0 mg), measured for body length ($\pm$ 0.01 mm) and a small fin clip taken for later

187 parental assignment (see SI). By conducting triple-pass electro-fishing (see SI), we were able to

188 estimate the densities of age 0+ and age 1+ focal fish based on the removal method and analysed

189 (using maximum-likelihood) by Microfish software (Van Deventer & Platts 1989; Dochtermann

190 & Peacock 2013).

2.5 | Statistical analyses

192 Effects of parental nutrient levels on juvenile salmon and their macroinvertebrate prey were tested

193 using a series of linear mixed models. All models included treatment (low versus high parental

194 nutrients) and salmon age (age 0 and age 1+ for invertebrate analyses and age 0+ and 1+ for fish

195 analyses) as categorical fixed effects and stream as a random effect in cases where the dependent

196 variable was measured more than once. Residuals were not normally distributed for most

197 dependent variables, so we used a hierarchical bootstrapping approach to generate mean effects

198 and p-values (Adèr & Adèr 2008). For analyses of macroinvertebrate abundance and biomass and

199 fish fork length, body mass, density and biomass, the bootstrap procedure first sampled with

200 replacement among values within each stream and age, then streams and age within each treatment.

201 Models were rerun 20,000 times. Significance values were then calculated as a two-tailed *P*-value

202 from the bootstrapped distribution of the treatment effect. Results from models that included or

203 excluded fish of unknown parentage were qualitatively the same since there were few fish with

204 unknown parentage, so only results from models that excluded fish of unknown parentage are

205   reported. The same analyses, albeit without age as a fixed effect, were run for body size, density

206   and biomass of the second fish cohort.

207   Effects of parental nutrient levels on family-level diversity were examined using a bootstrap

208   procedure that sampled, with replacement, values for the numbers of families represented in the

209   captures from each of the streams per treatment and fish age. The model was rerun 20,000 times,

210   and p-values were calculated as above. The number of fish captured per stream was included as a

211   covariate in the analysis of family diversity, but was not statistically significant ($p > 0.05$;

212   presumably because similar numbers were collected in each stream) so was dropped from the

213   model. We ran two models, one that included and one that excluded fish captured outside the

214   experimental reach section (see above).

215   Finally, differences in fork length between treatments (low versus high parental nutrients) at age 2

216   were tested using a linear mixed model that included stream and family ID as random effects. The

217   residuals from this model were normally distributed; therefore, the bootstrapping approach was

218   not considered necessary.

219   **3 | RESULTS**

220   Both macroinvertebrate abundance (treatment $p < 0.001$; season $p = 0.18$; season x treatment $p =$

221   0.336) and biomass (treatment $p < 0.001$; season $p < 0.001$; season x treatment $p = 0.462$) were

222   higher in streams with high compared to low parental nutrient levels. These differences were

223   consistent across both the spring and the following winter, when juvenile salmon were age 0 and

224   1+, respectively (Fig. 2).

225   Differences in prey availability among stream types were associated with distinct differences in

226   juvenile salmon body size at both age 0+ and age 1+. Specifically, fork length increased with age

227   as expected (Fig. 3; $p < 0.001$), but juvenile salmon in high nutrient streams were also

228   consistently larger than their siblings in low nutrient streams (treatment: $p = 0.001$; age x

229   treatment: $p = 0.609$). Likewise, body mass increased with age (Fig. 3; $p < 0.001$), but juvenile

230   salmon in high nutrient streams were consistently larger than their siblings in low nutrient

231   streams (treatment: $p < 0.001$; age x treatment: $p = 0.681$). Fish density declined with age (Fig. 4;

232   $p < 0.001$) but was not affected by nutrient level (treatment: $p = 0.966$; age x treatment: $p =$

233   0.495). Fish biomass had declined at age 1+ (Fig. 4; $p = 0.012$) but was consistently greater in

234   high compared to low nutrient streams (treatment: $p = 0.034$; age x treatment: $p = 0.364$). Results

235   for body size, density, and biomass were qualitatively the same for the second cohort of fish at

236   age 0+ (Fig. S3). Both length ($p < 0.001$) and biomass ($p = 0.012$), but not density ($p = 0.200$) were

237   higher in streams with high compared to low parental nutrient levels.

238   Significant treatment differences in fork length were also observed when focal fish were age 2 (Fig

239   5, $p = 0.008$). Importantly, 89.6% of the captured individuals in high nutrient streams had reached

240   the minimum fork length of 100mm required for smolt transformation in this river catchment

241   (Malcolm, Millar & Millidine 2015), and so had a high likelihood of migrating to sea as an age 2

242   smolt. In contrast, only 38.3% of the fish in the low nutrient streams had fork lengths above this

243   threshold size (Fig 5).

244   Finally, parental nutrient levels also influenced the genetic diversity of surviving fish (Fig. 6).

245   Specifically, there was a trend for surviving fish to be drawn from a higher mean number of

246   families in streams with high compared to low parental nutrient levels ($p = 0.111$) at both age 0+

247   and age 1+ (age: $p < 0.001$, age x treatment: $p = 0.559$) when the analysis excluded age 1+ fish

248   captured outside the bounds of the experimental reach. These differences in family-level diversity

249   among stream types were statistically significant when these extra-limital fish were included

250   (treatment: $p = 0.016$; age: $p = 0.127$; age x treatment: $p = 0.559$).

251   **4 | DISCUSSION**

252   **4.1 | Sustained effects on prey availability and salmon growth rate**

253   Previous studies have shown that the addition of marine-derived nutrients via carcasses or carcass

254   analogues increases invertebrate abundance and biomass (Wipfli, Hudson & Caouette 1998;

255   Claeson *et al.* 2006; Nislow *et al.* 2010). Marine-derived nutrients from salmon carcasses may

256   become incorporated at multiple trophic levels within a stream (Nislow *et al.* 2010; Samways, Soto

257   & Cunjak 2018). Therefore, a short-term increase in invertebrate abundance and biomass is

258   perhaps unsurprising, given that many invertebrates feed directly on the carcass analogues and/or

259   benefit from bottom-up effects of a nutrient pulse (Nislow *et al.* 2010). We show here that these

260   effects can be both persistent and substantial: a year after the carcass addition (just prior to the

261   second deposit of analogue carcasses) both the abundance and biomass of macroinvertebrate prey

262   types for juvenile salmon were around 5 times higher than in streams receiving no carcasses. Since

263   salmon carcasses are naturally deposited at yearly intervals, our results indicate that these natural

264   annual nutrient pulses may sustain macroinvertebrate prey numbers at higher levels throughout the

265   year. Scottish upland streams are often naturally nutrient poor (Elliott *et al.* 1998; Nislow,

266   Armstrong & McKelvey 2004) and this has been further exacerbated by anthropogenic influence

267   (Williams *et al.* 2009). Therefore, it is perhaps not surprising that even a relatively modest addition

268   of simulated carcasses could significantly boost the productivity of the food webs that exist within

269   these otherwise nutrient limited habitats.

270   This sustained increase in prey availability may explain why we also found that the salmon in these

271   nutrient-supplemented streams were significantly larger at the end of the experiment, when they

272   were two years old. While there is evidence of correlations between prey availability and the

273   growth rate of age 0+ salmon in the field (Kennedy, Nislow & Folt 2008), less is known about the

274   older freshwater life stages, when other sources of growth limitation (e.g. enhanced risk averse

275   behaviour; see Nislow, Armstrong and Grant (2011)) may act to decouple individual growth from

276   the availability of prey. Here we have shown a similar growth response to a nutrient pulse and

277   associated enhanced prey availability among the different freshwater age classes of salmon.

278   Previous studies that report a positive effect of parental nutrients on fish biomass have been limited

279   in determining the underlying mechanisms of such an effect (e.g. Williams *et al.* 2009). In the

280   present study, all matings occurred at the same time and all streams received the same genetic mix

281   of eggs, so the only explanation for the larger juvenile size-at-age in high nutrient streams is a

282   faster growth rate. Likewise, the greater biomass of juvenile salmon in the high parental nutrient

283   streams was driven by differences in growth rate since we found no effect on their density. Other

284   studies have found a positive effect on fish density from adding carcasses (e.g. Williams et al.

285   2009), but this could be caused by increased immigration (Bilby *et al.* (1998), which might imply

286   no overall increase in population size. Our study reduced the noise in estimating the effects of

287   nutrients on juvenile salmon by seeding all streams with the same number and genetic diversity of

288   salmon eggs, and then genotyping the surviving offspring, so allowing us to show that levels of

289   immigration were trivial. The upper density limit (i.e. carrying capacity) of juvenile salmon

290   populations is linked to size of feeding territory that the fish defend (Grant & Kramer 1990).

291   However, stream salmonids (including Atlantic salmon) make only minor adjustments to territory

292 size in response to changes in food supply (Grant, Weir & Steingrímsson 2017), which could

293 explain the lack of difference in density between streams despite the difference in food abundance.

294 **4.2 | Sustained effects on genetic diversity**

295 We have demonstrated previously that higher parental nutrient levels lead to the survival of a

296 higher number of salmon families through to 3 months of age (Auer *et al.* 2018). Here we show

297 that this higher genetic diversity persists until at least 15 months of age: of the original families

298 present in a stream at the egg stage (which was the same for all streams), more families had

299 surviving representatives in the high compared to low parental nutrient streams. This indicates

300 stronger selection against particular families when nutrient levels are low (Auer et al. 2018). For

301 this experiment, eggs were artificially planted in mixed family nests. While this may have possibly

302 altered early life competition dynamics between the focal families, juveniles from different nests

303 would have invariably become mixed (and so run into competition with each other), since average

304 dispersal distances are likely to be much greater than average distances between the nests.

305 The importance of including genetic diversity in conservation management plans is becoming

306 increasingly recognised (Garcia de Leaniz *et al.* 2007; Kahilainen, Puurtinen & Kotiaho 2014).

307 Though clearly a complex issue, genetic diversity is generally linked to the adaptive potential of a

308 population (Kahilainen, Puurtinen & Kotiaho 2014), which is of particular current importance

309 given increasingly unstable environmental conditions and associated new selection pressures

310 (Hoffmann & Sgrò 2011). An additional reason for considering genetic diversity in conservation

311 management plans is that low genetic diversity can be linked to inbreeding depression and

312 population extinction (O'Grady *et al.* 2006). We still know very little about how Atlantic salmon

313 populations are impacted by genetic loss, in part due to our limited knowledge of historic genetic

314 diversity levels (Wang, Hard & Utter 2002). It has been suggested that salmon populations are

315 already under inbreeding risk by evolutionary design, since they exhibit a high degree of natal

316 homing, which in turn restricts the genetic make-up of spawning aggregates (Allendorf & Waples

317 1996). There is evidence that Atlantic salmon have evolved inbreeding avoidance mechanisms

318 and, in part, choose mates based on MHC dissimilarity (Landry *et al.* 2001). However, while

319 mechanisms such as these can help to increase the genetic diversity of offspring, they rely on there

320 being sufficient existing variation in MHC alleles, which may not be the case if low nutrient levels

321 create selection pressures that reduce the genetic variation in already small populations. Restoring

322 nutrient levels can thus have the benefit of improving genetic diversity and so increasing

323 population resilience, even if it does not boost the size of the juvenile population size.

324 **4.3 | Possible effects on later life stages**

325 The age at which juvenile Atlantic salmon transform into the smolt stage of the life cycle and

326 migrate to sea is variable, but since the seaward migration only takes place in the spring this

327 generates discrete year classes of smolts. Across the geographic range of the species, migrants

328 range in age from 1-8 years, with most rivers containing 1-4 year classes (Metcalfe & Thorpe

329 1990); those in the River Conon catchment are generally either 2 or 3 years old (McLennan et al.

330 2017). The probability of an individual smolting is governed by whether it is on course to exceed

331 a threshold body size by the time of the smolt migration; presumably because survival rates of the

332 smolts are strongly dependent on body size at the time of the migration (Jokikokko *et al.* 2006;

333 Armstrong *et al.* 2018). Those individuals that are projected to fall short of the threshold will

334 exhibit suppressed growth over winter and will delay smolting for at least a further year (Metcalfe

335 1998; Dodson *et al.* 2013). These differences in growth strategy make it is possible (with up to

336 90% accuracy) to differentiate those fish that will/will not migrate, several months prior to the

337 migration (Pearlstein, Letcher & Obedzinski 2007). Therefore, although the last sampling was

conducted in March, prior to smolts developing the characteristic silver body coloration, we can presume that the larger individuals would have smolted. Given the size distribution of migrating smolts in this river system (Malcolm, Millar & Millidine 2015), it is reasonable to assume that the majority of fish larger than 100mm when captured in March would have smolted that spring. Therefore, it is likely that the nutrient manipulation greatly boosted the proportion of fish that would have become 2- rather than 3-year-old smolts, since almost 90% of individuals in the high nutrient streams were $\geq$ 100mm in March, compared to less than 40% in the low nutrient streams.

If the simplifying assumption is made that all fish $\geq$ 100mm in March smolted at age 2, and that 80% of the remaining fish smolted at age 3 (the remainder having died or were males that failed to smolt after becoming sexually mature), then the addition of nutrients reduced the mean age of smolts from 2.56 to 2.09. In an analysis covering the geographic range of the Atlantic salmon, Metcalfe and Thorpe (1990) showed that the mean age of smolts in a river was closely related to growth conditions (measured in 'degree-hours' during which foraging could occur, being the annual sum of monthly mean temperatures above a baseline of 5.5°C (the threshold for growth) multiplied by daylight hours each month). Using the same approach as used in Metcalfe and Thorpe (1990), we calculate that at the latitude of the Conon river system the nutrient addition was the equivalent of an average increase in annual mean water temperature of 1.3°C.

How this acceleration of the freshwater phase of the life cycle might influence overall population dynamics is not clear, since there are a number of potential interacting factors. Individuals that migrate to sea after only two years in fresh water have an increased likelihood of surviving the freshwater phase of their life cycle, since age-3 smolts are subjected to freshwater mortality for an additional year. All else being equal, an increased smolt yield due to a greater proportion of age-2 smolts could cause a substantial decrease in generation time, with an associated increase in the

361   population growth rate. However, age-2 smolts are also typically smaller than those migrating a

362   year later from the same river (Jonsson, Jonsson & Hansen 1998), and so may experience higher

363   mortality during migration (Jokikokko *et al.* 2006; Armstrong *et al.* 2018), which could then

364   counteract the positive effect of increased smolt production on population size. Aspects of growth,

365   size and age during the freshwater stage of the Atlantic salmon life cycle are often correlated with

366   subsequent marine growth dynamics and the numbers of years spent at sea (Hutchings & Jones

367   1998). These interactions are complex; however, if there were a negative relationship between

368   smolt age and the duration of the marine phase (i.e. younger smolts spending an extra year or more

369   at sea), the overall generation time would not be reduced and there would be no effect on

370   population growth rate. This considered, an increased proportion of age-2 smolts could still result

371   in a net population gain via reduced inter-cohort competition within the stream (Einum *et al.* 2011).

372   Older fish are likely to be competitively superior to those of younger age classes but have differing

373   microhabitat requirements, so the impact of a reduction in the size of older cohorts is not

374   straightforward (Nislow, Armstrong & Grant 2011). However, it is possible that reduced

375   competition from older cohorts could increase the carrying capacity of younger cohorts (Nordwall,

376   Näslund & Degerman 2001), which might also increase the number of smolts being produced.

377   Though clearly complex, the results of our study suggest that restoring nutrients to the spawning

378   grounds of Atlantic salmon could have significant implications for both population dynamics and

379   fisheries management. Further work is needed to evaluate whether the gains from both increasing

380   genetic diversity and reducing the time spent by fish in fresh water are actually sufficient to offset

381   the higher mortality that individuals may then experience as a result of potentially being smaller

382   at the time of migration. However, the groundwork is now partially laid for encouraging a greater

383 consideration of how the nutrient decline of fresh waters may affect the species that live within

384 these ecosystems, and how such effects may be better managed and mitigated.

AUTHOR CONTRIBUTIONS

D.M, S.K.A., S.M., K.H.N., J.D.A. and N.B.M. conceived the ideas and designed the methodology; D.M., S.K.A., G.J.A., T.C.R., S.M., K.H.N., R.D.B., D.C.S, E.C, J.S and N.B.M collected the data; D.M., S.K.A. and R.D.B. analysed the data; D.M., S.K.A. and N.B.M. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Should this paper be accepted for publication, data sets corresponding to the statistical models would be deposited in a Dryad repository.

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585   Multiple Trophic Levels in Streamside Experimental Channels. *Transactions of the American*
586   *Fisheries Society,* **139,** 824-839.

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588 **Figure 1.** Map of River Conon catchment in Northern Scotland, including the location of study
589 streams (green circle = high parental nutrients and blue circle = low parental nutrients), key
590 hydroelectric dams, and trap for collecting returning adult salmon on their spawning migration.
591 Figure taken from Auer et al 2018.
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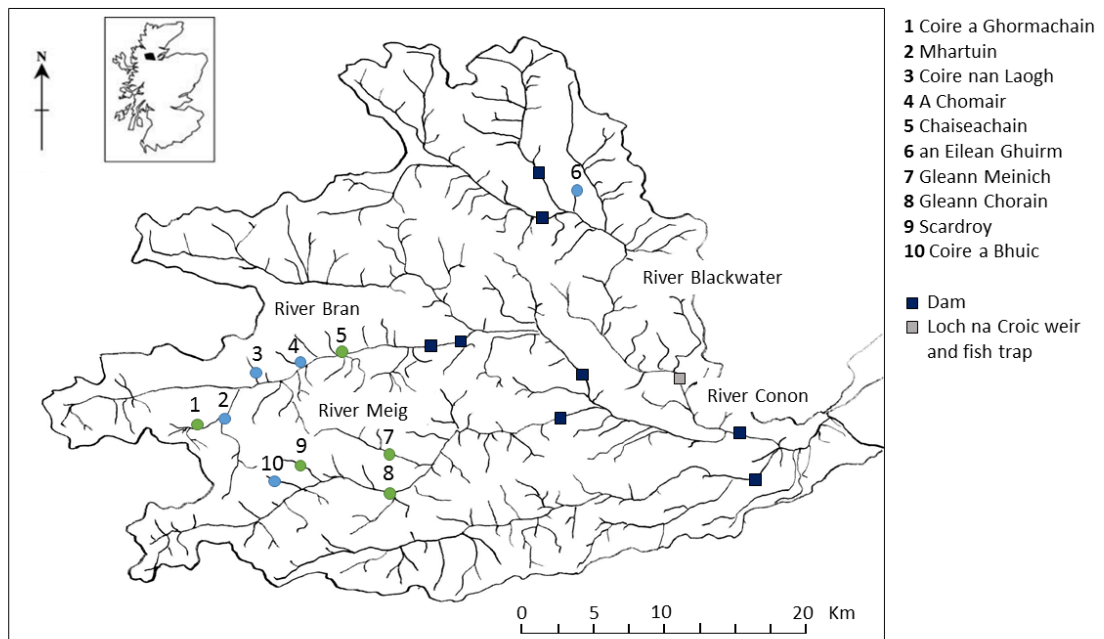
Figure 2. Mean (±1SE) macroinvertebrate prey abundance and biomass when fish were age 0 (May-June 2016) and age 1+ (March 2017) in streams with low (blue, n = 5) and high (green, n = 5) parental nutrient levels. Only invertebrates equal to or less than 1mm in width during Spring 2016 and equal to or less than 2.5 mm in width in Winter 2017 were included since they represent the maximum prey size for age 0 and wintering age 1+ juveniles, respectively. Estimates are given as the mean catch per unit effort for 1-minute samples taken at three locations at each of 50, 25, and 0 m above the downstream limit of each experimental reach. Data for May-June 2016 are from Auer et al. 2018.
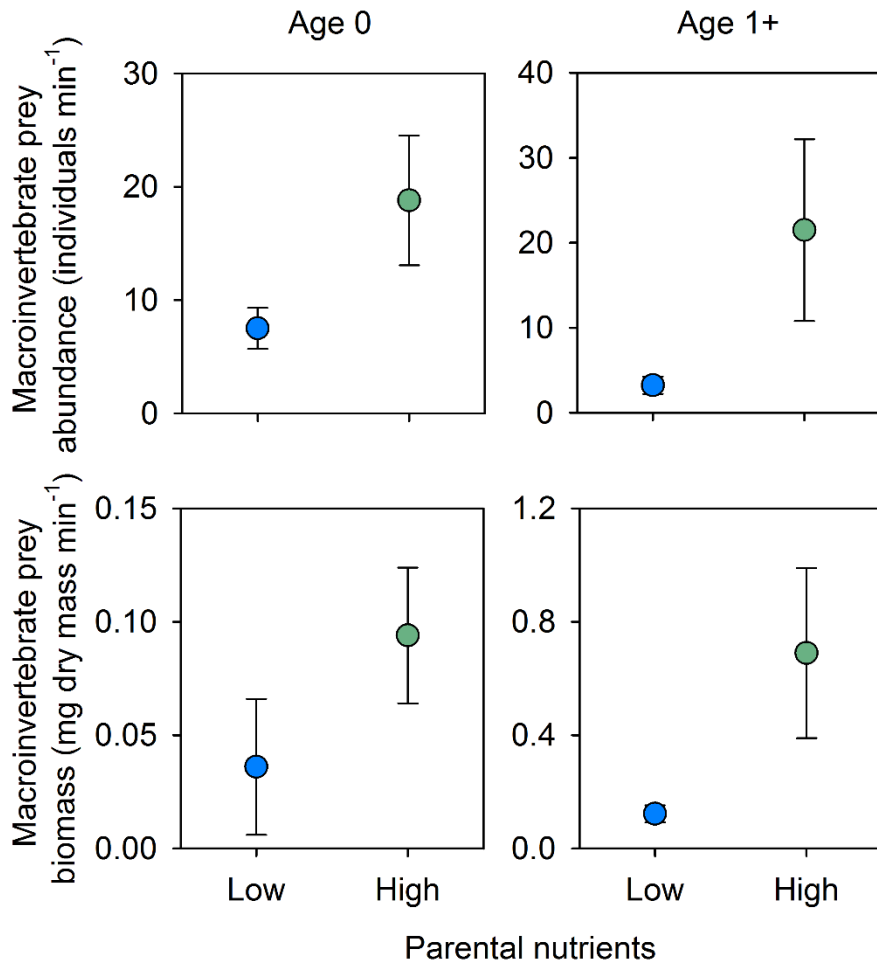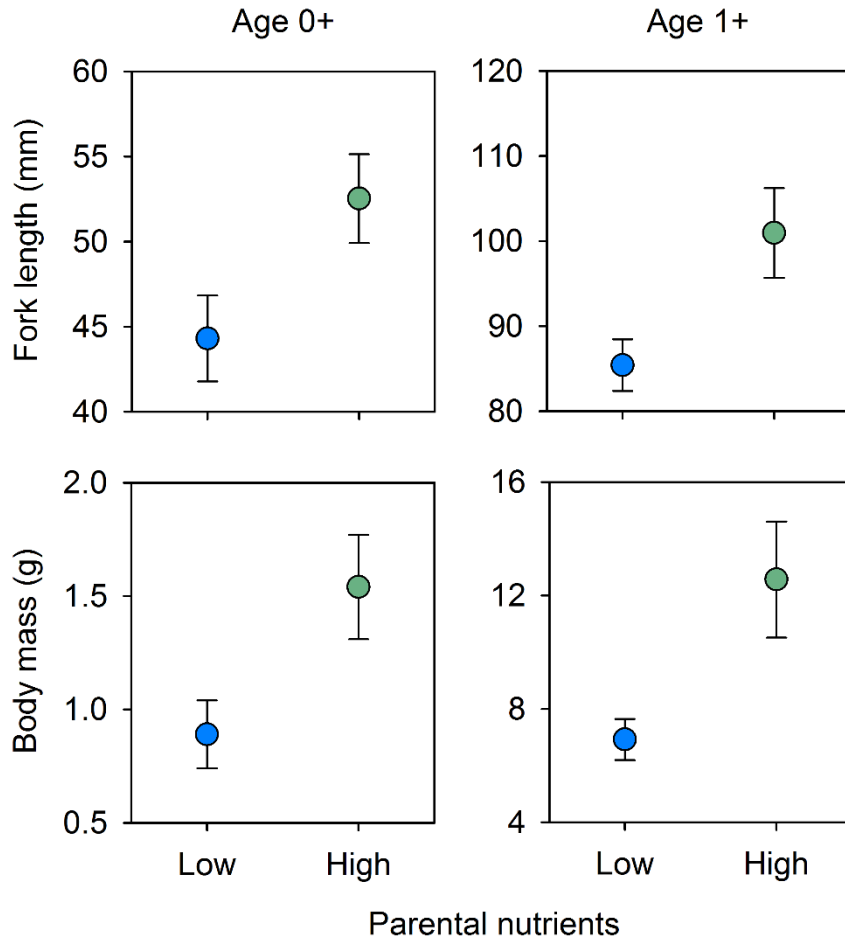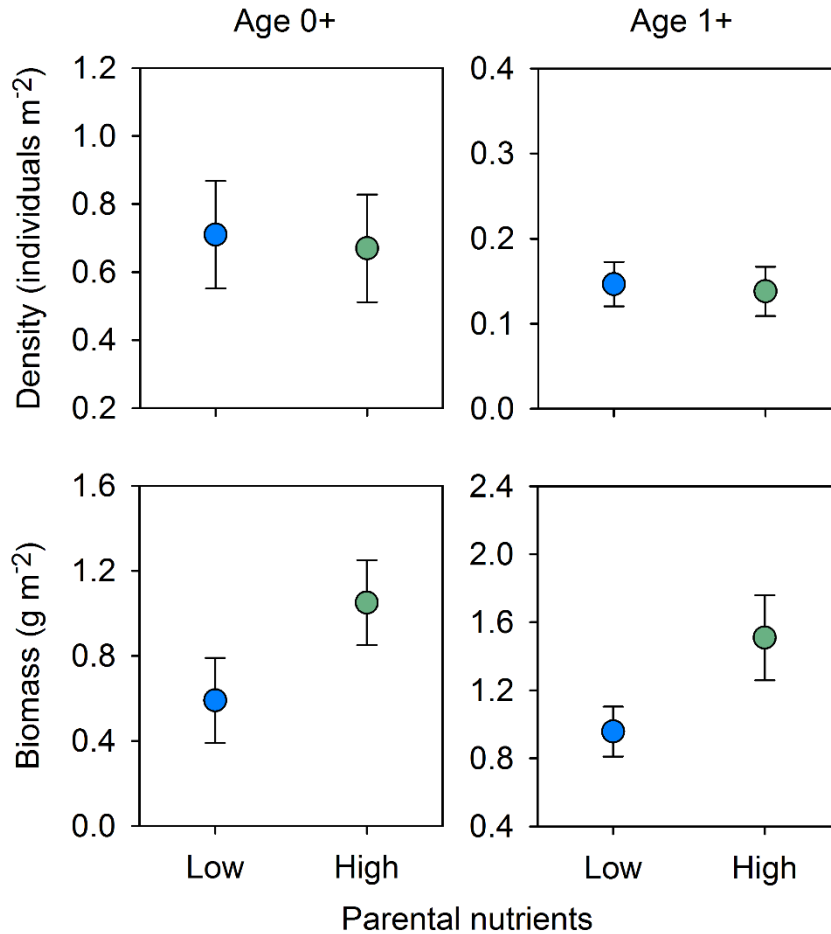
606   **Figure 3.** Mean (±1SE) fork length and body mass of juvenile Atlantic salmon (*Salmo salar*) in
607   streams with low (blue, n = 5) and high (green, n = 5) parental nutrient levels recaptured at age
608   0+ (July 2016) and age 1+ (July 2017). Data for age 0+ fish are from Auer et al. 2018.

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Figure 4. Mean (±1SE) density and biomass of juvenile Atlantic salmon (*Salmo salar*) captured at age 0+ (July 2016) and again at age 1+ (July 2017) in streams with either low (blue, n = 5) and high (green, n = 5) parental nutrient levels. Fish density for each age class was estimated from depletion curves of the number of fish captured during triple-pass electrofishing. Fish biomass was calculated as the product of the average fish body mass and the estimated density for each stream. Data for age 0+ fish are from Auer et al. 2018.

Figure 5. Distribution of fork length in streams with low (blue, n = 5) and high (green, n = 5)
parental nutrient levels. Blue represents low parental nutrients, green represents high parental
nutrients and purple represents overlaid data. The red dotted line corresponds to the minimum
smolting size for this river system.
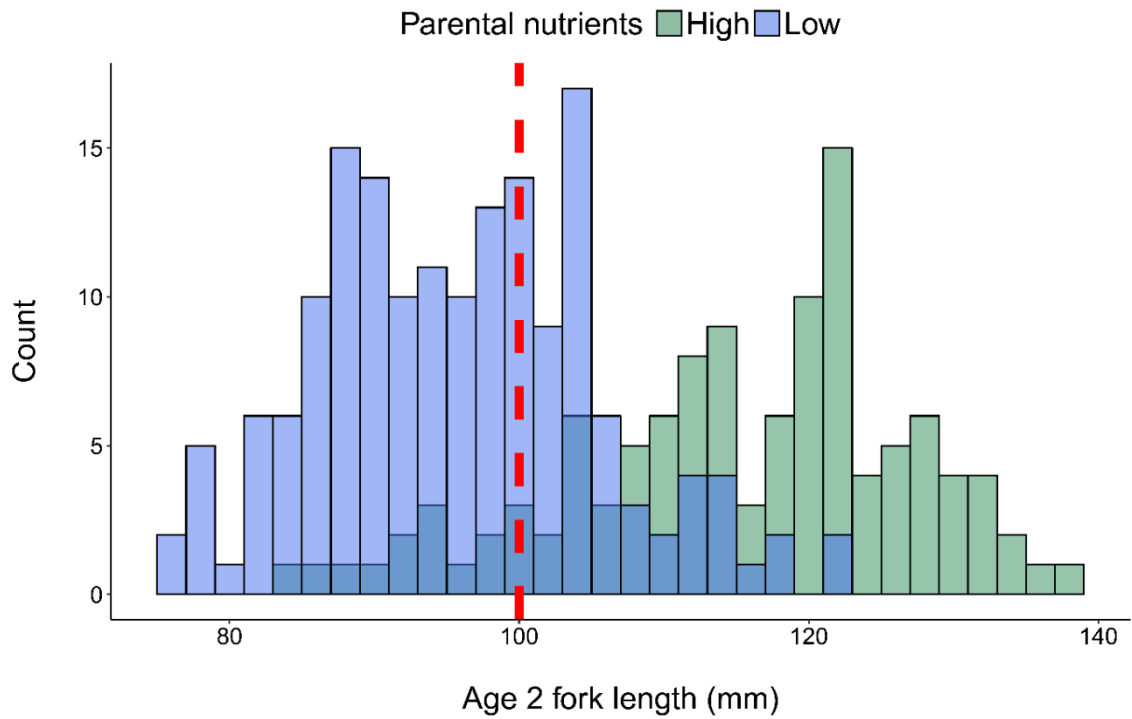
628 **Figure 6**. Genetic diversity of Atlantic salmon (*Salmo salar*) from streams with low (blue, n = 5)
629 and high (green, n = 5) parental nutrient levels. Plotted are raw estimates for the mean (±1SE)
630 number of surviving families among fish captured at age 0+ (July 2016) and age 1+ (July 2017).
631 Data include fish captured in the experimental reach as well as extra-limital areas 50m up and
632 downstream of the experimental reach. Data for age 0+ fish are from Auer et al. 2018.
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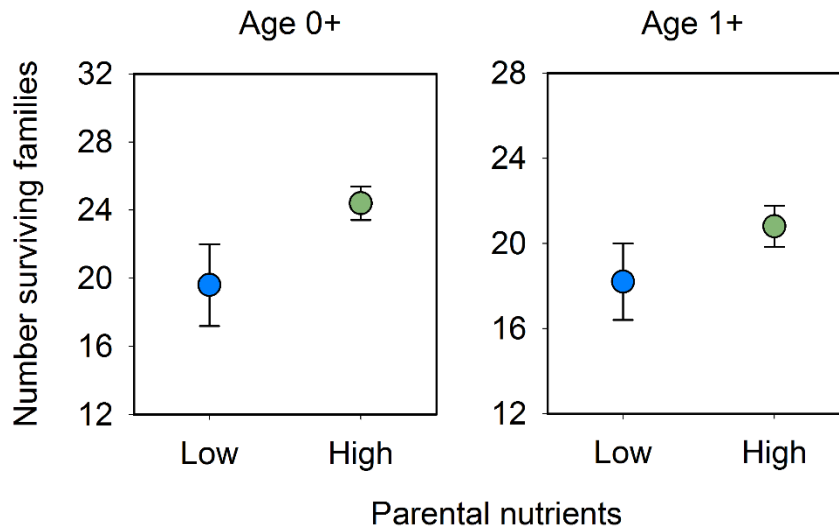
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637 # Supplementary Information corresponding to:

638 ## Simulating nutrient release from parental carcasses increases the growth,

639 ## biomass and genetic diversity of juvenile Atlantic salmon

640

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SUPPLEMENTARY METHODS

Macroinvertebrate collection and analysis

Invertebrates were collected using the electrobugging technique (Taylor, McIntosh & Peckarsky 2001), in which an electrofisher (500W electrofishing backpack system from E-fish ltd, Grange-over-Sands, UK; 350 V, 60 Hz and a 10% duty cycle) was operated for 60 s with its anode held stationary and immediately upstream from a Surber sampler (250 μm filter; EFE and GB Nets, Totnes, UK) resting on the stream substrate. This procedure was carried out at three contiguous locations at each of three positions (0, 25, and 50 m up from the downstream limit of the experimental reach) in each of the 10 streams. Macroinvertebrates collected from the three contiguous locations were preserved together in 70% ethanol. They were later identified to the family level under a dissecting microscope, enumerated to determine their abundance, and measured to the nearest 0.5mm. Length measurements were then used to determine their biomass using published length-mass regressions for the relationship between dry mass and length for each taxonomic family (Table S2; Bird & Prairie 1985; Benke *et al.* 1999; Baumgärtner & Rothhaupt 2003; Mroczyński & Daliga 2016). The area upstream from the anode was not blocked off, so abundance and biomass were defined as catch per unit effort. Only those macroinvertebrates equal to or smaller than 1 mm and 2.5 mm in width were included in estimates of prey abundance and biomass for age 0+ and 1+ fish, respectively, since those prey sizes are the maximum that gape-limited juvenile salmon can consume at their respective body sizes (Wankowski 1979). These were primarily macroinvertebrate from the Orders Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, and Diptera, all of which are known to be in the diet of juvenile salmon in the Conon and other Scottish river catchments (Table S2; Mills 1964; Maitland 1965).

Electrofishing protocols

680   A total of 907 age 0+ focal fish were captured via triple-pass electrofishing in two different sections

681   (each at least 10m in length) of riffle habitat within the experimental reach of each stream. At the

682   same time age 0+ fish outside these two sections, but within the boundaries of the experimental

683   reach (n = 365), were also captured via single-pass electrofishing to increase the family-level

684   sample sizes needed to estimate genetic diversity. In July 2017, focal age 1+ fish and non-target

685   0+ fish from the next cohort were captured using the same triple-pass electrofishing technique but

686   now covering the entire experimental reach (n = 458 1+ focal fish). Again, age 1+ fish within 50m

687   upstream and 50m downstream (n = 292) were also captured via single pass electrofishing to

688   increase family-level samples sizes needed to estimate genetic diversity. The area that was

689   electrofished increased from the first to the second year due to the decrease in density with age,

690   also since territory size increases and microhabitat use shifts from shallower riffles to deeper pools

691   as the fish grow larger (Armstrong *et al.* 2003), so requiring a larger sampling area to obtain

692   adequate sample sizes of focal fish.

693   During each sampling period, captured fish were anaesthetized with clove oil (20 ppm), weighed

694   (± 1.0 mg) and then measured for body length (± 0.01 mm). In the case of focal fish, a small section

695   of anal (age 0+) or adipose (age 1+) fin was clipped and stored in ethanol for later parental

696   assignment (see SI)). Densities of age 0+ and age 1+ fish were estimated using Microfish (Van

697   Deventer & Platts 1989).

698   The final electrofishing when the focal fish were age 2 allowed assessment of the proportion of

699   focal fish that had reached the minimum size (100mm for this river system (Malcolm, Millar &

700   Millidine 2015)) at which fish would undergo smolt transformation and migrate to sea as two year

701   olds. To maximise capture numbers, but at the expense of estimating density, we captured fish via

702   single-pass electrofishing, both within and ~100m below the experimental reaches. Captured fish

703   were anaesthetized, weighed and measured as before, and a small fin clip was again taken for

704   parental assignment. This survey was timed to be at least one month prior to the onset of the main

705   smolt migration period in the River Conon catchment (McLennan *et al.* 2017; McLennan *et al.*

706   2018), but it is possible that smolting individuals may have already started moving down from our

707   study streams into the main river (McCormick *et al.* 1998), so results need to be interpreted with

708   caution. In total, 306 age-2 fish were captured across our 10 experimental streams, and genotyping

709   (see SI) confirmed that 282 of these individuals came from our focal families.

710   **Genotyping and parental assignment**

711   Captured fish of age classes 0+ and 1+ were genotyped and assigned to a specific focal family by

712   commercial suppliers (Landcatch Natural Selection Ltd, Stirling, Scotland). In brief, an E-Z 96

713   tissue DNA Tissue kit (Omega Bio-Tek, Georgia, USA) was used to extract DNA from the fin

714   clips of parental fish and captured progeny. Fish were then genotyped using a panel of 110

715   informative SNP markers scattered across the genome using individual end point PCR assays

716   (KASP TM technology, UK). Parentage assignment by exclusion was carried out blind to

717   experimental treatments with the programme Vitassign 8.3 (Vandeputte, Mauger & Dupont-Nivet

718   2006) with some modifications to allow the analysis of more than 100 markers.

719   Captured fish of age class 2+ were genotyped and assigned to a specific focal family by

720   Marine Scotland – Science, Pitlochry, Scotland. DNA was extracted from fin clips using a Chelex

721   protocol (Walsh, Metzger & Higuchi 1991). Samples were screened at a panel of 96 polymorphic

722   SNPs, using a SNPtype assay on a Fluidigm EP1 platform (Fluidigm, UK), according to

723   manufacturer's protocols. Parentage assignment was carried out in Colony 2.0.5.0 (Jones & Wang

724   2010) assuming polygamy, using a 0.95 probability of the parent being included in the dataset and

725   allowing for 0.001 marker error rate. Genotyping successfully assigned almost all fish to parents

726   (98% at 0+, 97% at 1+ and 92% at age 2, the decrease presumably a consequence of greater

727   movements of fish from other stocked non-experimental streams).

728   **Second fish cohort**

729   Methods used to create families for the second cohort were the same as used for the focal cohort,

730   with the exception that parents were not genotyped. Eggs were drawn from a random sample of

731   eggs in the hatchery and planted out planted out in the same numbers and locations as described

732   for focal fish. At age 0+, fish were captured via triple-pass fish (n = 1830) of the entire experimental

733   reach (as described for age 1 fish). Analyses of growth, density, and biomass were the same as

734   described for focal cohort.

SUPPLEMENTAL TABLES AND FIGURES

736

737 **Table S1**. Location, sampling times, and characteristics of ten study streams in the northern highlands of Scotland. Nutrients refers to
738 whether streams had low or high parental nutrient levels; the latter each received carcass-analogs in an amount equivalent to 25 salmon
739 carcasses.

740

Stream	Location (N Lat./W Long.)	Nutr-ient	Egg / Carcass 2016	Invert 2016	Fish 2016	Egg/ Carcass / Invert 2017	Fish 2017	Fish 2018
A Chomair	57.595, 5.002	Low	7 Mar	31 May	15 July	13 Mar	19 July	8 March
An Eilean Ghuirm	57.706, 4.696	Low	25 Feb	31 May	11 July	1 Mar	12 July	17 March
Chaiseachain	57.598, 4.949	High	25 Feb	8 June	12 July	3 Mar	17 July	9 March
Coire a Ghormachain	57.549, 5.129	High	3 Mar	4 June	12 July	3 Mar	22 July	13 March
Coire nan Laogh	57.581, 5.059	Low	3 Mar	4 June	16 July	3 Mar	16 July	14 March
Coire a Bhuic	57.513, 5.001	Low	26 Feb	2 June	13 July	1 Mar	14 July	22 March
Gleann Chorain	57.500, 4.916	High	26 Feb	7 June	21 July	1 Mar	21 July	16 March
Mhartuin	57.555, 5.096	Low	3 Mar	4 June	24 July	3 Mar	20 July	12 March
Scardroy	57.519, 4.992	High	26 Feb	2 June	22 July	1 Mar	18 July	22 March
Gleann Meinich	57.543, 4.935	High	26 Feb	10 June	14 July	28 Feb	13 July	25 March

741

Table S2. Published coefficients for length-mass equations (DM = aLb where DM = dry mass in mg and L = length in mm) for aquatic larvae from macroinvertebrate families in five different taxonomic orders.

	Coefficients		
	a	*b*	Reference
Ephemeroptera			
Baetidae	0.00530	2.875	Benke et al. 1999
Heptageniidae	0.01080	2.754	Benke et al. 1999
Plecoptera			
Nemouridae	0.00560	2.762	Benke et al. 1999
Taeniopterygidae	0.00720	2.665	Benke et al. 1999
Leuctridae	0.00280	2.719	Benke et al. 1999
Chloroperlidae	0.00650	2.724	Benke et al. 1999
Trichoptera			
Hydropsychidae	0.00460	2.926	Benke et al. 1999
Hydroptilidae	0.01220	2.570	Baumgartner and Rothhaupt 2003
Psychomyiidae	0.00390	2.873	Benke et al. 1999
Coleoptera			
Elmidae larvae	0.00740	2.879	Benke et al. 1999
Hydrophilidae	0.01760	2.580	Mroczyński and Daliga 2016
Diptera			
Chironomidae	0.00180	2.617	Benke et al. 1999
Simuliidae	0.00200	3.011	Benke et al. 1999
Empididae	0.00540	2.546	Benke et al. 1999

759 **Figure S1. Outline of experimental timeline.**

| December 2015	March 2016	May/June 2016	July 2016	December 2016	March 2017	July 2017	March 2018
IVF Cohort 1	Plant out eggs and carcasses	Invertebrate sampling	Age 0+ electro-fished	IVF Cohort 2	Plant out eggs and carcasses; invert sampling	Age 0+ and 1+ electro-fished	Age 2+ electro-fished
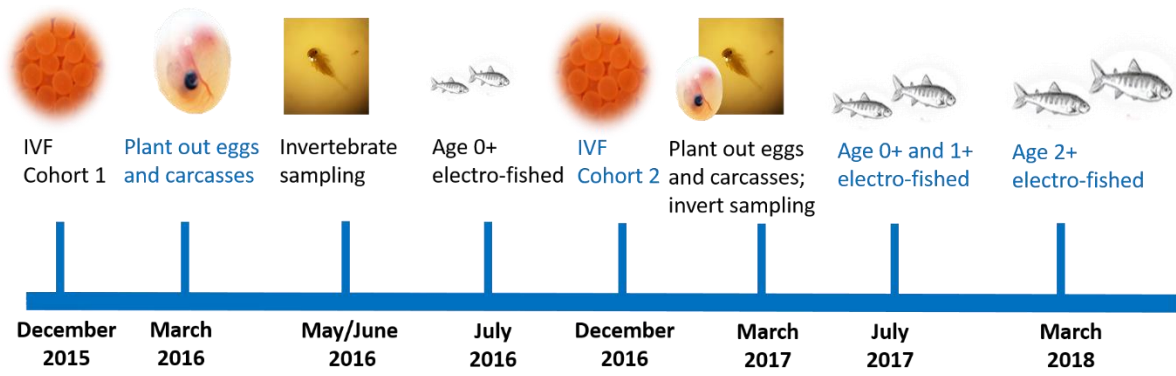
760

761 **Figure S2.** Temporal changes in mean (±1SE) daily water temperature in 8 study streams with
762 either low (n = 4, blue) or high (n = 4, green) nutrient levels in the northern highlands of
763 Scotland. Temperatures were measured from the time eggs were planted out to their recapture
764 as age 1+ parr.

765
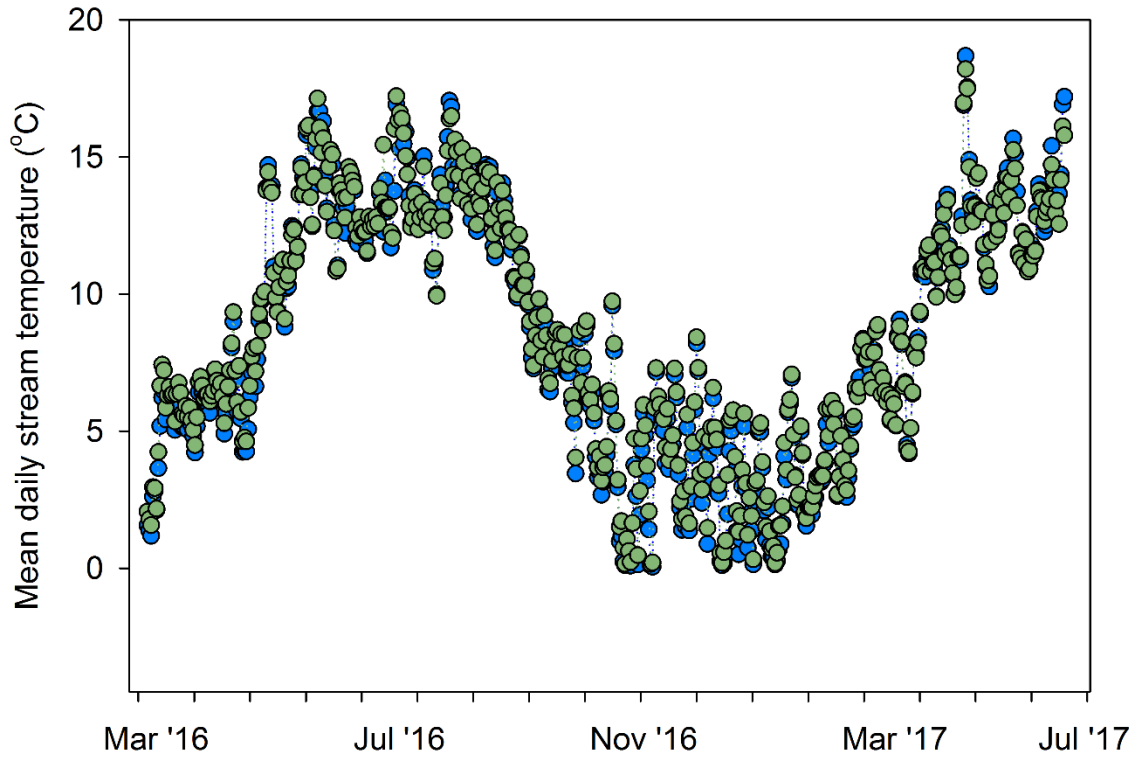
766

767 **Figure S3**. Mean (±1SE) (a) fork length as an index of growth, (b) density, and (c) biomass of
768 a second cohort of juvenile Atlantic salmon (*Salmo salar*) in streams with either low (blue, n
769 = 5) or high (green, n = 5) parental nutrient levels. Fish were captured in July 2017 at age 0+.
770 Fish density was estimated from depletion curves of the number of fish captured during triple-
771 pass electrofishing. Fish biomass was calculated as the product of the average fish body mass
772 and the estimated density for each stream. Both length (p < 0.001) and biomass (p = 0.012),
773 but not density (p = 0.200) were higher in streams with high compared to low parental nutrient
774 levels.

775
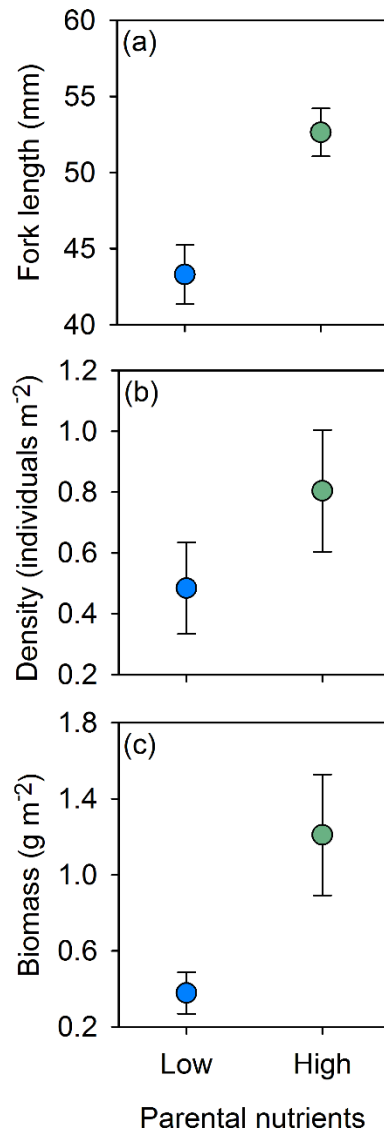
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