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1 **Influence of feed ration size on somatic and muscle growth in farmed and**
2 **landlocked dwarf Atlantic salmon (*Salmo salar*)**

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20 **ABSTRACT**

21 The dwarf Bleke population of Atlantic salmon (*Salmo salar*) inhabiting the oligotrophic Lake
22 Byglandsfjord in southern Norway were isolated from the sea after the last glacial period about
23 9000 years ago. This landlocked salmon reaches sexual maturity after 4-5 years at a body weight
24 of about 150 g and 30 cm in length. We examined the possible adaptation of the dwarf salmon
25 strain to limited food resources by studying the growth performance and muscle development
26 in juvenile Bleke salmon and farmed salmon under satiate or restricted (50%) feeding for ten
27 months starting three weeks after first-feeding stage. Totally 4000 fish were divided into four
28 replicated groups and random samples of 16-40 fish per group were measured six times during
29 the experiment. The two strains showed no significant difference in mean body weight when
30 fed restricted ration, but the individual variation was considerably higher in the farmed fish.
31 Both Bleke and farmed salmon grew significantly faster when fed to satiation, but the farmed
32 salmon showed much higher weight gain and were three times heavier than landlocked salmon
33 after ten months (201.5 g *versus* 66.7 g). Farmed fish fed full ration displayed both hypertrophic
34 and hyperplasic muscle growth, while the increased growth in Bleke salmon was entirely
35 associated with a larger fibre diameter. The landlocked Bleke strain has apparently adapted to
36 low food availability by minimizing the metabolic costs of maintenance and growth through
37 reduced dominance hierarchies and by an increase in average muscle fibre diameter relative to
38 the ancestral condition.

39 Key words: dwarfism, feed restriction, landlocked salmon, muscle fibre, hypertrophy,
40 adaptation

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44 INTRODUCTION

45 Wild European populations of Atlantic salmon are commonly anadromous and return to their
46 native freshwater streams to spawn after one, two- or three-years growth at sea. Additional
47 complexity to the lifecycle is provided by male parr that sexually mature in freshwater and
48 attempt to fertilise the eggs of sea-run females using “sneaky mating” behaviour (Hutchings &
49 Myers, 1988; Letcher & Gries, 2003; Hutchings & Jones, 1998). Non-anadromous populations
50 of Atlantic salmon are common in parts of North America, but are also found in a few localities
51 in Europe (Berg, 1985). Landlocked salmon complete their life cycle in freshwater above
52 impassable waterfalls formed after postglacial rebound. The Bleke population in the
53 oligotrophic Lake Byglandsfjord of southern Norway was isolated from sea migration about
54 9000 years ago and show reduced osmoregulatory capacity when moved to seawater (Dahl,
55 1928; Lande, 1973; Berg, 1985; Nilsen *et al.*, 2003). This dwarf salmon strain is resource
56 restricted, feeding mainly on zooplankton such that it only reaches a body size of about 150 g
57 and 30 cm after 5-6 years. Female Bleke salmon become sexually mature at 4-5 years and spawn
58 in December with a fecundity of about 250 eggs (Barlaup *et al.*, 2005). In comparison, farmed
59 Atlantic salmon of the same age would be expected to reach 12-16 kg with a fecundity of around
60 15,000 eggs (Kittelsen, 1986). Atlantic salmon have been farmed on an industrial scale since
61 the 1970s. Domestication has included genetic selection programmes for increased growth rates
62 realising trait gains of 10-15% per generation, which have been linked to increased appetite and
63 food conversion efficiency (Thodesen *et al.*, 1999; Thodesen & Gjedrem, 2006; Gjedrem, 2010).
64 Selection for fast growth in farmed salmon also seems to be associated with inadvertent
65 selection to the captive environment, e.g. constant availability of food from the surface reduces
66 awareness of predators a trait which may be maladaptive in the wild (Skaala *et al.*, 2012;
67 Solberg *et al.*, 2013).

68

69 Myogenesis is a trait that is closely associated with intraspecific and population differences in
70 body size (Johnston *et al.*, 2003a, 2012). Growth is associated with the continuous production
71 of myotubes which differentiate to form muscle fibres that subsequently expand in length and
72 diameter (reviewed Johnston *et al.*, 2011). Individual muscle fibres have a maximum diameter
73 which is limited by diffusional constraints and that varies with environmental conditions,
74 chiefly temperature (Johnston *et al.*, 2003b). Using phylogenetic comparative methods
75 maximum body size is a highly significant predictor of species variation in the maximum
76 number of muscle fibres (FN_{max}) (Johnston *et al.*, 2003a). FN_{max} of Bleke salmon was found
77 to be only 30% and 21% of that found in wild anadromous and farmed salmon, respectively,
78 indicating that fibre recruitment is under strong divergent selection (Johnston *et al.*, 2005). A
79 similar reduction in muscle fibre number has been observed in post-glacial populations of
80 Icelandic arctic charr (*Salvelinus alpinus*) and threespine stickleback (*Gasterosteus aculeatus*)
81 (Johnston *et al.*, 2012). Theoretically, the energy cost of maintaining a negative membrane
82 potential is proportional the surface to volume ratio of the individual muscle fibres because
83 active ion pumping is required to counteract passive leak of ions across the muscle sarcolemma.
84 According to the optimum size hypothesis divergent selection acts on fibre recruitment to
85 produce the mix of fibre size that minimises the routine energy costs of maintaining ionic
86 homeostasis (Johnston *et al.*, 2004). The maximum diameter of fast muscle fibres in 4 year-old
87 Bleke salmon was 118 µm which is similar to that of immature farmed salmon of the same body
88 size (Johnston *et al.*, 2005). This suggests the possibility that resource-limited Bleke salmon
89 could growth to larger size under optimal feeding conditions even with the reduced number of
90 muscle fibres. In the present study, we therefore compared somatic and muscle growth in
91 farmed and Bleke Atlantic salmon either fed to satiation or a restricted (50%) ration.

92

93

94 MATERIAL AND METHODS

95 FISH MATERIAL

96 The Bleke salmon examined were offspring of four females and two males caught in Lake
97 Byglandsfjord, southern Norway, and kept at the local Syrtveit Fishery Station. The farmed
98 Atlantic salmon were generated from seven females and two males of the Bolaks strain, which
99 had been selected for high growth rates for seven generations. This late-spawning farmed strain
100 was chosen to match the spawning time of Bleke, thus ensuring concomitant development of
101 the offspring from the two strains. The Bleke eggs were fertilized in early December 2005 and
102 incubated at about 2°C at Syrtveit Fishery Station, while the Bolaks eggs were fertilized two
103 weeks later and incubated at about 3°C at Erfjord Brood Stock Station in south-western Norway.
104 The Bleke and Bolaks eggs were transport to Akvaforsk Breeding Station, Sunndalsøra, in April
105 2006 and incubated at 7.0-7.2°C until hatching. The majority of the eggs hatched at 422.5
106 (Bleke) and 424.5 (Bolaks) day degrees (number of days multiplied by temperature degrees in
107 °C), and all eggs hatched within three days in late April.

108

109 ETHICAL CONSIDERATIONS

110 The experiment protocol was authorized by the University's Ethical Review Committee and the
111 feeding study was performed in strict accordance with the Norwegian Welfare Act to secure
112 fish welfare. The personnel involved had undergone training approved by the Norwegian Food
113 Safety Authority. Tagging, sampling and sacrifice of the fish were performed under anaesthetics
114 using metacain according to the supplier's instructions. As the wild Bleke salmon population is
115 threatened, only fish raised at Syrtveit Fishery Station were used in this study.

116

117 FEED RATIONS

118 Totally 4000 fish of the landlocked Bleke and farmed Bolaks strains of Atlantic salmon were
119 divided into eight 500-L indoor fibreglass tanks each of 500 fish and kept at 7.5-7.9°C in
120 continuous light. Oxygen levels were measured 2-3 times weekly and did not drop below 80 %
121 saturation. The fish were fed commercial salmon feed (Nutra 0.5-3.0 mm, Skretting) 3-4
122 times/hour diurnally using automated feeders developed in-house. Start feeding was initiated in
123 June 6th (Bleke) and June 8th (Bolaks) at 662 and 683 day degrees, respectively, and all groups
124 were fed full ration for three weeks prior to the experimental study. The trial lasted from June
125 29th 2006 to May 9th 2007, and replicated groups of Bleke and Bolaks salmon kept at 11.5-
126 12.4°C were either fed to satiation or fed a restricted ration of 50% satiation level. Satiated
127 ration was 10-20% above commercial salmon feed ration (Skretting V3 feeding table). Daily
128 feed ration was recalculated at 2-weeks intervals throughout the study by batch weighing 50-
129 100 fish from each tank, and the restricted ration was corrected for differences in body weight
130 between the groups. The mortality in the eight tanks during the experiment ranged from 0.6 and
131 3.4 % recorded in Bleke and Bolaks salmon, respectively, fed restricted ration.

132 BODY GROWTH AND MUSCLE FIBRE ANALYSIS

133 Body weight and fork length (FL) were measured to the nearest 0.1 g and 0.1 cm, respectively,
134 in fish randomly sampled from each tank during the first week of August, September and
135 December in 2006, and of February, April and May in 2007 (n=16-40 per group, see FIG. 1 and
136 FIG. 2). The lightly anaesthetized (MS-222) fish were returned to the tanks, except for the fish
137 analysed for muscle fibre number and diameter in September 2006 and May 2007 (n=8-11 per
138 group, see TABLE I) as previously described (Johnston *et al.*, 2004, 2005). Briefly, the fish
139 were over-anaesthetized in MS-222 and killed by a blow to the head. A 0.5 cm thick steak
140 through the trunk muscle on the right-hand side was prepared at 0.7 fork length, photographed

141 at high resolution, and frozen in isopentane cooled to freezing in liquid nitrogen. Frozen
142 sections were cut at 8 μm on a cryostat (Leica Microsystems, CM1850), mounted on poly-L-
143 lysine-coated slides and air dried for storage at -80°C . Sections were stained in modified Harris
144 haematoxylin solution (Sigma-Aldrich), and the total cross-section area (CSA) and the outlines
145 of 800 randomly selected fast muscle fibres were digitized using Sigma ScanPro software. The
146 total number of fast muscle fibres per trunk cross section was estimated as previously described
147 (Johnston *et al.*, 2003a).

148 CALCULATIONS AND STATISTICAL ANALYSIS

149 The coefficient of variation (CV) was calculated as: $(\text{standard deviation} \times \text{mean value}^{-1}) \times 100$.
150 Statistical significance was determined by two-way analysis of variance (ANOVA) followed
151 by Duncan multiple range test, using SAS 9.4 computer software (SAS Institute Inc., Cary, NC,
152 USA). Strain and ration were used as variables and individuals fish as experimental units. The
153 non-parametric Kruskal Wallis test was conducted to verify the results of the parametric test.
154 To test differences in CV, a paired t-test was conducted using strain within each ration as a
155 nominal variable and CV at the different time points as the measurement variable. Differences
156 were considered significant when $P < 0.05$ and results are shown as mean \pm standard deviation
157 (SD).

158

159 RESULTS

160 BODY GROWTH AND FEED RATION

161 Hatchery-reared offspring of the landlocked Bleke strain and the farmed Bolaks strain of
162 Atlantic salmon were either fed to satiation or a restricted (50%) ration for ten months starting
163 three weeks after first-feeding stage. No significant difference in body weight and fork length
164 was shown between the two strains under restricted feeding, except for the longer farmed fish

165 in April 2007 (FIG. 1). Both strains had a significantly ($P < 0.01$) higher body weight when fed
166 to satiation, but the farmed salmon were significantly ($P < 0.01$) heavier than Bleke salmon at
167 each sampling point and 3-folds higher body weight was found at the final measurement (201.5
168 g *versus* 66.7 g) (FIG. 1). The body growth in the four groups were consistent with the results
169 from the batch weightings to recalculate feed rations (data not shown). Farmed salmon fed to
170 satiation were significantly longer than the other groups throughout the study possessing a fork
171 length of 25.6 cm after ten months compared to 18.7 cm in Bleke salmon under satiate feeding
172 (FIG. 1). Intriguingly, farmed salmon fed restricted ration showed consistently higher
173 individual variation (CV) within body weight than the other groups (paired t-test: $P = 0.03$, FIG.
174 2). Loss of the parr marks indicating onset of smoltification was observed by the end of the
175 experiment only in the larger individuals of the farmed salmon fed to satiation (FIG. 3).

176 MUSCLE CELLULARITY

177 Bleke salmon and farmed salmon showed no significant difference in fast muscle fibre number
178 and fibre diameter after ten months on restricted feed ration (TABLE I). When fed to satiation,
179 farmed salmon showed a 2-fold increase in number of fibres, while the fibre number in Bleke
180 salmon did not differ between the satiate and restricted feeding groups. However, the mean
181 fibre diameter increased almost 30% in both Bleke salmon and farmed salmon by increasing
182 the feed ration. The hypertrophic and hyperplastic muscle growth in the satiated farmed salmon
183 resulted in a total muscle cross section area of 411.0 mm² compared to only 177.8 mm² in the
184 landlocked salmon (TABLE I).

185

186 DISCUSSION

187 The weight gain displayed by Bleke salmon fed restricted ration for ten months was similar to
188 that found in the wild population, which reach the body size of about 30 g and 15 cm after one
189 year in its natural habitat of Lake Byglandsfjord (Barlaup *et al.*, 2005). The growth potential of

190 this dwarf salmon strain was indicated by a 2-fold higher body weight when fed to satiation
191 compared to fish under restricted feeding. Accordingly, landlocked salmon from the Swedish
192 Klarälvs river grew faster at 100 % than at 50 % ration, and the difference was stronger at high
193 lipid (2.4-fold) than low lipid levels (1.4-fold) after 19 months (Norrgård *et al.*, 2014). The
194 considerably heavier farmed salmon than Bleke salmon fed full ration is consistent with the up
195 to 3-fold higher body weight in farmed salmon than wild salmon examined in a hatchery
196 environment (Glover *et al.*, 2009; Solberg *et al.*, 2013). In contrast, farmed and Bleke salmon
197 fed half ration showed no difference in body weight that agrees with the modest or marginal
198 growth difference between farmed and wild salmon in wild stream environments (Fleming *et*
199 *al.*, 2000; Skaala *et al.*, 2012) or in a semi-natural environment with restricted food (Solberg *et*
200 *al.*, 2013). Competition for limited resources is expected to favour the most aggressive and
201 territorial individuals (Ruzzante, 1994). Consistently, the farmed salmon fed restricted ration
202 showed large individual variation in body weight, and fin damages were only observed in this
203 group (Erlend Stubø, pers. comm.). Einum and Fleming (1997) reported that wild Atlantic
204 salmon parr were outcompeted and displaced by the more aggressive farmed salmon in captivity.
205 Farmed salmon parr were also more aggressive than wild parr when released into the river, but
206 the wild fish fed more effectively by choosing habitats with preferred sizes of food items, while
207 the diet of cultured fish comprised food items and inedible particles avoided by the wild fish
208 (Orlov *et al.*, 2006). The landlocked Bleke salmon seem to have adapted to the low food
209 availability in the Lake Byglandsfjord by foraging together with brown trout on pelagic and
210 benthic prey items, respectively (Barlaup *et al.*, 2005). The effective foraging of the dwarf
211 Bleke salmon was supported by the present study showing that farmed salmon, which had been
212 selected for high growth rates during seven generations, did not outgrow the Bleke salmon when
213 fed a restricted ration.

214

215 The polygenic nature of body growth was evidenced by genome mapping multiple
216 quantitative trait loci (QTL) for body weight and length in Atlantic salmon using F2 families
217 from founding generations consisting of Bleke males and farmed females (Baranski *et al.*,
218 2010). Studying the endocrine mechanisms underlying the faster growth in domesticated fish,
219 Fleming *et al.* (2002) measured significantly higher pituitary and plasma growth hormone
220 (GH) levels in the seventh-generation of farmed Atlantic salmon compared to the wild
221 principal founder population. GH is also an osmoregulatory hormone, and a role of GH in the
222 development of seawater tolerance in salmonids independent of fish size was recently shown
223 in size-matched groups of transgenic and wild-type coho salmon (*Oncorhynchus kisutch*)
224 (Bystriansky *et al.*, 2017). The reduced osmoregulatory capacity of Bleke salmon was
225 associated with the lack of increased plasma GH and gill GH receptor mRNA levels as found
226 in anadromous salmon during the smoltification period (Nilsen *et al.*, 2008). Reduced GH
227 activity may partly explain the lower growth rate of Bleke salmon compared to farmed fish,
228 and plasma IGF-1 levels were reported to respond to changed feed rations in coho salmon
229 (Pierce *et al.*, 2001). IGF-1 and myostatin are conserved stimulatory and inhibitory factors
230 regulating myoblast proliferation and muscle growth (Garikipati & Rodgers, 2012; Gabillard
231 *et al.*, 2013; Li *et al.*, 2014; Retamales *et al.*, 2015). Low genetic variation of the myostatin
232 gene paralog *Ssa-mstn1b* was found in Bleke salmon compared to farmed salmon by analysing
233 an internal polymorphic microsatellite (Østbye *et al.* 2007), while several private alleles of
234 lysyl oxidase required to initiate muscle cross-link formation was reported in Bleke salmon
235 (Consuegra & Johnston, 2008). Relative low genetic variation was shown in the landlocked
236 Namsblank salmon population (Sandlund *et al.* 2014), but genome sequences from a single
237 dwarf fish revealed no loss of coding regions suggesting that fine-scale (epi)genetic alterations
238 and population genetic processes underlie adaption to the landlocked life-style (Hauge *et al.*,
239 2016). In support of this idea, we recently reported that effects of genetic background and

240 embryonic temperature on muscle development in Atlantic salmon were associated with
241 altered DNA methylation and gene expression of the myogenic regulatory factor myogenin
242 (Burgerhout *et al.*, 2017).

243

244 Muscle enlargement in teleosts with indeterminate growth, such as salmonids, occurs by both
245 hypertrophy and hyperplasia during a large part of post-hatching life (Rowlerson & Veggetti
246 2001; Johnston *et al.*, 2011). Large muscle fibre size is metabolically advantageous, and
247 dwarfism in landlocked populations of salmonid and stickleback has been associated with a
248 reduction in fibre numbers compared to anadromous populations (Johnston *et al.*, 2004, 2012;
249 Jimenez *et al.*, 2013). Our study showed similar muscle fibre number in Bleke and farmed
250 salmon fed a restricted ration, whereas the fibre number in farmed salmon was more than
251 twice that of Bleke salmon after ten months on full ration. The increased growth of the Bleke
252 salmon fed to satiation was entirely due to fibre hypertrophy. It should be noted that had the
253 trial gone on longer, fibre number would have been expected to increase further based on
254 results for mature adults (Johnston *et al.*, 2005). However, FNmax in Bleke salmon is
255 nevertheless only 21% of that of adult farmed salmon (Johnston *et al.*, 2005). Resource
256 limitation in the landlocked Lake Byglandsfjord salmon is likely the primary cause of
257 dwarfism which resulted in divergent selection to reduce fibre number and increase fibre
258 diameter relative to the ancestral condition. In addition, dominance hierarchies also seemed
259 to be reduced contributing to a further reduction in the metabolic costs of maintenance and
260 growth. These adaptive traits are apparently maintained during conditions of surplus food
261 suggesting the involvement of genetic or epigenetic mechanisms.

262

263

264

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269

270 **CONTRIBUTIONS**

271 OA and IAJ designed the study. OA was responsible for the experiment. VV performed the
272 laboratory analyses. IAJ and JED analysed the data. OA wrote the manuscript with
273 contributions from IAJ and JED.

274

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458

Table 1. Fast muscle growth in juvenile landlocked Bleke and farmed Atlantic salmon (*Salmo salar*) after two (Sept 06) and ten months (May 07) on full or restricted (50 %) feed ration. Body weights of the four groups at the two sampling points are included. Mean \pm SD are indicated. TCA (total cross section area). Different lower case superscript letters in the same column indicate significant difference.

Strain	Ration	Sampled	N muscle samples	TCA	Diameter (μm)	Fibre number	Body weight (g) N = 16
Landlocked	50 %	Sep 06	8	17 \pm 1	35.0 \pm 0.5	14300 \pm 500 ^a	3.4 \pm 0.6 ^c
		May 07	10	113 \pm 12	37.0 \pm 1.0	82000 \pm 5590 ^a	33.6 \pm 12.4 ^c
Farmed	50 %	Sep 06	8	16 \pm 1	33.4 \pm 0.3	14600 \pm 1100 ^a	2.8 \pm 1.0 ^c
		May 07	9	131 \pm 11	37.5 \pm 1.0	90700 \pm 7963 ^a	32.0 \pm 20.4 ^c
Landlocked	100 %	Sep 06	8	22 \pm 1	35.0 \pm 0.6	17600 \pm 1100 ^a	4.7 \pm 0.9 ^b
		May 07	11	78 \pm 11	47.8 \pm 2.0	84800 \pm 9186 ^a	66.7 \pm 23.5 ^b
Farmed	100 %	Sep 06	8	31 \pm 2	36.4 \pm 0.4	23200 \pm 1600 ^b	7.8 \pm 2.2 ^a
		May 07	10	411 \pm 15	48.0 \pm 1.0	179700 \pm 11926 ^b	201.5 \pm 48.9 ^a

1 **Figure legends**

2

3 **Figure 1.** Body weight (A) and fork length (B) of juvenile landlocked Bleke and farmed
4 Atlantic salmon (*Salmo salar*) fed full or restricted (50%) ration for ten months. Error bars
5 indicate standard deviation (SD), and different letters at each sampling point show significant
6 difference. Number of fish measured at the six sampling points were 20, 16, 30, 16, 40 and 16,
7 respectively.

8

9 **Figure 2.** Individual variation in body weight expressed as coefficient of variation (CV) in
10 juvenile landlocked Bleke and farmed Atlantic salmon (*Salmo salar*) under full or restricted
11 (50 %) feeding for ten months. Number of fish measured at the six sampling points were 20,
12 16, 30, 16, 40 and 16, respectively.

13

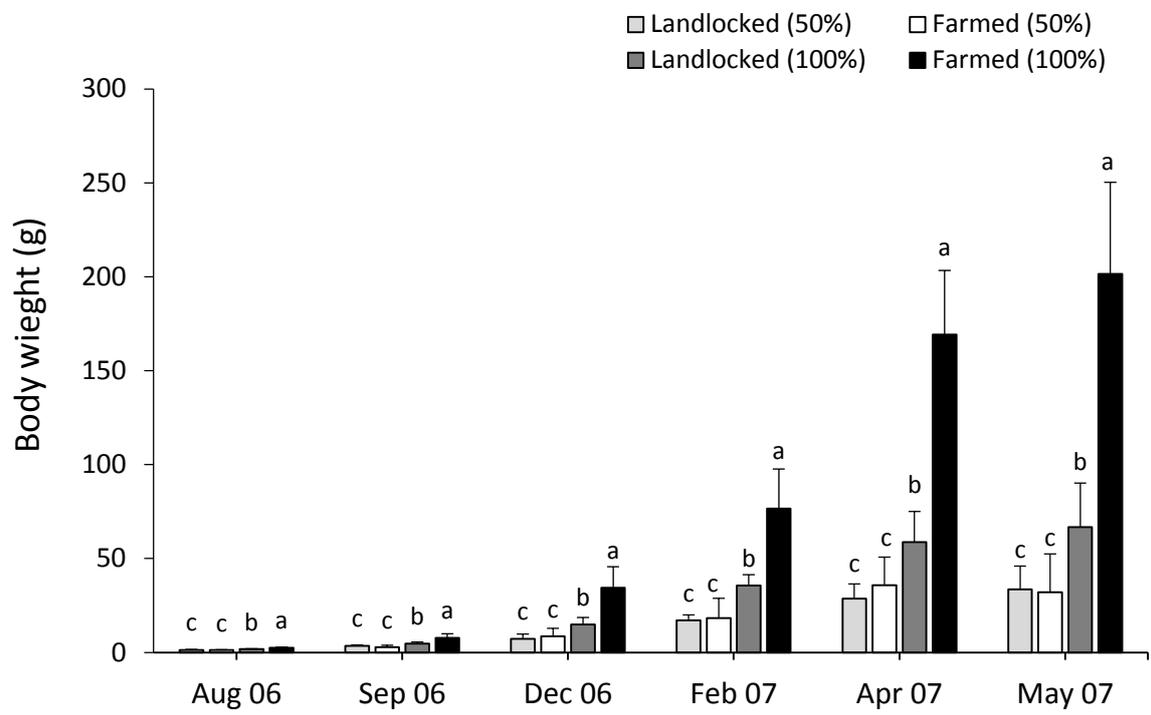
14 **Figure 3.** Typical specimen of Atlantic salmon (*Salmo salar*) representing (from top):
15 Landlocked salmon – restricted feeding, farmed salmon – restricted feeding, landlocked salmon
16 – satiate feeding, farmed salmon – satiate feeding. The fish was photographed at the end of the
17 experimental study.

18

19

Figure 1

(a)



(b)

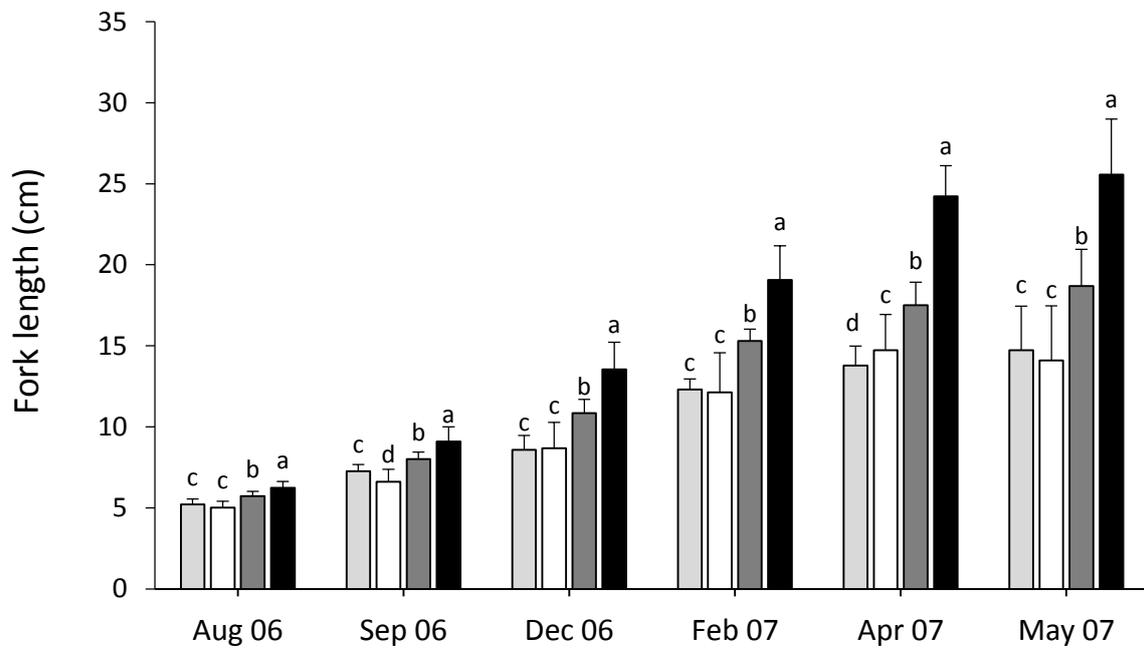


Figure 2

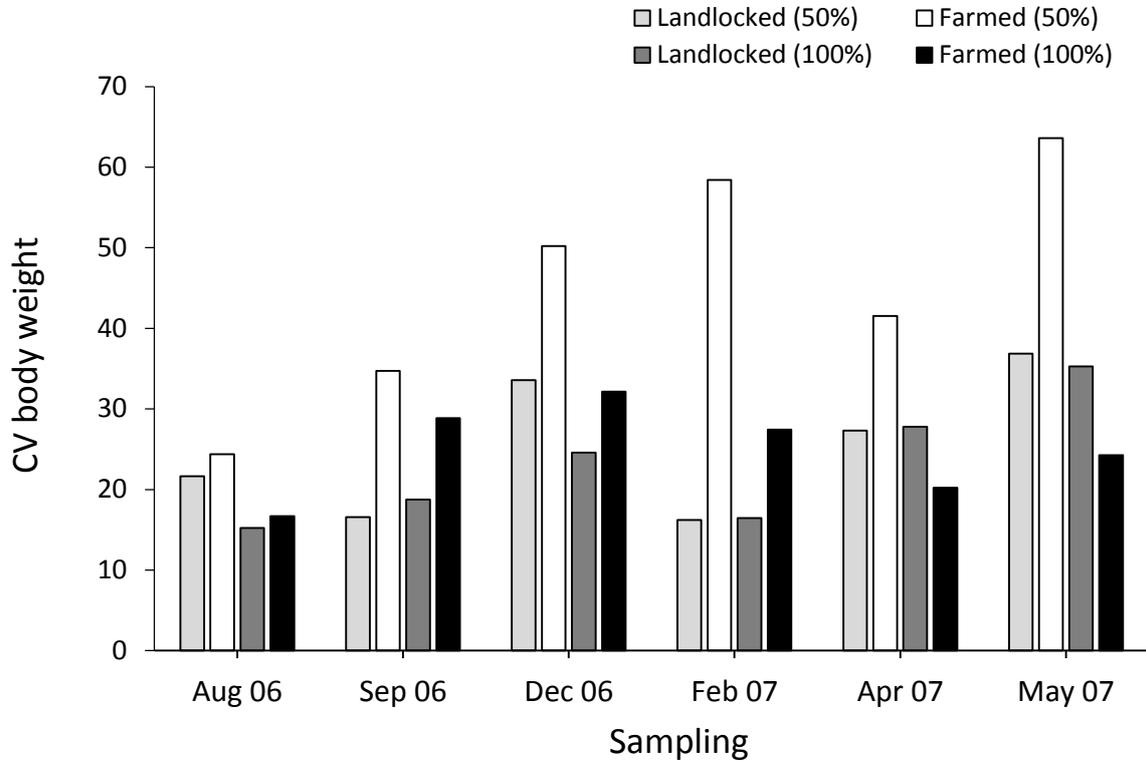


Figure 3

