

2018-08-16

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

Lane, SM

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

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10.1242/jeb.187740

Journal of Experimental Biology

Company of Biologists

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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

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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

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  $15^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  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  $\text{O}_2$  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  $\text{O}_2$  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 (Brookes 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

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

#### 455 **Acknowledgements**

456 We thank Michael Collins for laboratory assistance and Ann Torr for help collecting  
457 anemones.

#### 458 **Competing interests**

459 No competing interests declared.

#### 460 **Funding**

461 This study was supported by a BBSRC grant awarded to M.B. (grant no. BB/M019772/1).

#### 462 **Data availability**

463 Data will be available from the Dryad Data Repository.

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#### 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.

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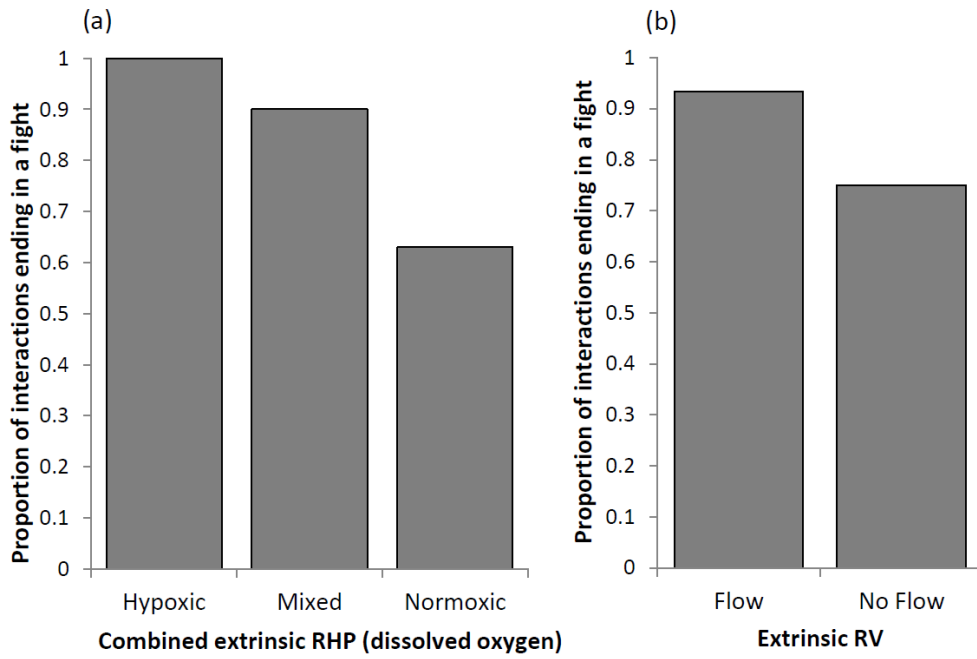
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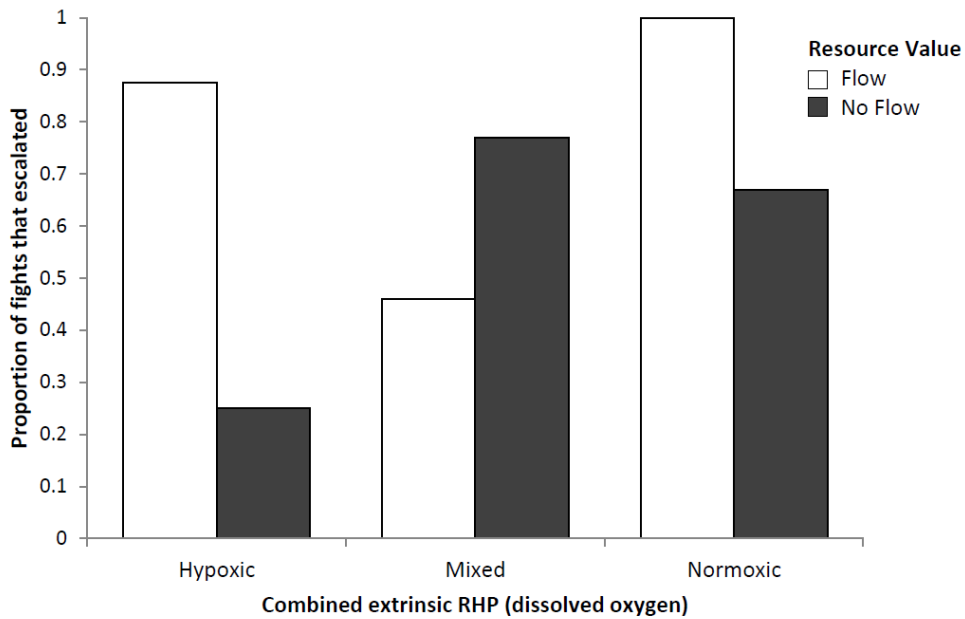
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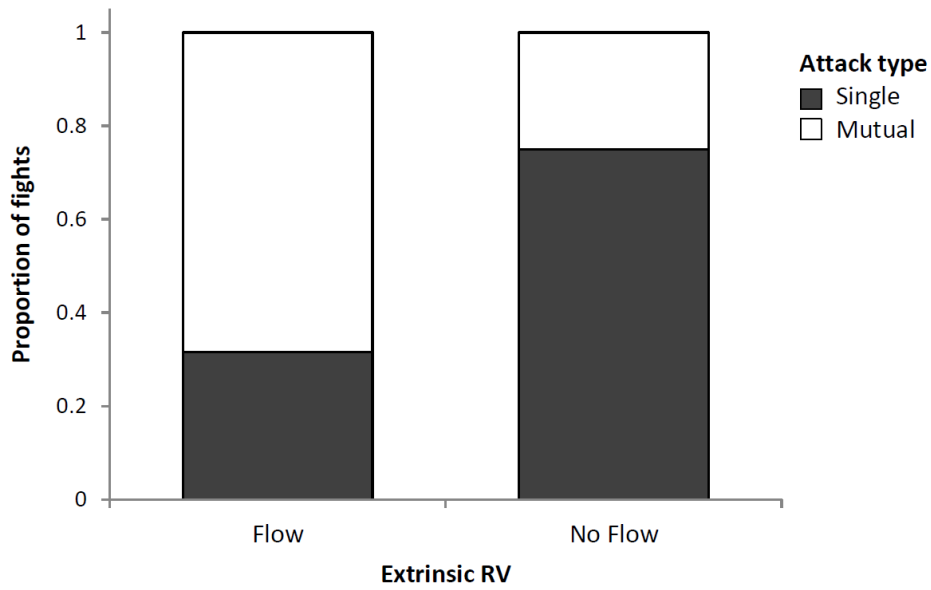


**Figure 1** Effect of the (a) combined resource holding potential (RHP) of a pair of individuals ( $n =$  hypoxic, 16; mixed, 31; normoxic, 19) and (b) resource value (RV) ( $n =$  flow, 30; no flow, 36) on the likelihood of a fight occurring.

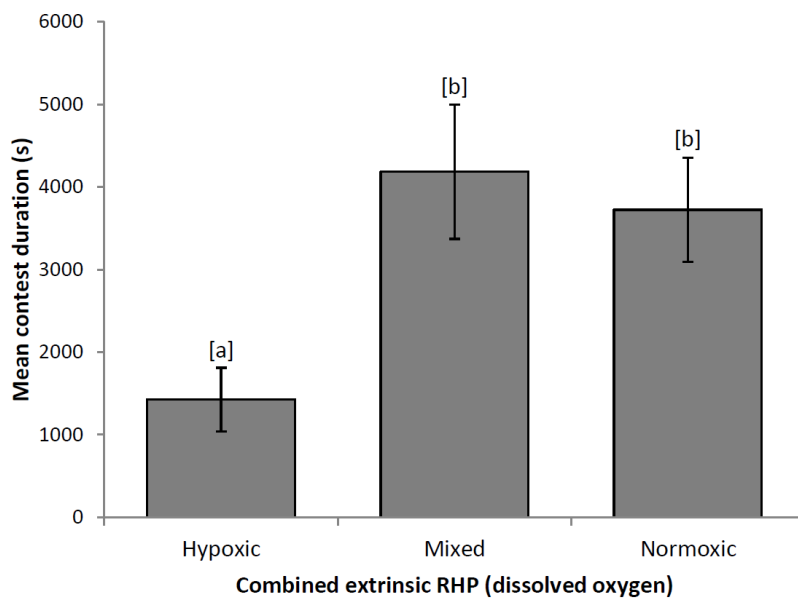


**Figure 2** Interaction between combined extrinsic RHP and RV on the proportion of fights that escalated ( $n =$  hypoxic: - flow, 8; no flow, 8; mixed: - flow, 14; no flow, 13; normoxic: - flow, 6; no flow, 6).

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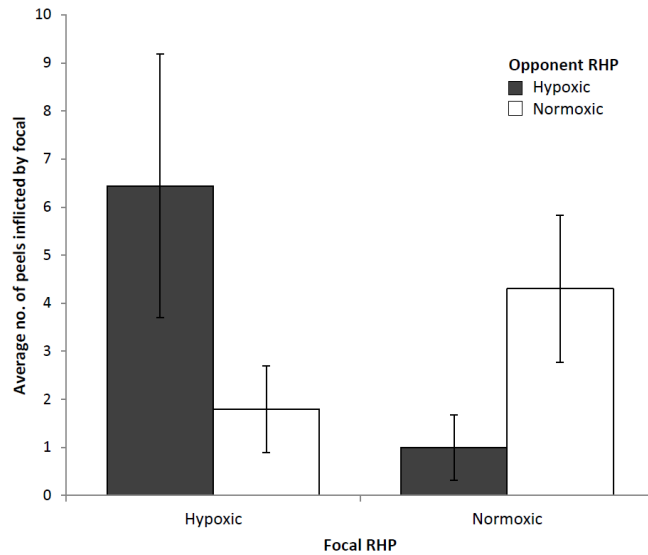


**Figure 3** Effect of resource value (RV) on the type of attack exhibited during escalated fights ( $n = \text{flow}, 30; \text{no flow}, 36$ ).



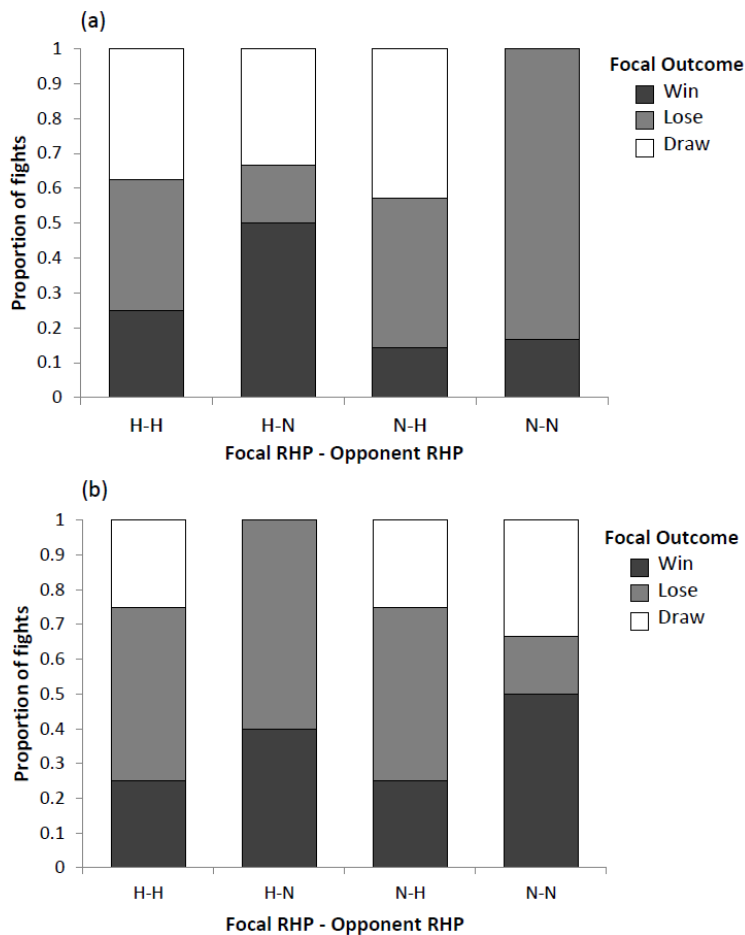
**Figure 4** Effect of combined RHP on mean contest duration (seconds). Letters indicate significant differences at  $P < 0.05$  ( $n = \text{hypoxic}, 16; \text{mixed}, 27; \text{normoxic}, 12$ ).

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662 **Figure 5** Interaction between focal and opponent extrinsic RHP on the mean number of  
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664 normoxic-hypoxic, 10; normoxic-normoxic, 7).

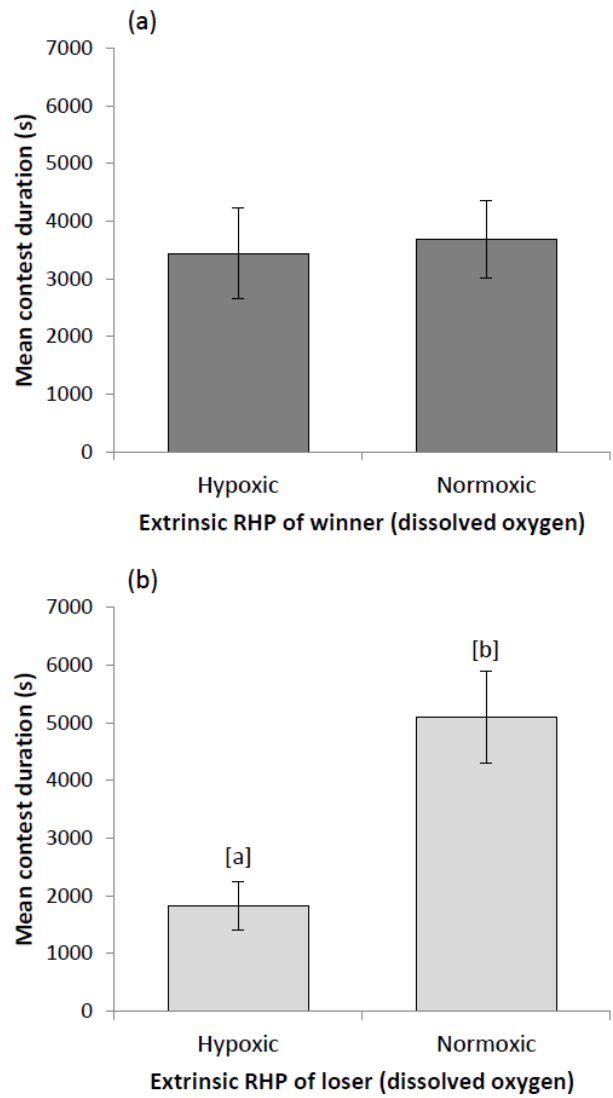
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680 **Figure 6** Interaction between focal RHP, opponent RHP and RV on focal outcome under (a)  
681 high extrinsic RV (flow) ( $n =$  H-H, 8; H-N, 7; N-H, 7; N-N, 6) and (b) low extrinsic RV (no flow)  
682 ( $n =$  H-H, 8; H-N, 5; N-H, 8; N-N, 6).

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**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  $P < 0.05$ .

