1 **Collective action reduces androgen responsiveness with implications for shoaling**

2 **dynamics in stickleback fish**

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Abstract

Androgens, traditionally viewed as hormones that regulate secondary sexual characteristics and reproduction in male vertebrates, are often modulated by social stimuli. High levels of the 'social hormone' testosterone (T) are linked to aggression, dominance, and competition. Low T levels, in contrast, promote sociopositive behaviours such as affiliation, social tolerance, and cooperation, which can be crucial for group-level, collective behaviours. Here, we test the hypothesis that, in a collective context, low T levels should be favourable, using non-reproductive male and female stickleback fish (*Gasterosteus aculeatus*) and non-invasive waterborne hormone analysis. In line with our predictions, we show that the fishes' T levels were significantly lower during shoaling, with high-T individuals showing the largest decrease. Ruling out stress-induced T suppression and increased T conversion into oestradiol, we find evidence that shoaling directly affects androgen responsiveness. We also show that groups characterized by lower mean T exhibit less hierarchical leader-follower dynamics, suggesting that low T promotes egalitarianism. Overall, we show that collective action results in lower T levels, which may serve to promote coordination and group performance. Our study, together with recent complementary findings in humans, emphasizes the importance of low T for the expression of sociopositive behaviour across vertebrates, suggesting similarities in endocrine mechanisms.

1. Introduction

Androgens are a group of steroid hormones which play an important role in vertebrate male reproductive physiology and behaviour (Nelson, 2000), and are often modulated by social stimuli (Wingfield et al., 1990, Oliveira, 2004, Oliveira et al., 2002; Hirschenhauser and Oliveira, 2006, van Anders, 2013; Goymann et al., 2019; Moore et al., in press). Changes in androgens in response to social stimuli are viewed to be "a mechanism for adjusting androgen-dependent behaviours to the current social environment of the individual'' (Oliveira, 2009) and a large body of work across vertebrate taxa has therefore studied if and how the major androgen testosterone (T) is modulated according to social behaviour or context (for recent reviews see e.g. Oliveira, 2004, Oliveira et al., 2002; Hirschenhauser and Oliveira, 2006, van Anders, 2013; Goymann et al., 2019; Moore et al., in press). The 'challenge hypothesis' predicts that male T varies as a function of mating system, paternal investment, and aggression (Wingfield 1987, 1990; Goymann et al., 2019; for a recent meta-analysis see Moore et al., in press). First, males from paternal/biparental species with monogamous pair bonds typically have lower breeding baseline T-levels than males from species without paternal investment and polygynous mating behaviour. Second, T responsiveness above baseline in response to social (male-male and/or male-female) challenges is larger in species with lower breeding baseline T (Wingfield 1987, 1990; Goymann et al., 2019; for a recent meta-analysis see Moore et al., in press; but see e.g. Ros et al., 2002 for support of the challenge hypothesis in a non-reproductive context). The majority of studies have therefore focussed on males and their variation in T responsiveness in reproductive contexts.

72   The present study, in contrast, focuses on social modulation of T in both sexes and

73   outside a reproductive context to help broaden our understanding of the ecological

74   relevance of rapid changes in androgen levels during day-to-day social interactions.

75   Specifically, we are interested in possible advantages of low T in non-

76   competitive/cooperative situations. Whilst high levels of T in vertebrates are generally

77   linked to aggression, dominance, and competition and are therefore important for

78   mediating status-related behaviours (see above; reviewed by Wingfield 1987, 1990;

79   Goymann et al., 2019; Mazur and Booth, 1998, Mehta et al., 2008, Eisenegger et al., 2011,

80   Mehta and Josephs, 2011, van Anders, 2013, Hamilton et al., 2015; Figure 1), high T can also

81   suppress affiliation, constrain social tolerance, and disrupt collaboration in humans (e.g.

82   Mehta et al., 2009, Bos et al., 2010, van Honk et al., 2011, Wright et al., 2012, van Anders,

83   2013). Similarly, social birds with experimentally elevated T are found to decrease levels of

84   cooperation (wire-tailed manakins, *Pipra filicauda*: Ryder et al., 2018). Indeed, low T levels

85   promote affiliation, social tolerance, and cooperation (Mehta et al., 2009, Mehta and

86   Josephs, 2011, Hamilton et al., 2015, Lozza et al., 2017; Cieri et al., 2014; Figure 1).

87   Whilst previous studies thus provide evidence for a positive feedback loop between

88   low T levels and sociopositive/cooperative interactions outside reproductive contexts,

89   empirical data on this interrelationship are generally scarce. Studies that do exist mainly

90   focus on humans (but see e.g. Ryder et al., 2018) and have yielded inconsistent results,

91   indicating that T can both inhibit and promote sociopositive behaviour/cooperation (e.g.

92   Mehta et al., 2009, Eisenegger et al., 2011, Mehta and Josephs, 2011, Wright et al., 2012).

93   Given the (i) dynamic nature of the T- behaviour relationship and complexities of human

94   social interaction (see e.g. Mehta et al., 2009), and (ii) the similarity in biological

95   mechanisms that underpin social behaviour across vertebrate taxa (reviewed by Mehta and

96   Gosling, 2006, Mehta and Josephs, 2011), we propose that investigations of non-

97   reproductive sociopositive and cooperative behaviours and T in non-humans are likely to be

98   informative.

99   Tendency to approach conspecifics and social tolerance form the basis of

100   cooperation (Soares et al., 2010). Consider self-organising vertebrate groups like fish schools

101   or bird flocks, for instance, where social attraction can result in complex patterns of social

102   and cooperative behaviours (Sumpter 2006; Couzin & Krause 2003; Herbert-Read et al.

103   2016) and fast and accurate shared decision-making (Couzin, 2009). However, if there is

104   within-group conflicts of interest, e.g. individuals with different motivation or information,

105   social attraction and tolerance decreases (Conradt et al. 2009), and this can result in

106   reduced decision-making accuracy (e.g. Woolley et al., 2010), and group fission (e.g. King et

107   al. 2009). Thus, in a collective context, where individuals can gain mutual benefits from

108   coordination, low T levels should be favourable and positively affect group performance. We

109   suggest that the coordinated behaviours of shoaling fish (which have recently been linked to

110   metabolic traits; e.g. Killen et al. 2012, 2017; McLean et al. 2018) present an ideal

111   opportunity to study if and how 'collective action' modulates androgen levels and whether

112   collective (i.e. group-level) hormone profiles are linked to group coordination and

113   performance (see e.g. Akinola et al., 2016, Akinola et al., 2018).

114   We test this "collective action hypothesis", studying adult, reproductively quiescent,

115   male and female three-spined stickleback fish (*Gasterosteus aculeatus*). Sticklebacks are

116   member of a large and extremely diverse group of ray-finned fishes, the teleosts, which

117   have been key models for our understanding of the reciprocal interactions between

118   androgens and behaviour (reviewed by Oliveira et al., 2002; Moore et al., in press). As a

119   small, highly gregarious freshwater fish, sticklebacks have become a major model organism

120 for behavioural biologists (for reviews see e.g. Huntingford & Ruiz-Gomez, 2009; Östlund-

121 Nilsson et al., 2007). Sticklebacks exhibit a pronounced social plasticity ranging from

122 territoriality (e.g. during breeding) to forming mixed-sex shoals of varying sizes (e.g. 4-44,

123 Ward et al., 2017) and can be housed singly or in groups (Huntingford & Ruiz-Gomez, 2009).

124 A positive feedback loop between low T levels and sociopositive interactions (Mehta et al.,

125 2009, Mehta and Josephs, 2011, Hamilton et al., 2015, Lozza et al., 2017; Figure 1), should

126 result in lower androgen levels where fish are engaged in sociopositive interactions

127 (shoaling), compared to when they are alone.

128 To test this, we experimentally manipulated the fishes' social environment by

129 switching non-shoaling ('individual') and shoaling ('collective') contexts and assess

130 associated changes in androgen levels to provide insight into the physiological bases of

131 social plasticity outside a reproductive context. To obtain repeated hormone measures for

132 the same individuals, we use non-invasive waterborne hormone analysis (see e.g. Fürtbauer

133 et al., 2015a,b; Fürtbauer and Heistermann, 2016). First, we predicted that T levels should

134 be lower in a collective than an individual context, and consequently, we expected high T

135 individuals to show the largest decrease in T responsiveness when switching to a collective

136 context. Because collective (i.e. group-level) hormone profiles have recently been linked to

137 group coordination and performance (e.g. Akinola et al., 2016, Akinola et al., 2018) we also

138 predicted that groups with lower average T should be less hierarchical/more egalitarian in

139 their leadership given that high T is linked to social dominance (see above). To investigate

140 this, we use a measure of shoal egalitarianism based on leader-follower dynamics (see e.g.

141 Nagy et al., 2010, Strandburg-Peshkin et al., 2018) derived from automated video-tracking.

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143 **2. Materials and Methods**

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(a) Subjects and experimental procedures

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147   All procedures were approved by Swansea University's Ethics Committee (IP-1213-3). N=30

148   adult, non-reproductive, male (N=17) and female (N=13) three-spined sticklebacks were

149   used in this study (body mass: mean±SD = 1.09±0.16g). Fish were kept at a constant

150   temperature/photoperiod regime (16°C/8L:16D) in which they remain reproductively

151   quiescent (e.g. Katsiadaki et al., 2006; King et al., 2013). One week before the experiments,

152   subjects were transferred from their holding tank (30 x 39 x 122 cm) into individual 2.8 litre

153   gravel-lined tanks, were they were housed throughout the study. Fish were fed defrosted

154   bloodworm (*Chironomus sp.*) daily. For individual identification, fish were tagged using

155   circular, spine-mounted tags (Webster and Laland, 2009, Hansen et al., 2016).

156   On the first day (trial 1), waterborne hormone samples were collected from each

157   singly housed fish ("Individual context"). To this end, fish were netted from their individual

158   holding tanks and placed into individual 150 mL glass beaker (rinsed with 99.9% methanol

159   and distilled water prior to use) filled with 50 mL water for 1 hour. Fish were then randomly

160   assigned to six shoals of n=5 individuals and placed in a "starting box" inside a rectangular

161   tank (73 x 42.5 cm) to acclimatise for five minutes. The starting box was then removed and

162   the fish were able to swim around freely for 20 minutes. Subsequently, individual

163   waterborne hormone samples were collected ("collective context") by again placing each

164   fish in an individual 150 mL glass beaker filled with 50 mL water for 1 hour. Following

165   hormone sample collection, subjects were returned to their individual holding tanks. The

166   same protocol was repeated 24h later, with groups being tested in a different order (trial 2).

167   Group composition remained the same in both trials. This sampling design allowed us to

168   examine each individual's hormone levels twice in both contexts and to test for repeatability

169   in hormone responses (see Fürtbauer et al., 2015a).

170   During free-swimming trials fish were filmed by a Panasonic HDC-SD60 HD video

171   camera (Panasonic Corporation of North America, Secaucus, NJ, USA) positioned above the

172   tank. The tank was surrounded by an aluminium frame and white screen (PhotoSEL BK13CW

173   White Screen) enabling optimum conditions for video recording. Fish positions were tracked

174   from video using custom MATLAB code (Supplementary Information), and we used this

175   positional information to calculate the time delay τ(s) between changes in direction (i.e.

176   normalised velocity, $\boldsymbol{v}$) of pairs of fish (Strandburg-Peshkin et al., 2018). For each pair ($i \neq$

177   j) the directional correlation function was calculated as: $C_{ij}(\tau)$ is $\langle \boldsymbol{v}_i(t) \cdot \boldsymbol{v}_j(t + \tau) \rangle$ $\langle$where

178      $\langle ... \rangle$ denotes time average (Nagy et al., 2010, Strandburg-Peshkin et al., 2018). We used

179   time values where $C_{ij}(\tau)$ is maximised for each dyad to calculate a shoal-averaged

180   directional correlation delay time within trials, $\bar{\tau}$, for each fish (Nagy et al., 2010). Positive

181   values of $\bar{\tau}$ indicate the fish tends to lead (i.e. others copy its direction) whilst negative

182   values indicate following (i.e. the focal fish copies directional changes of others) (see

183   Supplementary Information; Nagy et al., 2010). We then calculated the variance in averaged

184   directional correlation delay time for each shoal, $\sigma^2 \bar{\tau}$, representing a measure of "shoal

185   egalitarianism in leader-follower dynamics" since small variance in $\bar{\tau}$ scores would indicate

186   fish do not have consistent leader-follower roles, whilst a high variance would represent

187   shoals where one or more fish has large, and different, $\bar{\tau}$ scores compared to shoal-mates.

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189   *(b) Hormone sample collection, extraction, and analysis*

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191   Water hormone samples (total n=120 samples; N=30 individuals, each individual was

192   sampled twice in the individual context and twice in the collective context samples) were

193   collected using the procedure described above (*"Subjects and experimental procedures"*)

194   which is a common and validated method for the collection of waterborne sample for

195   steroid hormone analysis in fish including sticklebacks (see e.g. Fürtbauer et al., 2015 a,b,

196   Fürtbauer and Heistermann, 2016; for a review see Scott et al., 2008). All subjects were

197   habituated to handling and beaker confinement prior to experiments (see Fürtbauer et al.,

198   2015a) in order to exclude potential handling effects on hormone secretion (Scott et al.,

199   2008). Water samples were processed and extracted, following published procedures

200   (Fürtbauer et al., 2015a,b, Fürtbauer and Heistermann, 2016), In brief, samples were

201   extracted using Waters Sep-Pak Plus C18 (Waters, Milford, MA, USA) solid phase extraction

202   cartridges placed onto a 12-port vacuum manifold. Cartridges were primed with 5 mL

203   methanol followed by 5 mL of distilled water. After samples had been passed through, the

204   cartridges were washed with 5 mL distilled water, followed by air to remove water. Steroids

205   were eluted with 5 mL absolute methanol, collected in a glass tube and evaporated under a

206   stream of nitrogen at 45 °C. The dried extracts were shipped to the Endocrinology

207   Laboratory of the German Primate Center, Göttingen, Germany, where they were stored at -

208   20°C until steroid hormone analyses using enzyme immunoassays.

209   In teleost fish, the major circulating androgens are testosterone and 11-keto

210   testosterone. The latter is important with respect to male reproduction (Borg, 1994) but is

211   often undetectable in females and reproductively quiescent males (Sebire et al., 2007).

212   Testosterone (which usually correlates with 11-keto testosterone; e.g. Kidd et al., 2010), in

213   contrast, is ubiquitous in males and females (Borg, 1994, Sebire et al., 2007) and, because

214   we were interested in both sexes (and potential differences between them) studied in a

215   non-reproductive context, was chosen as the target androgen in this study. Prior to

216   hormone analyses, all samples were redissolved in assay buffer (Fürtbauer et al., 2015a,b).

217   All samples were run in duplicate, and samples with a CV above 7% between duplicates

218   were re-measured. Samples were analysed for T concentrations using an antibody that has

219   been raised in a rabbit against testosterone-3-CMO-BSA. The cross-reactivities of the T

220   antibody are described by Palme and Möstl (1994). Sensitivity of the assay at 90% binding

221   was 0.5 pg. Intra- and inter-assay coefficients of variation, calculated from replicate

222   determinations of high- and low- value quality controls were 6.6% (n=16) and 10.8% (n=8)

223   (high) and 8.9% (n=16) and 12.8% (n=8) (low).

224   To investigate potential mechanisms of changes in T between individual and

225   collective contexts, we also analysed samples for: (i) cortisol (which may suppress T

226   production), and (ii) oestradiol (E2) to calculate an index of T aromatisation to E2. Cortisol

227   was measured according to Fürtbauer et al. (2015a) using an antibody raised in a rabbit

228   against cortisol-3-CMO-BSA. The cross-reactivities of this antibody are described by Palme

229   and Möstl (1997). Sensitivity of the assay was 0.6 pg. For the cortisol assay, intra- and inter-

230   assay coefficients of variation were 7.4% (n=16) and 8.3% (n=8) (high), 8.9% (n=16) and

231   13.2% (n=8) (low). E2 was measured using a commercially available 17β-oestradiol saliva

232   enzyme immunoassay (IBL International; RE52601). For E2, intra-assay variability ranged

233   from 2.6% to 6.9% according to the assay manufacturer, and inter-assay CVs were 7.3%

234   (n=8) (high) and 6.9% (n=8) (low). All hormone data are expressed as ng/g/h.

235

236   *(c) Statistical analysis*

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238   Data were analysed with linear mixed models (LMMs) in R (R Development Core

239   Team, 2010) using the function lmer of the R package lme4 (Bates et al., 2014). Model

240   diagnostics for all LMMs were performed using graphical procedures (Q-Q plot and

241   standardized residuals vs. fitted values). The level of significance was set at $p < 0.05$. To test

242   our prediction that T levels are lower in a collective than an individual context, we used a

243   model (LMM1), including testosterone levels (log-transformed which satisfied the

244   assumptions of normal distribution of residuals) as the response, context (individual versus

245   collective), sex, and trial as fixed effects, and "ID" and "group" as random effects. To test

246   our prediction that high T individuals would show the largest decrease in T responsiveness

247   during shoaling (collective context), we tested whether changes in T were linked to

248   individual context T levels and included the relative change in T as response, individual

249   context T, sex, and trial as fixed effects, and ID and group as random effects (LMM2).

250   Because cortisol may suppress T production, we also ran a model (LMM3), testing whether

251   cortisol levels differed between individual and collective contexts, and included cortisol

252   levels (log-transformed) as response, context, sex (to control for potential sex differences in

253   HPI axis activity), and trial as fixed effects, and ID and group as random effects. Two-way

254   interactions between context and sex were initially added (models 1 and 3) but excluded

255   from final models due to non-significance. To investigate whether groups with lower mean T

256   are more egalitarian/less hierarchical in their leader-follower dynamics, we run a model

257   (LMM4) testing for a relationship between the groups' mean T levels and egalitarianism

258   ($\sigma^2 \bar{\tau}$) in leadership. Spearman's rank correlations were used to test for correlations

259   between individual and collective context T levels. Finally, to test the possibility that

260   aromatisation of T to E2 is responsible for the change in waterborne T from the individual to

261   the collective context, we calculated the ratio E2:(E2 +T), an index of T aromatisation to E2,

262 following Ramallo et al. (2015). We then ran a model (LMM5) including the index of T

263 aromatization to E2 as the response, context (individual versus collective), sex (to control for

264 the fact that females have higher E2 levels than males), and trial as fixed effects, and ID and

265 group as random effects.

266

267 **3. Results**

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269   In line with our predictions, we found that fishes' T levels were significantly lower in

270 a collective context compared to an individual context (LMM1: estimate±SE=0.33±0.06,

271 t=5.69, p<0.001, Figure 2; Table 1) in both trials. Moreover, those individuals with higher T

272 concentrations also showed a larger decrease in T responsiveness, with T levels in the

273 individual context being significantly correlated with the change in T levels in the collective

274 context (LMM2: estimate±SE=-0.51±0.10, t=-5.07, p<0.001; Figure 3; Table 1). Sex and trial

275 had no effect on T levels (LMM1: sex: estimate±SE=-0.08±0.05, t=-1.53, p=0.14; trial:

276 estimate±SE=0.01±0.05, t=0.32, p=0.75; Table 1). Individuals differed significantly in their T

277 levels (LMM1: random effect "ID": χ^2=48.2, df=1, p<0.001) and individual and collective

278 context T levels were significantly correlated (Trial 1: r_s=0.6-4, p<0.001, n=30; Trial 2:

279 r_s=0.59, p=0.001, n=30; LMM: estimate±SE=0.68±0.06, t=10.89, p<0.001), indicating

280 consistent individual differences in T concentrations across the two contexts. Cortisol levels

281 did not differ significantly between individual and collective contexts (LMM3: estimate±SE=-

282 0.04±0.11, t=-0.38, p=0.70), ruling out cortisol suppressing T production, but were

283 significantly lower in trial 2 than in trial 1, suggesting a habituation effect (LMM3:

284 estimate±SE=-0.32±0.11, t=-2.84, p=0.006). The index of T aromatisation to E2 was

285 significantly higher in the individual compared to the collective context (LMM5:

286   estimate±SE=0.14±0.06, t=2.41, p=0.018; Table 1), indicating that the observed decrease in

287   T responsiveness during the collective context is not due to increased aromatisation of T to

288   E2.

289   To explore whether T levels relate to group-level, collective behaviour, we used each

290   groups' variance in time delay between fishes velocity ($\sigma^2 \bar{\tau}$) and found that mean group T

291   predicts $\sigma^2 \bar{\tau}$ (LMM4: estimate±SE=2.02±0.69, t=2.92, p=0.018; Figure 4), suggesting that

292   groups with higher collective T concentrations are less egalitarian/more hierarchical in their

293   leadership.

294

295   **Table 1.** Results of Linear Mixed Models investigating hormonal changes between individual

296   and collective contexts in three-spined sticklebacks. In all models, group was included as

297   random effect. "ID" was included as random effect in models 1-3 and 5. Statistically

298   significant values are shown in bold.

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Model	Response variable	Predictor variable	Estimate±SE	t-value	p-value
1	*Testosterone*	Context (individual)	0.33±0.06	5.69	**<0.001**
		Sex	0.09±0.16	0.58	0.573
		Trial	-0.07±0.06	-1.2	0.252
2	*Change in T*	Individual context T	-0.51±0.10	-5.07	**<0.001**
		Sex	-0.08±0.05	-1.53	0.136
		Trial	0.01±0.05	0.32	0.752
3	*Cortisol*	Context	-0.04±0.11	-0.38	0.703
		Sex (male)	-0.37±0.17	-2.14	**0.042**

		Trial	-0.32±0.11	-2.84	**0.006**
4	$\sigma^2 \bar{\iota}$	Mean group T	2.02±0.69	2.92	**0.018**
		Trial	0.01±0.03	0.18	0.864
5	*E2:(E2 + T)*	Context (individual)	0.14±0.06	2.41	**0.018**
		Sex (male)	-0.79±0.10	-8.19	**<0.001**
		Trial	0.09±0.06	1.59	0.116

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301

302 **4. Discussion**

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304 In the present study we examined the responsiveness of testosterone (T) to group-level,

305 collective behaviour, i.e. shoaling, in reproductively quiescent, male and female three-

306 spined stickleback. Our overall aim was to further our understanding of the physiological

307 bases of social plasticity outside reproductive contexts and to elucidate potential benefits of

308 acute changes in androgen levels during social encounters (Moore et al., in press). In line

309 with our predictions, the fishes' T levels were significantly lower during shoaling and the

310 magnitude of this change in T levels depended on an individual´s T concentration in an

311 individual (non-collective) context. Our results suggest that shoaling directly reduces

312 androgen responsiveness which may serve to promote group formation and coordination as

313 indicated by our finding that groups characterized by lower mean T levels exhibited less

314 hierarchical leader-follower dynamics, suggesting that low T concentrations promote

315 egalitarianism.

316 Our finding that collective action has modulating effects on androgen levels in

317 gregarious, non-reproductive three-spined stickleback fish supports our "collective action

318   hypothesis". Across two trials, on two consecutive days, fishes' waterborne T levels were

319   lower during shoaling, and high-T individuals showed the largest decrease in T

320   responsiveness. Higher T levels in single compared to group contexts have also been

321   reported for mice (Mucignat-Caretta et al. 2014), and in black howler monkeys (*Alouatta*

322   *pigra*), males living in single-male groups have higher T levels than males in multi-male

323   groups which is believed to be associated with perceived future danger (Rangel-Negrín et

324   al., 2011). Higher T levels are also found in human singles compared to individuals living in a

325   relationship (Gray et al., 2004, van Anders and Watson, 2007).

326   Generally, individuals, when introduced to a novel environment often exhibit an

327   increase in glucocorticoid levels which, in turn, can reduce T production (e.g. Hu et al.,

328   2008). In our study, however, cortisol-induced T suppression is unlikely to explain the

329   observed difference in T given that cortisol levels did not increase during shoaling (Table 1).

330   Furthermore, cortisol levels were lower during the second trial, suggesting habituation to

331   the social and/or physical environment. Testosterone levels, in contrast, did not differ

332   between trials (Table 1).

333   Alternatively, lower T in the collective context could potentially be related to

334   increased aromatase activity, an enzyme that modulates rapid changes in social behaviours

335   of birds, mammals, and fish via conversion of T to oestradiol (E2) (e.g. Ramallo et al., 2015).

336   In the Neotropical cichlid *Cichlasoma dimerus*, for instance, non-territorial males have

337   higher E2 levels as well as higher T conversion to E2 than territorial males, and E2 is

338   positively correlated with submissive behaviour (Ramallo et al., 2015). Similarly, in peacock

339   blenny (*Salaria pavo*) males, E2 implants decrease the frequency of aggressive displays

340   (Gonçalves et al., 2007). Following removal of the dominant male in the sex-changing fish

341   *Lythrypnus dalli*, increases in aggression of the dominant (sex-changing) female are related

342   to lower aromatase activity in the brain (Black et al., 2005). Likewise, in male California mice

343   (*Peromyscus californicus*), aggressive behaviour and aromatase activity are inversely related

344   (Trainor et al., 2004). To test the possibility that the conversion of T to E2 is responsible for

345   the observed decrease in T responsiveness in this study, we analysed our samples for

346   waterborne E2 concentrations and calculated an index of T aromatisation to E2 (Ramallo et

347   al., 2015). The index was significantly higher in the individual compared to the collective

348   context, suggesting that the observed decrease in T during shoaling is not due to increased

349   aromatisation of T to E2 (see Ramallo et al., 2015). However, given that rapid behavioural

350   changes often occur due to local E2 synthesis in the brain (for a review see e.g. Balthazart &

351   Ball, 2013) and our study measured hormone concentrations from water samples, reflecting

352   concentrations in the general circulation, future work should investigate brain aromatase

353   activity during shoaling. Furthermore, we did not consider potential oxidization of T to 11KT

354   because T to 11KT conversion rates (Ramallo et al., 2015) and high 11KT levels are usually

355   linked to territoriality and aggression (reviewed by Moore et al., in press) whereas sub-

356   ordinance results in a blockage of 11KT production (reviewed by Oliveira 2004). In our study,

357   no behaviours associated with increased 11KT were observed (pers. obs.).

358   A number of studies have reported decreased steroid hormone levels after repeated

359   handling (reviewed by Scott et al., 2008). In our study, all subjects were habituated to

360   handling and beaker confinement prior to experiments, and we have previously shown that

361   T levels in sticklebacks do not change significantly across 5 days of handling (whilst cortisol

362   levels decrease significantly after 2 exposures; see Fig. 5 in Fürtbauer et al., 2015a).

363   Given that neither handling effects, increased HPI-axis activity, or conversion of T to

364   E2 or 11KT are likely to explain reductions in T levels, the observed difference in T

365   concentrations between individual and collective contexts is likely to be the result of a direct

366   negative feedback of social stimuli on androgen responsiveness. But what are the potential

367   functions of low T levels during shoaling in sticklebacks? Across vertebrates, low T has been

368   linked to affiliation, social tolerance, cooperation motivation, and fatherhood (Lynn, 2016,

369   Mehta et al., 2009, Gettler et al., 2011, Mehta and Josephs, 2011, Hamilton et al., 2015).

370   Here, we show that in stickleback fish T is linked to the dynamics of group coordination. We

371   find a group's mean T level was significantly and positively related to the groups' variance in

372   time delay between fishes velocity correlation. In practice, this meant that the behavioural

373   interactions of fish in low T shoals were less variable and resulted in more egalitarian leader-

374   follower patterns (i.e., lower T fish shoals were composed of individuals that responded to

375   and followed all or most individuals' movements, whereas higher T fish shoals were

376   composed of individuals that were likely to respond to the movements of just one or a few

377   fish). In groups which have high phenotypic variation (e.g. in morphology, behaviour, or

378   physiology) cohesion can break down and groups fission (Delgado et al., 2018), resulting in

379   phenotypic assortment within and among groups (Gueron et al., 1996). In contrast, groups

380   of individuals with similar traits exhibit greater coordination, enhanced information transfer,

381   and improved predator avoidance (reviewed by Killen et al., 2017). We therefore speculate

382   that low T in fish shoals may serve to promote coordination and ultimately improve

383   collective action.

384   Future work can now test this hypothesis by presenting low and high T groups with

385   tests related to predator avoidance (Ward et al., 2011) or acquiring food (Hansen et al.,

386   2016); the over-arching "collective action hypothesis", and the results we present here,

387   predict that low T shoals should respond more quickly and efficiently to such tasks.

388   Moreover, if such differences (or lack thereof) provide benefits, we would predict that fish

389   may show (active or passive) assortment within and among groups according to T levels,

390   and/or plasticity in T in response to their social environment given new evidence for co-

391   regulatory processes on fish hormone responses (Fürtbauer & Heistermann, 2016). Overall,

392   our findings provide opportunities for understanding the physiological bases of social

393   plasticity outside a reproductive context and resemble earlier findings emphasizing the

394   importance of low T for the expression of non-reproductive sociopositive/cooperative

395   behaviour in humans (e.g. Mehta et al., 2009, Mehta and Josephs, 2011, Hamilton et al.,

396   2015, Lozza et al., 2017), suggesting similarities in endocrine mechanisms. However, given

397   the lack of non-human studies investigating social modulation of T in this context, a large

398   amount of future work is needed to confirm the generality of these findings.

399   In this study, we deliberately focussed on a neutral, i.e. resource-free environment

400   to minimise competition. However, assessing endocrine shifts surrounding both non-

401   competitive and competitive situations (e.g. by introducing resources such as food or shelter

402   over which individuals are likely to compete) in the same individuals could provide further

403   insight into social modulation of androgen levels in collective contexts. There may be a

404   trade-off between competition and the innate behaviour to aggregate, similar to the trade-

405   off between territorial aggressiveness and paternal care as shown in numerous species (see

406   e.g. Oliveira et al., 2002; Lynn 2016). In line with this, including reproductively active

407   individuals could be another potentially useful direction of future research. Both

408   reproductive male and female sticklebacks exhibit higher T levels and are more aggressive

409   towards conspecifics than non-reproductive ones (Sebire et al., 2007).

410   Overall, our results suggest a negative feedback of shoaling on T responsiveness,

411   which may serve to promote coordination and ultimately facilitate collective action. Future

412   research should investigate whether groups with higher collective T "perform worse", e.g.

413   are less coordinated/efficient than groups with lower collective T (see e.g. Akinola et al.,

414   2016, Akinola et al., 2018), e.g. through grouping individuals of known T levels.

415   Furthermore, experimental administration/blocking of T before shoaling as well as tests in

416   reverse order (i.e. testing fish in the collective followed by the individual context) could

417   further elucidate social modulation of T in non-reproductive collective contexts. Our fish

418   model system therefore provides a powerful framework for testing theories of the

419   development of social tolerance and collective intelligence and performance.

420

428

429   **Author contributions**

430

431   IF designed and performed the experiments, extracted water samples, analysed the data,

432   prepared figures, and wrote the manuscript. MRB tracked the fish, calculated fish position

433   and time delay between fishes' velocity. MH performed hormone analyses and contributed

434   to the writing of the manuscript.

435

436   **Competing interests**

437

438   The authors state no competing interests.

439

440   **References**

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628 **Figure legends**

629

630 **Figure 1.** Behavioural contexts linked to low and high levels of testosterone (T). Low T

631 promotes affiliation, social tolerance, and cooperation, whereas high T is linked to

632 aggression, dominance, and competition (see text for details and references).

633

634 **Figure 2.** Differences in waterborne log testosterone levels in individual and collective

635 contexts across two trials (LMM1: p<0.001).

636

637 **Figure 3.** The relationship between the relative change in T levels from an individual to a

638 collective context and individual log T levels preceding collective action across two trials

639 (LMM2: p<0.001).

640

641 **Figure 4:** Egalitarianism in leader-follower dynamics as a function of groups' mean

642 testosterone (LMM4: p=0.018). Higher $\sigma^2\bar{\tau}$ indicates less egalitarian/more hierarchical

643 leader-follower dynamics. The line and grey band represent the predicted effect and
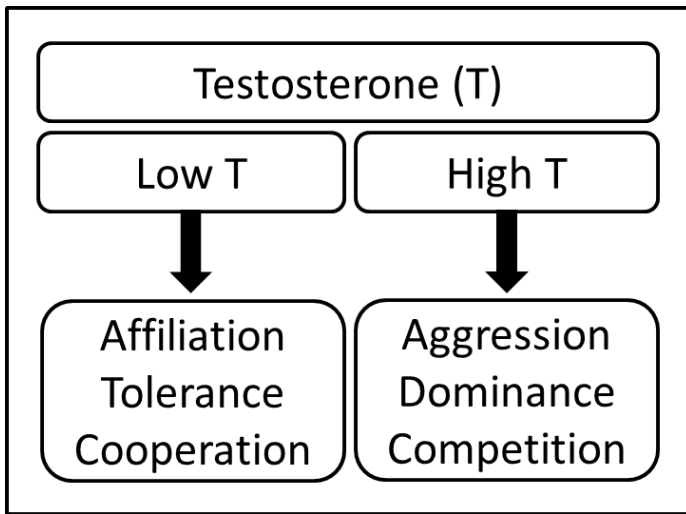
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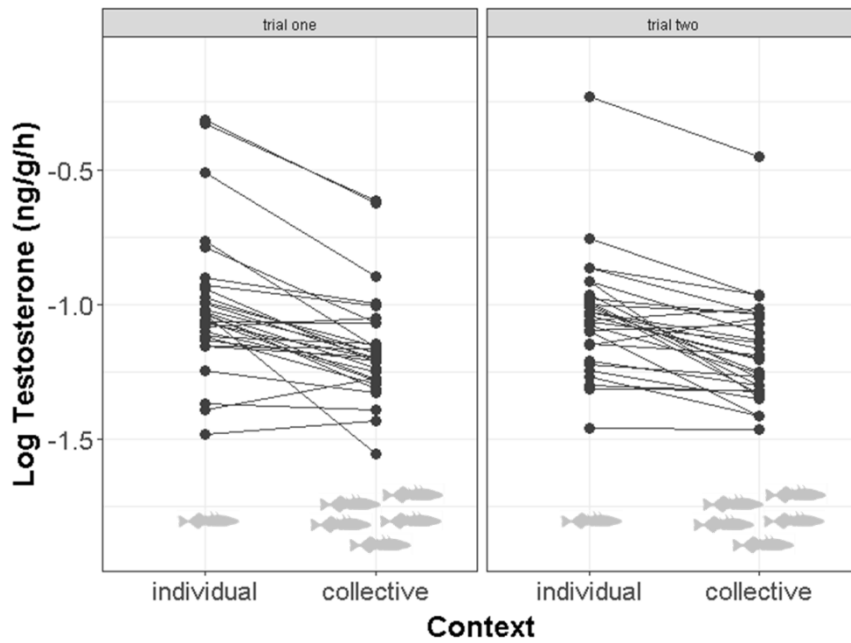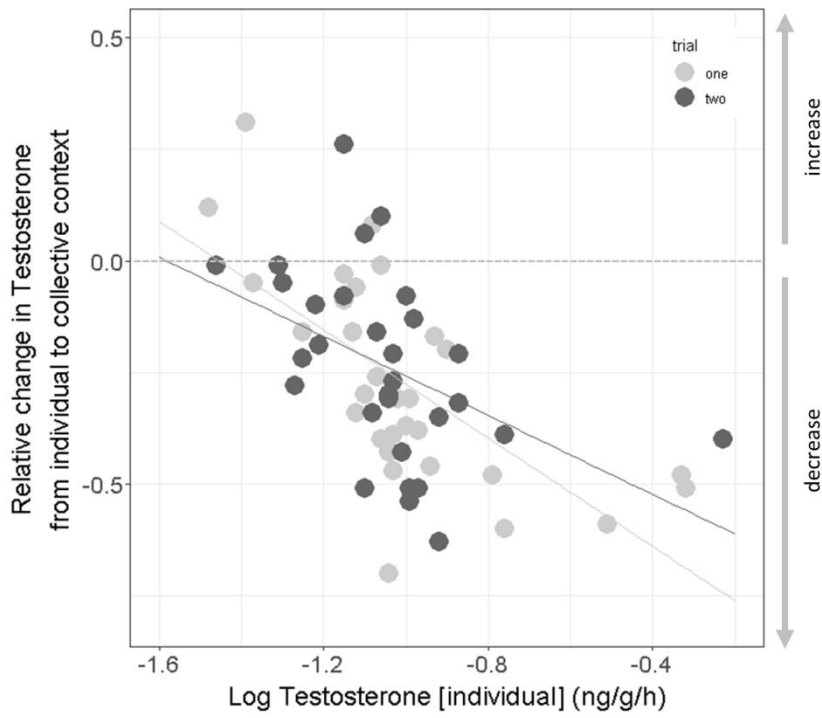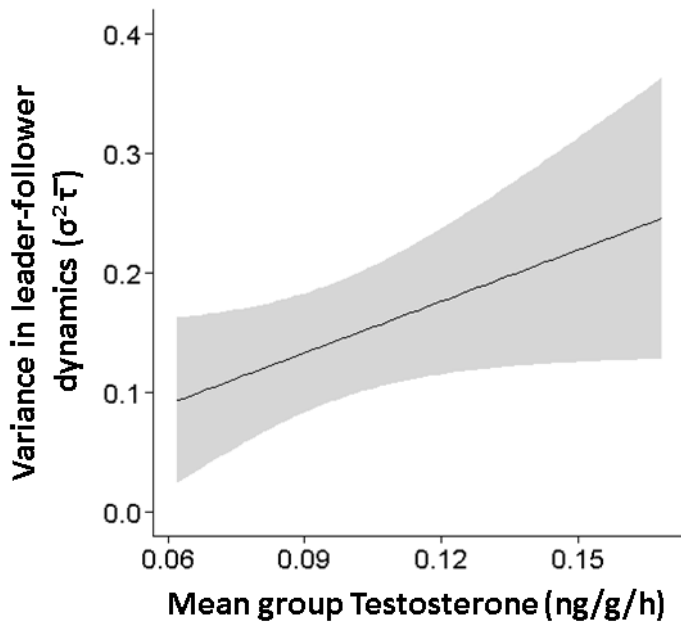
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Figures

Figure 1

Figure 2

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