2018-08-16

How does the environment affect fighting? The interaction between extrinsic fighting ability and resource value during contests.

Lane, Sarah

http://hdl.handle.net/10026.1/12376

1 **HOW DOES THE ENVIRONMENT AFFECT FIGHTING? THE INTERACTION BETWEEN**

2 **EXTRINSIC FIGHTING ABILITY AND RESOURCE VALUE DURING CONTESTS**

3 **Running title:** Environmental effects on fighting

4 **Sarah M. Lane* & Mark Briffa**

5 Marine biology and ecology research centre, Plymouth University, Drake Circus, Plymouth,

6 Devon, PL4 8AA, UK.

7 *corresponding email: sarah.lane@plymouth.ac.uk

8

9

10

11

12

13

14

15

16

17

18

19

20   **Summary statement**

21   Here we demonstrate how environmental variation can affect an individual's willingness and

22   capacity to engage in fights over indivisible resources.

23   **Abstract**

24   An individual's performance during a fight is influenced by a combination of their capacity

25   and willingness to compete. While willingness to fight is known to be determined by both

26   intrinsic and extrinsic drivers, an individual's capacity to fight is generally thought of as

27   solely intrinsic, being driven by a host of physiological factors. However, evidence indicates

28   that variation in fighting ability can also be generated through exposure to different

29   environmental conditions. Environmental contributions to fighting ability may be

30   particularly important for animals living in spatially and temporally heterogeneous habitats,

31   in which fights can occur between rivals recently exposed to different environmental

32   conditions. The rapidly changing environment experienced within intertidal zones, for

33   example, means that seawater parameters, including dissolved oxygen content and

34   temperature, can vary across small spatial and temporal scales. Here we investigate the

35   relative importance of these extrinsic contributions to fighting ability and resource value on

36   contest dynamics in the beadlet sea anemone *Actinia equina*. We manipulate the extrinsic

37   fighting ability of both opponents (through dissolved oxygen concentration prior to fights)

38   and resource value (through seawater flow rate during the fight). Our results indicate that

39   the extrinsic fighting ability of both opponents can interact with resource value to drive

40   escalation patterns and that extrinsic drivers can be more important in determining contest

41   dynamics than the intrinsic traits commonly studied. Our study highlights the need to

42   combine data on intrinsic state and extrinsic conditions in order to gain a more holistic view

43   of the factors driving contest behaviour.

44

45   **Keywords:** Animal contests; Assessment; Fighting behaviour; Hypoxia; Resource holding

46   potential; Resource value

47

48   **1. Introduction**

49   Traditional and recent contest theory predicts that injurious fighting is more likely to occur

50   when the potential benefits to be gained exceed the potential costs (Maynard Smith, 1973;

51   Parker, 1974; Lane and Briffa, 2017). Operationally this means that fighting behaviour is

52   driven by two main variables, fighting ability or resource holding potential (RHP) and

53   resource value (RV). The costs of entering a fight will be driven by differences in RHP

54   between the opponents (e.g. energy expended, injuries incurred) while the potential

55   benefits to be gained from fighting will equate to the value of the contested resource (RV).

56   Although RHP and RV have been the subject of many studies on contest dynamics (i.e.

57   patterns of escalation and duration), most work examines either RHP (Briffa and Elwood,

58   2000; Dissanayake et al., 2009) or RV (Mohamed et al., 2010; Stockermans and Hardy, 2013;

59   Palaoro et al., 2017). In reality, these factors will affect contest behaviour simultaneously

60   and thus it is important that we understand their additive and interactive effects.

61   Furthermore, variation in RHP and RV can be influenced by both extrinsic and

62   intrinsic factors. Extrinsic (or objective) sources of variation in RV (Stockermans and Hardy,

63   2013) derive from the absolute properties of the resource unit such as the size of a territory

3

64   or the number of calories in a piece of food, while intrinsic (subjective) RV reflects the

65   subjective value different individuals place on the same resource. Fights can be affected by

66   one or both of these RV components. For instance, the intensity of fights between female

67   parasitoid wasps, *Goniozus legneri*, is driven by both extrinsic (host size) and intrinsic

68   (female age) factors, with intrinsic RV having the greatest overall impact as the value of

69   finding a host increases dramatically with female age (Stockermans and Hardy, 2013).

70   Meanwhile variation in RHP is generally considered to be determined only by intrinsic

71   factors such as body size, weapon size, condition and metabolic rate, factors driven by

72   genes, development and the effects of prior contest experiences e.g. damage sustained. Yet

73   contest intensity can also be affected by rapidly fluctuating extrinsic factors such as

74   environmental conditions, particularly those expected to affect an individual's capacity for

75   performing energetically demanding aggression, (e.g. oxygen availability [Briffa and Elwood,

76   2000; Sneddon et al., 1999] and the presence of environmental toxins [Dissanayake et al.,

77   2009]). Thus variation in RHP may also be driven by extrinsic RHP components (henceforth

78   'extrinsic RHP') via their influence on physiological factors that drive fighting performance.

79   Despite the potential for extrinsic effects on RHP, contests are usually studied in

80   experimental set-ups in which environmental conditions are held constant. While this may

81   allow the effects of intrinsic RHP to be investigated, by ignoring extrinsic drivers of RHP we

82   may be overestimating the importance of these intrinsic RHP traits. Furthermore,

83   experiments in which external conditions are manipulated could allow us to test key ideas

84   about the evolution of fighting behaviour. For example, experiments designed to distinguish

85   between the assessment rules used by individuals during fights (mutual versus self-

86   assessment –Payne and Pagel, 1997; Payne, 1998) typically test for correlations between

87   some continuous measure of intrinsic RHP (e.g. body size) and contest duration. As losers

88   decide when a contest ends, contest duration should always increase with the RHP of the

89   loser, but if mutual assessment is being used there should also be a negative correlation

90   with the RHP of the winner (Taylor and Elwood, 2003; Arnott and Elwood, 2009). There are,

91   however, limits to this correlative approach (Briffa and Elwood, 2009) and manipulating

92   extrinsic RHP offers an alternative way of probing assessment rules. Providing that the

93   extrinsic RHP of each opponent can be manipulated independently, we could incorporate a

94   categorical extrinsic RHP predictor into analyses that are analogous to the correlative tests

95   currently used.

96   In nature, extrinsic sources of RHP variation may be particularly important for

97   animals living in spatially and temporally heterogeneous habitats, in which fights can occur

98   between rivals that have recently been exposed to different environmental conditions. The

99   rapidly changing environment experienced within intertidal zones, for example, means that

100   seawater parameters, including dissolved oxygen content and temperature, can vary across

101   small spatial and temporal scales. Furthermore the exposed nature, particularly on the

102   upper shore, provides motivation for conflict as individuals vie to gain suitably sheltered

103   territory before the tide goes out. Exposure to low dissolved oxygen levels (hypoxia) has

104   been shown to reduce the fighting ability of marine invertebrates, by reducing their capacity

105   to meet the energetic demands of fighting. For example, hermit crabs *Pagurus bernhardus*

106   exposed to hypoxic conditions fight with less vigour and are less likely to win fights

107   compared with crabs exposed to normoxic seawater (Briffa and Elwood, 2000). Another

108   intertidal marine invertebrate, the beadlet sea anemone *Actinia equina*, fights over limited

109   space on rocky shores, using specialised stinging structures called acrorhagi to attack rivals

110   and convince them to relinquish their territory (Williams, 1978; Brace et al., 1979; Bigger,

111   1982). Although anemones are sedentary, conflicts over territory cause individuals to move

112   across the rocks and between the microclimates created by the changing tides. Thus

113   anemones are likely to come into contact with individuals that have recently experienced

114   different levels of dissolved oxygen, and consequently differ in their extrinsic RHP.

115   Furthermore, exposure to different environmental conditions is known to drive variation in

116   extrinsic RV in *A. equina*, with individuals exposed to flowing seawater demonstrating

117   increased persistence during fights in comparison with individuals exposed to still water

118   (Palaoro et al., 2017), reflecting a higher value placed on territories that experience greater

119   flow rates. Thus, sea anemones represent an ideal system with which to simultaneously

120   investigate the effects of extrinsic drivers of fighting ability and resource value.

121   With the exception of Briffa and Elwood (2000) studies on the effects of the abiotic

122   environment on fighting have involved fights where both individuals have been subjected to

123   the same conditions during the fight. This paradigm limits our ability to determine whether

124   extrinsic variables contribute to RHP as it is not possible to separate the effects of winner

125   and loser RHP on the outcome of the contest (i.e. which individual wins). Therefore in this

126   study we manipulate the extrinsic RHP of each individual separately in order to test for the

127   potential of additive and interactive effects of both individuals' extrinsic RHPs. Furthermore,

128   we test the idea, for the first time to our knowledge, that extrinsic variation in RHP

129   (manipulated through dissolved oxygen concentration prior to fights) and RV (manipulated

130   through seawater flow rate during the fight), and the interaction between them, should

131   influence the intensity and outcome of contests. If dissolved oxygen represents an extrinsic

132   source of RHP difference, anemones exposed to higher levels of dissolved oxygen should

133   escalate more and persist for longer than those exposed to low dissolved oxygen, and

134   ultimately should win more fights. Similarly, as flow rate represents an extrinsic RV variable

135   (Palaoro et al., 2017), anemones exposed to flowing water should escalate more and persist

136   for longer than those exposed to still water and should defeat opponents of similar RHP. If

137   these two factors have an interactive effect, the most intense fights are predicted to occur

138   when both opponents are exposed to high oxygen and high flow, and the least intense fights

139   occur when both are exposed to low oxygen and still water. Thus, the chance of victory

140   should be greatest for focal individuals exposed to higher dissolved oxygen fighting against

141   opponents exposed to low dissolved oxygen under high flow conditions. We also

142   incorporate intrinsic RHP traits into our analysis in order to determine how their influence

143   on contest behaviour may be modified by the external environment, and to determine the

144   relative importance of intrinsic and extrinsic RHP. Finally, as described above, we use the

145   data from this experiment to demonstrate how manipulation of extrinsic RHP can be used

146   as an alternative means of probing assessment rules during animal contests that avoids the

147   need for correlative analyses based on intrinsic RHP variation.

148

149   **2. Materials and Methods**

150   *Animal collection and husbandry*

151   *Actinia equina* (*N* = 132) of the red/brown colour morph were collected intertidally from

152   Portwrinkle (Cornwall UK; grid reference: SX 357539) between September and December

153   2017 and taken back to the lab within 1-2 hours of collection. All anemones collected were

154   visually inspected for injury and only anemones without injury were brought back to the lab.

155   Once in the lab anemones were housed individually in plastic tanks (23 x 16 x 17.5cm)

156   containing 700ml of filtered seawater (pumped from Mount Batten, Plymouth, UK; grid

157   reference: SX 48715319; Average seawater quality: pH = 8 - 8.2; salinity = 34psu [HI-96822

158   seawater refractometer, Hanna Instruments, RI, USA]; ammonia = 0ppm; nitrite = 1ppm;

159   nitrate = 10ppm [API saltwater master test kit, API Fishcare]) along with an air stone for

160   constant aeration. Anemones were maintained at 15°c ± 0.5°c and fed ad libitum on aquaria

161   marine flakes every 2-3 days. Tank seawater was topped up daily and replaced fully every 7

162   days with fresh filtered seawater.

163

164   *Manipulating resource holding potential and resource value*

165   All anemones were given a 7-14 day acclimatisation period before they were dislodged from

166   their position in the tank and provided with stones to attach to. Anemones were then

167   randomly allocated to one of two treatments – hypoxic (H) or normoxic (N) seawater. The

168   following day, anemones allocated to the hypoxic treatment were exposed to hypoxic

169   conditions for 30 minutes prior to being introduced to an opponent. Hypoxic conditions

170   were produced by bubbling nitrogen (rather than the usual air) into the anemone's tank

171   until O_2 levels reached 30%. The oxygen levels in the tank were then kept at 30% for 30

172   minutes by covering the tank with a piece of Perspex and monitoring O_2 levels with an

173   oxygen probe (YSI Pro2030, YSI Inc., OH, USA). Normoxic individuals were maintained under

174   normal seawater conditions before the fight. In order to create a fully orthogonal design,

175   anemones were allocated into size-matched pairs (estimated visually) according to

176   treatment and assigned at random as either the focal or opponent individual (focal-

177   opponent: - H-N, N-H, H-H and N-N). As anemones rely on water flow in the wild to find

178   food, we also manipulated resource value by altering flow conditions within the fighting

179   tanks. All fights were performed in freshly aerated sea water to control for effects of oxygen

180   content during the fight itself, then in order to create a high resource value environment,

181   half of the tanks were supplied with a small water pump (L/min) (EHEIM compactON 300,

182   EHEIM GMbH & Co., Deizisau, Germany) (flow (F)) while the other half were not (no flow

183   (NF)). The pump was fully submerged to eliminate the possibility that anemones under flow

184   conditions were receiving more oxygen, and the levels of dissolved oxygen monitored in

185   each tank type prior to the experiment to confirm this assumption. Pairs were then

186   randomly allocated to one of these two resource value conditions in a fully orthogonal

187   manner, resulting in a total of 8 treatment groups (H-N (F); H-N (NF); N-H(F); N-H(NF); H-

188   H(F); H-H(NF); N-N(F); N-N(NF)).

189

190   *Staging contests*

191   Fights in *A. equina* take two forms (1) non-injurious contact of the feeding tentacles or (2)

192   one or both anemones inflict injurious attacks using acrorhagi leaving behind acrorhagial

193   stinging 'peels' on the opponent. These 'peels' cause localised necrosis on the recipient but

194   are not fatal in *A. equina*. In order to stimulate agonistic behaviour, anemones were

195   positioned such that their body columns were touching. Fights were recorded from this

196   initial contact until one anemone (the loser) either: (a.) moved an approximate distance of

197   one pedal disc away from its opponent (estimated visually) or (b.) retracted its tentacles

198   completely for at least 10 minutes. If both opponents performed these retreating

199   behaviours, the outcome of the fight was classified as a draw. Similarly, if neither individual

200   retreated after 3 hours, the fight outcome was classed as a draw. At the end of the contest

201   individuals were checked for the presence of acrorhagial peels, separated and returned to

202   their tanks. If one or both anemones failed to open their tentacles within the three hour

203   observation period, the interaction was categorised as a 'no fight' and the anemones were

204   removed from the study. All fights were recorded using a Canon LEGRIA HF R706 High

205   Definition Camcorder and scored blind manually for contest behaviour and duration. A total

206   of 66 interactions were observed with an average of 8 interactions per treatment

207   combination, see table 1 in appendix for a full breakdown of sample sizes.

208   *Measuring intrinsic RHP traits*

209   After the fights the minimum and maximum pedal disc diameters of each anemone were

210   measured using callipers to the nearest 0.1mm. As pedal disc shape is elliptical, body size

211   was then calculated for each anemone as the average of the minimum and maximum

212   diameter (Brace and Quicke, 1986). Tissue samples from acrorhagi that had not been used

213   in the contest were taken from each anemone using forceps, spread onto a glass slide and

214   stained using 1% methylene blue solution (Manuel, 1988). Anemones are capable of rapidly

215   regenerating body parts (Brockes and Kumar, 2008; Leclère and Röttinger, 2017) and thus

216   this removal of acrorhagi only damages the animals temporarily. Nematocysts were imaged

217   using a Leica M205 FA stereo microscope equipped with a camera (Leica DFC7000 T, Leica

218   microsystems Ltd. CH-9435, Heerbrugg) connected to a computer. Nematocyst length was

219   then measured blind, using point-to-point measurements in ImageJ (version 1.50i).

220   Nematocyst length for each individual anemone was then calculated as the average length

221   of 10 randomly selected nematocysts.

222   At the end of the experiment, all anemones were returned to the shore they were

223   collected from.

224

225   *Statistical analyses*

226   We approached the analysis in two ways. First, we tested for the effects of extrinsic RHP and

227   RV on overall contest dynamics, in order to determine how these factors would influence (i)

228   the occurrence of fights, (ii) the occurrence of escalated fights involving injuries, (iii) for

229   fights that escalated, the type of fight in terms of whether one or both individuals deployed

230   their acrorhagi (attack type), and (iv) the duration of the contest. Second, we analysed the

231   effects of extrinsic RHP and RV from the perspective of focal individuals to determine the

232   effects of these factors on (v) the likelihood of focal individuals deploying their acrorhagi,

233   (vi) for those focal individuals that did attack the opponent, the number of peels that they

234   inflicted and (vii) the chance of victory for focal individuals. In analyses (i-iv) focal and

235   opponent extrinsic RHP conditions were combined to give an overall extrinsic RHP factor

236   (henceforth 'combined RHP'), with three levels: both hypoxic (H-H), both normoxic (N-N)

237   and mixed (H-N and N-H). We then analysed the effects of combined RHP and extrinsic RV

238   (henceforth 'RV'; flow or no flow) and their interaction on the binary measures of fight

239   occurrence, escalation, and attack type (escalated fights only) using generalised linear

240   models (GLMs) with a binomial error distribution. To determine the effect of the same two

241   predictors on contest duration (which was log-transformed) we used a linear model. In

242   analyses (v to vii) we used two factors to account for the distinct extrinsic RHP conditions of

243   focal and opponent individuals; 'focal RHP' (H or N) and 'opponent RHP' (H or N). We then

244   used binomial GLMs to analyse the effect of these two RHP factors, and the RV factor (flow

245   or no flow), and their interactions, on the probability that the focal anemone attacked the

246   opponent (i.e. deployed its acrorhagi) and on the probability of victory for focal anemones.

247   We used a GLM with a quasipoisson error distribution (accounting for overdispersion in the

248   data) to analyse the effect of these three predictors on the number of peels the focal

249   individual inflicted. Two measures of intrinsic RHP, relative size difference (RSD) and relative

250   nematocyst length (RND) (both calculated as described in Rudin and Briffa (2011)) were

251   included in the analyses as covariates. RSD was included as a covariate in all analyses while

252   RND was only included as a covariate in analyses of escalated fights (RND has previously

253   been shown to only be of importance for determining outcome in escalated fights – see

254   Rudin and Briffa, 2011). In order to explore significant effects further, we performed post-

255   hoc linear contrasts using the glht function of the R package multcomp (Hothorn et al.,

256   2008). Finally, to examine the assessment rules used by anemones, we performed two t-

257   tests with contest duration as the response variable and winner or loser RHP as the

258   explanatory variable, respectively. All analyses were carried out in R Studio v.1.0.136 (R Core

259   Team, 2016).

260

261   **3. Results**

262   *Contest dynamics*

263   The likelihood of a fight occurring was significantly affected by combined extrinsic RHP

264   (hypoxic, normoxic or mixed) (X^2 = 10.55, P = 0.005), with fights being less likely to occur

265   when both individuals had been exposed to normoxic seawater (figure 1a). Fights also

266   occurred more often under the high resource value (flowing seawater) treatment (X^2 = 4.21,

267   P = 0.04) (figure 1b), but there was no interaction between combined extrinsic RHP and RV

268   (X^2 = 1.93, P = 0.38).

269   When fights did occur, the probability of escalation was significantly influenced by an

270   interaction between RHP and RV (X^2 = 11.97, P = 0.0025). When both anemones were evenly

271   matched in extrinsic RHP (i.e. when the combined extrinsic RHP was either hypoxic or

272   normoxic) escalation was more likely under high RV (flow) than under low RV (no flow) but

273   when anemones were mismatched (combined extrinsic RHP was mixed) the opposite

274   pattern was seen, with fights being more likely under low RV conditions (figure 2). For fights

275   that did escalate, there was a further effect of resource value on whether single or mutual

276   attacks occurred (X^2 = 6.80, P = 0.009). Mutual attacks occurred more often under high

277   resource value (flow) while single attacks occurred more often under low resource value (no

278   flow), suggesting that opponents were more likely to strike back when the contested

279   resource was of high value (figure 3). Attack type (single or mutual attack), was not affected

280　by combined extrinsic RHP (X^2 = 4.38, P = 0.11) and there was no interaction between

281　combined extrinsic RHP and RV (X^2 = 4.56, P = 0.10). Contest duration was significantly

282　affected by the combined RHP of pairs (X^2 = 8.43, P = 0.004). Post-hoc analyses revealed that

283　fights between pairs of hypoxic individuals (H-H) were significantly shorter than fights

284　between normoxic individuals (N-N; P = 0.02) and mixed pairs (H-N or N-H; p = 0.02) (figure

285　4), but duration was not effected by RV (X^2 = 0.04, P = 0.83) and there was no interaction

286　between combined RHP and RV (X^2 = 0.88, P = 0.58). There was no effect of relative size

287　difference or relative nematocyst length on any of the factors analysed (table 2 in

288　appendix).

289

290　*Focal agonistic behaviour*

291　There was no effect of focal RHP, opponent RHP, RV, or their interactions on whether or not

292　the focal individual attacked (table 3 in appendix). Furthermore, resource value and its

293　interactions with focal and opponent RHP had no effect on the number of peels inflicted by

294　focal individuals in escalated fights (table 4 in appendix). However an interaction between

295　focal RHP and opponent RHP (X^2 = 42.11, P = 0.01) indicates that focal individuals inflicted

296　more peels on opponents when both had received the same RHP treatment (H-H or N-N),

297　compared with pairs that had received different treatments (H-N or N-H) (figure 5). Finally,

298　contest outcome for focal individuals was significantly affected by a three-way interaction

299　between focal RHP, opponent RHP and RV (X^2 = 4.25, P = 0.039) (figure 6). However, this

300　effect was lost when individuals that drew were removed from the dataset (X^2 = 1.31, P =

301　0.25), indicating that this interaction was driven by differences in the distribution of fights

302　that ended in draws across treatment combinations. Under flowing seawater conditions

13

303   every fight involved a clear outcome when both opponents were pre-treated with normoxic

304   seawater. In contrast, under still seawater conditions, every combination of focal and

305   opponent pre-treatment yielded a proportion of contests that resulted in a draw. There was

306   no effect of relative size difference or relative nematocyst length on any of the factors

307   analysed (table 1 in appendix).

308

309   *Assessment rules*

310   Contest duration was significantly affected by the extrinsic RHP of losers ($t = 3.67$, $P < 0.001$)

311   such that fights were resolved more quickly when losers had been subject to the hypoxic

312   treatment. In contrast, the treatment of winners had no effect on contest duration ($t = 0.24$,

313    $P = 0.811$) (figure 7).

314

315   **4. Discussion**

316   In this study we have demonstrated that contest dynamics and decisions can be significantly

317   impacted by extrinsic sources of variation in both resource holding potential and resource

318   value. Furthermore, our results indicate that some aspects of fighting behaviour are

319   affected by interactions between an individual's extrinsic RHP, the extrinsic RHP of its

320   opponent and the value of the contested resource, while others are subject only to additive

321   effects of RHP and RV.

322   Fights between hypoxic individuals, where the extrinsic component of RHP had been

323   experimentally reduced, were significantly shorter than fights in which both individuals had

324   been pre-treated with normoxic seawater and fights in which each individual had received a

325   different pre-treatment (i.e. normoxic and hypoxic seawater). Similarly, fights in shore

326   crabs, *Carcinus maenas*, have been shown to be shorter under hypoxic conditions (Sneddon

327   et al., 2009) and hermit crabs, *P. bernhardus*, pre-exposed to hypoxic seawater fight less

328   intensely than those exposed to normoxic seawater (Briffa and Elwood, 2000). As in the

329   cases of these decapod crustaceans, it also appears that that exposure to hypoxia caused a

330   reduction in RHP in *A. equina*, since exposed individuals persisted for less time. However,

331   despite this reduced fighting ability, the amount of damage (number of peels) inflicted by

332   focal individuals was significantly higher in hypoxic pairs than in mixed pairs, indicating that

333   hypoxic individuals fought more aggressively but only when matched with their opponents

334   in terms of extrinsic RHP. Similarly, encounters involving at least one hypoxic individual

335   were significantly more likely to result in a fight than those containing two normoxic

336   individuals. These findings suggest that contrary to expectations, individuals with reduced

337   fighting ability had an increased motivation to fight. Similar results were found in a study of

338   shore crabs *Carcinus maenas* in which starved and pyrene-exposed crabs fought with

339   greater vigour and spent more time in possession of the contested resource than control

340   individuals (Dissanayake et al., 2009). In contrast, previous studies investigating the effect of

341   hypoxia on fighting behaviour have found that the reduction in RHP elicited by low oxygen

342   levels leads to a decrease in competitive ability (Sneddon et al., 1999; Briffa and Elwood,

343   2000). Dissanayake et al. (2009) suggested that the discrepancy between these findings

344   could be explained by the presence of a high value resource in their study which increased

345   the motivation to fight, a possibility that also seems likely in the current study. Although

346   100% of interactions between hypoxic individuals resulted in a fight, the likelihood of these

347   contests escalating to injurious fighting was dependent upon resource value. Contests

348   between hypoxic pairs were significantly more likely to escalate when resource value was

349 high (i.e. in the presence of flowing water). Thus, low extrinsic RHP appears to increase the

350 motivation to escalate but only when the contested resource is of high value.

351   Since we manipulated the extrinsic RHP independently for each opponent, we could

352 also test for its effects on tactical (i.e. escalation) and strategic (i.e. giving up) decision-

353 making. Furthermore, we were able to ask whether these effects were modified by

354 resource value. During escalated fights, focal individuals inflicted a higher number of peels

355 on their opponent when both individuals had experienced the same pre-treatment prior to

356 the contest. This result was expected as a general prediction of theory is that contests

357 should be more intense, in terms of the agonistic tactics used, when opponents are

358 matched in RHP (e.g. Enquist and Leimar, 1983). The outcome of a contest is expected to be

359 driven by a similar interaction between contestant RHPs, such that (regardless of whether

360 self or mutual-assessment is being used) an individual's chance of winning should ultimately

361 be driven by the difference between its own RHP and that of the opponent, but we did not

362 find this result here. Rather, there was a three way interaction between resource value, the

363 extrinsic RHP of focal individuals and the extrinsic RHP of their opponents. Furthermore, this

364 interaction was driven primarily by the distribution of draws across the treatments, rather

365 than by the distribution of victories and losses. Under conditions of high RV (flowing water),

366 clear outcomes (fights in which there was a clear winner) were more likely when both

367 individuals were of high extrinsic RHP (normoxic pre-treatment). In contrast, if resource

368 value was low (still seawater) draws were only recorded in pairs in which the focal had low

369 extrinsic RHP (hypoxic pre-treatment) and the opponent had high extrinsic RHP (normoxic

370 pre-treatment). In general, our ability to interpret contests that end in draws is limited by

371 the fact that predictions from contest theory are based on the assumption of clear

372 outcomes. Nevertheless, Jennings et al. (2005) make the point that draws may be common

373   in nature, and might be underrepresented in datasets obtained from fights staged under

374   controlled conditions, often within a constrained space. The prevalence of draws in the

375   current data, where fights were observed under conditions that simulated natural abiotic

376   variation, support this view. Furthermore, in a previous study on *A. equina* (Lane and Briffa,

377   2017b) in which the same individuals fought twice, draws were more prevalent in the

378   second fight than in the first. Again, repeated fights, within a short time frame, are likely in

379   nature whereas in lab studies individuals often only engage in a single fight. In their study of

380   fighting fallow deer, *Dama dama*, Jennings et al. (2005) concluded that drawn encounters

381   were more likely when opponents were evenly matched in terms of RHP, which they

382   inferred from the use of specific agonistic tactics. Here, we found a different pattern, where

383   a clear outcome was more likely when both opponents had high RHP (i.e. both were pre-

384   treated with normoxic seawater) and when fights took place under conditions of high RV

385   (flowing seawater).

386   It appears then, that although extrinsic RHP contributes to the dynamics of fighting,

387   it cannot fully explain fight outcomes. On the other hand, contest outcomes were also not

388   explained by our intrinsic measures of RHP (body size and nematocyst length), which were

389   shown to differ between winners and losers in previous studies (e.g. Rudin and Briffa, 2011;

390   2012). In those studies, extrinsic components of RHP and RV were not manipulated, so it is

391   possible that in the current study the effects of these extrinsic factors have over-ridden the

392   effects of intrinsic RHP. This still leaves the question of what might have differed between

393   winners and losers in contests where both opponents had been treated identically. One

394   possibility is that winners and losers differed physiologically such that winners were best

395   able to take advantage of the normoxic conditions. In giant freshwater prawns,

396   *Macrobrachium rosenbergii*, (Brown et al., 2003) and the ectoparasitoid wasp *Eupelmus*

397   *vuilleti* (Boisseau et al., 2017), for example, winners of fights had higher resting metabolic

398   rates compared with losers. Similarly, in the damselfish, *Pomacentrus amboinensis*, winners

399   had greater aerobic scope compared with losers (Killen et al., 2014). Although the idea that

400   fighting can be energetically demanding is widely appreciated (Briffa and Sneddon, 2007)

401   and links between metabolic rate and aggressiveness have been proposed (Reale et al.,

402   2010), relatively few studies have directly measured the effects of variation in metabolic

403   rate on fight outcomes (Earley and Hsu, 2013). Although we did not measure metabolic rate

404   in the current study, our data suggest that the effect of variation in metabolism on fight

405   outcomes might be dependent on external conditions. In *C. maenas*, for example, low

406   oxygen leads to changes in the degree to which glycogen is mobilised during fights and the

407   extent to which glycogen concentration differs between winners and losers (Sneddon et al.,

408   1999). Thus, we suggest that extrinsic components of RHP, such as oxygen tension, might

409   determine the relative importance of intrinsic RHP traits (e.g. body size, weapons size,

410   energy reserves, metabolic rate, boldness).

411   What seems apparent is that, as in other marine species, dissolved oxygen (perhaps

412   in conjunction with intrinsic physiological traits) represents an extrinsic source of variation

413   in RHP for sea anemones. Since we manipulated dissolved oxygen independently for each

414   opponent there is the potential to use the two RHP levels (normoxic = high RHP; hypoxic =

415   low RHP) to probe the assessment rules used in the fights. In the case of losers, fights lasted

416   longer when they had been pre-treated with normoxic sea water, whereas the pre-

417   treatment of winners had no effect on contest duration. This pattern indicates that losers

418   give up when they cross a threshold of persistence but that this decision is not influenced by

419   the RHP of the opponent. In a previous study (Rudin and Briffa, 2011) we found an

420   analogous result based on intrinsic RHP measures in *A. equina* that also indicated the use of

421   self-assessment. In that case, however, the ability to identify an assessment rule was

422   dependent on the choice of intrinsic RHP trait used in the correlative analysis. When

423   nematocyst length was chosen as the measure of RHP, the data clearly indicated self-

424   assessment but when dry mass was used there was no correlation between contest

425   duration and either winner or loser RHP. An explanation for that discrepancy was that the

426   importance of each intrinsic RHP trait depended on the level of escalation reached during

427   the fight. Understanding how different RHP traits contribute across escalation levels in a

428   contest is important but at the same time these differences in the importance of RHP traits

429   can hinder our ability to probe assessment rules. Furthermore, by relying on correlative data

430   there is the risk that additional unmeasured variables that co-vary with an assumed

431   predictor (i.e. body size or weapon size) might drive or obscure the patterns of interest. The

432   current data, where extrinsic RHP appears to override the intrinsic traits that normally

433   predict victory, show how manipulation of fighting ability offers an alternative approach

434   that can potentially be used to clarify conclusions based on intrinsic RHP traits.

435   While many studies have investigated the effects of RHP and RV on animal contests,

436   relatively few have directly tested the interactions between these factors. An exception is

437   Stockermans and Hardy (2013), who investigated the effects of subjective (i.e. intrinsic) RV,

438   objective (i.e. extrinsic) RV and intrinsic RHP, revealing additive rather than interactive

439   effects between the RV and RHP components studied. A potential difficulty in identifying

440   interactions between RHP and RV is that intrinsic RHP components are difficult to

441   manipulate. While extrinsic sources of RHP have been manipulated previously (Sneddon et

442   al., 1999; Briffa and Elwood, 2000), this is the first study to our knowledge to vary extrinsic

443   RHP independently for each opponent in conjunction with manipulating extrinsic RV. In

444   systems where it is feasible, manipulation of extrinsic RHP may be a useful step in probing

445　or confirming contest assessment rules. Furthermore, differences in extrinsic RHP are likely

446　to be important for animals living in heterogeneous environments, especially if they come

447　into contact with individuals that have recently experienced different environmental

448　conditions. Here we have shown how the extrinsic RHP of both opponents can interact with

449　extrinsic RV and, in sea anemones, over-ride the effects of the intrinsic RHP traits that are

450　normally studied. Thus, it seems probable that fights in a natural setting are governed by a

451　set of interactions between intrinsic and extrinsic components of RHP and RV. In order to

452　fully understand the evolution of fighting behaviour, further experiments that investigate

453　the interactions between these factors will be needed.

454

464

465　**References**

466   **Arnott, G., and Elwood, R. W.** (2009) Assessment of fighting ability in animal contests.

467   Animal Behaviour **77**, 991-1004. doi:10.1016/j.anbehav.2009.02.010

468

469   **Bigger, C. H.** (1982) The cellular basis of the aggressive acrorhagial response of sea

470   anemones. *J. Morph*. **173**, 259-278.

471

472   **Boisseau, R. P., Woods, H. A., and Goubault, M.** (2017) The metabolic costs of fighting and

473   host exploitation in a seed-drilling parasitic wasp. *J. Exp. Biol.* **220**, 3955-3966.

474   doi:10.1242/jeb.160887

475

476   **Brace R. C., and Quicke, L. J.** (1986) Dynamics of colonization by the beadlet anemone,

477   *Actinia equina*. *J. Mar. Biol. Assoc. UK* **66**, 21-47.

478

479   **Brace, R. C., Pavey, J., and Quicke, D.L. J.** (1979) Intraspecific aggression in the colour

480   morphs of the anemone *Actinia equina*: The 'convention' governing dominance ranking.

481   *Anim. Behav.* **27**, 553-561.

482

483   **Briffa, M., and Elwood, R. W.** (2000) Cumulative or sequential assessment during hermit

484   crab fights: effects of oxygen on decision rules. *Proc. R. Soc. B* **267**, 2445-2452. doi:

485   10.1098/rspb.2000.1304

486

487   **Briffa, M., and Elwood, R. W.** (2009) Difficulties in distinguishing between mutual and self-

488   assessment in animal contests. *Anim. Behav.* **77**, 759-762.

489   doi:10.1016/j.anbehav.2008.11.010

490

491   **Briffa, M., and Sneddon, L. U.** (2007) Physiological constraints on contest behaviour. *Funct.*

492   *Ecol.* **21**, 627-637. doi: 10.1111/j.1365-2435.2006.01188.x

493

494   **Brockes, J.P., and Kumar, A.** (2008) Comparative aspects of animal regeneration. *Annu. Rev.*

495   *Cell. Dev. Biol.* **24**, 525-549. doi: 10.1146/annurev.cellbio.24.110707.175336

496

497   **Brown, J.H., Ross, B., McCauley, S., Dance, S., Taylor, A. C., and Huntingford, F. A.** (2003)

498   Resting metabolic rate and social status in juvenile giant freshwater prawns, Macrobrachium

499   rosenbergii. *Mar. Freshw. Behav. Phy.* **36**, 31-40. doi: 10.1080/1023624021000054307

500

501   **Dissanayake, A., Galloway, T. S., and Jones, M. B**. (2009) Physiological condition and

502   intraspecific agonistic behaviour in *Carcinus maenas* (Crustacea: Decapoda). *J. Exp. Mar.*

503   *Biol. Ecol*. **375**, 57-63. doi: 10.1016/j.jembe.2009.05.007

504

505   **Earley, R. L., and Hsu, Y.** (2013) Contest behaviour in fishes. In *Animal Contests* (eds ICW

506   Hardy, M Briffa), pp. 199-227. Cambridge, UK: Cambridge Univeristy Press.

507

508 **Enquist, M., and Leimar, O.** (1983) Evolution of fighting behaviour: Decision rules and

509 assessment of relative strength. *J. Theor. Biol.* **102**, 387-410.

510

511 **Hothorn, T., Bretz, F., and Westfall, P.** (2008) Simultaneous Inference in General Parametric

512 Models. *Biometrical J.* **50**, 346-363.

513

514 **Jennings, D. J., Gammell, M.P., Carlin, C.M., and Hayden, T. J.** (2005) Win, lose or draw: a

515 comparison of fight structure based on fight conclusion in the fallow deer. *Behaviour* **142**,

516 423-439.

517

518 **Killen, S. S., Mitchell, M. D., Rummer, J. L., Chivers, D. P., Ferrari, M. C. O., Meekan, M. G.,**

519 **and McCormick, M. I.** (2014) Aerobic scope predicts dominance during early life in a tropical

520 damselfish. *Funct. Ecol.* **28**, 1367-1376. doi: 10.1111/1365-2435.12296

521

522 **Lane, S. M., and Briffa, M.** (2017) The price of attack: Rethinking damage costs in animal

523 contests. *Anim. Behav.* **126**, 23-29. doi: 10.1016/j.anbehav.2017.01.015

524

525 **Lane, S. M., and Briffa, M.** (2017b) Boldness is for rookies: prefight boldness and fighting

526 success in a sea anemone. *Anim. Behav.* **132**, 13-20. doi:10.1016/j.anbehav.2017.07.012

527

528   **Leclère, L., and Röttinger, E.** (2017) Diversity of Cnidarian muscles: Function, anatomy,

529   development and regeneration. *Front. Cell. Dev. Biol.* **4**:157. doi: 10.3389/fcell.2016.00157

530

531   **Manuel, R. L.** (1988) British Anthozoa. London, UK: Academic Press

532

533   **Maynard Smith, J., and Price, G. R.** (1973) The logic of animal conflict. *Nature* **246**, 15-18.

534

535   **Mohamed, R., Monge, J-P., and Goubault, M.** (2010) Can subjective resource value affect

536   aggressiveness and contest outcome in parasitoid wasps? *Anim. Behav.* **80**, 629-636.

537   doi:10.1016/j.anbehav.2010.06.022

538

539   **Palaoro, A. V., Velasque, M., Santos, S., and Briffa, M.** (2017) How does environment

540   influence fighting? The effects of tidal flow on resource value and fighting costs in sea

541   anemones. *Biol. Lett.* **13**: 20170011.doi: 10.1098/rsbl.2017.0011

542

543   **Parker, G. A.** (1974) Assessment strategy and the evolution of fighting behaviour. *J. Theor.*

544   *Biol.* **47**, 223-243.

545

546   **Payne, R. J. H., and Pagel, M.** (1997) Why do animals repeat displays? *Anim. Behav.* **54**, 109-

547   119.

548

549   **Payne, R. J. H.** (1998) Gradually escalating fights and displays: The cumulative assessment

550   model. *Anim. Behav.* **56**, 651-662. doi:10.1006/anbe.1998.0835

551

552   **R Core Team** (2016). R: A language and environment for statistical computing. R Foundation

553   for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

554

555   **Reale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., and Montiglio, P-O.**

556   (2010) Personality and the emergence of the pace-of-life syndrome concept at the

557   population level. *Philos. T. R. Soc. B* **365**, 4051–4063. doi: 10.1098/rstb.2010.0208

558

559   **Rudin, F. S., and Briffa, M.** (2011) The logical polyp: Assessments and decisions during

560   contests in the beadlet anemone Actinia equina. *Behav. Ecol.* **22**, 1278-1285.

561   doi:10.1093/beheco/arr125

562

563   **Rudin, F. S., and Briffa, M.** (2012) Is boldness a resource-holding potential trait?

564   Fighting prowess and changes in startle response in the sea anemone *Actinia*

565   *equina*. *Proc. R. Soc. B* **279**, 1904-1910. doi:10.1098/rspb.2011.2418

566

567   **Sneddon, L. U., Taylor, A. C., and Huntingford, F. A.** (1999) Metabolic consequences of

568   agonistic behaviour: crab fights in declining oxygen tensions. *Anim. Behav.* **57**, 353–363.

569

570 **Stockermans, B. C., and Hardy, I. C. W.** (2013) Subjective and objective components of

571 resource value additively increase aggression in parasitoid contests. *Biol. Lett.* **9**, 20130391.

572 doi: 10.1098/rsbl.2013.0391

573

574 **Taylor, R. W., and Elwood, R. W.** (2003) The mismeasure of animal contests. *Anim. Behav.*

575 **65**, 1195-1202. doi:10.1006/anbe.2003.2169

576

577 **Williams, R. B.** (1978) Some recent observations on the acrorhagi of sea anemones. *J. Mar.*

578 *Biol. Assoc. UK* **58**, 787-788.

579

580

581

582

583

584

585

586

587

588

589

590

591

592
593
594
595
596
597
598
599
600
601
602

(a)

(b)
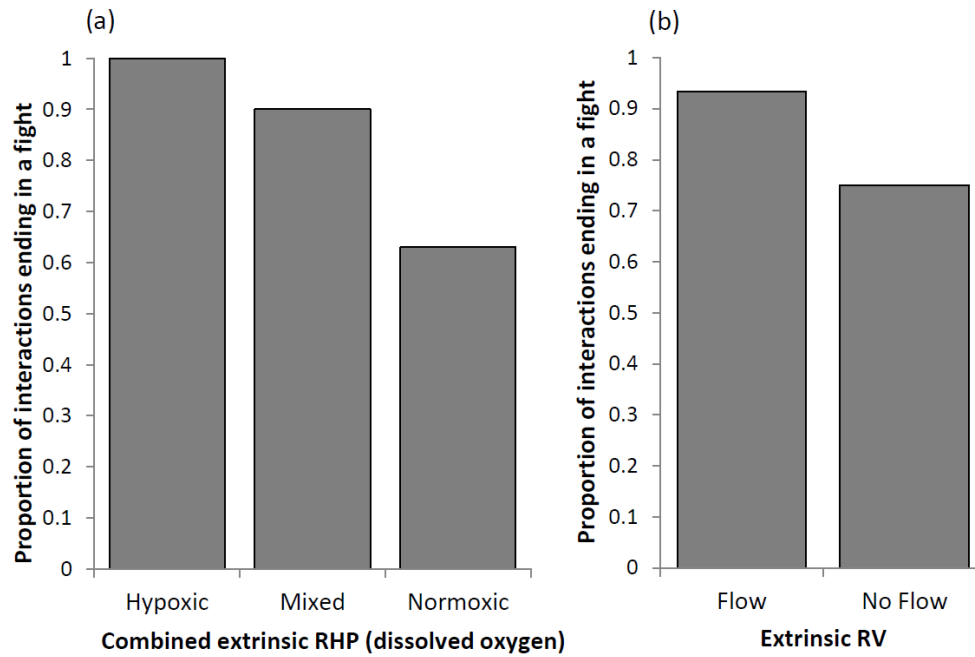
Figure 1 Effect of the (a) combined resource holding potential (RHP) of a pair of individuals (*n* = hypoxic, 16; mixed, 31; normoxic, 19) and (b) resource value (RV) (*n* = flow, 30; no flow, 36) on the likelihood of a fight occurring.
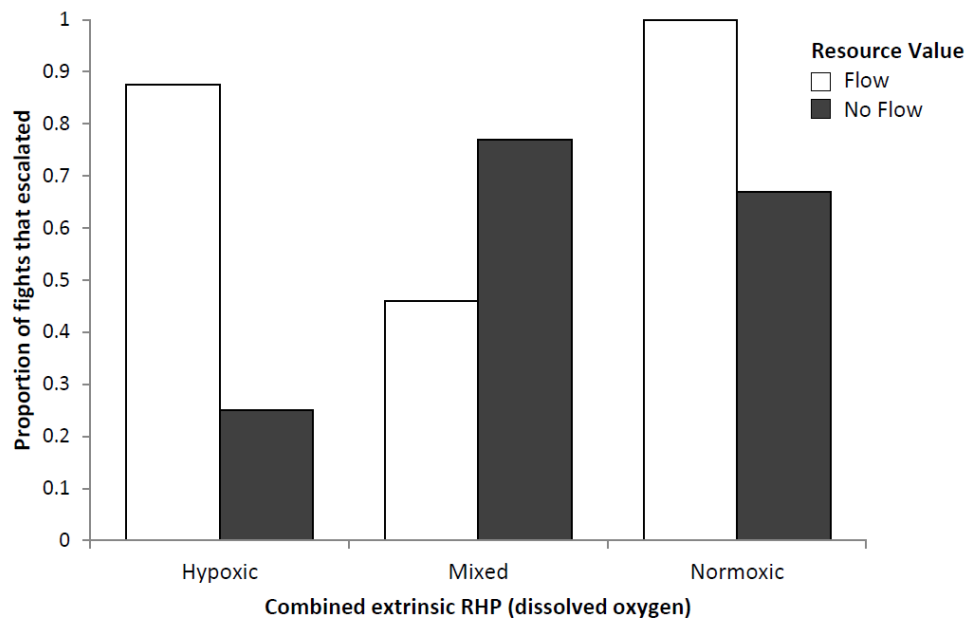
Figure 2 Interaction between combined extrinsic RHP and RV on the proportion of fights that escalated (*n* = hypoxic: - flow, 8; no flow, 8: mixed: - flow, 14; no flow, 13: normoxic: - flow, 6; no flow, 6).

623
624
625
626
627
628
629
630
631
632
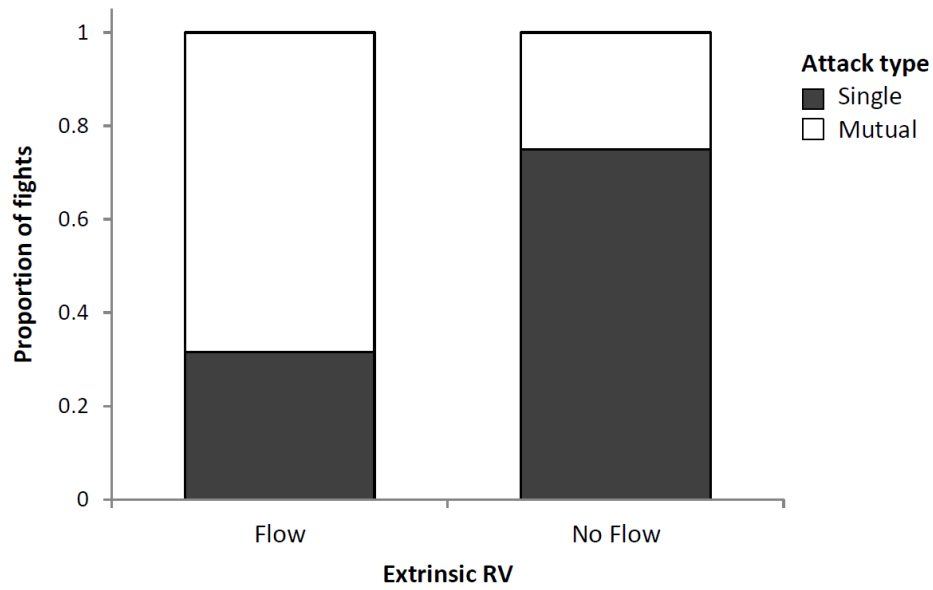
Figure 3 Effect of resource value (RV) on the type of attack exhibited during escalated fights (*n* = flow, 30; no flow, 36).
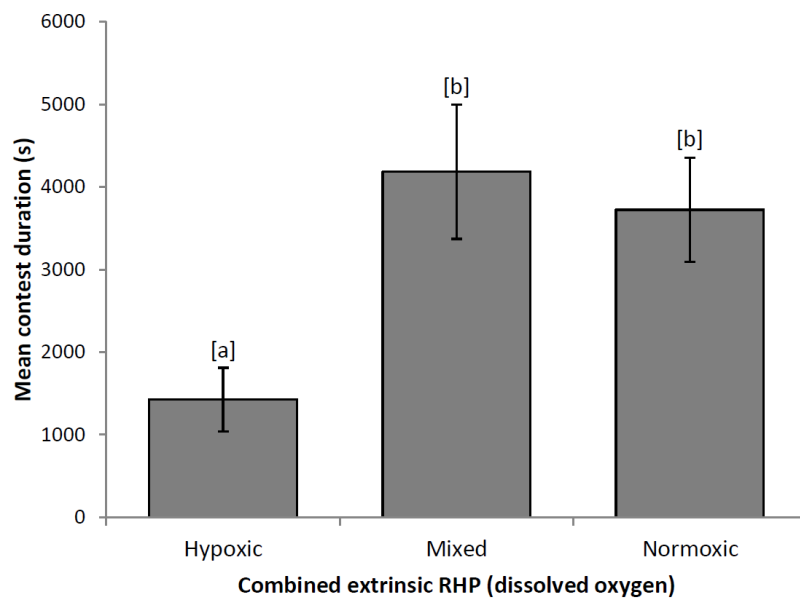

Figure 4 Effect of combined RHP on mean contest duration (seconds). Letters indicate significant differences at *P* <0.05 (*n* = hypoxic, 16; mixed, 27; normoxic, 12).
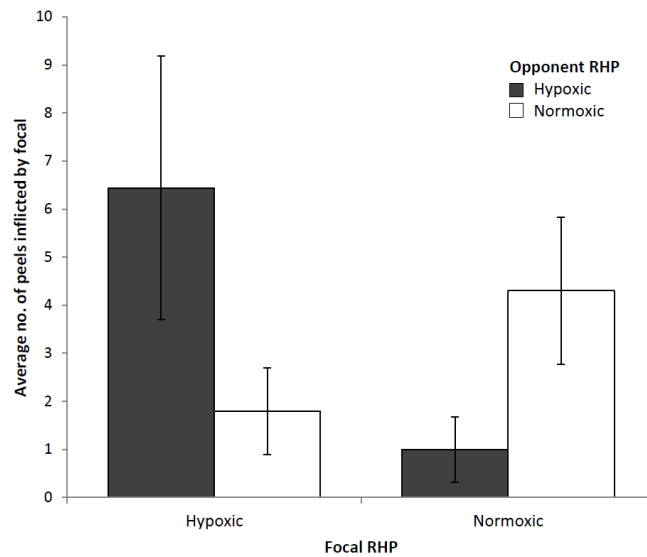
653
654
655
656
657
658
659
660
661

662 **Figure 5** Interaction between focal and opponent extrinsic RHP on the mean number of
663 peels inflicted by the focal individual (n = hypoxic-hypoxic, 7; hypoxic-normoxic, 6;
664 normoxic-hypoxic, 10; normoxic-normoxic, 7).

665
666
667
668
669
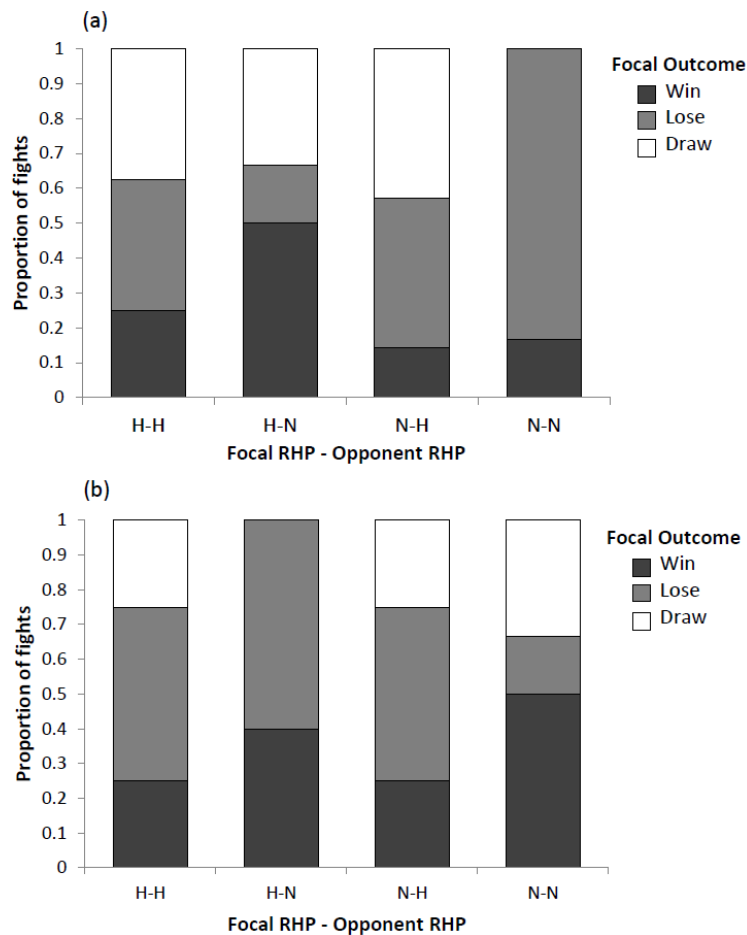670
671
672

673
674
675
676
677
678
679

680 **Figure 6** Interaction between focal RHP, opponent RHP and RV on focal outcome under (a)
681 high extrinsic RV (flow) (n = H-H, 8; H-N, 7; N-H, 7; N-N, 6) and (b) low extrinsic RV (no flow)
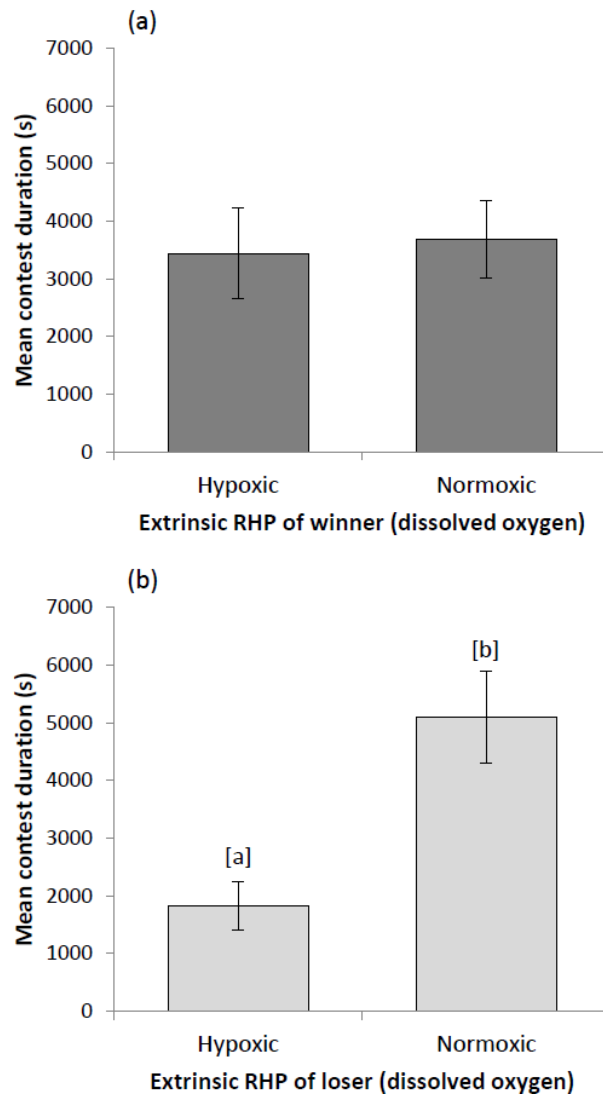682 (n = H-H, 8; H-N, 5; N-H, 8; N-N, 6).

683

Figure 7 Effect of (a) winner (*n* = hypoxic, 23; normoxic, 17) and (b) loser (*n* = hypoxic, 19; normoxic, 21) extrinsic RHP on contest duration. Letters indicate significant differences at *P* <0.05.