Plasticity in response to feed availability - does feeding regime influence the relative growth performance of domesticated, wild and hybrid Atlantic salmon *Salmo salar* parr?

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Interactions of farm and wild Atlantic salmon

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ABSTRACT

Growth of farmed, wild and F1 hybrid Atlantic salmon parr, *Salmo salar*, was investigated under three contrasting feeding regimes in order to understand how varying levels of food availability affects relative growth. Treatments consisted of standard hatchery feeding (*ad libitum*), access to feed for 4h every day, and access to feed for 24h on three alternate days weekly. Mortality was low in all treatments, and food availability had no effect on survival of all groups. The offspring of farmed *S. salar* significantly outgrew the wild *S. salar*, while hybrids displayed intermediate growth. Furthermore, the relative growth differences between the farmed and wild *S. salar* did not change across feeding treatments, indicating a similar plasticity in response to feed availability. Although undertaken in a hatchery setting, these results suggest that food availability may not be the sole driver behind the observed reduced growth differences found between farmed and wild fishes under natural conditions.

Key words: Escapees, Farmed, Food availability, Genetic interaction, Hybridisation, Reaction norms

INTRODUCTION

Aquaculture is undergoing rapid expansion on a global scale. However, there is increasing evidence of a diverse array of negative consequences on both the natural environment and wild fish stocks (Naylor *et al.*, 2000; McGinnity *et al.*, 2003; Heuch *et al.*, 2005). To ensure the sustainability of aquaculture, especially at a time when many natural populations continue to decline, greater understanding of the threats to wild populations and potential mitigation strategies is required. Specifically for Atlantic salmon *Salmo salar* (Linnaeus 1758) aquaculture, one of the world´s most socio-economically important farmed fishes, several challenges to sustainability have been identified, including, parasitic sea lice *Lepeophtheirus salmonis* (Krøyer 1837) and farm escapees (Taranger *et al.*, 2014).

Each year, numerous farmed *S. salar* escape into the wild. While most escapees fail to recruit (Skilbrei *et al.*, 2014), some enter rivers and attempt to spawn with wild *S. salar* (Lura and Saegrov, 1991; Webb *et al.*, 1993; Saegrov *et al.*, 1997). Following successful spawning, genetic changes in native salmonid populations have been demonstrated in Ireland (Crozier, 1993; Clifford *et al.*, 1997), Canada (Bourret *et al.*, 2011) and Norway (Skaala *et al.*, 2006; Glover *et al.*, 2012; 2013). Wild salmonid populations may be locally adapted to their native rivers (Taylor, 1991; Garcia de Leaniz *et al.*, 2007; Fraser *et al.*, 2011), and experimental studies have demonstrated that offspring of farmed *S. salar* display significantly reduced survival in the wild compared to wild *S. salar* offspring (McGinnity *et al.*, 1997; Fleming *et al.*, 2000; McGinnity *et al.*, 2003; Skaala *et al.*, 2012). Such findings indicate that interbreeding of farmed escapees with wild fishes is likely to inflict a negative fitness effect upon the native population.

In addition to domestication selection (Glover *et al.*, 2004), aquaculture species typically undergo directional selection for a variety of commercially important traits, for example increased growth and late maturation (Gjedrem, 2000; 2010; Thodesen and Gjedrem, 2006). The hatchery environment is typically characterised by high densities, a lack of predation, and continuous feed availability. Farmed *Salmo salar* have exhibited changes in behavioural traits such as increased aggression, higher stress resistance and decreased predator awareness that are attributed to inadvertent selection resulting from the artificial hatchery environment (Einum and Fleming, 1997; Fleming and Einum, 1997; Houde *et al.*, 2010*a*; *b*; Solberg *et al.*, 2013*a*; Debes and Hutchings, 2014). Thus, direct and indirect selection has resulted in domesticated fishes that are adapted to their captive environment and that typically display traits which may be maladaptive in the wild relative to their wild counterparts.

Since *S. salar* farming began in the late 1960s, domestication selection has been primarily directed at growth, with gains of up to 15% per generation seen in farmed *S. salar* (Gjedrem *et al.*, 1991; Thodesen and Gjedrem, 2006). Increased growth has been linked to an increased appetite and food conversion efficiency in farmed *S. salar* (Thodesen *et al.*, 1999; Gjedrem, 2000). Growth is mediated by the growth hormone (GH) in most vertebrates, including fish (Björnsson, 1997). Studies have documented higher levels of GH (Fleming *et al.*, 2002) and IGF-I (insulin-like growth factor I) (Solberg *et al.*, 2012; although no changes were detected in Bicskei *et al.*, 2014) in farmed *S. salar* compared to wild conspecifics, suggesting that selection for growth in farmed fishes stimulates shifts in endocrine control. Growth hormone influences appetite, feed conversion efficiency, foraging behaviour (through increased movement and risk taking), and may influence aggression (Neregård *et al.*, 2008*a*; *b*). Farmed *S. salar* exhibit differences relative to wild *S. salar* in all of the above behavioural traits (Fleming and Einum, 1997; Thodesen *et al.*, 1999; Houde *et al.*, 2010*a*), supporting the endocrine findings of Fleming *et al*. (2002) and Solberg *et al*. (2012). Increased GH levels are also linked to a higher metabolism (Björnsson, 1997). It has been suggested that higher levels of growth may incur a metabolic cost when resources are low or predation levels are high, such as in the wild (Sundt-Hansen *et al.*, 2009). For example Sundt-Hansen *et al*. (2012) found that while GH-treated *S. salar* grew optimally under standard hatchery conditions (*ad libitum* feeding) their growth was negatively affected by the GH treatment under natural stream conditions.

When studied under hatchery conditions, growth differences of up to 2-3 fold exist between offspring of farmed and wild *S. salar* (Fleming and Einum, 1997; Glover *et al.*, 2009; Solberg *et al.*, 2013*a*; *b*). In contrast, studies in the wild have shown that growth differences between farmed and wild *S. salar* are lower than in hatchery-reared *S. salar* (Fleming *et al.*, 2000; Skaala *et al.*, 2012). Thus, the question arises: what causes such differences in the relative growth rates of wild and farmed *S. salar*? Several potential explanations exist, including behavioural changes associated with higher growth in farmed *S. salar*, such as less efficient foraging behaviour, increased aggression and higher risk behaviour. Such behaviours will incur a higher metabolic cost, thus, while faster growth is often linked to higher fitness, such behavioural-mediated trade-offs may limit growth and survival of individuals with higher growth rates in the wild through reduced starvation tolerance and increased predation risk (Martin-Smith *et al.*, 2004; Biro *et al.*, 2006). An especially pertinent factor influencing growth differences between farmed and wild fishes is variation in resource availability, specifically levels of food availability between the hatchery and the wild. Under standard hatchery conditions feed is readily available, and thus not limiting growth, while the frequency and nature of food in the wild is often more heterogeneous in time and space (Jonsson and Jonsson, 2011). It is possible that generations of direct and inadvertent domestication selection in farmed fishes will have resulted in a decreased ability to cope with the typically variable feed availability in the wild environment. Elucidating the factors influencing the ability of escaped farmed fishes in the wild to forage effectively crucially represents a key component of risk assessment.

In order to elucidate the potential mechanisms underlying the observed larger growth rate of farmed vs. wild fishes in the hatchery, contrary to trends detected in the wild, here the influence of varying levels of food availability on relative growth performance was examined. Growth of farmed, wild and F1 hybrid *S. salar* under three feeding regimes differing in availability and frequency of feed were examined under hatchery conditions. A gradient of feed availability were selected, ranging from the farmed environment (*ad libitum*) towards the wild environment (patchy and restricted).

MATERIALS & METHODS

FAMILY PRODUCTION

The farmed, hybrid and wild *S. salar* families used in this study were produced in November 2013 (week 46) at Matre Research station, Institute of Marine Research (IMR), Norway. *Salmo salar* originating from the commercial farmed Mowi strain, and wild *S. salar* caught in the River Etne (59°40'N, 5°56'E), were used to produce seven pure farmed, seven pure wild, and seven F1 hybrid families (SI Table I). Mowi represents one of the oldest Norwegian domestic *S. salar* strains (Gjedrem *et al.*, 1991) and has been selected for, among other traits, increased growth rate, and is known to display significantly higher growth rates under standard hatchery conditions in comparison with the offspring of wild *S. salar* (Glover *et al.*, 2009; Solberg *et al.*, 2013*a; b*). However, in the wild, this farmed strain only displays slightly higher growth rates than the offspring of wild *S. salar* (Skaala *et al.*, 2012). The three strains are from here on referred to as farmed, wild and hybrid groups.

The *S. salar* stock in the River Etne is the largest in Hordaland, Norway. Wild adult broodstock were collected in this river in the autumn of 2013 by angling, and transferred to the local hatchery and held until stripping of gametes. Fish scales were read on individuals to validate that they were indeed born in the wild and were not farmed escapees (Lund and Hansen, 1991). The F1 hybrid *S. salar* were produced by crossing farmed females and wild males (Mowi ♀ x Etne ♂). Five of the seven hybrid families were maternal and paternal half-siblings with the farmed and wild families, respectively. One hybrid family was paternal half-siblings to one wild family and one hybrid family was maternal half-siblings to one farmed family.

Eyed eggs from families were sorted into hatchery trays representing the single-strain replicate treatments in week 5 of 2014. Each replicate treatment consisted of 20 eggs per family of each group, yielding 140 eggs in each of 18 tanks. Each replicate was start-fed and thereafter reared in 1.5 m^3 tanks at ambient water temperature (varying from 12.5 to 13°C during the experimental period). The treatments began when start feeding commenced in week 18, with fish fed on Skretting Nutra pellets (www.Skretting.com), which were size adjusted according to manufacturer's tables. The *S. salar* were kept on a 24 h photoperiod from transfer to tanks until experiment termination as per standard hatchery conditions, also known to reduce the development of precocious males (Good *et al.*, 2015).

EXPERIMENTAL DESIGN

Salmo salar were reared in single-strain treatment tanks (two replicates/ treatment) with three contrasting feeding regimes (Table I). The first treatment was regarded as the standard hatchery control, and involved feeding *S. salar* continually with automatic feeders 24 h a day, every day, with an excess ration. The second treatment consisted of providing *S. salar* with an excess ration for 4 h every day (thus 20 h without any feeding each day), referred to as the daily restricted treatment. The third treatment involved feeding an excess ration for 24 h on three alternative days in a week (Monday, Wednesday, and Friday), referred to as the triweekly treatment. The selected gradient of feed availability, ranging from the farmed environment (*ad libitum*) towards the wild environment [patchy and restricted (Jonsson and Jonsson, 2011)] was designed in order to elucidate how growth differences between strains change with variable levels of food availability. Thus, treatments were chosen to represent a gradient in feeding opportunity from standard excess hatchery ration (treatment 1) to a more limited feed supply (treatment 3). Treatments are referred to as the control, daily restricted and triweekly treatments respectively.

The experiment was continued for 20 weeks, and terminated in week 37, 2014 *i.e.* *S. salar* were reared from egg to the parr stage. Upon termination, all *S. salar* in each tank replicate were euthanised with an overdose of Finquel® Vet anaesthetic (http://www.aqui-s.com, Årnes, Norway), and recordings of individual wet mass and fork length (L_F) were measured. A total of 2329 individuals were sampled.

STATISTICAL ANALYSIS

Statistical analysis was carried out using R version 3.2.2, and all critical P-values were set to 0.05 unless otherwise stated (R Core Team, 2014).

Mortality from week 5 (sorting into hatchery trays) to week 18 (commencement of experimental treatments) was low overall (<0.02%). Mortality for each tank was recorded during the experimental period. To investigate whether different feeding regimes or group origin had any effect on survival, a generalized linear mixed effect model (GLMM) was fitted using the *glmer* function in the *lme4* package (Bates *et al.*, 2014). The full model included the fixed covariates of group (G = representing the three groups; farmed, hybrid, and wild), treatment (T = representing the three feed treatments; control, daily restricted, and triweekly), and their interaction term (T_G). Tank was included in the model as a random intercept covariate (b_t):

$$\text{logit}(Y) = \beta_0 + \beta_1 T + \beta_2 G + \beta_3 T_G + b_t + \varepsilon \qquad (1)$$

where β_0 is the model intercept and ε is a random error term. The response variable, survival, was binary, and thus a binomial distribution was used, with the default logit link function. The random effect structure was investigated by plotting the 95% prediction intervals of the random effect using the *dotplot* function of the lattice package. If any of the tanks did not overlap zero, the effect was retained in the model. The *mixed* function from the afex package was used to investigate the significance of the fixed covariates (Singmann and Bolker, 2014). The function calculates type 3-like *P*-values for each fixed covariate based on parametric bootstrapping (Singmann and Bolker, 2014).

A linear mixed model (LME) was used to investigate the effect of group origin and feeding regime treatment on mass at termination. The response variable was logged mass at termination. The full model covariates were identical to the mortality model described above:

$$Y = \beta_0 + \beta_1 T + \beta_2 G + \beta_3 T_G + b_t + \varepsilon \text{ where } \varepsilon \sim N\ (0, \sigma 2) \qquad (2)$$

where β_0 is the model intercept and ε is the normally distributed error term. The LME model was fitted using *lmer* from the *lme4* package in R (Bates *et al.*, 2014). The random effects structure was investigated as described above; similarly the *P*–values for the fixed effects were calculated as above while using the Kenward-Roger approximation for degrees of freedom.

Post-hoc multiple comparisons were carried out using the function *pairs* in the *lsmeans* package with a Tukey adjustment for multiple comparisons, which calculates the differences of least squares means for the factor covariates of the fixed part of the final model (Lenth, 2015). The test computes all pair-wise comparisons of the interaction terms (Group x Treatment), and reports *P*-values and 95% confidence intervals for all comparisons (Lenth, 2015).

ETHICAL STATEMENT

The experimental protocol (permit number 6447) was approved 23 March 2014, by the Norwegian Animal Research Authority (NARA). All welfare and use of experimental animals was performed in strict accordance with the Norwegian Animal Welfare Act. In addition all personnel involved in this experiment had undergone training approved by the Norwegian Food Safety Authority, which is mandatory for all personnel running experiments involving animals included in the Animal Welfare Act.

RESULTS

SAMPLING & DATA

The experiment was terminated in week 37 of 2014, when 2329 *S. salar* were sampled from the 18 tanks. Five individuals were identified as outliers due to extreme condition factors caused by recording errors and removed from the dataset prior to statistical analysis, thus the final dataset consisted of 2324 *S. salar*.

MORTALITY

Overall, mortality within each treatment was low, ranging from 3.2 to 10.4 % (Table I), typically within the range observed from start-feeding to first autumn stage. None of the fixed effects were found to be significant (Table II), thus mortality did not differ between treatments or between strains. The random effect of tank replicate was found to be significant and thus controlled for by being retained in the final model.

GROWTH

All *Salmo salar* (*i.e.*, farmed, hybrid and wild) grew better in the control treatment than in the two more restricted treatments, and growth within all groups was observed to be lowest in the daily restricted treatment (Table III; Fig. 1). Farmed *S. salar* were larger than both hybrid and wild *S. salar* at each treatment, and the hybrids displayed intermediate growth (Fig. 1). There was a marginally significant treatment-by-group interaction effect detected (*P*=0.05); however the relative growth differences between the groups across treatments were very similar (SI Table II, Fig. 2). The relative growth differences between the wild and farmed *S. salar* were almost identical across treatments (1:1.5-1.6), as were the relative growth differences between hybrid and farmed *S. salar* (1:1.2-1.3) (SI Table II, Fig. 2). Relative growth differences between the wild and hybrid *S. salar* increase incrementally from the daily restricted treatment (1:1.2) through the control treatment (1:1.3) to the triweekly treatment (1:1.4) (Fig 2), which is probably driving the marginally significant interaction of group and treatment in the LME model (*P*=0.05). Possible variation between tank replicates was taken into account in the initial model by including replicate as a random effect which was retained in the final model despite the model output suggesting it be dropped due to lack of effect.

DISCUSSION

The present study investigated the effect of feed variability on growth and survival of farmed, wild and F1 hybrid *S. salar* reared in single strain tanks. Understanding how farmed escapees interact with wild conspecifics is an important part of developing management and mitigation efforts for both conservationists and the aquaculture industry. In the hatchery, farmed *S. salar* typically outgrow wild *S. salar* markedly (Fleming and Einum, 1997; Glover *et al.*, 2009; Solberg *et al.*, 2013*a; b*), while in the wild, corresponding growth differences are much lower

(Fleming *et al.*, 2002; Skaala *et al.*, 2012). A striking difference between the farm and wild environments is the levels of food availability; constant *versus* varying in time and space (Jonsson and Jonsson, 2011). It is possible that plasticity in response to variable feed supply differs between farmed and wild fishes, which may potentially contribute to the contrast in growth differences observed between farmed and wild fishes in each environment. Here, although a marginally significant interaction was found between group and treatment, similar growth differences were observed between the farmed and wild *S. salar* across the feed availability gradient ranging from the farmed environment (*ad libitum*) to conditions more resembling the wild environment (patchy and restricted). Thus *S. salar* of both origins responded in a comparable manner relative to the varying levels of food availability, indicating a similar plasticity in response to feed availability. Mortality was low both within and among the treatments, indicating no effect of treatment or group origin on survival.

River environmental conditions, such as fluctuating natural food availability, can adversely affect the growth of fast growing fishes due to metabolic costs (Sundt-Hansen *et al.*, 2012). In the present study growth of the farmed, hybrid and wild *S. salar* decreased along a food availability gradient ranging from the farmed environment to conditions more resembling the fluctuating levels in the wild. Lowest growth was observed in the daily restricted feeding regime -the most variable food availability. Growth was significantly different between the groups at all treatments, indicating an effect of feed availability on growth in all groups. Farmed *S. salar* were significantly larger than the wild *S. salar* in all treatments, and hybrid growth was intermediate between the farmed and wild *S. salar*. Despite differing growth rates, farmed and wild *S. salar* responded identically to the increasingly variable food supply, as shown by the similar relative growth differences and low mortality observed across the treatments. This indicates that more than 10 generations of directional selection with contentious access to feed has not resulted in farmed *S. salar* displaying reduced abilities to cope with fluctuating and/or restricted levels of feed by not being able to maintain their elevated growth rate as compared to wild *S. salar*. Morris *et al*. (2011) found that the response to compensatory growth (CG) in farmed, wild and hybrid (including backcrossed) *S. salar* was similar between the groups, although the mean control and CG growth rates were highest in the farmed group. This indicates that although selection has acted on growth, farmed *S. salar* have not lost their plastic ability to respond to a lack of food through compensation by increasing their growth rates when food becomes available (Morris *et al.*, 2011).

The growth differences between farmed and wild *S. salar* observed in all treatments were, on average, less than previously documented in hatchery studies (Glover *et al.*, 2009; Solberg *et al.*, 2013*a; b*). It is still evident however that multiple generations of selection for growth in farmed *S. salar* have resulted in significant elevated growth relative to wild *S. salar*. Under typical hatchery conditions, where food supply is constant, generally uniform and plentiful, growth differences between farmed and wild *S. salar*, as much as 3- to 5-fold, have been observed (Solberg *et al.*, 2013*a; b*). Glover *et al*. (2009) investigated various trait differences between farmed, wild and F1 hybrid *S. salar* throughout the farming production cycle, including growth. For two experimental cohorts they found that at the freshwater stage the wild *S. salar* had mean weights of 1:1.6 and 1:2.4 relative to the farmed *S. salar*. However in nature, farmed and wild *S. salar* grow more similarly. For example, Skaala *et al*. (2012) found growth differences within three year classes of wild and farmed *S. salar* in the wild to be just 1:1.07, 1:1.25 and 1:1.06 respectively. In an attempt to understand these growth differences, Solberg *et al*. (2013*b*) investigated the competitive balance between farmed, wild and hybrid *S. salar* by comparing growth in standard hatchery conditions, and restricted feed conditions in the hatchery and semi-natural environments. They found that the growth of farmed, hybrid and wild *S. salar* became more similar as their environmental conditions approached natural conditions. They hypothesised that the reduced growth differences observed in their study and in the wild (Skaala *et al.*, 2012) could be due to size-selective mortality. The wild environment favours the survival of faster growing individuals which can out-compete smaller individuals for resources (negative size-selective mortality), while also selecting against larger risky individuals through mortality by predation (positive size-selective mortality). Positive size-selective mortality was, however, not tested directly in their study (Solberg *et al.*, 2013*b*). Biro *et al*. (2006) demonstrated under natural conditions that domestic rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792), were able to grow faster than their wild conspecifics due to increased foraging behaviour, and that these larger *O. mykiss* were more susceptible to predation due to higher risk behaviour linked to foraging (Biro *et al.*, 2006). Although studies indicate reduced predator awareness (Houde *et al.*, 2010*b*) and potentially increased tolerance to predation stress (Fleming and Einum, 1997; Debes and Hutchings, 2014) in farmed relative to wild salmonids, no explicit evidence has been found for increased predator susceptibility in farmed *S. salar* (Skaala *et al.*, 2014; Solberg *et al.*, 2015). In the wild, faster growing farmed *S. salar* may also incur a metabolic cost through behavioural changes such as increased appetite (Thodesen *et al.*, 1999) and foraging (Biro *et al.*, 2006) which result in their expending more energy searching for food under low food availability conditions, leading to lower growth (Sundt-Hansen *et al.*, 2009). The juxtaposition of these potential positive and negative size-selective forces may partly explain why growth differences seen in the wild are not as pronounced as in the hatchery environment (Solberg *et al.*, 2013*b*).

Growth in the wild may also be influenced by other environmental factors, such as density and competition (Einum and Fleming, 1997; Bohlin *et al.*, 2002), and even natural stream conditions like substrate composition and flow rate (Jonsson and Jonsson, 2011). In comparative studies inter-strain competition between farmed, wild and hybrid groups could potentially influence the levels of relative growth differences observed. Thus, as the groups were reared in separate tanks, the lack of inter-strain competition in the present study may potentially explain the lower relative growth differences observed. A study examining the relative growth differences of the same groups of farmed, hybrid and wild *S. salar* in both single strain and common garden experiments however found no difference in the relative growth differences across experimental designs (Solberg *et al.*, 2013*b*). Therefore, it is concluded that the present experimental design is unlikely to drive the lower relative growth differences, and any potential tank effects were controlled for in the statistical model.

Based upon population genetic analyses, genetic changes in the population inhabiting the River Etne have been observed (Glover *et al.*, 2012; 2013), and some level of admixture with farmed escapees has been demonstrated. It is therefore not possible to exclude the possibility that although the wild *S. salar* used in this study were indeed born in the wild (based upon scale reading), some individuals used as broodstock may represent some admixture with farmed escapees. This might explain why smaller growth differences were detected between the farmed and wild *S. salar* in this study, as compared to other studies of the same strains (Solberg *et al.*, 2013*a*).

In the present study the hybrids displayed intermediate growth relative to both their farmed and wild conspecifics. There were slight differences in the slopes between each treatment for the hybrids, *versus* the farmed and the wild *S. salar*, that likely resulted in the marginally significant (P= 0.05) group by treatment interaction. Intermediate hybrid growth relative to their parental strains has been observed in similar studies under hatchery (Glover *et al.*, 2009; Morris *et al.*, 2011; Solberg *et al.*, 2013*a*), semi-natural (Solberg *et al.*, 2013*b*), and wild conditions (McGinnity *et al.*, 1997). There was no evidence for hybrid vigour or outbreeding depression, whereby hybrids either perform better relative to their parents or display reduced fitness due to under-dominance, respectively. The hybrids in the present study were maternal half siblings to the farmed *S. salar*; therefore it is possible that maternal effects were influencing growth, although maternal effects are considered to be low at this life stage (Gilbey *et al.*, 2005). Bicskei *et al*. (2014) examined gene transcription in farmed, F1 hybrid and wild *S. salar* at two early life stages, and found fewer significantly differentially expressed transcripts between farmed and hybrid individuals than between hybrid and wild individuals. Their hybrid crosses were generated from the farmed females, suggesting that maternal effects might account for this bias (Bicskei *et al.*, 2014), highlighting the need for reciprocal hybrid crosses in comparative studies.

In summary, the results of the present study have demonstrated that the three feeding regimes implemented here did not influence the relative growth rate of farmed, hybrid and wild *S. salar* in the hatchery. Thus, while restricted to the hatchery, the present study provides evidence that variable food availability may not be the primary source governing the similar growth between farmed and wild *S. salar* in natural environments. Similarly, no evidence was found to indicate that more than 10 generations of adaption to the farmed environment, with continuous access to feed, has resulted in farmed *S. salar* exhibiting a reduced tolerance to limited or fluctuating levels of feed. Additional observations are required however that better simulate natural variation in food supply, which is typically not only variable in composition, but also varies markedly in time and space (Jonsson and Jonsson, 2011). It therefore remains a priority to elucidate further the nature of hybridisation and farm-wild interactions. Further studies in particular, exploring the key environmental differences between hatchery and wild environments (*e.g*., predation, density) are evidently required, in conjunction with direct comparison of performance in respective conditions.

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TABLES

Table I: Experiment design. Each treatment consisted of two replicate tanks for each single group = 18 tanks in total. Twenty eggs from each family of *Salmo salar* within a group were sorted into each replicate tank = 2250 eggs in total. Average mass (M), standard deviations (S.D.) and mortality are presented for each group within each tank replicate and pooled treatment. The pooled mass and mortality were calculated as averages of the total mass and mortality in the replicates of each treatment.

Treatment	Group	Tank	Initial *n*	Sampled *n*	Mean M (g)	S.D. (±)	Pooled W	Mortality *n*	Pooled mortality (%)
Daily Restricted	Farm	1	20 eggs per family - 140 fish per tank	125	24.5	4.9	24.5	15	9.3
		2		129	24.5	4.9		11	
	Hybrid	3		117	19.4	5.7	19.4	23	10.4
		4		134	19.3	5.4		6	
	Wild	5		136	15.8	6.2	16.1	4	3.2
		6		135	16.5	5.4		5	
Control	Farm	7	20 eggs per family - 140 fish per tank	127	43.7	12.0	43.1	13	9.6
		8		126	42.5	10.6		14	
	Hybrid	9		131	34.4	8.8	36.4	9	8.6
		10		125	38.3	9.2		15	
	Wild	11		125	28.7	9.3	28.9	15	9.3
		12		129	29.0	9.0		11	
Triweekly	Farm	13	20 eggs per family - 140 fish per tank	127	36.4	8.7	36.1	13	6.4
		14		135	35.8	7.9		5	
	Hybrid	15		130	31.9	8.0	30.9	10	7.1
		16		130	29.9	7.1		10	
	Wild	17		134	22.4	8.5	22.6	6	4.3
		18		134	22.9	9.1		6	

Table II: *P* values of the fixed effects of the GLMM model investigating survival. The Statistic represents a Chi-square value calculated as two- times the difference in likelihood between full and restricted model as specified by the *afex* package.

Effect	Statistic	*P* value
Treatment	1.47	0.57
Group	0.08	0.97
T x G	4.45	0.57

Table III: *P* values of the fixed effects of the LME model investigating growth. The F denotes the F statistic, Num Df denotes the numerator degrees of freedom and Den Df denotes the denominator degrees of freedom.

Effect	F	Num Df	Den Df	*P* value
Treatment	129.39	2	9.12	<0.0001
Group	74.32	2	9.25	<0.0001
T x G	3.67	4	8.99	0.05

FIGURE CAPTIONS

Figure 1: Average (a) mass and (b) log $_L$ mass ± S. E of each group (farm, hybrid and wild Atlantic *Salmo salar*) across each feeding treatment (triweekly, daily restricted, control/*ad libitum*). Log mass was examined in the statistical analysis.

Figure 2: (a) Relative growth reaction norms for each group (farm, hybrid and wild Atlantic *Salmo salar*) and (b) their average log mass across the feeding treatments. In (a) the hybrid and farmed groups are compared to the wild group within each treatment (based upon their untransformed mass). The x-axis shows the feeding treatments (triweekly, daily restricted, control/*ad libitum*).

FIGURES

Figure 1

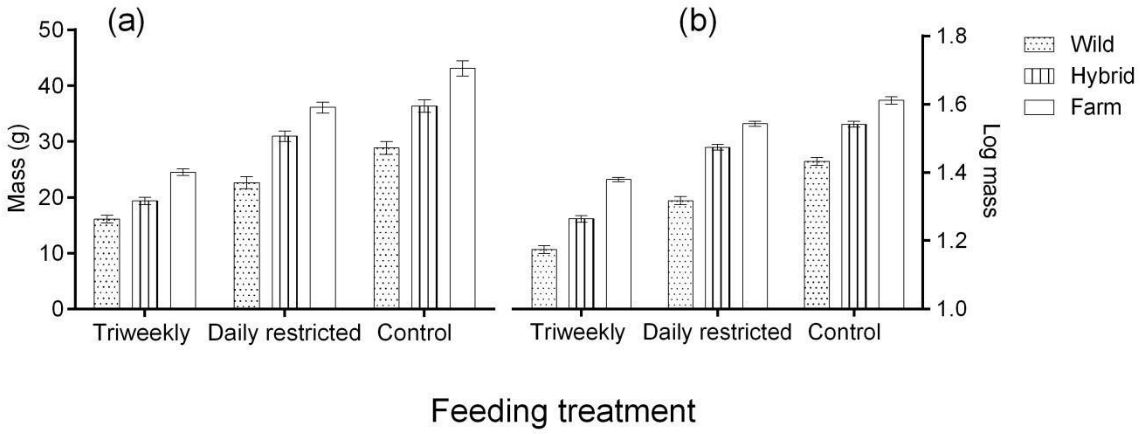
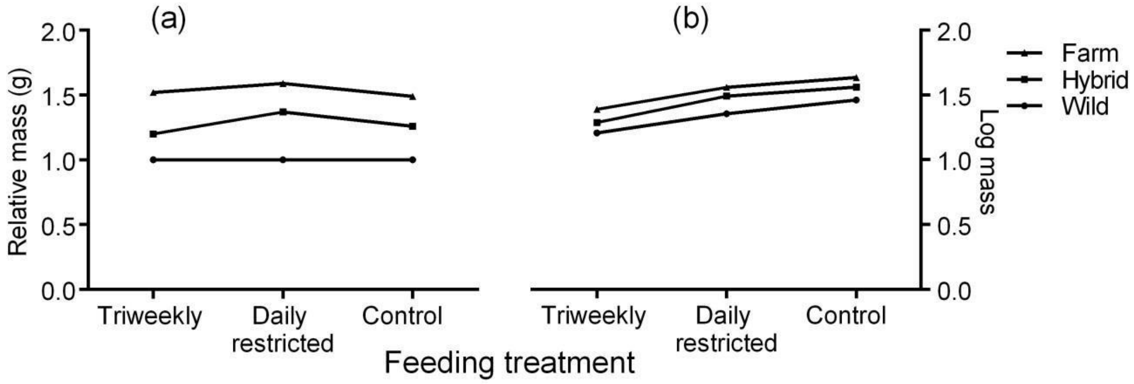


Figure 2


(a)
(b)
Relative mass (g)
Log mass
2.0
1.5
1.0
0.5
0.0
Triweekly
Daily restricted
Control
Feeding treatment
Farm
Hybrid
Wild

SUPPLEMENTARY TABLES

Table SI: Family crosses for the experiment. The commercial farmed strain Mowi and the wild strain Etne were used to make seven pure wild, seven pure farmed and seven hybrid F1 groups. The hybrid families were made by crossing a female farmed *S. salar* with a wild male. Five of the seven hybrid families are half-siblings to five wild and five farmed families, and one family is maternal half siblings to one farmed family and one family is paternal half siblings to one wild family.

Family	Dam	Sire	Group
1	M1	M9	Farm
2	M1	E11	Hybrid
3	M2	M10	Farm
4	M2	E12	Hybrid
5	M3	M11	Farm
6	M3	E13	Hybrid
7	M4	M12	Farm
8	M4	E14	Hybrid
9	M5	M13	Farm
11	M6	M14	Farm
12	M6	E16	Hybrid
14	M7	E17	Hybrid
15	M8	M16	Farm
16	M8	E18	Hybrid
17	E1	E11	Wild
18	E2	E12	Wild
20	E4	E14	Wild
21	E5	E15	Wild
22	E6	E16	Wild
23	E7	E17	Wild
24	E8	E18	Wild

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Table II: Relative growth differences between each group within each treatment and Tukey adjusted *P*-values for the multiple pair-wise comparisons of groups within each treatment. The *P* -values are shown in the bottom left diagonal, and the significance level was set to 0.05, with non-significant *P*-values indicated in bold. Each group within a treatment was significantly different to each other group within that treatment. The relative growth differences between each group within each treatment are shown in bold in the top right section. The average mass of each group was compared to the average mass of the other groups by dividing the larger mass by the smaller mass (*i.e.* farm to wild), creating a relative growth difference ratio. Relative growth differences were not compared across treatments. Daily R corresponds to the daily restricted treatment and triweek corresponds to the triweekly treatment.

	DAILY R Farm	DAILY R Hybrid	DAILY R Wild	CONTROL Farm	CONTROL Hybrid	CONTROL Wild	TRIWEEK Farm	TRIWEEK Hybrid	TRIWEEK Wild
Mass (g)	24.51	19.345	16.125	43.09	36.355	28.845	36.09	30.91	22.64
DAILY R Farm	/	1: 1.3	1: 1.5						
DAILY R Hybrid	0.0005	/	1: 1.2						
DAILY R Wild	<0.001	0.0033	/						
CONTROL Farm	<0.001	<0.001	<0.001	/	1: 1.2	1: 1.5			
CONTROL Hybrid	<0.001	<0.001	<0.001	0.0181	/	1: 1.3			
CONTROL Wild	**0.0825**	<0.001	<0.001	<0.001	0.0007	/			
TRIWEEK Farm	<0.001	<0.001	<0.001	0.0189	**1**	0.0007	/	1: 1.2	1: 1.6
TRIWEEK Hybrid	0.0022	<0.001	<0.001	<0.001	0.0203	**0.2231**	0.0199	/	1: 1.4
TRIWEEK Wild	0.0366	**0.0786**	0.001	<0.001	<0.001	0.0006	<0.001	0.001	/

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