Perch, *Perca fluviatilis* show a directional preference for, and increase predatory behaviour towards prey in response to water-borne cortisol

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In freshwater environments, chemosensory cues play an important role in predator/prey interactions. Prey use a variety of chemosensory cues to detect and avoid predators. However, whether predators use the chemical cues released by disturbed or stressed prey has received less attention. Here we tested the hypothesis that the disturbance cue cortisol, informs predatory behaviour when presented with prey. We presented predators (perch, *Perca fluviatilis*) with three chemosensory choice tests and recorded their location, orientation, and foraging behaviour. We compared the responses of our predators when provided with (i) visual cues of prey only (two adjacent tanks containing sticklebacks); (ii) visual and natural chemical cues of prey vs. visual cues only; and (iii) visual cues of prey with cortisol vs. visual cues only. Perch spent a significantly higher proportion of time in proximity to the prey, and orientated towards prey more, when presented with a cortisol stimuli plus visual cues, relative to natural chemical cues of prey, or visual cues of prey only. In addition, there was a trend that perch showed increased predatory behaviour (lunges) towards sticklebacks when presented with a cortisol stimuli plus visual cues, relative to visual cues only. This is the first evidence that water-borne cortisol, in conjunction with visual cues of prey, results in a directional preference and a possible increase in predatory behaviour toward prey. Our results support our hypothesis that cortisol can act as public information about prey state and/or disturbance, and that it may be involved in predator decision-making.

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2 **increase predatory behaviour towards prey in response to water-**

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5 Shot title: Hormonal cues and predation

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Abstract

In freshwater environments, chemosensory cues play an important role in predator/prey interactions. Prey use a variety of chemosensory cues to detect and avoid predators. However, whether predators use the chemical cues released by disturbed or stressed prey has received less attention. Here we tested the hypothesis that the disturbance cue cortisol, informs predatory behaviour when presented with prey. We presented predators (perch, *Perca fluviatilis*) with three chemosensory choice tests and recorded their location, orientation, and foraging behaviour. We compared the responses of our predators when provided with (i) visual cues of prey only (two adjacent tanks containing sticklebacks); (ii) visual and natural chemical cues of prey vs. visual cues only; and (iii) visual cues of prey with cortisol vs. visual cues only. Perch spent a significantly higher proportion of time in proximity to the prey, and orientated towards prey more, when presented with a cortisol stimuli plus visual cues, relative to natural chemical cues of prey, or visual cues of prey only. In addition, there was a trend that perch showed increased predatory behaviour (lunges) towards sticklebacks when presented with a cortisol stimuli plus visual cues, relative to visual cues only. This is the first evidence that water-borne cortisol, in conjunction with visual cues of prey, results in a directional preference and a possible increase in predatory behaviour toward prey. Our results support our hypothesis that cortisol can act as public information about prey state and/or disturbance, and that it may be involved in predator decision-making.

Key-words: *Gasterosteus aculeatus*; *Perca fluviatilis*; hormone; predation, sensory ecology

Introduction

43

44 The outcomes of predator-prey interactions are largely influenced by the ability of predators and

45 prey to detect and respond to one another (Lima & Dill, 1990; Endler, 1991). An organism that

46 perceives a predatory threat or potential prey before being detected itself gains a perceptive

47 advantage that can influence the result of a given encounter (Lunt & Smee, 2015). Prey can

48 successfully avoid predators when they have the sensory advantage, by detecting and avoiding

49 predators before being consumed (Mirza & Chivers, 2002). In contrast, predators prevail when

50 they hold the perceptual advantage and detect prey before they are detected and prey can escape.

51 There is a built-in imbalance between predator and prey in regard to the penalty of failure during

52 an encounter. This asymmetry in the selective pressure on predators and prey, known as the 'life-

53 dinner' principle (Dawkins & Krebs, 1979), is reflected in the greater research effort given to

54 prey responses over predator behavior (Ferrari, Wisenden & Chivers, 2010). While we know a

55 great deal about the sensory cues involved in the antipredator defences of prey, we know less

56 about the cues that predators use during foraging (Vavrek & Brown, 2009; Ferrari, Wisenden &

57 Chivers, 2010).

58

59 Prey use different sensory channels to detect predators, including visual, acoustic, chemical,

60 electrical and/or tactile cues (Ferrari, Wisenden & Chivers, 2010). Chemical cues are especially

61 common in aquatic systems (DeBose & Nevitt, 2008; Ferrari, Wisenden & Chivers, 2010). These

62 chemical cues can be produced directly by predators (e.g., kairomones), by prey that have been

63 injured by predators (e.g., damage released alarm cues, Mathis & Smith, 1993; Harvey & Brown,

64 2004; Chivers, Brown & Ferrari, 2012; Lonnstedt, McCormick & Chivers, 2012), and by prey

65   that have been disturbed or stressed by predators, without being injured or consumed (e.g.

66   disturbance cues, Chivers, Brown & Smith, 1996; Ferrari, Wisenden & Chivers, 2010). Prey

67   increase anti-predator behaviours in response to the chemical cues of predators, as well as the

68   chemical cues released by conspecifics in response to predatory attack (Ferrari, Wisenden &

69   Chivers, 2010). By responding to these chemical cues of predators, prey have increased chances

70   of survival during predator encounters (Mirza & Chivers, 2002). In contrast the responses of

71   predators to prey cues have received less research focus (but see Wisenden & Thiel, 2002;

72   Harvey & Brown, 2004; Elvidge et al., 2012). Predators that do not respond appropriately to

73   sources of information about their prey's location and state are likely to pay the costs of either

74   missed foraging opportunities, or time wasted pursuing vigilant prey. Therefore, a greater

75   understanding of how predators respond to the chemical cues released by prey would provide

76   insight into the selective forces that shape the chemical ecology of predator-prey interactions.

77

78   Given that water is a universal solvent, a vast number of chemicals released by prey could act as

79   potential chemical cues for predators (DeBose & Nevitt, 2008; Ferrari, Wisenden & Chivers,

80   2010). Predators are able to localise prey using the cues released by prey after attack (Mathis &

81   Smith, 1993; Harvey & Brown, 2004; Chivers, Brown & Ferrari, 2012; Lonnstedt, McCormick

82   & Chivers, 2012). However, there has been less research on cues that are released by disturbed

83   or stressed prey, and the response of predators to any such cues (Vavrek & Brown, 2009;

84   Chivers, Brown & Ferrari, 2012).

85

86   Disturbance cues, have been suggested to exist in a variety of systems, including invertebrates,

87   amphibians, and freshwater fish (Hazlett, 1990; Wisenden, Chivers & Smith, 1995; Kiesecker et

88   al., 1999; Bryer, Mirza & Chivers, 2001; Mirza & Chivers, 2002; Jordão, 2004; Brown et al.,

89   2012). It has been suggested that nitrogenous compounds in urine, and respiratory byproducts

90   could act as disturbance cues (Hazlett, 1990; Kiesecker et al., 1999; Vavrek & Brown, 2009;

91   Brown et al., 2012). One potential disturbance cue that has received less research focus is the

92   stress hormone cortisol (Olivotto et al., 2002; Vavrek & Brown, 2009), the principal

93   glucocorticoid in teleost fish (Brown, Gardner & Braithwaite, 2005).

94

95   Prey fish show an acute elevation of their circulating cortisol concentrations in response to the

96   presence of predators (Breves & Specker, 2005; Barcellos et al., 2007). Cortisol can pass across

97   the gills into the surrounding water (Scott & Ellis, 2007; Sebire, Katsiadaki & Scott, 2007), and

98   influences the stress responses of conspecifics (Barcellos et al., 2011). Elevated cortisol

99   concentrations are associated with reduced body mass and growth, as well as increased parasite

100 loads in fish species (Jentoft et al., 2005; Fast et al., 2006). These factors in turn have been

101 linked to reduced predator avoidance in prey (Sogard, 1997; Ness & Foster, 1999). It is therefore

102 possible that cortisol could act as source of public information about prey state and/or

103 disturbance, and that predators could become attuned to this physiological response of prey.

104 However, whether predators can detect and respond to the presence of cortisol released by prey,

105 or use the presence of the hormone to inform their foraging decisions is unknown.

106

107 To answer this question we investigated whether predatory fish show a preference for prey fish

108 associated with cortisol over prey fish with no chemical cue. We used the piscivorous European

109 perch (*Perca fluviatilis*) as model predator, and three-spined stickleback (*Gasterosteus*

110 *aculeatus*) as prey. We presented each perch with three chemosensory prey choice tests (i) prey

111 visual cues only, (ii) prey visual cues plus the natural chemical cues of prey, and (iii) visual cues

112 of prey plus a cortisol chemical cue. We predicted that if predators use cortisol to inform

113 predation decisions, a) if the presence of cortisol indicates prey that are vulnerable to attack, that

114 predators should show a preference for prey associated with cortisol over prey without the

115 chemical cue; (b) be indicative of prey that have increased vigilance, and so predators should

116 avoid such prey because of the reduced likelihood of attack success. We also hypothesise that (c)

117 if cortisol does not inform predator behaviour, predators would show no difference in preference

118 between the three treatments, or alternatively prefer the natural chemical cues of prey compared

119 to no chemical cues or cortisol. It is also possible that cortisol does not alter predatory behaviour,

120 but instead raises the predator's endogenous levels of cortisol. If this is the case then we

121 predicted that (d) the predator may show an undirected change in behaviour; i.e. increased

122 overall activity.

123

Materials and Methods

125 All fish were collected from water-bodies located in west central Scotland. Three-spined

126 sticklebacks (n = 150, ~1g) were collected in October-November 2011 from Balmaha Pond, a

127 small water body that does not contain piscivorous fish. Perch (~16-20cm length, 52.5±9.8g), a

128 natural predator of sticklebacks (Gross, 1978), were collected in October 2010 and 2011 from

129 Howend Trout Fishery. Perch were likely to have had experience of sticklebacks at Howend

130 Trout Fishery.

131

132   Fish were housed at indoor aquaria at the University of Glasgow, UK. Fish were fed daily with

133   frozen bloodworm (*Glycera dibranchiate*), and kept under a light regime of 12L:12D. Mains tap

134   water was used for holding water and water temperature was 16.5±1°C with a pH of 8-8.4.

135

136   All experiments were conducted between December 2011 and January 2012, using a tank design

137   that allows transfer of chemical and visual cues between fish (Le Vin, Mable & Arnold, 2010),

138   while preventing physical interaction for ethical reasons (Fig. 1)(Animal Behaviour, 2006).

139

140   Each perch was introduced into a 45x20x25cm experimental tank ~20h before a test to allow for

141   acclimation and to standardize hunger between individuals. Adjacent to the experimental tank on

142   either side were two flow-through stimulus tanks (10x20x25cm), each maintained with 2l of

143   water. Five sticklebacks were introduced into each stimulus tank, also ~20h before a test. Flow-

144   through stimulus tanks were used to avoid accumulation of cortisol in the water during

145   acclimation and after capture and handling (Pottinger et al., 2011; Archard et al., 2012).

146   Removable opaque barriers were positioned between the experimental and stimulus tanks to

147   prevent the perch and sticklebacks from seeing each other during acclimation. These were

148   removed at the start of a test to allow predators and prey to see one another.

149

150   **Predator responses to chemical cues**

151   To test the influence of hormonal cues on predator behaviour, each perch participated in three

152   chemosensory choice tests (number of perch = 18, total number of tests = 54): (i) a control test,

153   where fresh water was dripped into the perch tank on both sides directly in front of the stimulus

154   tanks holding sticklebacks (Fig. 1a); (ii) a natural chemical test, where water from one of the

155   stimulus tanks containing naïve sticklebacks (no previous exposure to perch) was dripped into

156   one side and fresh water dripped into the other (Fig. 1a and b); and (iii) a cortisol test, where we

157   provided our putative disturbance cue at a concentration that a predator might encounter in

158   nature (0.2ng/ml of cortisol; Sigma) on one side and fresh water on the other (Fig. 1a).

159   Sticklebacks have been documented to release up to 0.26ng/ml/hr cortisol into holding water

160   after capture and handling (Sebire, Katsiadaki & Scott, 2007; Pottinger et al., 2011).

161

162   The order of chemosensory tests and the side placement of the chemical stimulus were

163   randomized. Water was drawn from neighbouring tanks through a pump system with a flow rate

164   of 1.6 ml/min, which has been shown to induce a reaction to the chemical stimulus in other fish

165   species (McLennan & Ryan, 1997; Le Vin, Mable & Arnold, 2010). A previous study has shown

166   using colour-dyed water, that water dripped into a focal tank remained mainly localized within

167   the zone (13x8x19) immediately adjacent to where the water was dripped (Le Vin, Mable &

168   Arnold, 2010). Experimental tanks that held perch and sticklebacks were then drained and

169   cleaned between each test, and each perch had a two-day break between tests.

170

171   At the start of each test the opaque barriers were removed, the stimulus pumps were started and

172   the flow-through system in the stimulus tanks was stopped. We observed the perch for 60min

173   from behind a blind to avoid any effect of the observer on fish behaviour. We visually divided

174   the perch tank into three equal zones (15cm length), two 'preference zones' (chemical stimulus

175   zone and the opposite non-chemical stimulus zone) located adjacent to the front of each stimulus

176   tank and a 'non-preference' zone in between them (Fig. 1). For analysis in the control tests where

177   there were no chemical stimuli, the chemical stimulus zone was allocated to the left side in 50%

178   of tests and to the right for the other 50%.

179

180   To assess predatory behaviour, we used Observer 8 software to record the duration spent by

181   perch in each zone; the duration spent orientating towards sticklebacks; the number of predatory

182   attacks made towards the sticklebacks (hereon termed lunges, which were defined as rapid

183   forward movements towards sticklebacks); and the number of movements by perch between

184   zones.

185

186   **Cortisol concentration in experimental tanks**

187   To assess the concentration of cortisol released by stickleback into their holding water we

188   collected the water from their tanks in the natural chemical cue tests, 60min after presentation of

189   the perch ($n = 9$). To measure the level of cortisol released by perch into their holding water in

190   the experimental tanks, we collected water after 60mins when no stimulus water was being

191   pumped ($n = 4$).

192

193   To determine the cortisol concentrations in the water samples, cortisol was extracted by pulling

194   100ml under vacuum at a rate of ca. 5ml/min through primed SPE cartridges containing

195   octadecylsilane (Sep-pak Plus, Waters Ltd., Watford, UK). Water samples were not pre-filtered.

196   Priming involved 5ml methanol followed by 5ml distilled water. After the samples had been

197   pulled through, the cartridges were washed with 5ml distilled water followed by 20ml air (to

198   remove as much moisture as possible). The free steroids (not conjugated (Ellis et al., 2004)) were

199   eluted with 5ml ethyl acetate. This was collected in a glass tube and evaporated at 45°C under a

200 stream of air. Samples were then re-suspended in 250μl of assay buffer (tris buffered saline) and

201 cortisol concentrations were established using a Cortisol ELISA Kit (Enzo Life Sciences, Inc.).

202 Samples were run within two assays, all samples run in triplicate and recoveries calculated after

203 a known amount of cortisol was added to samples were >96% and the mean intra-and inter-assay

204 coefficient of variation were 8.6% and 8.4% respectively. 100ml water samples were used as

205 cortisol is found at low concentrations in holding water (Scott & Ellis, 2007), and this

206 methodology allowed the amount of cortisol to be concentrated so that values were measured on

207 the linear phase of the standard curve (sample value range: 14.2-4.4ng/ml, standard curve: 100-

208 0.78ng/ml). As the 100ml water samples were re-suspended in 250μl of buffer for the assay

209 (x400 concentration), cortisol values from the assay were divided by 400 to get the concentration

210 in the holding water (ng/ml).

211

212 Statistical analyses

213 To investigate whether perch behaviour differed between the three chemosensory tests, we

214 calculated the proportion of total time that perch spent in the chemical stimulus zone, the

215 proportion of time that perch spent orientating towards the sticklebacks in the chemical stimulus

216 zone, and the proportion of total lunges at sticklebacks in the chemical stimulus zone. We

217 calculated proportions as the total time spent in the chemical stimulus zone divided by the sum of

218 the time spent in or orienting towards sticklebacks in all zones. For lunges at stickleback we

219 calculated the proportion of lunges in the chemical stimulus zone divided by the sum of lunges in

220 the chemical stimulus zone and the opposite stimulus zone. All proportions were arcsine-

221 transformed to normalize data.

222

223   Linear Mixed Models (LMMs) were used to assess whether chemosensory test type (control,

224   natural cues, cortisol) influenced the location, orientation and lunging preference of perch, with

225   perch identity as a random factor (to account for repeated measures). The order chemosensory

226   tests were presented to perch did not influence their behaviour ($P > 0.70$), and so was not

227   included in final models. Chemosensory test type did not influence whether perch began the test

228   in the chemical stimulus zone (binomial GLMM $z = -0.76$, $P = 0.45$). At the start of each trial

229   perch could be located in any zone within the tank. Therefore, if the focal perch did not move for

230   the entire test, this could mask the effect of the chemosensory tests upon behaviour in actively

231   responded individuals, by assigning 100% of time to a single zone, and 100% of time orientating

232   in a single direction. Due to this, for behavioural analysis we only included trials where perch

233   were actively moving. Total movements between zones was square root transformed to

234   normalize data. All analyses were conducted in R 2.12.2 (R core team 2012). The data used for

235   analyses can be found in supporting information S1.

236

237   Ethics statement

238   This study was performed under a UK Home Office licence (60/4292) and was subject to review

239   by the University of Glasgow ethics committee. All experiments were conducted using a tank

240   design that allows transfer of chemical and visual cues between fish, while preventing physical

241   interaction for ethical reasons. The hormone concentrations used in this study are within the

242   natural levels experienced by fish in the wild. Prey fish were provided with shelter while in

243   visual contact with the predator. Permission was gained for catching perch from a privately

244   owned trout fishery (Howend) and sticklebacks were collected from Balmaha pond after

245   obtaining permission from the Scottish government. This study did not involve endangered or

246 protected species. This work was funded by an Association for the Study of Animal Behaviour

247 grant awarded to HMR.

248

Results

250 **Predator behaviour**

251 9 of 18 perch did not move during an entire test (n = 12 tests), and this was marginally, but non-

252 significantly, more likely to occur in trials when cortisol was the chemical cue (binomial GLMM

253 $z = 1.82$, $P = 0.06$). We removed these 12 tests from further analyses, so that only actively

254 moving individuals were included in the analyses of location, orientation, and movement. This is

255 because the fish that remained immobile could be located in any zone within the tank and could

256 therefore skew the analyses.

257

258 Perch spent a significantly higher proportion of their time in the chemical stimulus zone when

259 cortisol was added compared to natural chemical cues of sticklebacks and the water control

260 (Table 1 & Fig. 2a, LMM $t = 3.61$, $P = 0.001$). Perch also oriented towards sticklebacks for a

261 significantly higher proportion of time in the chemical stimulus zone when cortisol was added

262 compared to natural chemical cues of sticklebacks and the water control (Table 1 & Fig. 2b,

263 LMM $t = 3.93$, $P < 0.001$). There was a marginally non-significant trend that perch lunged more

264 frequently at sticklebacks at the side of the tank where the chemical stimulus was dripped in the

265 cortisol tests relative to the water control (Table 1 & Fig. 2c, LMM $t = 1.93$, $P = 0.07$). Perch

266 moved around the tank a similar amount in each chemosensory test (Table 1 & Fig. 2d, LMM $t = $

267 0.30, $P = 0.77$).

268

269 **Concentration of cortisol in experimental tanks**

270 The mean concentration of cortisol in the natural chemical tests was an order of magnitude lower

271 than the cortisol test; 0.02 ng/ml vs. 0.2 ng/ml, and was lower than the mean concentration of

272 cortisol in the perch holding water; 0.05 ng/ml.

273

274 # Discussion

275 We tested the hypothesis that cortisol elevates predatory behaviour, in the presence of visual

276 cues of prey (Vavrek & Brown, 2009). We found that predators showed increased prey

277 inspection behaviour (spending more time in close proximity, as well as more time orientated

278 towards prey) when prey were associated with cortisol, compared to prey with natural chemical

279 cues or prey with a water control. We also found that predators were marginally more likely to

280 increase their attack behaviour towards prey in the presence of cortisol compared to the other

281 chemical cues. We observed that some predators became inactive, and that this was marginally

282 more likely to occur in response to prey associated with cortisol. Despite the differences in

283 responsiveness of our predators to cortisol, the change in their behaviour supports our hypothesis

284 that cortisol can act as public information about prey disturbance (Wisenden, Chivers & Smith,

285 1995), but predatory fish may show a context-dependent behavioural response.

286

287 Our results are in line with the findings of other research that has shown predators respond to

288 chemical information to detect prey (Chivers, Brown & Smith, 1996; Wisenden & Thiel, 2002;

289 DeBose & Nevitt, 2008; Ferrari, Wisenden & Chivers, 2010; Elvidge & Brown, 2012; Lonnstedt,

290 McCormick & Chivers, 2012). Predators can use chemical information such as damage-released

291 alarm cues to capitalise on circumstances when the defences of prey are compromised

292   (Lonnstedt, McCormick & Chivers, 2012). Our study provides, to our knowledge, the first

293   evidence that cortisol could also provide cues to predators of disturbed or stressed prey.

294

295   Fish species show a behavioural and physiological response to the stress responses of

296   conspecifics (Toa, Afonso & Iwama, 2004; Barcellos et al., 2011; Oliveira et al., 2013) and

297   cortisol has been suggested to act as a disturbance cue to conspecific prey (Olivotto et al., 2002;

298   Vavrek & Brown, 2009). Prey fish retreat from water conditioned with chemical cues released by

299   stressed conspecifics, and it is proposed that these cues are interpreted by conspecifics as

300   threatening, unsafe, and/or undesirable situations (Abreu et al 2016). This is despite the fact that

301   cortisol is likely to be ubiquitous within the environment (Hazlett, 1990; Kiesecker et al., 1999;

302   Ferrari, Wisenden & Chivers, 2010). However, this is also the case for nitrogenous waste

303   products that are also suggested to act as disturbance cues, but are abundant within the

304   environment (Hazlett, 1990; Kiesecker et al., 1999; Ferrari, Wisenden & Chivers, 2010; Brown

305   et al., 2012).

306

307   Stressed prey may represent an easier target for predators because prey with higher levels of

308   cortisol also show reduced predator avoidance (Sogard, 1997; Ness & Foster, 1999). Therefore,

309   the presence of chemical cues such as cortisol may 'label' valuable food patches (Lonnstedt,

310   McCormick & Chivers, 2012), and piscivores may exploit these cues as a means to locate prey.

311   But a number of our perch predators responded to cortisol with immobility (freezing). It is

312   possible that cortisol could also indicate that other predators are already foraging in an area, and

313   that prey, having been disturbed, are now more vigilant. In this case, the costs of foraging

314   competition or of lost foraging opportunities may explain the difference in this subset of

315   predators who froze. The strength of both predator responses (attraction and freezing) was

316   greater in the cortisol test than the natural chemical test, supporting a role for the hormone in

317   predatory behaviour.

318

319   Our predators did not change their behaviour in the natural chemical test compared to the water

320   control. The concentration of cortisol in the natural chemical tests was an order of magnitude

321   lower than the cortisol test (0.02 vs. 0.2ng/ml), and was lower than the concentration of cortisol

322   in the perch holding water (0.05ng/ml). Therefore, the responses may be proportional to the

323   concentration of disturbance cue detected. This kind of graded response has also been shown in

324   prey (Vavrek & Brown, 2009; Brown et al., 2012). Alternatively, the responses of our predators

325   may have only occurred when the cue rose above the background levels of cortisol ('noise').

326   This is supported by the background noise hypothesis proposed by Vavrek and Brown (2009).

327   This hypothesis states that background levels of cues dictate how much cue is required to

328   actually elicit a response. Although this hypothesis relates to prey behaviour, we see that it could

329   also apply to predators.

330

331   An alternative explanation for our results is that cortisol does not increase predatory behaviours

332   *per se*, but that it elevated the endogenous cortisol levels in our perch due to uptake of the water-

333   borne cortisol across the gills (Scott & Ellis, 2007). Glucocorticoids are known to rapidly affect

334   behaviour (Moore & Evans, 1999). But perch moved around the tank a similar amount in each

335   chemosensory test, and it was specifically inspection and predatory behaviours that we elevated

336   in the cortisol tests. This finding is contrary to our expectation that predators would exhibit

337   undirected changes in behaviour if endogenous concentrations were elevated. Furthermore, the

338   concentration used in our cortisol tests is within the range released by sticklebacks after handling

339   stress (Both et al., 2004; Sebire, Katsiadaki & Scott, 2007; Pottinger et al., 2011), and could be

340   experienced by perch in the wild, so should be biologically meaningful (Sebire, Katsiadaki &

341   Scott, 2007; Pottinger et al., 2011). To establish whether cortisol strictly increased predatory

342   behaviour, rather than exploratory behaviour, it would be interesting to look at the behaviour of

343   perch when they are exposed to conspecifics rather than prey.

344

345   A limitation of our study is that we used predator naïve sticklebacks as prey. We found that

346   sticklebacks released less cortisol into their holding water following visual exposure to a

347   predator than sticklebacks that had experienced a handling stress (Sebire, Katsiadaki & Scott,

348   2007; Pottinger et al., 2011). This is likely because our sticklebacks originated from a water body

349   that did not contain piscivorous fish. Many prey species do not show innate recognition of

350   potential predators (Mathis, Chivers & Smith, 1993; Chivers & Smith, 1994), so a lack of

351   experience of predatory fish may explain the attenuated stress response. We did not examine

352   whether perch responded if given the choice between visual cues of sticklebacks when presented

353   with both elevated cortisol and natural "signature" chemical cues of experienced sticklebacks

354   (Wyatt, 2010). If experienced sticklebacks release cortisol at higher concentrations than the naïve

355   sticklebacks in our experiment (Mathis, Chivers & Smith, 1993; Chivers & Smith, 1994), we

356   could have explored the potential for graded responses, and the responses to chemical mixtures.

357   We also did not test whether perch showed a behavioural response to chemical cues alone. But as

358   perch are sight-dependent diurnal predators, they likely require both visual and chemical cue to

359   target prey (Turesson & Brönmark, 2004; Elvidge & Brown, 2012). Although this could have

360   provided information on the relative importance of visual and chemical cues, our design still

361   allowed for us to detect how chemical cues augment the behaviour of predators in comparison to

362   visual cues only.

363

364   The behavioural response of perch to a cortisol stimulus does suggest that this hormone could

365   influence predatory behaviour; therefore, there is the intriguing potential of a chemosensory arms

366   race between predator and prey. Populations of sticklebacks differ in their behavioural profiles

367   due to predation pressure (Bell, 2005; Bell & Sih, 2007), and fish from high predation

368   populations have been shown to have a lower cortisol release rate than those from low predation

369   populations (Archard et al., 2012). This has been suggested to be beneficial as it could prevent

370   excessive energy expenditure due to repeated stress responses caused by the presence of

371   predators (Brown, Gardner & Braithwaite, 2005). However, a reduced stress response could have

372   evolved due to predators selectively predating individuals that have a higher stress response.

373

374   Conclusions

375   Traditionally research has focused on the function of the stress response experienced by prey

376   animals during a predation event. However, in this study we show how an improved

377   understanding of how predators respond to the stress responses of their prey could be useful

378   when investigating the complexity of predator–prey interactions.

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380

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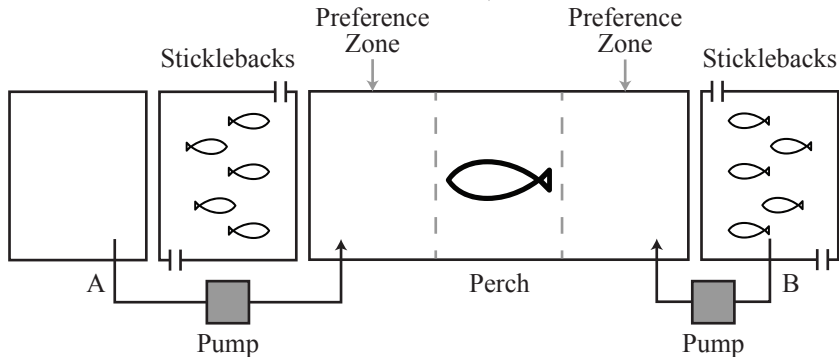
529

Schematic of experimental tanks.

Figure 1: Schematic of experimental tanks. Pump system A allowed transfer of fresh water or cortisol solution into the perch tank. Pump system B allowed transfer of the natural chemical cues from the stickleback holding water.

Bar charts showing how chemical cues influenced perch behaviour

Bar charts and captions showing mean±se of (a) the proportion of total time spent by perch in chemical stimulus zone, and duration in chemical stimulus zone (secs), (b) the proportion of time spent by perch orientating toward sticklebacks in the chemical stimulus zone, and duration orientating toward sticklebacks in chemical stimulus zone (secs), (c) the proportion of lunges at sticklebacks in the chemical stimulus zone, and the number of lunges in the chemical stimulus zone, and (d) total movements between zones by perch. Letters indicate significant differences (a: P < 0.01), and statistical trends (c: P = 0.07).

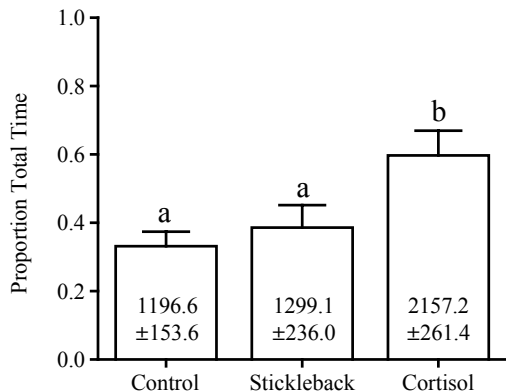
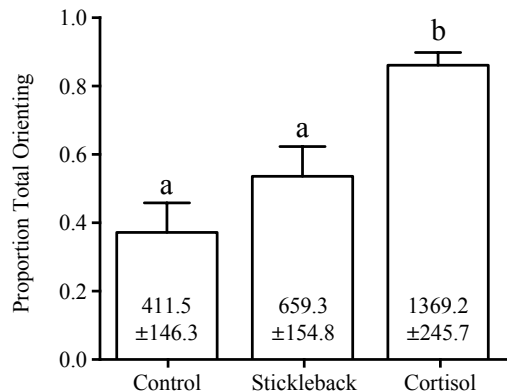
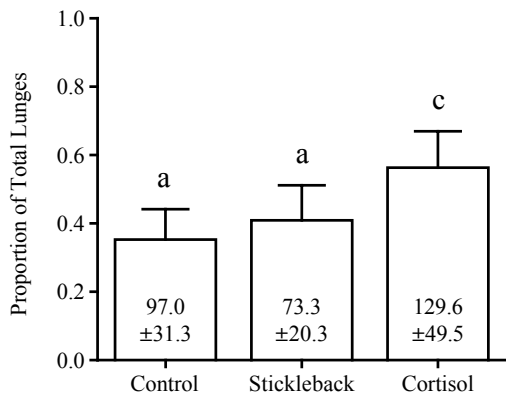
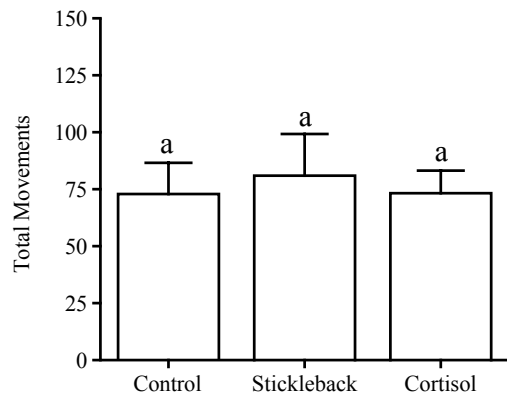



Table of LMMs results

Linear Mixed Models testing the effect of chemical trial upon proportion of time in chemical stimulus zone, proportion of time orienting in chemical stimulus zone, proportion of lunges in chemical stimulus zone, and total movements between zones. Individual ID was included as a random factor in all models. Values in bold denote statitsically significant factors, * denotes maginally non-significant factor.

	Fixed effects				Random intercept
	β	s.e.	*t*	P	%σ
Proportion of time in chemical stimulus zone	0.33	0.09	3.61	**0.001**	35.8
Proportion of time orienting in chemical stimulus zone	0.62	0.16	3.93	**< 0.001**	2.0e-03
Proportion of lunges in chemical stimulus zone	0.32	0.17	1.93	0.07*	50.8
Movements between zones	0.35	1.18	0.30	0.77	30.6