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Relationship of baseline and maximum glucocorticoid concentrations to migration propensity – a field test with wild sub-adult brown trout (Salmo trutta)

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3

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6

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23 ***Abstract***

24 There is considerable variation in individuals' glucocorticoid (GC) baseline status and stress

25 responses, yet the cause and consequence of this variation remains ambiguous. Attempts to

26 relate GC levels to fitness and life-history tradeoffs have yielded variable results. In this study,

27 we evaluated whether baseline and post-stressor GC hormone concentrations predicted

28 migration strategy (i.e., resident or migrant) and successful seaward migration in a partially

29 migrating population of juvenile brown trout (*Salmo trutta* (Linnaeus, 1758)). Baseline (N=99)

30 or post-stressor (N=102) plasma cortisol concentrations were obtained from trout and they

31 were tagged with passive integrated transponder (PIT) and released in a natural Danish stream.

32 Subsequently, fish were tracked with PIT reader systems and the stream was resampled for

33 resident individuals. GC levels were not found to be associated with recapture of resident

34 individuals or migration propensity to our first tracking station (S1), but increased baseline (and

35 not post-stressor) GC levels were associated with increased passage from S1 to our second

36 tracking station, which anecdotally was an area of high predation or challenge. Our study found

37 no evidence to suggest that cortisol regulates the migration life-history in juvenile brown trout,

38 but intermediate increases in baseline GC (and not post-stressor GC) levels may favor migration

39 performance.

40

41   ***Key Words***

42   Cortisol, Fish, Glucocorticoid, Migration, Stress response, Brown trout, *Salmo trutta*

43

44   ***Abbreviations***

45   GC, glucocorticoid; PIT, passive integrated transponder; S1, tracking station 1; S2, tracking

46   station 2; HPA, hypothalamic–pituitary–adrenal axis; HPI, hypothalamic–pituitary–interrenal

47   axis; K, Fulton's condition factor.

48 ***Introduction***

49 The study of the physiological basis of stress is useful for understanding physical, chemical,

50 social and perceived challenges faced by organisms, and the functional responses employed by

51 organisms to deal with those stressors (Denver 2009). In vertebrates, real or perceived

52 stressors stimulate the hypothalamic–pituitary–adrenal axis (HPA; or the hypothalamic–

53 pituitary–interrenal (HPI) axis in fish) resulting in the release of glucocorticoid (GC) hormones

54 into the blood stream (Sapolsky et al. 2000; Barton 2002). Thus, circulating concentrations of

55 GC hormones are often measured to understand an organism's stress response (Dantzer et al.

56 2014). Within a species, individuals often show repeatable baseline (prior to a stressor) GC

57 levels and a repeatable GC response following a stressor (Pottinger and Carrick 2001;

58 Ouedraogo et al. 2004; Wada et al. 2008; Rensel and Schoech 2011; Cook et al. 2012; Narayan

59 et al. 2013; Love et al. 2015) resulting in different reactive scope profiles (difference between

60 GC response and baseline) between individuals (Romero et al. 2009). The biological

61 functionality, as well as the individual and evolutionary consequences of such variability among

62 individua, are still debated (Breuner et al. 2008; Bonier et al. 2009).

63

64 To define the biological consequences of individual variation in GC levels during the stress

65 response, researchers have attempted to relate inter-individual differences in baseline and

66 post-stressor GC concentrations to performance (e.g., migration, parental care, territorial

67 behavior) and fitness (e.g. Comendant et al. 2003; Cabezas et al. 2007; Breuner et al. 2008).

68 From a theoretical point of view, it is often hypothesized that individuals with greater post-

69 stressor GC levels should have increased long term fitness (Breuner et al. 2008), because an

70   individual's stress response increases chances of surviving the stressor (e.g. predation attempt)

71   and mediates trade-offs between reproduction and survival. Indeed, experimental studies show

72   variability in the relationship between the magnitude of the stress response and performance

73   or fitness measures (reviewed by Breuner et al. 2008). This relationship is increasingly

74   understood as context dependent, because an elevated stress response in an emergency

75   situation shifts resources from reproduction to survival (Wingfield et al. 1998); however, an

76   elevated stress response when the perception of stressors is exaggerated can lead to the

77   misallocation of resources, thereby limiting investment in growth and reproduction (Hawlena

78   and Schmitz 2010). There is a paucity of studies directly examining inter-individual differences

79   in stress response while concurrently measuring performance or fitness (Romero and Wikelski

80   2001; Blas et al. 2007; Cook et al. 2011).

81

82   In contrast to the theorized positive association between elevated post-stressor GC levels and

83   performance or fitness, higher baseline GC levels are often inferred to indicate reduced

84   condition, because it suggests an organism is experiencing greater environmental stressors

85   (Cort-Fitness hypothesis; reviewed by Bonier et al. 2009). Higher GC levels are in circulation

86   when exposed to a stressor and are associated with the reallocation of resources from fitness-

87   related factors, such as growth and reproduction, towards the restoration of physiological

88   homeostasis. Despite the potential negative consequences associated with higher baseline GC

89   levels, some studies have found that moderate long term increases in GCs can actually increase

90   individual performance (Comendant et al. 2003; Pravosudov 2003; Cabezas et al. 2007). Further

91   investigation is therefore warranted to understand the relationship between baseline GC

92   hormones and performance.

93

94   Both baseline and post-stressor GC concentrations have a heritable component in some species

95   (Evans et al. 2006; Jenkins et al. 2014) and are associated with a suite of other traits that can

96   lead to different life history and coping strategies (Koolhaas et al. 1999; Pottinger and Carrick

97   1999; Blas et al. 2007; Øverli et al. 2007; Wada et al. 2008). Selection on heritable variation in

98   GC levels and response is thought to drive the evolution of life-history traits (Bolnick et al. 2003;

99   Williams 2008), such as migration behavior. Migration in particular, requires large amounts of

100   energy and often exposes individuals to many stressors including predation and changes in

101   environmental condition (Dingle 2014). Thus, GC levels have the potential to be an important

102   factor determining successful migration (Holberton 1999; Piersma et al. 2000; Angelier et al.

103   2009), which could affect fitness and potentially drive the evolution of migration strategies.

104   Migration success, meaning successfully reaching the migration destination, has been linked to

105   GC levels in a single species of fish, adult sockeye salmon (*Oncorhynchus nerka* (Walbaum,

106   1792)), where upriver migration success was found to increase with stress responsiveness but

107   not baseline stress levels (Cook et al. 2014). GC levels have also been related to the

108   smoltification process in anadromous salmonids. Smolting fish generally have higher baseline

109   and peak GC, which appears to induce the physiological changes required to cope with the

110   transition from freshwater to saltwater (e.g., increased osmoregularity capacity; Barton et al.

111   1985; Redding et al. 1991; Björnsson et al. 2011). Stressors that cause a GC response may cause

112   a reallocation of energy towards immediate survival resulting in a change in life history and

113   coping strategy (Landys et al. 2006; Shepard et al. 2008). Thus, GC variation resulting from past

114   or current conditionsmay influence the "decision" to migrate. Alternative migration strategies

115   have the potential to be heritable through heritable GC regulation (Zera and Harshman 2001;

116   Williams 2008).

117

118   For our study species, brown trout (*Salmo trutta* (Linnaeus, 1758)), migration success provides a

119   measure of performance, and also acts as an indicator of alternate life history strategies. More

120   specifically, European populations of brown trout show a continuum of migration strategies,

121   where subpopulations are capable of anadromous activity, smaller scale migrations within

122   freshwater systems (potamodromous), and residency in natal streams (Cucherousset et al.

123   2005). This phenomenon is termed partial migration and reflects the fact that only a

124   component of the population engages in migration, a common trait among some salmonid

125   species (Jonsson and Jonsson 1993). Previous studies on the propensity of migration in brown

126   trout have found that individuals with high metabolisms and high growth rates tend to migrate,

127   likely due to higher resource requirements (onsson and Jonsson 1993; Forseth et al. 1999).

128

129   The goal of this study is to define relationships between GC levels (baseline and post-stressor),

130   and migration propensity in juvenile brown trout. Given the role of GCs in smoltification, we

131   would expect to see higher post-stress and baseline GC levels in migrating individuals. The

132   effects of individual body condition and size (length, mass) in relation to migration propensity

133   will also be quantified.

134

135 ***Methods***

136 *Experimental Design*

137 Brown trout were captured using backpack electroshocking (Scubla ELT 60 II GI; run at 300

138 volts) in four regions of the Gudsø Stream, Jutland, Denmark (Figure 1) between 11 March and

139 18 March 2013. This time period was selected as it fell prior to the spring migration, which

140 typically occurs in mid-April (Midwood et al. 2014). Individuals between 120 and 182 mm in

141 total length (TL) were used to minimize the effects of allometry on results. To evaluate an

142 individual's resting plasma cortisol levels (baseline), blood was collected (~0.2-0.3 ml) from a

143 subset of fish within 3 min of capture via caudal puncture of the haemal arch using a 1 ml

144 syringe and 25-gauge needle. A separate subset of fish (Table 1) were exposed to a

145 standardized 3 minute air exposure and then held in a 20 L container with 5 L of freshwater for

146 30 min prior to the collection of a post-stressor blood sample. Blood was sampled in the same

147 manner as above at 30 ± 3 minutes post-air exposure corresponding with the approximate peak

148 in GCs in brown trout (Pickering and Pottinger 1989). Blood samples were held in a water-ice

149 slurry for no more than 2 hrs until centrifuged for 5 min (10,000 rpm) to isolate plasma. Plasma

150 isolate was decanted, flash frozen and stored (-80°C) for later analysis of plasma cortisol

151 concentrations. Total length and wet mass of each individual were then measured. Next, a

152 uniquely coded 23 mm PIT tag (RI-TRP-RRHP, half duplex, 134 kHz, diameter 3.85 mm and a

153 mass of 0.6 g in air; Texas Instruments, Plano, Texas, USA) was inserted into the body cavity

154 through a small incision made with a scalpel. Previous tag retention studies on salmonids have

155 found negligible effects on survival and growth (Larsen et al. 2013) and generally low PIT tag

156 rejection rates (< 4% after seven months; Ombredane et al. 1998). Following tagging fish were

157   then placed in a 60 L container of fresh river water and monitored for a minimum of 30 minutes

158   to ensure recovery before they were ultimately released back into the stream near their point

159   of capture. All fish recovered. Length and mass were used to calculate Fulton's condition factor

160   (K) for each sampled fish where K = mass (g) × 100 / length^3 (cm) (Ricker 1975).

161

162   *Cortisol Analysis*

163   Plasma cortisol (ng mL^{-1}) was quantified using a commercially available enzyme linked

164   immunosorbant assay (ELISA) (Enzo Life Sciences, Cortisol EIA Kit [901-071], BioAssay Systems)

165   previously validated for use in fishes (Sink et al. 2008). This assay has a detection limit of 0.0567

166   ng mL^{-1}, therefore, the 5 samples with undetectable cortisol concentrations were assigned

167   values equal to this detection limit (Haddy and Pankhurst 1999; Liss et al. 2013).

168

169   *Tracking*

170   To track movements of individuals from their point of release to the sea, two tracking stations

171   were placed approximately 1 km upstream of Kolding Fjord on Gudsø Stream, which was the

172   closest feasible place to the ocean. The first station (S1) was placed upstream of a millpond and

173   the second station (S2) was placed downstream of the millpond, approximately 150 m from S1.

174   Each station was comprised of a paired set of PIT gates spaced approximately 10-m apart. This

175   allowed for an assessment of the direction of movement for individuals at each station. The

176   same array was used by Midwood et al. (2015) and found to have a detection efficiency at S1 of

177   96.3%; detection efficiency could not be evaluated at S2, but missed-detections were assumed

178 to affect all trout equally and therefore should not bias conclusions related to baseline GC or

179 post-stressor GC correlations with relative migration success to S1 and S2.

180

181 To estimate the proportion of individuals that stayed resident and did not migrate to the ocean,

182 the stream was re-sampled using back-pack electrofishing on 18 and 19 June, 2013, at which

183 point migratory individuals should have left the stream. Surveys started near the antenna and

184 proceeded upstream through all areas where brown trout were initially captured (Figure 2). It

185 was not possible to resample the entire system as it is greater than 16 km in length; however,

186 given the shallow and narrow nature of the stream and the relatively high efficiency of capture

187 for salmonid species using electrofishing (Kennedy and Strange 1981; Haynes and Baird 1994),

188 efforts were likely sufficient to provide a relative estimate of residency.

189

190 *Statistical Analyses*

191 Multiple linear regression was used to quantify relationships between plasma cortisol

192 concentrations (for both post-stressor and baseline groups) and timing of migration (time from

193 sampling to S1 passage), while controlling for *K*, length or mass. Assumption of homogeneity of

194 variance and normal distribution for the models were ensured by visual inspection of residual

195 plots. If violated, the response variable was log or square root transformed. Multiple binomial

196 logistic regression was used to test for the effect of plasma cortisol concentration (baseline and

197 post-stressor) on the probability of successful migration to S1 (used as a proxy of migration

198 strategy) and on the probability of successful migration to S2 given successful passage of S1

199 (used as a measure of migration success through the millpond for migrating individuals). Again,

200   separate tests were conducted using mass, length or *K* as a covariate in the model. Length,

201   mass and *K* were also tested separately for effects on all migration response variables with the

202   baseline and post-stressor fish subsets pooled. Two outliers were removed before analyses as

203   they were outside the standard deviation of the mean: a baseline cortisol value of 44.5ng/mL

204   and a peak cortisol value of 3.43ng/mL. All significant results found without the outliers were

205   also found when the outliers were included in the analyses.

206

207   Variation in association with recorded mean values is given as standard error (± SE) throughout.

208   Statistical significance for all analyses was set at $\alpha = 0.05$ and all statistical analyses were

209   conducted in R (v 3.2.2; R Core Team, 2016).

210

211   ***Results***

212   Of the 198 fish tagged, 19 were recaptured in mid-June, 83 were detected passing S1 and 45

213   passing S2 (Table 2). All fish detected at S2 were also detected at S1, suggesting a detection

214   efficiency of 100% at S1. Mean baseline cortisol was 5.6 ± 6.7 ng/mL and post-stressor cortisol

215   was 69.2 ± 40.1 ng/mL (Table 1). Post-stressor (Multiple logistic regression: $Z_{2,96} = 1.44$, *p* =

216   0.15) and baseline (Multiple logistic regression: $Z_{2,96} = 0.15$, *p* = 0.89) plasma cortisol

217   concentrations did not have a significant effect on the probability of successful passage at S1

218   when controlling for K, mass, or length (*p*>0.1; Figure 2b and 2e). For fish that passed S1, the

219   probability of successful passage to S2 increased with increasing individual baseline cortisol

220   when controlling for mass (Multiple logistic regression: $Z_{2,27} = 1.96$, *p* = 0.05) and length

221   (Multiple logistic regression: $Z_{2,27} = 2.01$, p = 0.045), but not for *K* (*p*>0.05). For fish that passed

222 S1, the probability of successful passage to S2 showed no relationship with post stressor

223 cortisol (All: $p>0.1$)

224

225 Mean migration time from blood sampling to S1 passage was 33.4±19.1 days and mean time

226 from S1 to S2 was 3.2±7.6 days. Multiple regression showed no significant relationship between

227 migration duration or date of passage, and post-stressor or baseline cortisol when controlling

228 for mass, length or K (All: $p>0.1$). Migration time decreased with increasing fish length (Linear

229 regression: $F_{1,79}= 6.82$, p = 0.01, $R^2 = 0.8$) and fish mass (Linear regression: $F_{1,79} = 4.4$, $p = 0.04$,

230 $R^2 = 0.05$), but no relationship was found with K (Linear regression: $F_{1,79}= 3.14$, $p = 0.08$, $R^2 =$

231 0.04).

232

233 Multiple logistic regression revealed no effect of post-stressor and baseline cortisol on the

234 probability of recapture in resident streams when controlling for K, length or mass (Figure 2; All:

235 $p>0.1$). Length, mass and K also were found to have no effect on the probability of recapture in

236 resident streams (All: Logistic regression: $p>0.1$). No relationship was found between K, mass or

237 length and baseline or post-stressor cortisol (All: Linear regression: $p>0.1$). The probability of

238 S1 passage and the probability of passage between S1 and S2 had no relationship with K, fish

239 length and fish mass (All: $p>0.08$).

240

241 ***Discussion***

242 As previous studies have found cortisol to be elevated during smoltification (Barton et al. 1985),

243 we expected individual brown trout with higher baseline and post-stressor cortisol to have a

244   higher probability of migrating downstream to S1 and S2, coupled with a lower probability of

245   recapture as resident. However, no such relationships were found.

246

247   We found no evidence suggesting that migration is correlated with endocrine regulation in

248   juvenile brown trout, despite several studies having documented that cortisol levels rise during

249   smoltification in andromous fish (Barton et al. 1985; Redding et al. 1991; Björnsson et al. 2011),

250   and also that GC regulation has the potential to play a role in determining alternative coping

251   strategies (Zera and Harshman 2001; Landys et al. 2006; Williams 2008). Mass, length and body

252   condition were also not found to have a relationship with plasma cortisol (baseline or post-

253   stressor). Fish of greater mass and length (but not condition) were found to have shorter

254   migration times similar to previous findings (Bohlin et al. 1996). Body condition, length and

255   mass were not found to affect migration propensity to S1. the lack of correlation between our

256   condition metrics and migration propensity is counter to previous findings of reduced condition

257   leading to increased migration propensity (Peiman et al. 2017); however, it is possible that the

258   comparatively limited recapture rate in the present study (N=19) prevented the detection of a

259   size-based effect.

260

261   Baseline, but not post-stressor, cortisol concentration was found to be positively associated

262   with passage through the millpond (i.e., from S1 to S2). Previous studies have found brown

263   trout baseline cortisol without chronic stressors to be 0-5 ng/mL, while those exposed to

264   chronic stressors such as confinement or crowding have shown baseline cortisol elevated to 10

265   ng/mL (Pickering and Pottinger 1989). The average baseline cortisol found in individuals

266   recaptured in resident streams, individuals that passed S1 and individuals that passed S2 was

267   5.6 ng/mL, 5.8 ng/mL and 8.4 ng/mL, respectively (Table 2). Thus, individuals that passed

268   through the millpond had baseline cortisol levels of individuals approaching chronically stressed

269   levels on average, while other fish had cortisol levels closer to what would be expected for fish

270   not showing chronic stress.

271

272   The distance between S1 and S2 is only 150 m, thus it is unlikely that S1 versus S2 passage

273   would be indicative of migration strategy. The millpond may therefore be an area of increased

274   mortality, suggesting S1 passage is more indicative of migration strategy (resident and

275   potamodromous versus anadromous) while passage between S1 and S2 is more indicative of

276   migration success of migrant individuals. Forty five percent of all brown trout that passed S1

277   were not detected passing S2, which is only 150 meters downstream, and those that did

278   migrate through the millpond, took much longer than when migrating through the stream,

279   taking an average of 3.2 days to move 150 meters. Similar patterns are found in other studies of

280   migrating fish at Gudso (Midwood et al. 2014, 2015). This indicates the millpond was

281   challenging for migrating trout. We suggest that many of these individuals likely suffered

282   mortality, as it is unlikely for such a large proportion of migrants to take up residency in such a

283   small area. Furthermore, there is anecdotal evidence of high concentration of foraging grey

284   heron (*Ardea cinerea* Linnaeus, 1758) and other birds at the outflow of millpond. Previous

285   studies on salmonid smolts have documented large losses resulting from predation in lakes

286   (Jepsen et al. 1998; Koed et al. 2006) and at weirs and related millponds (Aarestrup and Koed

287   2003). For example, Jepsen et al. (1998) found 90% of smolts died while passing through Lake

288   Tange, Denmark, with 56 % of the mortality cause by pike (*Esox lucius* (Linnaeus, 1758)) and

289   31% by birds. Another possibility for the reduced passage and long migration time through the

290   millpond is that there is only one small exit and trout may have had a hard time getting out.

291   While it is possible that overall lower passage at S2 is driven by reduced detection efficiency at

292   this station, these missed-detections would affect all trout equally and thus would not affect

293   the relationship found between baseline cortisol and passage between S1 and S2 other than by

294   reducing statistical power. Our results, therefore, do not support the link between GC levels

295   and migration strategy in brown trout, but they do suggest that baseline GC (and not post-

296   stressor GC) is positively associated with migration success.

297

298   Generally it is hypothesized that increased baseline GC are associated with decreased

299   survivorship because increased baseline GC are associated with increased chronic stressors

300   (Cort-Fitness hypothesis: Bonier et al. 2009). Our findings appear to support the opposite trend

301   as migratory fish with lower baseline cortisol appear to have lower successful passage through

302   the millpond (Figure 2). Several other studies have also found higher baseline cortisol to be

303   correlated with increased survivorship: Cabezas et al. (2007) found moderately elevated

304   corticosterones were associated with decreased body condition but increased probability of

305   survival in European wild rabbits (*Oryctolagus cuniculus* (Linnaeus, 1758)); Comendant et al.

306   (2003) found higher corticosterone in female lizards (*Uta stansburiana* (Baird and Girard, 1852))

307   to be associated with increased survival to second ovulation; and Cote et al. (2006) found

308   increased corticosterone to modify behaviour (locomotor and social activity), increasing survival

309   in male adult common lizards (*Lacerta vivipara* (Jacquin, 1787)).

310   Another possible explanation for why our results are not consistent with the Cort-Fitness

311   hypothesis is that it is difficult to discern whether higher baseline cortisol indicates exposure of

312   the individual to greater chronic stress (which is assumed to be the case by the Cort-Fitness

313   hypothesis; Bonier et al. 2009), or indicates a more competent ability to cope with chronic

314   stressors. The first would likely lead to decreased survivorship with increasing baseline GC level,

315   while the later would likely result in the opposite trend. In our study, all individuals may be

316   subjected to similar chronic stressors as they were all reared in the same stream reach. Thus,

317   baseline stress may be a better indicator of better ability to cope with chronic stressors than

318   exposure to greater environmental stressors.

319

320   Post-stressor cortisol, a proxy for stress responsiveness, was not found to be a significant

321   predictor of migration timing, survival or success. Potentially, the effect of stress

322   responsiveness was masked, because we were not able to control for individual variability in

323   baseline as fish were too small to obtain both a baseline and a post-stressor cortisol sample.

324   However post-stressor cortisol was more variable (SD=40.7 ng/mL) than baseline cortisol

325   (SD=7.7 ng/mL). Cockrem (2013) reviewed studies on baseline and stress-induced GCs and

326   determined that there tended to be more variation in baseline GCs among individuals than in

327   stress-induced levels. Although our findings are not consistent with that generalization,

328   Cockrem (2013) did note that it was not ubiquitous with a number of exceptions. It is unclear

329   why that pattern was observed here but reasons, among others, could include inter-individual

330   differences in how the stressor was perceived or different stress histories. Despite the widely

331   accepted belief that acute stress responses are adaptive, similar to our study several other

332   studies have failed to find a relationship between stress responsiveness and performance

333   (Romero and Wikelski 2001; Blas et al. 2007), or may even reduce performance. These results

334   may be an indication that different reactive scopes profiles may allow individuals to adequately

335   cope with stressors. Additionally, the benefits of stress responsiveness are increasingly viewed

336   as context dependent, as traits induced by a stress response can have variable consequences to

337   an organisms' performance depending on the nature of the stressor (Breuner et al. 2008).

338

339   Similar to our findings in brown trout, the only other study we are aware of that examines

340   individual variation in endocrine regulation in relation to migration in fish (anadromous

341   semelparous sockeye salmon) found no correlation between GC levels and migration timing or

342   rate. They also found individuals with higher stress responsiveness to have greater migration

343   success, but that baseline cortisol not to be a significant predictor of migration success (Cook et

344   al. 2014). Differences in the relationship between GC secretion and migration success found by

345   our study may be attributed to differences in the stressors experienced. For example, salmon

346   may have more chronic stressors such that their baseline levels are at the turning point of the

347   baseline cortisol-survivorship curve hypothesized by Cabezas et al. (2007), where differences in

348   baseline levels have negligible effects on survivorship. The inconsistency between findings may

349   also be reflective of differences in physiology between species.

350

351

352   ***Conclusions***

353   In summary, we found no evidence suggesting that cortisol regulates the migration life history

354   trade off in juvenile brown trout. But, slightly higher levels of baseline GC (and not post-stressor

355   GC) may favor migration performance. Further study is warranted to help determine the

356   relationship between migration success and GC, with special focus on stress responsiveness

357   within an individual.

358

359

360   ***Acknowledgements***

368

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549   Table 1: The mean (with SD) and range of plasma cortisol, individual fish length, mass and

550   Fulton's Condition factor (*K*), for juvenile brown trout (*Salmo trutta*) collected from the Gudsø

551   Stream, Denmark. Collected fish were blood sampled either within three minutes of capture

552   (baseline) or 30 minutes following a standardized three minute air exposure (post-stressor).

Treatment	Baseline	Post-Stressor
Sample size	99	99
Cortisol (ng/mL)	5.6 ± 6.7	69.2 ± 40.1
	(0.1 to 25.5)	(7.2 to 197.6)
Length (mm)	146±126	147±13
	(120 to 182)	(122 to 171)
Mass (g)	28.2±7.6	29.2±8.1
	(15.8 to 48.5)	(17.1 to 45.8)
K	0.90±0.07	0.90±0.06
	(0.66 to 1.06)	(0.72 to 1.09)

553

554

555   Table 2. Number of individuals recaptured, number of sampled individuals that were detected

556   at S1 and the mean cortisol concentration (with SD) of these individuals for baseline (sampled

557   within three minutes of capture) and post-stressor (sampled 30 min minutes following a

558   standardized three minute air exposure) treatment groups of juvenile brown trout (*Salmo*

559   *trutta*) collected from the Gudsø Stream, Denmark.

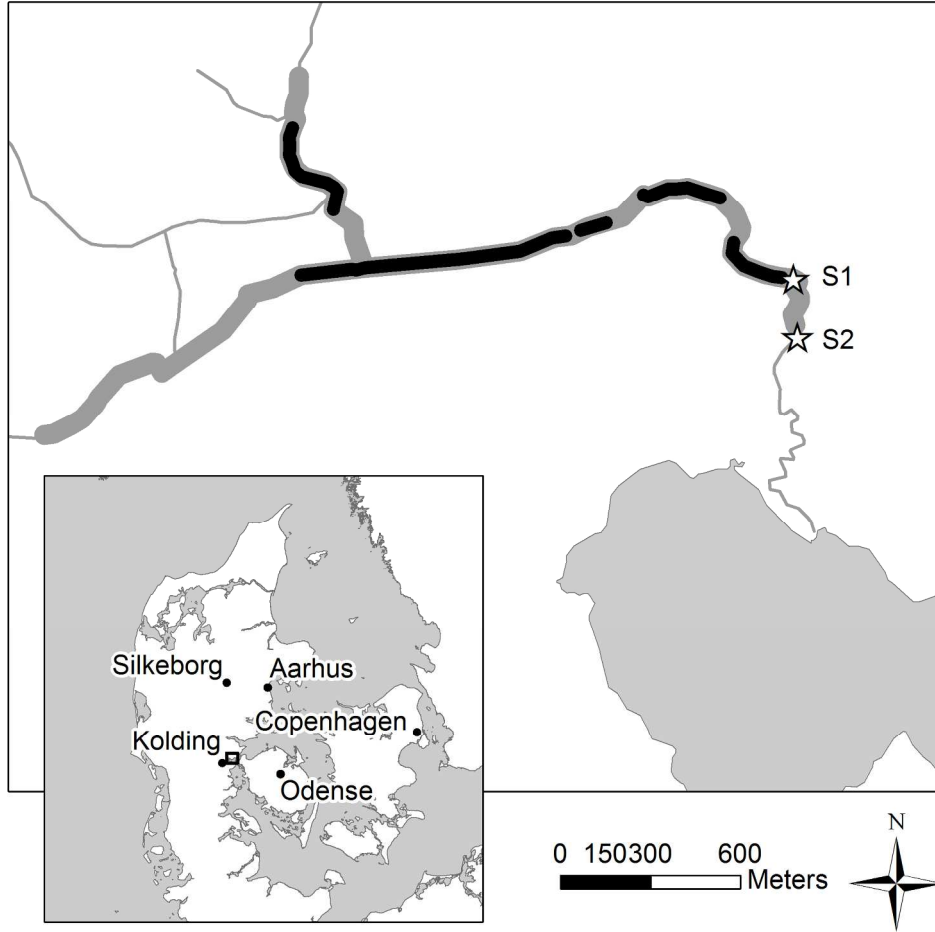| Treatment	Baseline	Post-Stressor
Number recaptured	13	6
Cortisol of recaptured (ng/mL)	5.6±5.0	69.9±40.1
Number S1 passage	37	46
Cortisol Passing S1(ng/mL)	5.8±7.7	76.2±42.3
Number S2 passage	19	26
Cortisol Passing S2 (ng/mL)	8.4±9.0	79.2±42.4

560

561

562   Figure 1. Location of Gudsø Stream in Denmark is shown as a black box in the bottom left panel

563   (north-east of Kolding). The portions of the Stream where brown trout (*Salmo trutta*)were

564   captured are shown as thick black lines and the areas re-sampled in June 2013 are shown as

565   thick grey lines. The positions of the two PIT antennas, S1 and S2, are also shown in the larger

566   map. Light gray areas represent water and the outflow location of Gudsø Stream into Kolding

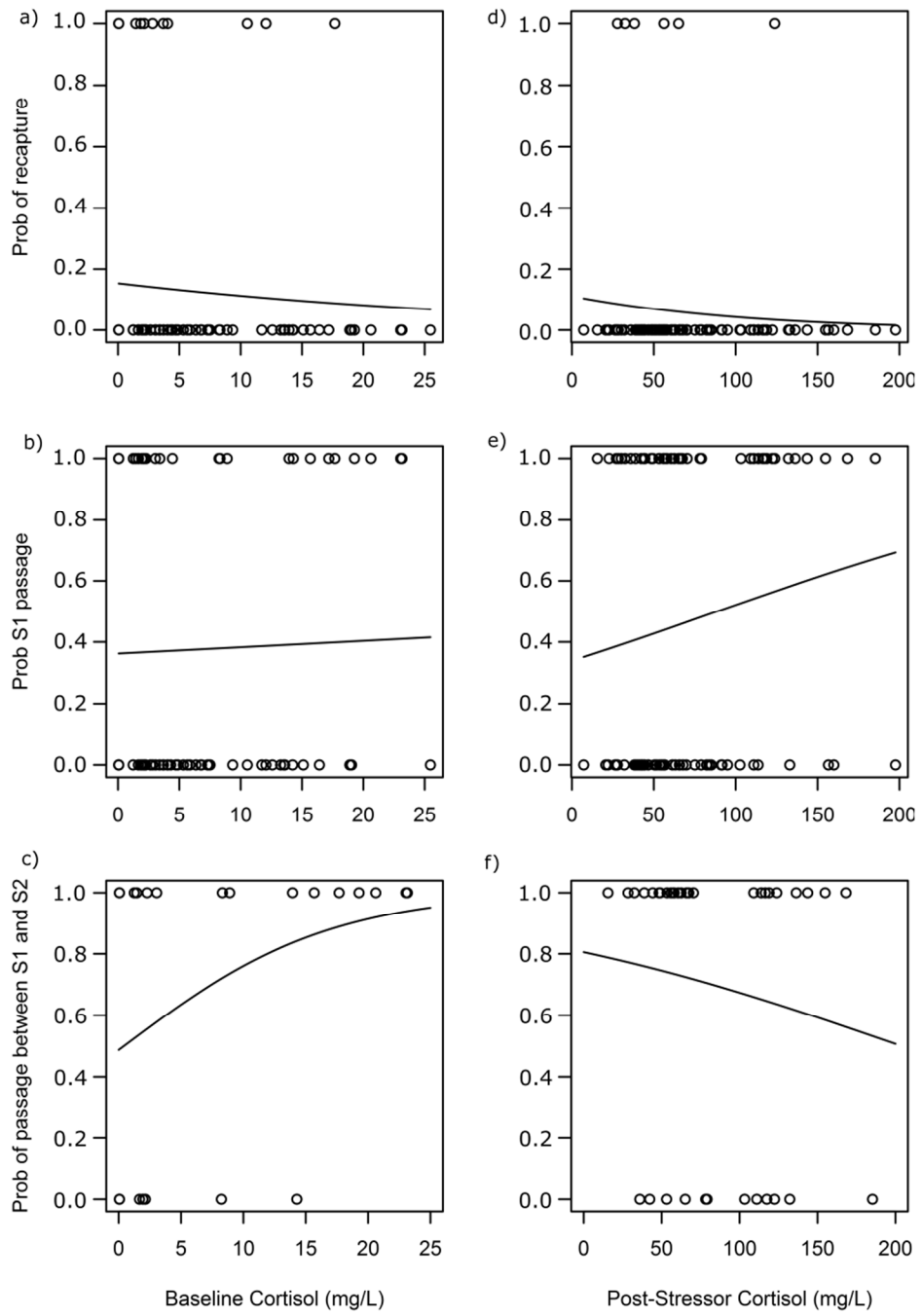567   Fjord. Map data from the European Environment Agency.

568

569   Figure 2. The probability of recapture (a), successful S1 passage (b), and successful passage

570   between S1 and S2 (c) as a function of individual baseline cortisol plasma concentration

571   collected within 3 minutes of capture, for individual juvenile brown trout (*Salmo trutta*) on the

572   Gudsø Stream, Denmark on the left, and on the right the probability of recapture (d), successful

573   S1 passage (e), and successful passage between S1 and S2 (f), as a function of individual cortisol

574   plasma concentration 30 minutes post 3 minute air exposure. Open circles represent actual

575   values found for individuals and the line represents the values predicted using a binary logistic

576   regression with no covariates. Binary logistic regression found no significant relationships

577   except for (c): increased baseline cortisol was associated with greater probability of successful

578   S2 passage for fish that had completed S1 passage, with marginal significance when mass

579   (p=0.05) or length (p=0.04) was included in the model as a covariate, but not when Fulton's

580   condition factor was included as a covariate. Significant relationships are indicated by three

581   stars.

582

583   Figure 1

584

585   Figure 2