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1 **Title**: HPI reactivity does not reflect changes in personality among trout introduced to bold or

2 shy social groups

3

4 **Short Title**: Effect of social interaction on trout coping style

5

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25 **Summary**

26 Physiological stress responses often correlate with personalities (e.g. boldness). However,

27 this relationship can become decoupled, although the mechanisms underlying changes in this

28 relationship are poorly understood. Here we quantify (1) how an individual's boldness

29 (response to novel objects) in rainbow trout, *Oncorhynchus mykiss*, changes in response to

30 interactions with a population of either bold or shy conspecifics and we (2) measured

31 associated post-stress cortisol levels. Initially-bold trout became shyer regardless of group

32 composition, whereas shy trout remained shy demonstrating that bold individuals are more

33 plastic. Stress-induced plasma cortisol reflected the original personality of fish but not the

34 personality induced by the treatment, irrespective of population personality. Change in

35 boldness of bold trout may indicate preference towards initially subordinate behaviour when

36 joining a new population. However, here we provide further evidence that behavioural and

37 physiological parameters of coping styles may become uncoupled whereby behavioural

38 changes are not correlated with stress responsiveness.

39

40 **Keywords:** coping style; boldness; behavioural plasticity; *Oncorhynchus mykiss*; cortisol;

41 group influence

42

43

44 1. **Introduction**

45

46 Variation in personality – consistent between-individual differences in behaviour

47 (Adriaenssens & Johnsson, 2012) – has clear fitness consequences and is, therefore,

48 evolutionarily important. However, the fitness benefits of behavioural traits depend on the

49 environmental and social context (Sih & Watters, 2005), and the extent to which individuals

50 can modulate their personality to match new challenges thus has important evolutionary

51 implications. While between-individual differences in behaviour generally are consistent

52 through time (Sih et al., 2004), personalities may change dependent upon environmental

53 context (Dingemanse et al., 2010). One key personality axis is boldness, the propensity for

54 taking risks in novel situations (Sneddon, 2003; van Oers et al., 2005). Boldness typically

55 correlates with behaviours such as aggression, activity and exploration (Lima & Dill, 1990;

56 Sneddon et al., 2003; Bell, 2005; van Oers et al., 2005; Adriaenssens & Johnsson, 2012).

57 Interactions with exogenous stimuli often lead to shifts in boldness (Cockrem, 2007; Frost et

58 al., 2007); the extent of behavioural change may, however, be limited by the costs of

59 information gathering and integration, usually in the form of detecting environmental cues

60 and enacting any relevant physiological adjustments associated with such change (DeWitt et

61 al., 1998).

62

63 Personality has been linked with the magnitude of an individual's physiological response to

64 stress (e.g. Koolhaas et al., 1999) such that individuals can be described according to coping

65 style. Proactive animals respond to stress with relatively low hypothalamo-pituitary-

66 adrenal/interrenal (HPA/I) axis activity and tend to exhibit bold behaviour, while reactive

67 individuals are characterised by a higher HPA/I response, inactivity and shyness.

68 Additionally, proactive and reactive animals have different capacities for behavioural change,

69 with proactive animals more likely to form routines and having more rigid personalities than

70 the more behaviourally labile reactive animals (Koolhaas et al., 1999; Cockrem, 2007; Ruiz-

71 Gomez et al., 2011). These individual differences are heritable and likely provide populations

72 with the requisite behavioural variation to cope with a variety of environmental pressures

73 (Ruiz-Gomez et al., 2011). However, recent data suggests that proactive and reactive profiles

74 are not rigid *per se*. For instance, though boldness is often strongly linked with HPA/I

75 reactivity, the relationship could be context-dependent or decoupled under particular

76 environments (e.g. Vaz-Serrano et al., 2011). Likewise, incidents of high stress have also

77 been observed to induce behavioural change in trout without a shift in underlying

78 physiological parameters (Ruiz-Gomez et al., 2008) . Recent work has explored whether

79 underlying physiology drives behavioural differences (Koolhaas et al., 2010), whether

80 behaviour instead drives physiological differences through encouraging exposure to

81 particular stress-inducing challenges (Carere et al., 2010), or indeed whether such

82 relationships are linked through underlying factors such as a common pathway or pleiotropic

83 effects (Carere et al., 2010). Currently, none of these relationships appears to be particularly

84 strongly supported over any other.

85

86 Although personality of an individual strongly defines their responses to stimuli, a common

87 question, particularly among social animals, is how much of any behavioural response can be

88 attributed to an individual's personality compared to the influence of other group members

89 (Magnhagen & Bunnefeld, 2009). The contribution of individual and social factors may

90 depend on context: in some instances, individuals of a particular personality may take a

91 particular role (e.g. initiative and leadership by bold individuals; LeBlond & Reebs, 2006;

92 Harcourt et al., 2009; Favati et al., 2014); alternatively, the group's actions may be influenced

93 by the average behaviour of the group (Sih & Watters, 2005). However, individual fish may

94 be expected to adapt their behaviour to conform to their group behavioural profile to

95 minimise the potential increase in risk (associated with behaving differently to the rest of the

96 group) whilst simultaneously maximising the benefits of working together and sharing

97 information, particularly to more quickly acclimate to a new environment with unknown risks

98 and resource availability (Krause & Ruxton, 2002; Magnhagen, 2012; Castanheira et al.,

99 2016). Thus the expectation is that there may be a shift in some behaviours as individuals join

100 a group. However, how socially-induced changes in personality are reflected in underlying

101 physiological mechanisms appear to be little explored.

102

103 Thus, the aims of this study were (1) to determine whether boldness in rainbow trout

104 *Oncorhynchus mykiss* is influenced by the behavioural composition of a social group, and

105 whether such behavioural change was linked to the personality of the fish itself; and (2) to

106 determine whether post-stress cortisol profiles reflect any such behavioural change. The

107 relationship between personality and stress responsiveness has been well-explored in rainbow

108 trout (Øverli et al., 2005; Thomson et al., 2011) and *O. mykiss* therefore provides a robust

109 model for investigating animal personality and the influence of personality on how

110 individuals respond to social and environmental stimuli.

111

112 **2. Material and methods**

113

114 *2.1 General animal husbandry*

115

116 Rainbow trout, *Oncorhynchus mykiss* (approx. 7.5-12.5 cm total length) were obtained from a

117 commercial supplier and housed in stock tanks (2 x 2 x 0.5 m) at approximately 170 fish per

118 tank (not all of which were used in this project). Trout were maintained on a semi-

119 recirculating system on a 14:10 h light:dark cycle at 13±1°C, and fed 1% body weight per

120 day on commercial trout feed (Skretting, UK). Half of the tank had an opaque cover for

121 shelter. For experimental procedures, fish were caught at random and transferred to

122 individual glass aquaria (90 x 50 x 45 cm; termed the home aquaria) which were screened

123 from visual disturbance (Figure 1). All home aquaria were supplied with filtered freshwater

124 and maintained at 10 ± 1°C with constant aeration and 1% body weight of food per day at the

125   same time each day. Fish to be used as a focal individual were anaesthetised, weighed and

126   tagged one day after transferal to the home aquarium (Section 2.4). Otherwise, fish were

127   allowed to acclimatize for at least one week before being evaluated for boldness (Section 2.2)

128   to ensure that behavioural responses to stimuli are not been impaired by the stress associated

129   with moving (Beitinger, 1990). Fish that did not resume feeding within two weeks of moving

130   were excluded from the experiment.

131
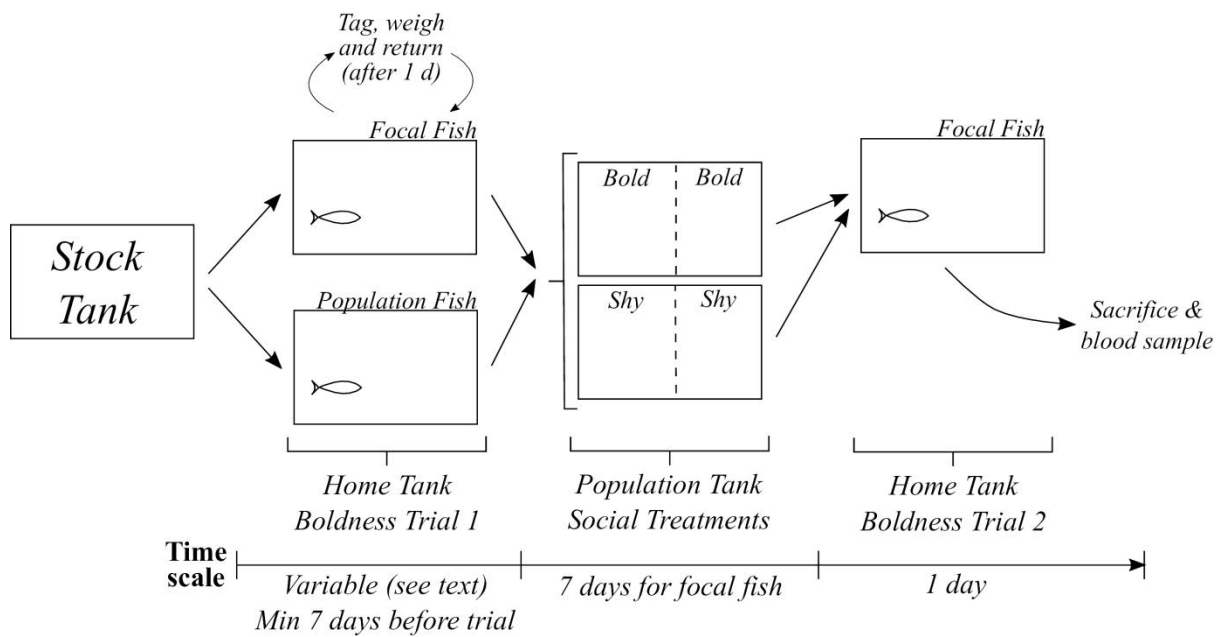
Figure 1: Schematic of the experimental procedure, indicating the experimental set-up and the time-scale of experiments. Dotted lines indicate dividers within the Population Tanks which housed two fish populations simultaneously (see Section 2.3 for details).

138   *2.2 Individual boldness trial I*

140   Boldness was assessed using standard novel object tests used to distinguish bold and shy

141   behaviour in rainbow trout (Frost et al., 2007; Thomson et al., 2011), and which correlate

142 with other measures of boldness, e.g. latency to emerge into a novel arena (Brown et al.,

143 2007; Adriaenssens & Johnsson, 2012), latency to taste novel prey (Frost et al., 2007), and

144 the degree of active / passive) behaviour (Thomson et al., 2011). A low-light video camera

145 was positioned in front of the home aquarium that was lined with rulers to quantify the

146 position of any fish and object. After 10 min, to allow fish to recover from any disturbance of

147 setting up the equipment, a novel object was placed into the centre of the aquarium and the

148 behaviour of the fish was recorded for 10 min (see also Section *2.7*). Boldness was scored

149 according to three factors: latency to approach to within 5 cm of a novel object (*approach*

150 *latency*), duration of time that individuals were passive (*passive duration*), and the frequency

151 with which an individual initiated passive behaviour (*passive frequency*). Passive behaviour

152 included the subject resting at the base of the tank, pivoting on its own axis, and drifting

153 across the tank, but excluded active swimming for more than one body length. Including

154 measures of passive behaviour provides further justification of our selection criterion for

155 identifying bold and shy fish.

156

157 Concordant with previous studies (Frost et al., 2007; Thomson et al., 2011; Thomson et al.,

158 2012), the main measure of boldness was latency to approach the novel object; fish that

159 approached the novel object within 180 s were considered bold (*n*=13) and those that did not

160 approach the object within 300 s as shy (*n*=21), as trout that do not approach an object within

161 600s are unlikely to approach it ever (Frost et al., 2007). No fish had an *approach latency*

162 between 180-300 s in the first trial. Trout classified as bold or shy exhibit clear differences in

163 personality (e.g. Thomson et al., 2011; Thomson et al., 2012), with such measures of

164 behaviour consistent over time in constant conditions (Thomson et al., 2011). After this test

165 for boldness, fish were moved into population tanks (Figure 1) either as a member of the

166 population (Section 2.3) or as a focal fish (Section 2.4).

168 *2.3 Population tanks*

169

170 Separate social populations of either nine bold or nine shy trout were set up in opaquely-sided

171 tanks (~149x92x37 cm) that were otherwise identical to the stock tanks. Populations

172 consisted of entirely bold or shy groups to maximise the impact of social cues on focal

173 individuals and to limit potential conflicting cues that may arise from mixed groups. A total

174 of four populations (two bold and two shy; Figure 1) were established in two aquaria, with

175 populations within the same aquarium separated by a divider to prevent direct interactions.

176 As populations within the same tank shared the same flow-through of water, the same

177 behavioural types, both the social population and the focal individual, were maintained in the

178 same aquarium to ameliorate transfer of chemical cues associated with specific behaviours

179 among populations during the experiments. Populations were established over a one-month

180 period, with the same fish utilized throughout the experiment to reduce variation in the

181 population composition between focal fish, and to minimize the total numbers of animals

182 used in accordance with Home Office guidelines.

183

184 *2.4 Focal fish*

185

186 The day after the transfer from stock tanks to home aquaria, focal trout (n=34, 35.64±3.09 g)

187 were netted, anaesthetized (benzocaine: Sigma-Aldrich Co., UK) at 0.033 g l^{-1}) and tagged

188 using yellow visible implant elastomer (VIE, Northwest Marine Technology Inc.) behind the

189 eye. Tagging with VIE has no effect on fish behaviour or growth (Olsen & Vøllestad, 2001).

190 Each fish was weighed (to 0.01 g), returned to its home aquarium and allowed to acclimate

191 for one week or until resumption of feeding. Focal fish were then given the novel object test

192   (Section *2.2*) to assess boldness before being placed into one of the population tanks. Only

193   one focal fish was placed into each population at a time.

194

195   *2.5 Social treatment*

196

197   Focal fish spent 1 week in the population tank (Figure 1), during which time the group was

198   exposed (in random order) to three cues, each on a single occasion: (1) novel object exposure

199   - a novel object was placed centrally into the tank and left for 15 min before being retrieved

200   with a net (see also *2.8 Novel Objects*); (2) simulated predator attack – food pellets were

201   presented to the population and when one fish attempted to feed, a predator threat was

202   simulated using a model heron (*Ardea cinerea*) head that was thrust towards the group of fish

203   twice in succession (Jönsson et al., 1996; Johnsson et al., 2001); (3) Net chase - the

204   population was chased with a hand-net (15x10 cm) for 2 min, with the net moved around the

205   tank to ensure that each fish was chased; net-chasing is thought to simulate chasing by a

206   predator (Brown et al., 2007). These cues were intended to encourage a population response

207   to an environmental challenge, thereby reinforcing interactions within the population to

208   facilitate possible transmission of boldness-related behaviour between the focal fish and other

209   trout within their populations.

210

211   During husbandry procedures two bold focal fish in a bold population were observed to be

212   chased by the population animals; this would be expected, since rainbow trout form

213   dominance hierarchies through agonistic interactions (Pottinger & Carrick, 2001; Gilmour et

214   al., 2005), and bold animals are more aggressive and tend to become dominant (Sih et al.,

215   2004; Huntingford & Adams, 2005). Animals that experienced excessive chasing and which

216   exhibited signs of stress were removed from the experiment within 24 hours; there was no

217 evidence that this biased our sample, since our data is fully representative for bold and shy

218 fish, with no systematic removal of fish that responded during a certain time period (Section

219 *3.1*). To minimise the impact upon focal fish, the sample size for the bold focal fish in bold

220 population treatment group was capped at *n*=5. Aggressive interactions were not quantified as

221 part of the experiment. No chasing behaviour was observed among the shy population, nor of

222 shy focal fish held within the bold population, and thus sample sizes were not capped for

223 these treatments.

224

225 *2.6 Individual boldness trial II*

226

227 After one week, the focal fish was identified by its VIE tag, retrieved and replaced in its

228 home aquarium. After a 24 hour recovery period, focal fish were re-tested for boldness

229 (Section *2.7*): a 24 hour period was considered sufficient time to allow the fish to recover

230 from the immediate stress of handling, but not enough time to re-habituate to solitary

231 conditions. Physiological habituation to stimuli takes upwards of 7 days in trout (Moreira et

232 al., 2004) whilst behavioural conditioning also takes more than 2 or 3 days to achieve

233 (Sneddon, 2003). Trout are routinely tested for boldness immediately after movement

234 between tanks or locations as part of novel- or open-field tests (e.g. Schjolden et al., 2005),

235 suggesting that relocation does not appreciably alter behaviour. Therefore the second measure

236 of boldness was considered to accurately reflect the animal's behaviour after co-habitation

237 with the population, and extending the recovery period would have a detrimental impact on

238 our estimate of the influence of social exposure to behaviour.

239

240 *2.7 Novel objects*

241

242 Each focal fish was exposed to three novel objects throughout the study.

243 1. Boldness trial I (section 2.2): Orange rubber stopper (7.1 x 4.9 cm)

244 2. Social Treatment (section 2.5): Lego Duplo[TM] construct with shapes and colours altered

245   between every trial and each used just once throughout the study (objects constructed

246   from a combination of 4-8 blocks, either 2x2 or 2x4; mean $\pm$ SE volume of objects=137.7

247      $\pm$ 9.1cm^3; colours of bricks were red, blue and black)

248 3. Boldness trial II (section 2.6): Weighted blue box (7.5 x 5.3 x 3.8cm)

249 Use of three objects prevented individuals from habituating to one object, although this

250 procedure was precautionary as it is unlikely that fish will habituate unless an object is

251 presented regularly and repeatedly (Sneddon et al., 2003); here, an object was presented at a

252 maximum rate of once per week.

253

254 *2.8 Stress physiology measurements*

255

256 On the same day as the final behavioural trial focal fish were exposed to an emersion stressor

257 by being netted and lifted from the water for 60 s to induce an acute physiological stress

258 response (Pickering & Pottinger, 1989). Focal fish were then returned to the home aquaria for

259 15 min (to allow a cortisol response to the stressor to develop), after which time the fish was

260 netted and euthanized by concussion. This procedure was completed at the same time each

261 day for every focal fish to ensure that hormonal levels were not compromised by diel

262 fluctuations (Pickering & Pottinger, 1983). Immediately after euthanasia, fish were weighed

263 and a blood sample was taken from the caudal vessels into a 2 ml heparinised syringe. The

264 blood was centrifuged at 3,500 RPM for 5 min at 4°C, and the supernatant plasma aspirated

265 and frozen at -20°C until analysis. Cortisol concentrations were determined using a validated

266 radioimmunoassay procedure (Pottinger & Carrick, 2001) with sample order randomised and

267   the assays conducted blind. Overall, 34 focal fish were tested (in bold populations: 5 bold and

268   11 shy; in shy populations: 8 bold and 10 shy). Given the size of the fish and the possible

269   effect of blood removal on blood volume and associated tissue damage it was considered too

270   much of a risk to the well-being and survival of the fish to collect blood for cortisol

271   measurements before the Social Treatment.

272

273   *2.9 Statistical Analyses*

274   All analyses were completed in R (v. 3.2.3; R Core Team, 2015) and in all cases *p* values

275   were compared to α=0.05.

276

277   Mann-Whitney tests were used to compare the *passive duration* (s) and *passive frequency*

278   between ostensibly bold and shy trout in the first trial, and thus demonstrate that *approach*

279   *latency* alone (Section *2.2*) could be used to discriminate between discrete bold and shy

280   personalities. Post-stress plasma cortisol concentration (ng ml^{-1}) was analysed using ANOVA

281   with focal boldness, group boldness, and their interaction as fixed factors. Analysis of

282   specific growth rate is detailed in Supplementary Material.

283

284   Change in *passive behaviour* and *passive frequency* across the two trials were analysed using

285   a mixed-model approach (Zuur et al., 2009). For *passive duration* a linear mixed effects

286   model (lmer) was used whereas *passive frequency* was modelled using a generalized linear

287   mixed effects model (glmer) approach assuming a Poisson distribution (using the package

288   lme4, v. 1.1-12; Bates et al., 2015). In each case, individuals were considered as a random

289   effect (with random intercepts), and focal personality, group personality and trial number as

290   fixed effects. Initially, full models were constructed using all main effects and their full

291   interactions. Models were reduced with step-wise removal of non-significant terms and

292   comparisons of BIC (using the nlme package; Pinheiro et al., 2016) until a minimum

293   adequate model (MAM) was developed (Table 1); *p* values for individual terms in the final

294   models were then obtained through a Likelihood Ratio Test procedure, comparing the model

295   with the term to the MAM under a Chi-squared distribution. Assumptions of normality and

296   heteroscedasticity were checked by plotting the residuals.

297

298   *Approach latency* was subject to strong floor- and ceiling-effects and analysis using the

299   above method resulted in an error structure which indicated the data did not meet the

300   assumptions of these models. Modelling using the above approach was therefore

301   inappropriate for these data. Instead, a binomial logit regression model was used to determine

302   whether there was any effect of either the original personality of the focal fish or the

303   population personality, or their interaction, on whether individuals changed their boldness. A

304   change in boldness was considered to be a change in *approach latency* that crossed the

305   selection criterion boundary e.g. a change of *approach latency* from 10s to 150s was not

306   considered a change in boldness, since both fall within the definition of 'bold', but a change

307   from 10s to 200s was considered a change since the upper limit for bold behaviour was 180s.

308   As per previous models, a model reduction approach was taken, removing non-significant

309   terms in the model and comparing BIC between models (using the nlme package; Pinheiro et

310   al., 2016) to generate a MAM (Table 1).

311

312   **Table 1:** Full and Minimum Adequate Models (MAM) and BIC for each behavioural

313   response variable used in analyses. Parameters are abbreviated as pop (boldness of the

314   population into which the focal fish was placed: bold or shy), foc (boldness of the focal fish:

315   bold or shy) and trial (behavioural trial before or after the focal fish spent one week with the

316 population fish), along with an error term indicating repeated measurements on individual

317 fish (id).

Model Parameters		BIC
Passive Duration (s)		
Linear Mixed Effects Model (lmer)		
Full	pop × foc × trial +(1|id)	856.97
MAM	foc × trial + (1|id)	810.11
Passive Frequency		
Generalized Linear Mixed Effects Model (glmer)		
Full	pop × foc × trial + (1|id)	524.14
MAM	pop + foc + trial + (pop × trial) + (foc × trial) + (1|id)	518.16
Change in boldness		
Binary Logistic model (glm)		
Full	pop × foc	41.00
MAM	pop + foc	39.55

318

319

320 **3. Results**

321

322 *3.1 Initial Behaviour and Justification of Selection Criterion*

323

324 The Results from this study are comprised from a total of *n*=70 fish, involved either within

325 the populations (*n*=36) or as focal fish (*n*=34). Personality descriptors of focal fish were

326 defined by *approach latency*, with *a priori* definitions of bold fish approaching to within 5cm

327 of the novel object within 180s and shy fish approaching after 300s or not at all. Here fish

328 exhibited a similar distribution: in the first trial, bold focal fish approached the object very

329 quickly (mean ± SE *approach latency* for bold fish=42.1 ± 12.5s) whereas most (18 of 21)

330 shy fish did not approach the object at all (Figure 2a). No fish exhibited an *approach latency*

331 between 180 – 300s. Fish selected for boldness on this criterion also exhibited differences in

332   activity levels: bold fish were initially less passive than shy fish ($W=72$, $p=0.023$; Figure 2b).

333   Likewise, bold fish more frequently switched between passive and active behaviour than shy

334   fish ($W=198.5$, $p=0.029$; Figure 2c). These data therefore justify the selection criterion and

335   binning of fish into bold or shy categories.

336

337   *3.2 Physiology*

338

339   Irrespective of the behavioural type of the population that focal fish were placed into

340   ($F_{1,30}=0.08$, $p=0.78$), bold trout consistently had significantly lower post-stress plasma

341   cortisol concentrations than shy trout (mean $\pm$ SE $=36.18\pm5.93$ ng ml^{-1} for bold and

342   89.82 ± 11.40 ng ml^{-1} for shy; $F_{1,30}=7.92$, $p=0.009$; Figure 2). The interaction of personality

343   and population was not significant ($F_{1,30}=0.05$, $p=0.83$). Specific growth rate did not differ

344   between any treatment groups; see Supplementary Material for further information.

345

346   *3.3 Behavioural Change*

347

348   *Passive duration* differed between trials dependent on the personality of the focal fish

349   ($\chi^2_1=6.60$, $p=0.010$). Bold fish in the initial trial were, on average, the least passive; however,

350   after the trial bold focal fish were at least as passive as shy fish, which did not appear to

351   change in this behaviour across the two trials (Figure 2b). Frequency of passive behaviour

352   also differed between trials, with the change in frequency dependent on both the personality

353   of the group ($\chi^2_1=18.03$, $p<0.0005$) and of the focal individual ($\chi^2_1=5.72$, $p=0.0168$), but

354   there was no significant interaction between all three parameters. Initially bold focal fish

355   switched between active and passive behaviours most frequently; both bold and shy focal fish

356   reduced the frequency of switching behaviours in the second trial (Figure 2c). However, in

357 the second trial fish which had spent time in a bold population tended to switch behaviour

358 more frequently than those which had spent time in the shy population (Figure 2d).

359

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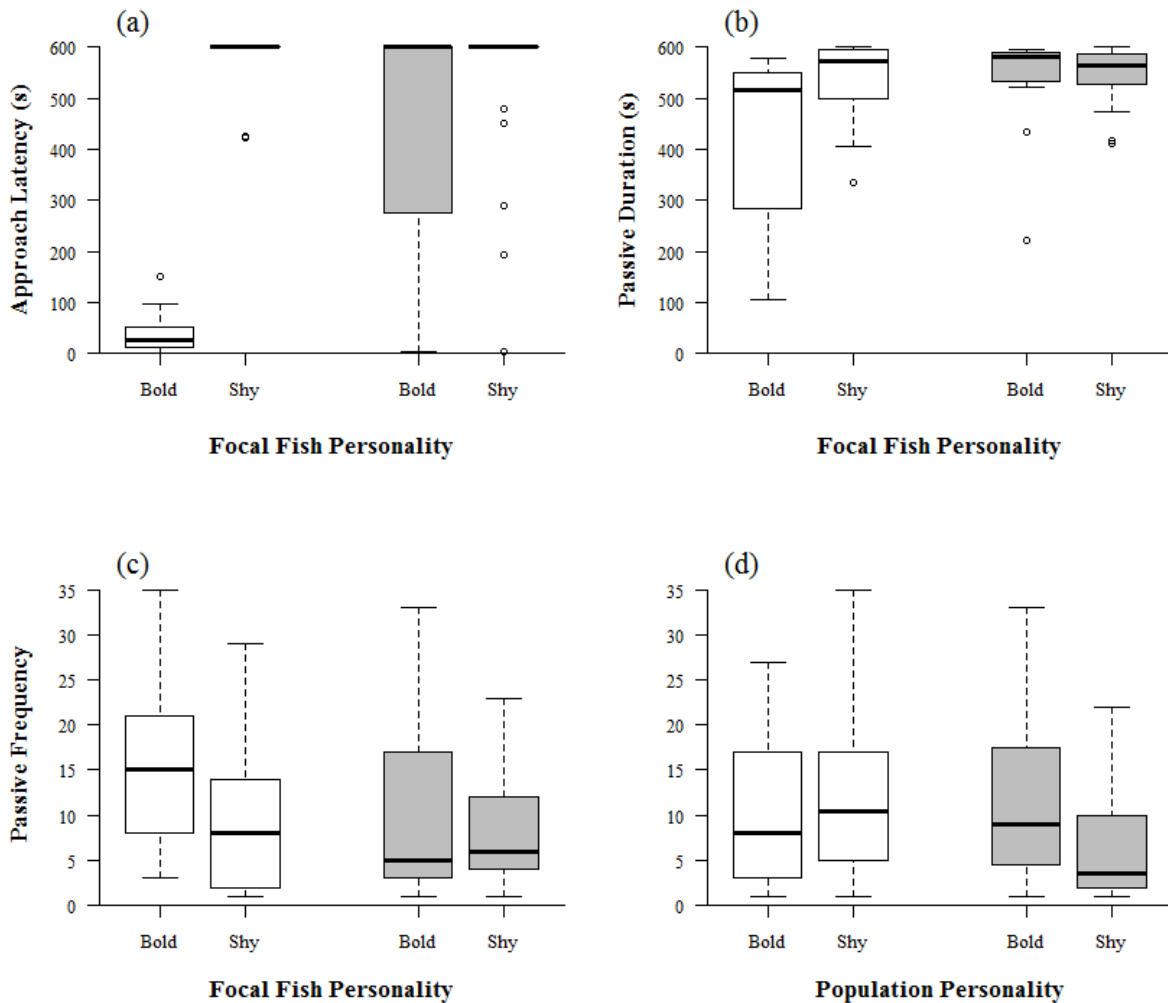
361

362 **Figure 2:** Behaviour of rainbow trout, *Oncorhynchus mykiss*, before (white boxes) and after

363 (grey boxes) a week spent with a population of either bold or shy trout (where boldness was

364 measured as latency to approach within 5cm of a novel object, and where bold fish

365 approached in <180s and shy fish approached in >300s or not at all). Figures indicate

366 different measures of behaviour: (a) latency to approach to within 5cm of a novel object (s)

367 for bold and shy focal fish; (b) duration of passive behaviour (s) of bold and shy focal fish;

368 and frequency of changing between passive and active behaviour (see text for details)

369 between (c) bold and shy focal fish and (d) comparisons of all focal fish compared between

370 populations. For all boxes: line=median, box=interquartile range, whiskers indicate extent of

371 data <1.5×IQR, dots=outliers (greater than 1.5×IQR outside the box).

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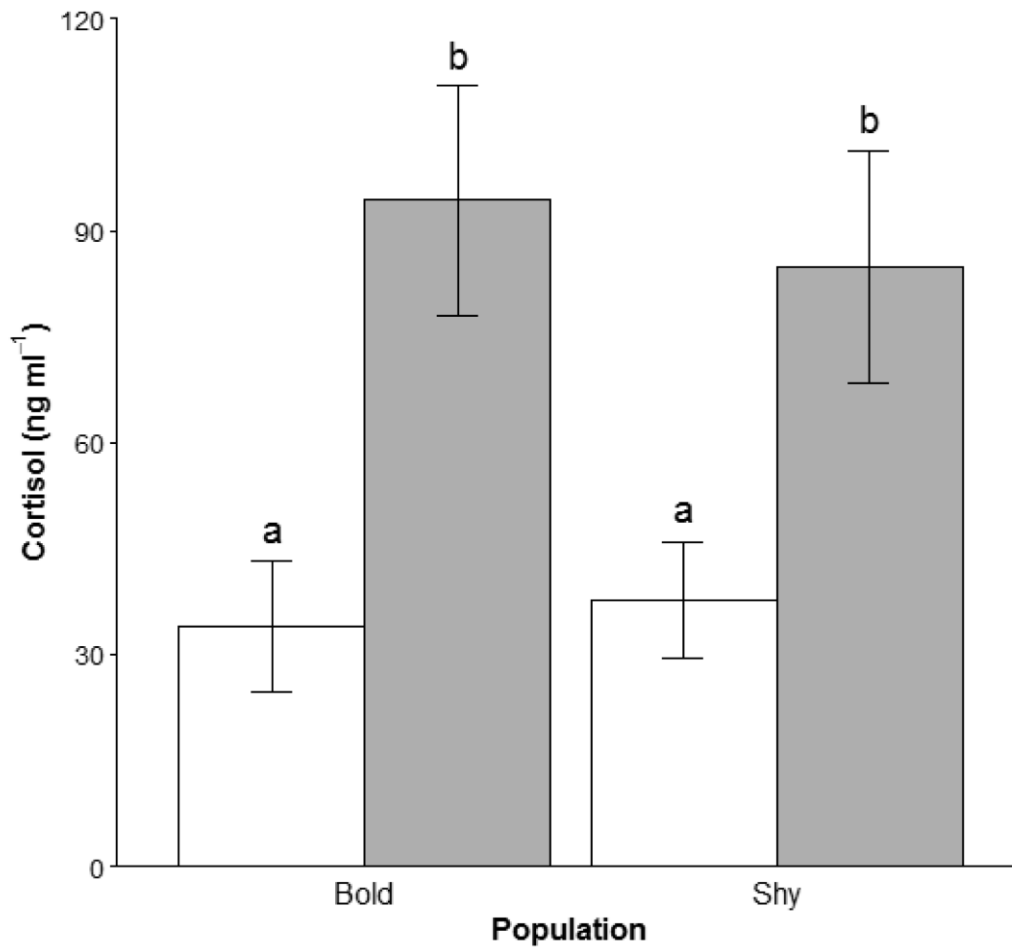
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375 **Figure 3:** Mean (±SE) plasma cortisol concentrations in bold (white) and shy (grey) rainbow

376 trout, *Oncorhynchus mykiss*, 15 min after an emersion stress. Individual trout were placed

377 into a group of either nine bold (Bold Population) or nine shy conspecifics (Shy Population)

378 for one week before sampling. Groups which do not share a common lower case letter were

379 significantly different ($p<0.05$). $n=5,8$ for bold trout and $n=11,10$ for shy trout in a bold and

380 shy population respectively.

381

17

382   Initially-bold fish were more likely to change their boldness than shy fish (z_2=-3.08, p=0.002;

383   Figure 2a): 77% of initially bold fish changed their behaviour towards either an intermediate

384   or shy type, whereas only 14% of initially shy focal fish changed behaviour. There was,

385   however, no effect of the personality of the population in determining whether focal fish

386   changed boldness (z_2=-1.36, p=0.175).

387

388   **Discussion**

389

390   Plasticity of behaviour and coping style allows individuals flexibility to deal with rapid

391   changes in environment. Here, we show that trout tended towards a shy behavioural strategy

392   after time spent with a population of conspecifics, regardless of their original personality or

393   that of the population with which they cohabited. Of significant interest, however, was that

394   stress physiology reflected the original personality type of the focal fish rather than the

395   personality after removal from the population: initially-bold fish exhibited lower plasma

396   cortisol concentrations than initially-shy trout regardless of any behavioural change.

397

398   *4.1 Physiology*

399

400   Post-stress cortisol profiles differed between initially bold or shy focal fish, regardless of any

401   change in behavioural profile. The link between HPI reactivity and boldness is well-

402   established (Koolhaas et al., 1999; Øverli et al., 2005) yet studies are beginning to highlight

403   occasions where this link is disrupted, either through behavioural inconsistency or a

404   combined behavioural and physiological inconsistency over time under certain conditions

405   (e.g. Ruiz-Gomez et al., 2008; Vaz-Serrano et al., 2011; Boulton et al., 2015). We took only

406   one blood sample, and thus could only detect stress-induced cortisol secretion at the end of

407 the experiment rather than quantify temporal changes (an issue that may have been resolved

408 by using larger fish); however, our data imply that whilst boldness may change under social

409 conditions in rainbow trout, the underlying physiological components reflect an innate

410 behavioural type (i.e. bold or shy). Thus whilst coping styles may provide an excellent model

411 of the relationship between personality traits and underlying physiological mechanisms,

412 particularly among rats and mice (Koolhaas et al., 1999), emerging evidence indicates that

413 this association may be lost during development (Vaz-Serrano et al., 2011), periods of stress

414 (Ruiz-Gomez et al., 2008; Boulton et al., 2015), or because the relationship is only evident

415 under certain contexts (Øverli et al., 2007; Castanheira et al., 2016).

416

417 Recent studies have focused on the mechanisms underlying differences in stress physiology

418 and personality. For instance, Carere et al. (2010) highlight three proximal explanations for

419 the relationship between stress physiology and personality: (1) stress physiology determines

420 behaviour, (2) additional factors underlie both physiology or behaviour, or (3) behaviour

421 determines physiology. However, a correlation between between stress physiology and

422 personality (potentially due to pleiotropy) may be more likely than a direct cause-effect

423 relationship (Carere et al., 2010). Indeed, greater plasticity in personality than physiology

424 (e.g. Ruiz-Gomez et al., 2008) may indicate one or more additional, unmeasured, factor(s), to

425 explain the decoupling between behaviour and physiology. However, what should also be

426 considered are the broad suite of hormones, proteins and pathway interactions which exert

427 some control over the HPI axis (and thus cortisol secretion) and the roles that they might also

428 have in the control of behaviour. For instance, corticotropin releasing factor initiates the HPI

429 axis by binding to receptors in the pituitary, but is also implicated in control of appetite

430 (Bernier & Craig, 2005), aggression (Backström et al., 2011) and locomotor activity

431 (Clements et al., 2002). Also, serotonin influences agonistic interactions (LePage et al., 2005)

432 but has roles in regulation of adrenocorticotropic hormone and cortisol secretion and is likely

433 regulated itself by corticosteroids (Dinan, 1996; Kreke & Dietrich, 2008). Such direct

434 interactions between elements of the HPI axis and behaviour are numerous, and suggest that

435 if behaviour can change whilst HPI reactivity remains static further underlying mechanisms

436 mediating the relationship are likely to be present and require further study. Importantly,

437 these data have potential implications for how individuals cope with stress whilst

438 simultaneously interacting, often competitively, with an established group of conspecifics.

439

440 *4.2 Behavioural flexibility*

441

442 Trout adjusted levels of neophobia and activity a suite of behaviours when placed into a

443 population of conspecifics. However, the change was largely independent of the population-

444 level behaviour and, instead, was associated with the original personality of the focal fish: the

445 implication is that bold and shy trout may react to a population differently. Furthermore, this

446 would suggest that behavioural change under these conditions is not driven by a requirement

447 for social cohesiveness: behavioural homogeneity within a group increases information

448 sharing, for instance, and may limit opportunities for predators to single out potential prey in

449 an extension of the oddity effect (which usually applies to how different individuals look

450 compared to their group rather than how they behave; Krause & Ruxton, 2002). Thus other

451 factors may contribute to observed behavioural changes, though further studies should

452 investigate how behaviour changes over various time scales within, and after removal from,

453 the population.

454

455 Particularly among animals which form dominance hierarchies (Winberg & LePage, 1998;

456 Sneddon et al., 2005) entry into a group may provide a distinct challenge for a newcomer, as

457   the hierarchy must be resettled, often through agonistic interactions (e.g. trout; Barnard &

458   Burk, 1979), and where the new member may be the focus of aggression (Johnsson, 1997;

459   Höjesjö et al., 1998). Effects of prior residency (e.g. Deverill et al., 1999) and experience

460   within the group naturally provide a competitive advantage for group members over new

461   entrants, even moreso than the experience of winning a contest (Kim & Zuk, 2000).

462   Furthermore, group members encountering a new individual may only need to resolve this

463   single contest whereas the new member will likely be required to resolve contests against all

464   the members of the group which, coupled with transport between tanks, will be extremely

465   stressful, particularly if the focal fish is inherently aggressive. Thus new members may need

466   to utilise, or be coerced into utilising, a subordinate or shy strategy (Huntingford, 1976;

467   Sundström et al., 2004; Bell, 2005) to accommodate themselves within the group (Øverli et

468   al., 2004). Indeed, dominant individuals tend to exert a behavioural influence rather than

469   allow group behaviour to be defined by consensus (Sih & Watters, 2005; Magnhagen &

470   Bunnefeld, 2009).

471

472   An alternative explanation for differences in behavioural change between bold and shy focal

473   fish may derive from known differences in cognitive ability between fish, where

474   bold/proactive individuals tend to have greater learning ability than and shy/reactive fish

475   (Sneddon et al., 2003; Moreira et al., 2004). Such differences in learning ability also manifest

476   within social contexts (Magnhagen & Staffan, 2003). Thus, if shy fish take longer to learn

477   than bold fish, then any change in behaviour may not have occurred within the timespan of

478   this experiment. Furthermore, the duration such changes last after removal from the group

479   may also differ; bold animals retain responses to a conditioned stimulus for longer (Moreira

480   et al., 2004), and this may impact on how these fish respond in the future both in a social

481   context but, potentially, to unrelated environmental stimuli.

482

483   Behavioural change (in bold focal fish) or lack of change (in shy fish) was not ubiquitous.

484   Some bold fish (approximately 20-25%) remained bold, possibly because these fish were

485   particularly aggressive or large relative to the population into which they were placed and

486   thus able to compete successfully under difficult conditions. Unfortunately, no direct

487   measurements of the behaviour of focal fish within the population tank were made, only

488   observations of behaviour during regular husbandry procedures, nor were measurements of

489   the size of population fish recorded. Shy fish can become bolder after watching other fish or

490   winning competitive interactions (Frost et al., 2007). Whether individuals changed in

491   behaviour may depend upon the degree with which they observed or directly interacted with

492   other fish in the group. We did not measure behaviour or behavioural change within the

493   population itself and thus the mechanisms of any behavioural change within this study are

494   difficult to explain, but are an important consideration for future studies. Furthermore,

495   addressing whether the population exhibited the expected bold or shy personalities as a group

496   for the duration of the experiment should be measured. We chose to use the same populations

497   throughout to reduce the number of fish used in the experiment for ethical reasons and to

498   ensure each focal individual had the same experience rather than each individual being

499   presented with a different group which may have confounded our results; however, this is

500   certainly a factor of interest.

501

502   *4.4 Conclusions and implications*

503

504   Our data support emerging evidence that the relationship between stress physiology and

505   personality, as accepted through coping style theory, can become decoupled. Whilst the

506   change in behaviour observed, principally in bold focal fish becoming shyer, has implications

507 for our understanding of how fish may integrate into social groups, that stress responses were

508 linked to individuals' initial personality may indicate alternative and unexplored factors link

509 physiology and behaviour and mediate the personality change within this particular context.

510 Furthermore, these data may also help us improve the social environment and integration for

511 fish held in captivity, particularly in compliance with regulations on use of animals in

512 scientific research to ensure social animals are housed appropriately.

513

522

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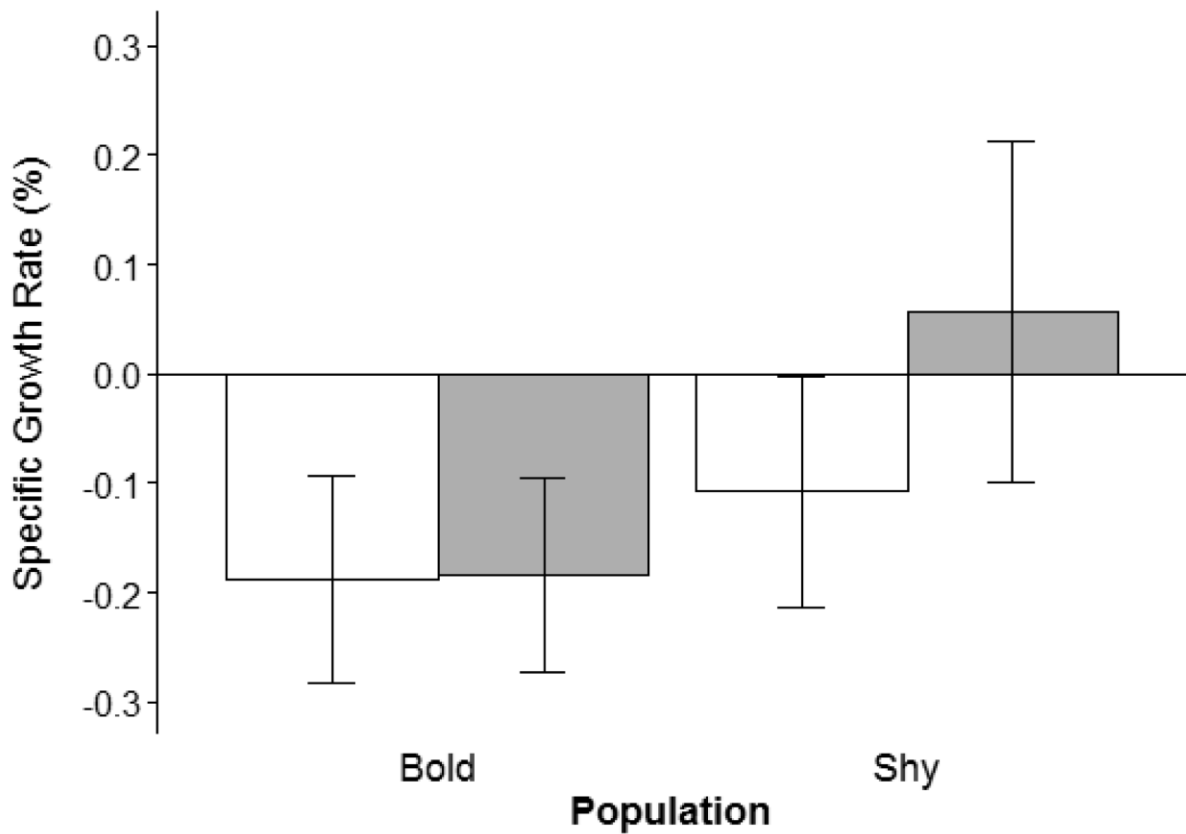
Figure S1: Mean (±SE) specific growth rate (G) of bold (white) and shy (grey) rainbow trout,

Oncorhynchus mykiss, placed for one week into a population of nine bold or shy trout. $n = 5,8$

for bold trout and $n = 10,10$ for shy trout in a bold and shy population respectively.