1 **Selection for improved stress tolerance in rainbow trout (*Oncorhynchus mykiss*)**

2 **leads to reduced feed waste**

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Abstract

The magnitude of the cortisol response to a standardised stressor is a heritable trait in salmonid fishes, and selection for stress responsiveness induces differences in both behaviour and neuroendocrine function. For instance, in laboratory studies, fish with a high cortisol response show a greater propensity for stress induced anorexia. Some authors have, however, commented that controlled studies encompassing relatively small groups of fish hold little or no relevance to practical aquaculture. This notion may be flawed, since understanding the mechanisms behind the behaviour of individuals is a proviso to predict behaviour in groups even with the caveat that some behaviors may be modified by group size. As an example, optimal feeding regimes should be easier to predict in a population consisting of individuals whose appetite is relatively less affected by external stressors. In a fluctuating and potentially stressful environment, such a population should also generate less feed waste, if kept on fixed rations. In the present experiment, we tested this hypothesis by monitoring feed waste and feed conversion efficiency in lines of rainbow trout selected for a low (LR) or high (HR) cortisol response to stress. The study was carried out after fish had been transported between rearing sites in the United Kingdom and Norway. There was significantly more feed waste from rearing units containing HR fish, and these fish also showed lower feed efficiency (growth per unit feed consumed). The difference in feed waste became more apparent with increasing time after transport, as rations increased. Simultaneously, size was more variable and growth was slower in HR rearing units. These results suggest that there are several potential benefits of selection for low stress responsiveness in aquaculture.

1. Introduction

Intensive aquaculture of salmonid fish is a relatively new innovation, compared to the cultivation of most other animals. The process of domestication has only just begun in salmonids, and considerable effort is devoted to selecting for and estimating the heritability of economically important traits in these fish, such as growth rate, size at maturity, muscle composition, and disease resistance (see e.g. Gjerde, 1993; Gjøen and Bentsen 1997; Gjedrem, 2000; Midtlyng et al., 2002).

Fish under intensive culture conditions are exposed to a regime of both acute and chronic stressors, which have adverse effects on growth, immunocompetence, and flesh quality, among other things (Barton et al., 1987; Barton and Iwama, 1991; Balm, 1997; Sigholt et al., 1997; Schreck et al., 2001; Pottinger, 2001). As in other vertebrates, the blood concentration of corticosteroid hormones is used as an index of stress in fish, and elevated levels of these hormones arise from activation of the hypothalamus-pituitary-interrenal (HPI) axis (Wendelaar-Bonga, 1997). The main corticosteroid in teleosts is cortisol (Kime, 1987), and this steroid is a causal factor in many of the deleterious effects of stress (Barton et al., 1987; Barton and Iwama, 1991; Harris and Bird, 2000; Pankhurst and Van der Kraak, 2000; Schreck et al., 2001; Bernier et al., 2004).

In rainbow trout the magnitude of the cortisol response to stress is an individual characteristic which is stable over time, with a moderate to high degree of heritability (Pottinger et al., 1992, 1994; Fevolden et al., 1999). Thus, post-stress cortisol levels provide a trait of functional significance upon which selection pressure can be directed, and lines of high- (HR) and low-responsive (LR) trout have been established at the Windermere laboratory of the UK Natural Environment Research Council, Centre for Ecology and Hydrology (Pottinger and Carrick, 1999).

Selection for cortisol responsiveness is associated with a range of behavioural differences between the HR and LR trout lines (reviewed by Øverli et al., 2005). Specifically, LR fish show a tendency to become socially dominant (Pottinger and Carrick, 2001), a rapid recovery of food intake after transfer to a novel environment (Øverli et al., 2002), and a reduced locomotor response in a territorial intrusion test (Øverli et al., 2002). Experiments with non-selected aquaculture populations of rainbow trout later showed that large variation

77   in the cortisol response to stress also exists in such populations, and cortisol responsiveness

78   predicts behaviour in a number of situations (Øverli et al., 2004, 2006).

79   The above associations between individual variation in stress responsiveness and

80   behaviour have all been revealed in laboratory studies encompassing relatively low numbers

81   of fish. There seems to be a strong notion, at least in some research milieus, that tightly

82   controlled small-scale studies hold little or no relevance to practical aquaculture. We will

83   argue, however, that proper understanding of mechanisms behind the behaviour of individual

84   fish is a proviso to comprehend behaviour in groups. The latter a viewpoint is supported by

85   recent work on the relative growth of the HR and LR selected fish over several years and two

86   generations at near-production scale (Pottinger, 2006). In this study, the performance of the

87   two lines in co-culture was found to be consistent with what might be predicted on the basis

88   of the relative competitiveness/aggressiveness of the fish in smaller scale studies.

89   While the conclusions are so far equivocal with regard to the value of directly

90   exploiting the LR line, several potential benefits of selection for reduced stress

91   responsiveness in aquaculture can be nonetheless be identified. For instance, the amount of

92   feed waste from a rearing unit, and the efficiency with which feed is transformed to somatic

93   growth, are economically and environmentally important factors that may be affected by

94   stress responsiveness. Optimal feeding regimes should be easier to predict in a population

95   consisting of individuals whose appetite is less affected by external stressors. In a fluctuating

96   and potentially stressful environment, such a population should also generate less feed waste,

97   if kept on fixed rations.

98   Transport of live fish is a common procedure in aquaculture and fisheries

99   management that exposes fish to multiple stressors, which may in turn cause production loss

100   (see e.g. Iversen et al., 2005, and references therein). It is likely that an effect of heritable

101   variation in stress responsiveness on feed utilisation would be particularly evident following

102   exposure to a stressful procedure such as transport. To test this hypothesis, we monitored

103   feed waste, growth, and conversion efficiency in HR and LR trout rearing units during twelve

104   days after fish had been transported between the original rearing site (Windermere, UK) and

105   a research station in Norway.

106

107   **2. Material and methods**

108 Experimental fish and procedures: The selection procedure yielding the HR and LR trout

109 lines and the effect of the breeding program on cortisol responsiveness has been described in

110 detail elsewhere (Pottinger and Carrick 1999, 2001), and will only be briefly reviewed here.

111 The parental generation of the HR and LR trout lines was established in 1996 by repeated

112 stress testing (3h confinement in 50 L water in groups of 6-7 individuals once monthly) of

113 passive integrated transponder (PIT) tagged 2-year-old rainbow trout. The mean post-stress

114 plasma cortisol content across five episodes of confinement was calculated for each fish, and

115 individuals were ranked accordingly. Between testing, fish were kept in groups of 25 in 1500

116 L holding tanks, and the four most high-responding (HR) and the four most low-responding

117 (LR) fish in each tank were removed from their home tank, segregated by sex, and kept

118 separate in 4 tanks based on group (HR, LR) and sex until maturation.

119   The 1st generation offspring (F1) consisted of 15 HR and 14 LR families each

120 resulting from a unique male-female crossing. A highly significant regression of mid-parent

121 cortisol response on progeny response was seen (estimated r^2 [h^2] value = 0.41), and the six

122 LR families with the lowest mean cortisol response and the six HR families with the highest

123 mean cortisol response were identified and used for further work (Pottinger and Carrick,

124 1999). In the current experiment, 150 PIT-tagged fish from the third generation of offspring

125 from each population (weight HR: 493±12 g, LR: 477±9 g, mean ± SE) were starved for 5

126 days and loaded on a tank truck equipped to keep water fully oxygenated during transport

127 (Donslund Special Transport, Hejnsvig, Denmark, http://www.donslund.dk). Water

128 temperature was 14ºC at departure from Windermere, increasing to 18ºC during transport. On

129 arrival at the Norwegian Institute for Water Research marine research station (Solbergstrand,

130 Akershus county, Norway) two days later, all fish except 12 HR and 12 LR individuals used

131 for another study were again weighed and distributed in 3 HR and 3 LR rearing units (tank

132 volume 0.9 m^3, water flow 30 l / min, 15-17ºC). HR and LR fish were not separated during

133 the transport, but were sorted upon arrival based on PIT-tag readings. No mortalities

134 occurred during the transfer.

135   Three days after arrival, a feeding regime was initiated in which all rearing units were

136 hand fed a ration corresponding to 0.5% of the biomass (B.M.) present in that tank

137 (Skretting, Standard Expanded 60). Feed was distributed once daily over a 20 min period.

138 After three days, rations were increased to 1% B.M. / day, and after another 3 days to 2%

139 B.M. / day. All rearing units were equipped with PVC grid pellet traps at the water outlet,

140 and uneaten pellets were collected and counted each day 15 min after termination of feeding.

141 At this point in time, it was visually confirmed that all food had either been consumed or

142 trapped. Quantification of uneaten food was stopped after 6 days of feeding at the 2% level,

143 i.e. 12 days after feeding was initiated. At this point, all fish were again weighed and growth

144 rates were calculated.

145   Data analysis: The feed waste from each rearing unit during the course of the

146 experiment was recorded daily. Mean values for the amount of accumulated feed waste

147 released by HR (n=3) and LR (n=3) rearing units were then expressed as a regression over

148 time. The rate of increase in accumulated feed waste was compared between HR and LR

149 lines using the method suggested by Armitage (1980) to test whether the regression slopes

150 were different (using the t-statistics), and whether the lines were significantly separated

151 (using the t-statistics for adjusted constants). The latter test corresponds to a co-variance

152 analysis using lineage (HR, LR) as independent variable, accumulated feed waste as

153 dependent variable, and time (days of feeding) as co-variate. Total feed waste from the two

154 populations during the whole experiment was also calculated as the percentage of the food

155 delivered to each tank, and compared using two-sided t-test. Two-sided t-tests were also used

156 to compare biomass, size variation (coefficient of variance, CV), growth rates, and feed

157 efficiency ratio (FER = growth / feed consumed) between the two lines. Relationships

158 between relevant variables (see the results section) were tested using Pearsson correlation.

159

160 **3. Results**

161 Fish size, growth rates, and feed efficiency: In the following, data are reported as mean ±

162 S.E.M., and p values are the result of two-sided t-test unless otherwise stated. There was no

163 significant difference in average fish size between the two populations prior to transport (HR:

164 493±12 g, LR: 477±9 g, p=0.25), or upon arrival in Norway (HR: 456±12 g, LR: 442±9 g,

165 p=0.44). There was a trend towards larger CV's for initial size in HR than LR rearing units

166 (HR: 26.6±1.9, LR: 23.2±0.6, p=0.16). Growth rates during the feeding experiment were, on

167 average, higher in the LR than in the HR rearing units (HR: 2.1±0.1 % B.W. / day, LR:

168 2.6±0.05 % B.W. / day, p=0.02). This difference was not fully explained by higher feed

169 waste in HR rearing units (see below), because there was also a significant difference in feed

efficiency (growth/feed consumed) between the two populations (FCR HR: 1.53±0.1, LR:1.86±0.04, p=0.03).

Feed waste: Accumulation of feed waste under the three different feeding regimes is shown in figure 1. Data for the two populations were distributed along two separate regression lines under all three feeding regimes, with significant differences between mean y in all cases, and slopes significantly different at the 1.0% and 2.0% feeding regimes, but not under the initial 0.5% regime. Hence, feed waste accumulated more rapidly from HR rearing units in the final stages of the experiment, when rations were higher. Total feed waste during the whole experiment was also calculated in relative terms (percentage of distributed feed lost) and compared between populations by unpaired t-test (figure 2). Despite the relatively low number of rearing units, total feed waste was also significantly higher from the HR rearing units (p=0.03).

Relationship between size variation and feed waste: The fact that the cumulative difference in feed waste between the two lines increased with time after transport suggests that stress induced reductions in feed intake were not the only factor underlying the greater feed waste from the HR rearing units. As noted above, there was a trend towards a larger variation in fish size in the HR rearing units. We therefore analysed whether there was a correlation between initial size variation and total feed waste from the different rearing units. There was a strong trend towards such a relationship in pooled data from HR and LR rearing units (p=0.08, figure 3).

Discussion

These data demonstrate conclusively that during a 12 day period after transport between two rearing sites, feed waste from rearing units holding LR fish was lower than that from units containing HR fish. This observation is consistent with an earlier study, in which it was demonstrated that LR fish tend to resume feed intake more rapidly than HR fish in a new environment (Øverli et al., 2002). Furthermore, in the present study LR fish yielded more growth from each unit of food they consumed. Previous studies carried out at the original Windermere rearing site found no significant difference in growth between the lines when they were reared in separate tanks (Pottinger, 2006). Hence, at present it is not known if LR and HR fish also utilize feed resources differently under more undisturbed and stable

201 conditions. It should be noted, however, that in the present study the difference in feed waste
202 between the two lines appeared to increase with time after transport. This may be a
203 consequence of the fact that rations were increased with time, or indicate that different feed
204 utilisation is a permanent characteristic of the HR and LR rainbow trout lines. The latter
205 explanation may seem the less likely, since in a previous study no significant difference in
206 growth was detected over time between HR and LR lines held separately for longer periods
207 of time (Pottinger, 2006). An alternative explanation for this discrepancy could be that
208 divergence in trait characters has been amplified in the subsequent generation of HR and LR
209 fish that was utilised in the present study.

210   In salmonids, appetite inhibition is a prominent behavioural response to stress and
211 aversive experience, which is in part mediated by neurotransmitters and hormones that are
212 also involved in control of the physiological stress response (Øverli et al., 1998; Bernier and
213 Peter, 2001; Carr, 2002; Bernier et al., 2004). It is, however, not immediately clear that
214 increased feed waste from the HR rearing units depends only on stress induced anorexia in
215 these fish. Stress responsiveness is also linked to aggressive behaviour and social dominance
216 (Pottinger and Carrick, 2001; Øverli et al., 2004, 2005; Schjolden et al., 2005), and may
217 thereby affect the structure of social hierarchies. Social interactions are in turn an important
218 factor in the distribution and utilisation of feed resources (e.g. Jobling and Wandsvik, 1983;
219 McCarthy et al., 1992). In the present experiment, there was no initial difference in mean
220 body weight between HR and LR fish. On the other hand, HR fish were somewhat more
221 heterogeneous in size at the beginning of the experiment. Furthermore, the data suggested a
222 positive relationship between initial variance in body weight and feed waste resulting from a
223 rearing unit (c.f. figure 3).

224   In light of the above, it would appear that homogenous populations generate less feed
225 waste. It should, however, be noted that the number of rearing units in this experiment was
226 too low to reveal whether there also was such a relationship within each population. Hence,
227 these data only demonstrate that there was some correspondence between the magnitude of
228 the difference in feed waste and enhanced size heterogeneity in the HR population, and does
229 not constitute proof of a cause-effect relationship. The correlation should nevertheless be
230 taken in concern to remind us that there are several plausible backgrounds for the association
231 between increased feed waste and high stress responsiveness.

232   It should also be kept in mind that these measurements were made in a period
233 following transport and starvation. Compensatory growth in salmonid fish appears to involve
234 both increased feed intake and improved feed efficiency (Miglavs and Jobling, 1989; Jobling
235 and Koskela, 1996, Boujard et al., 2000). Hence, our results are in line with recent reports
236 demonstrating that feed conversion efficiency may have a substantial genetic component
237 (Thodesen et al., 2001; Silverstein et al., 2005; Mambrini et al., 2006).

238   In summary, the present study suggests that both improved FCR and reduced feed
239 waste are among the potential benefits selection for stress responsiveness could have on the
240 production of salmonid fish in aquaculture. It should be kept in mind, however, that the present
241 study was carried out after a significant transport stressor, coupled with exposure to a wholly
242 novel environment. In this situation, the results clearly showed that LR fish were able to regain
243 growth more rapidly after transport than HR fish. A reduction in the adverse effects on
244 performance induced by the imposition of unavoidable stressors is likely to be among the
245 potential benefits selection for stress responsiveness offers for the production of salmonid fish
246 in aquaculture. Finally, it should be noted that there was close correspondence between the
247 actual results obtained under conditions of intensive group rearing and the predictions made
248 from previous, small-scale laboratory research. This observation underlines the importance of a
249 functional interface between applied research and fundamental studies on the biology of
250 species currently undergoing domestication.

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260   **References**

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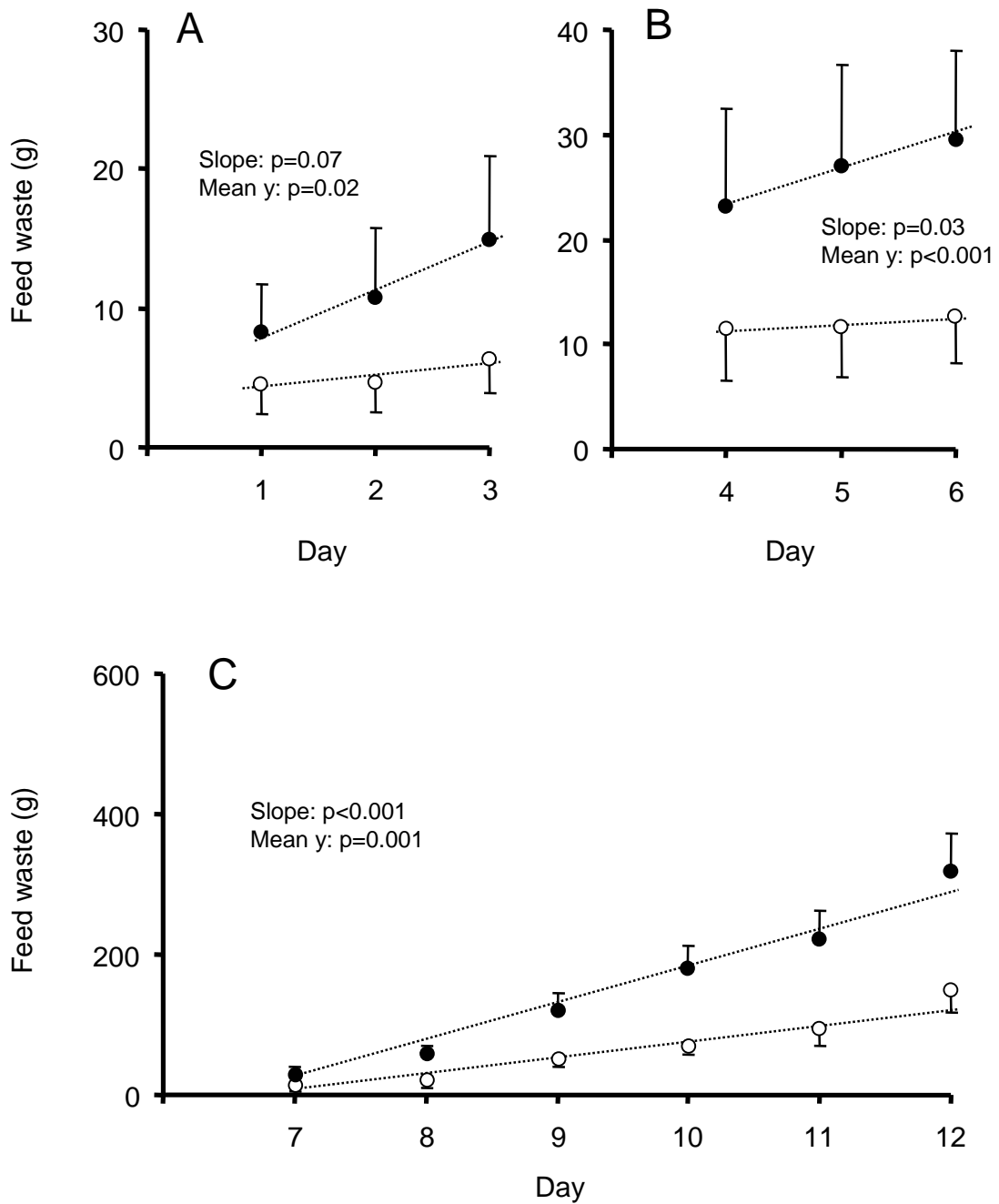
367   Figure 1: Accumulation of feed waste from 3 HR (filled circles) and 3LR (open circles)

368   rearing units in A) 0.5%, B) 1.0%, and C) 2.0% B.M. / day feeding regimes (mean $\pm$ S.E.).

369   Note that fish biomass and hence distributed feed was slightly higher in the LR rearing

370   units (see text for details), while feed waste was higher in HR units. Likelihoods that slope

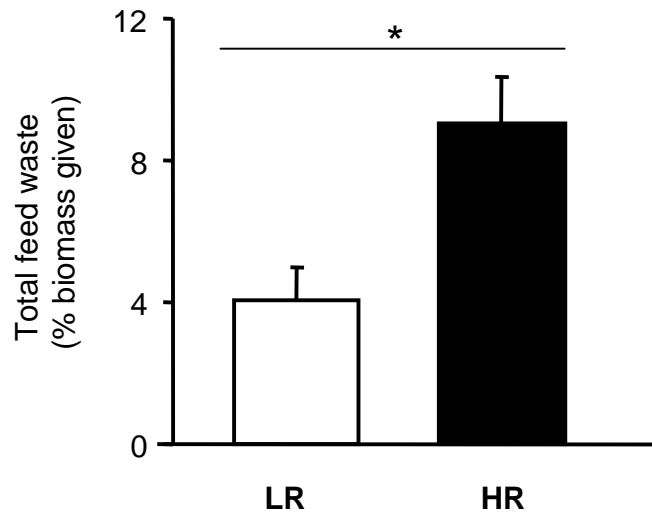371   and mean y are similar are given in each panel (ANCOVA).

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373   Figure 2: Total feed waste over the 12 day observation period expressed as the proportion

374   of food not eaten ($* = p>0.05$, two-sided t-test).

375

376   Figure 3: Relationship between initial size variation and total feed waste in HR and LR

377   rearing units (Pearsson r^2 and p values).

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381   Øverli et al., figure 3

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