

## RESEARCH ARTICLE

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

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## ABSTRACT

An individual's performance during a fight is influenced by a combination of their capacity and willingness to compete. While willingness to fight is known to be determined by both intrinsic and extrinsic drivers, an individual's capacity to fight is generally thought of as solely intrinsic, being driven by a host of physiological factors. However, evidence indicates that variation in fighting ability can also be generated through exposure to different environmental conditions. Environmental contributions to fighting ability may be particularly important for animals living in spatially and temporally heterogeneous habitats, in which fights can occur between rivals recently exposed to different environmental conditions. The rapidly changing environment experienced within intertidal zones, for example, means that seawater parameters, including dissolved oxygen content and temperature, can vary across small spatial and temporal scales. Here, we investigated the relative importance of these extrinsic contributions to fighting ability and resource value on contest dynamics in the beadlet sea anemone *Actinia equina*. We manipulated the extrinsic fighting ability of both opponents (through dissolved oxygen concentration prior to fights) and resource value (through seawater flow rate during the fight). Our results indicate that the extrinsic fighting ability of both opponents can interact with resource value to drive escalation patterns and that extrinsic drivers can be more important in determining contest dynamics than the intrinsic traits commonly studied. Our study highlights the need to combine data on intrinsic state and extrinsic conditions in order to gain a more holistic view of the factors driving contest behaviour.

**KEY WORDS:** Animal contests, Assessment, Fighting behaviour, Hypoxia, Resource holding potential, Resource value

## INTRODUCTION

Traditional and recent contest theory predicts that injurious fighting is more likely to occur when the potential benefits to be gained exceed the potential costs (Maynard Smith and Price, 1973; Parker, 1974; Lane and Briffa, 2017). Operationally, this means that fighting behaviour is driven by two main variables: fighting ability or resource holding potential (RHP) and resource value (RV). The costs of entering a fight will be driven by differences in RHP between the opponents (e.g. energy expended, injuries incurred) while the potential benefits to be gained from fighting will equate to

the value of the contested resource (RV). Although RHP and RV have been the subject of many studies on contest dynamics (i.e. patterns of escalation and duration), most work has examined either RHP (Briffa and Elwood, 2000; Dissanayake et al., 2009) or RV (Mohamed et al., 2010; Stockermans and Hardy, 2013; Palaoro et al., 2017). In reality, these factors will affect contest behaviour simultaneously and thus it is important that we understand their additive and interactive effects.

Furthermore, variation in RHP and RV can be influenced by both extrinsic and intrinsic factors. Extrinsic (or objective) sources of variation in RV (Stockermans and Hardy, 2013) derive from the absolute properties of the resource unit such as the size of a territory or the number of calories in a piece of food, while intrinsic (subjective) RV reflects the subjective value different individuals place on the same resource. Fights can be affected by one or both of these RV components. For instance, the intensity of fights between female parasitoid wasps *Goniozus legneri* is driven by both extrinsic (host size) and intrinsic (female age) factors, with intrinsic RV having the greatest overall impact as the value of finding a host increases dramatically with female age (Stockermans and Hardy, 2013). Meanwhile, variation in RHP is generally considered to be determined only by intrinsic factors such as body size, weapon size, condition and metabolic rate, factors driven by genes, development and the effects of prior contest experiences, e.g. damage sustained. Yet, contest intensity can also be affected by rapidly fluctuating extrinsic factors such as environmental conditions, particularly those expected to affect an individual's capacity for performing energetically demanding aggressive behaviours [e.g. oxygen availability (Briffa and Elwood, 2000; Sneddon et al., 1999) and the presence of environmental toxins (Dissanayake et al., 2009)]. Thus, variation in RHP may also be driven by extrinsic RHP components (henceforth 'extrinsic RHP') via their influence on physiological factors that drive fighting performance.

Despite the potential for extrinsic effects on RHP, contests are usually studied in experimental set-ups in which environmental conditions are held constant. While this may allow the effects of intrinsic RHP to be investigated, by ignoring extrinsic drivers of RHP we may be overestimating the importance of these intrinsic RHP traits. Furthermore, experiments in which external conditions are manipulated could allow us to test key ideas about the evolution of fighting behaviour. For example, experiments designed to distinguish between the assessment rules used by individuals during fights (mutual versus self-assessment: Payne and Pagel, 1997; Payne, 1998) typically test for correlations between some continuous measure of intrinsic RHP (e.g. body size) and contest duration. As losers decide when a contest ends, contest duration should always increase with the RHP of the loser, but if mutual assessment is being used, there should also be a negative correlation with the RHP of the winner (Taylor and Elwood, 2003; Arnott and

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Elwood, 2009). There are, however, limits to this correlative approach (Briffa and Elwood, 2009) and manipulating extrinsic RHP offers an alternative way of probing assessment rules. Providing that the extrinsic RHP of each opponent can be manipulated independently, we could incorporate a categorical extrinsic RHP predictor into analyses that are analogous to the correlative tests currently used.

In nature, extrinsic sources of RHP variation may be particularly important for animals living in spatially and temporally heterogeneous habitats, in which fights can occur between rivals that have recently been exposed to different environmental conditions. The rapidly changing environment experienced within intertidal zones, for example, means that seawater parameters, including dissolved oxygen content and temperature, can vary across small spatial and temporal scales. Furthermore the exposed nature, particularly on the upper shore, provides motivation for conflict as individuals vie to gain suitably sheltered territory before the tide goes out. Exposure to low dissolved oxygen levels (hypoxia) has been shown to reduce the fighting ability of marine invertebrates, by reducing their capacity to meet the energetic demands of fighting. For example, hermit crabs *Pagurus bernhardus* exposed to hypoxic conditions fight with less vigour and are less likely to win fights compared with crabs exposed to normoxic seawater (Briffa and Elwood, 2000). Another intertidal marine invertebrate, the beadlet sea anemone, *Actinia equina*, fights over limited space on rocky shores, using specialised stinging structures called acrorhagi to attack rivals and convince them to relinquish their territory (Williams, 1978; Brace et al., 1979; Bigger, 1982). Although anemones are sedentary, conflicts over territory cause individuals to move across the rocks and between the microclimates created by the changing tides. Thus, anemones are likely to come into contact with individuals that have recently experienced different levels of dissolved oxygen, and consequently differ in their extrinsic RHP. Furthermore, exposure to different environmental conditions is known to drive variation in extrinsic RV in *A. equina*, with individuals exposed to flowing seawater demonstrating increased persistence during fights in comparison with individuals exposed to still water (Palaoro et al., 2017), reflecting a higher value placed on territories that experience greater flow rates. Thus, sea anemones represent an ideal system with which to simultaneously investigate the effects of extrinsic drivers of fighting ability and resource value.

With the exception of Briffa and Elwood (2000), studies on the effects of the abiotic environment on fighting have involved fights where the two contestants have been subjected to the same conditions during the fight. This paradigm limits our ability to determine whether extrinsic variables contribute to RHP as it is not possible to separate the effects of winner and loser RHP on the outcome of the contest (i.e. which individual wins). Therefore, in this study we manipulated the extrinsic RHP of each individual separately in order to test for the potential of additive and interactive effects of both individuals' extrinsic RHPs. Furthermore, we tested the idea, for the first time to our knowledge, that extrinsic variation in RHP (manipulated through dissolved oxygen concentration prior to fights) and RV (manipulated through seawater flow rate during the fight), and the interaction between them, should influence the intensity and outcome of contests. If dissolved oxygen represents an extrinsic source of RHP difference, anemones exposed to higher levels of dissolved oxygen should be more likely to escalate fights and persist for longer than those exposed to low dissolved oxygen, and ultimately should win more fights. Similarly, as flow rate represents an extrinsic RV variable (Palaoro et al., 2017), anemones

exposed to flowing water should escalate fights more often and persist for longer than those exposed to still water and should defeat opponents of similar RHP. If these two factors have an interactive effect, the most intense fights are predicted to occur when both opponents are exposed to high oxygen and high flow, and the least intense fights should occur when both are exposed to low oxygen and still water. Thus, the chance of victory should be greatest for focal individuals exposed to higher dissolved oxygen fighting against opponents exposed to low dissolved oxygen under high flow conditions. We also incorporated intrinsic RHP traits into our analysis in order to determine how their influence on contest behaviour may be modified by the external environment, and to determine the relative importance of intrinsic and extrinsic RHP. Finally, as described above, we used the data from this experiment to demonstrate how manipulation of extrinsic RHP can be used as an alternative means of probing assessment rules during animal contests that avoids the need for correlative analyses based on intrinsic RHP variation.

## MATERIALS AND METHODS

### Animal collection and husbandry

*Actinia equina* (Linnaeus 1758) ( $N=132$ ) of the red/brown colour morph were collected intertidally from Portwrinkle (Cornwall, UK; grid reference: SX 357539) between September and December 2017 and taken back to the lab within 1–2 h of collection. All anemones collected were visually inspected for injury and only anemones without injury were brought back to the lab. Once in the lab, anemones were housed individually in plastic tanks ( $23\times16\times17.5$  cm) containing 700 ml of filtered seawater [pumped from Mount Batten, Plymouth, UK; grid reference: SX 48715319; average seawater quality: pH 8–8.2; salinity 34 psu (HI-96822 seawater refractometer, Hanna Instruments, Woonsocket, RI, USA); ammonia 0 ppm; nitrite 1 ppm; nitrate 10 ppm (API saltwater master test kit, API Fishcare)] along with an air stone for constant aeration. Anemones were maintained at  $15\pm0.5^{\circ}\text{C}$  and fed *ad libitum* on aquaria marine flakes every 2–3 days. Tank seawater was topped up daily and replaced fully every 7 days with fresh filtered seawater.

### Manipulating RHP and RV

All anemones were given a 7–14 day acclimatisation period before they were dislodged from their position in the tank and provided with stones to attach to. Anemones were then randomly allocated to one of two treatments: hypoxic (H) or normoxic (N) seawater. The following day, anemones allocated to the hypoxic treatment were exposed to hypoxic conditions for 30 min prior to being introduced to an opponent. Hypoxic conditions were produced by bubbling nitrogen (rather than the usual air) into the anemone's tank until  $\text{O}_2$  levels reached 30%. The oxygen levels in the tank were then kept at 30% for 30 min by covering the tank with a piece of Perspex and monitoring  $\text{O}_2$  levels with an oxygen probe (YSI Pro2030, YSI Inc., Yellow Springs, OH, USA). Normoxic individuals were maintained under normal seawater conditions before the fight. In order to create a fully orthogonal design, anemones were allocated into size-matched pairs (estimated visually) according to treatment and assigned at random as either the focal or opponent individual (focal–opponent: H–N, N–H, H–H and N–N). As anemones rely on water flow in the wild to find food, we also manipulated RV by altering flow conditions within the fighting tanks. All fights were performed in freshly aerated seawater to control for effects of oxygen content during the fight itself, then, in order to create a high RV environment, half of the tanks were supplied with a small water

pump (EHEIM compactON 300, EHEIM GmbH & Co., Deizisau, Germany) (flow, F) while the other half were not (no flow, NF). The pump was fully submerged to eliminate the possibility that anemones under flow conditions were receiving more oxygen, and the levels of dissolved oxygen were monitored in each tank type prior to the experiment to confirm this assumption. Pairs were then randomly allocated to one of these two RV conditions in a fully orthogonal manner, resulting in a total of 8 treatment groups [H–N (F); H–N (NF); N–H (F); N–H (NF); H–H (F); H–H (NF); N–N (F); N–N (NF)].

### Staging contests

Fights in *A. equina* take two forms: (1) non-injurious contact of the feeding tentacles or (2) one or both anemones inflict injurious attacks using acrorhagi, leaving behind acrorhagial stinging ‘peels’ on the opponent. These ‘peels’ cause localised necrosis on the recipient but are not fatal in *A. equina*. In order to stimulate agonistic behaviour, anemones were positioned such that their body columns were touching. Fights were recorded from this initial contact until one anemone (the loser) either: (i) moved an approximate distance of one pedal disc away from its opponent (estimated visually) or (ii) retracted its tentacles completely for at least 10 min. If both opponents performed these retreating behaviours, the outcome of the fight was classified as a draw. Similarly, if neither individual retreated after 3 h, the fight outcome was classed as a draw. At the end of the contest, individuals were checked for the presence of acrorhagial peels, separated and returned to their tanks. If one or both anemones failed to open their tentacles within the 3 h observation period, the interaction was categorised as a ‘no fight’ and the anemones were removed from the study. All fights were recorded using a Canon LEGRIA HF R706 High Definition Camcorder and scored blind manually for contest behaviour and duration. A total of 66 interactions were observed with an average of 8 interactions per treatment combination; see Table S1 for a full breakdown of sample sizes.

### Measuring intrinsic RHP traits

After the fights, the minimum and maximum pedal disc diameters of each anemone were measured using callipers to the nearest 0.1 mm. As pedal disc shape is elliptical, body size was then calculated for each anemone as the average of the minimum and maximum diameter (Brace and Quicke, 1986). Tissue samples from acrorhagi that had not been used in the contest were taken from each anemone using forceps, spread onto a glass slide and stained using 1% Methylene Blue solution (Manuel, 1988). Anemones are capable of rapidly regenerating body parts (Brockes and Kumar, 2008; Leclère and Röttinger, 2017) and thus this removal of acrorhagi only damages the animals temporarily. Nematocysts were imaged using a Leica M205 FA stereo microscope equipped with a camera (Leica DFC7000T, Leica Microsystems Ltd, Heerbrugg, Switzerland) connected to a computer. Nematocyst length was then measured blind, using point-to-point measurements in ImageJ (version 1.50i). Nematocyst length for each individual anemone was then calculated as the average length of 10 randomly selected nematocysts.

At the end of the experiment, all anemones were returned to the shore they were collected from.

### Statistical analyses

We approached the analysis in two ways. First, we tested for the effects of extrinsic RHP and RV on overall contest dynamics, in order to determine how these factors would influence (i) the

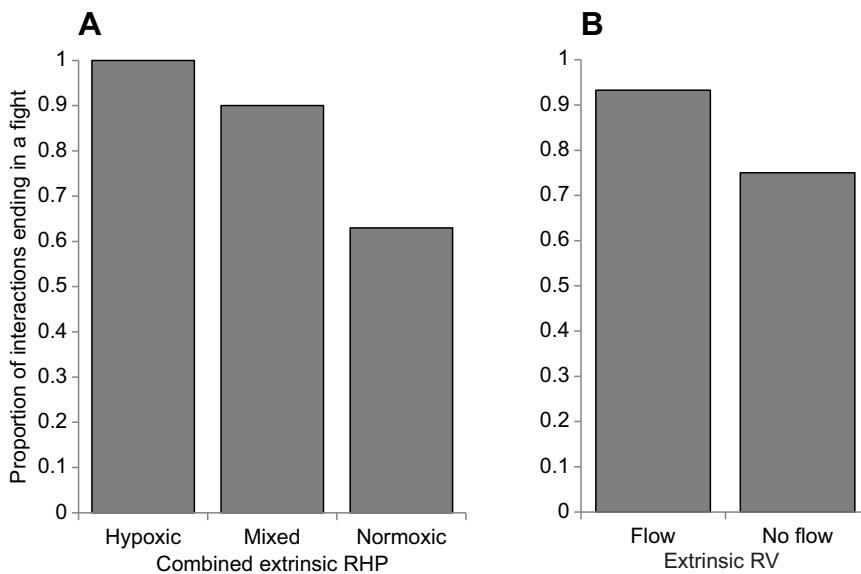
occurrence of fights, (ii) the occurrence of escalated fights involving injuries, (iii) the type of fight (for fights that escalated) in terms of whether one or both individuals deployed their acrorhagi (attack type) and (iv) the duration of the contest. Second, we analysed the effects of extrinsic RHP and RV from the perspective of focal individuals to determine the effects of these factors on (v) the likelihood of focal individuals deploying their acrorhagi and, for those focal individuals that did attack the opponent, (vi) the number of peels that they inflicted and (vii) the chance of victory for focal individuals. In analyses i–iv, focal and opponent extrinsic RHP conditions were combined to give an overall extrinsic RHP factor (henceforth ‘combined RHP’), with three levels: both hypoxic (H–H), both normoxic (N–N) and mixed (H–N and N–H). We then analysed the effects of combined RHP and extrinsic RV (henceforth ‘RV’; flow or no flow) and their interaction on the binary measures of fight occurrence, escalation and attack type (escalated fights only) using generalised linear models (GLMs) with a binomial error distribution. To determine the effect of the same two predictors on contest duration (which was log transformed), we used a linear model. In analyses v–vii, we used two factors to account for the distinct extrinsic RHP conditions of focal and opponent individuals: ‘focal RHP’ (H or N) and ‘opponent RHP’ (H or N). We then used binomial GLMs to analyse the effect of these two RHP factors, and the RV factor (flow or no flow), and their interactions, on the probability that the focal anemone attacked the opponent (i.e. deployed its acrorhagi) and on the probability of victory for focal anemones. We used a GLM with a quasipoisson error distribution (accounting for overdispersion in the data) to analyse the effect of these three predictors on the number of peels the focal individual inflicted. Two measures of intrinsic RHP, relative size difference (RSD) and relative nematocyst length difference (RND) (both calculated as described in Rudin and Briffa, 2011) were included in the analyses as covariates. RSD was included as a covariate in all analyses while RND was only included as a covariate in analyses of escalated fights (RND has previously been shown to only be of importance for determining outcome in escalated fights – see Rudin and Briffa, 2011). In order to explore significant effects further, we performed *post hoc* linear contrasts using the `glht` function of the R package `multcomp` (Hothorn et al., 2008). Finally, to examine the assessment rules used by anemones, we performed two *t*-tests with contest duration as the response variable and winner or loser RHP as the explanatory variable, respectively. All analyses were carried out in R Studio v.1.0.136 (<https://www.R-project.org/>).

## RESULTS

### Contest dynamics

The likelihood of a fight occurring was significantly affected by combined extrinsic RHP (hypoxic, normoxic or mixed;  $\chi^2=10.55$ ,  $P=0.005$ ), with fights being less likely to occur when both individuals had been exposed to normoxic seawater (Fig. 1A). Fights also occurred more often under the high RV (flowing seawater) treatment ( $\chi^2=4.21$ ,  $P=0.04$ ) (Fig. 1B), but there was no interaction between combined extrinsic RHP and RV ( $\chi^2=1.93$ ,  $P=0.38$ ).

When fights did occur, the probability of escalation was significantly influenced by an interaction between RHP and RV ( $\chi^2=11.97$ ,  $P=0.0025$ ). When the two anemones were evenly matched in extrinsic RHP (i.e. when the combined extrinsic RHP was either hypoxic or normoxic), escalation was more likely under high RV (flow) than under low RV (no flow), but when anemones were mismatched (combined extrinsic RHP was mixed), the opposite pattern was seen, with fights being more likely under low RV conditions (Fig. 2). For fights that did escalate, there was a



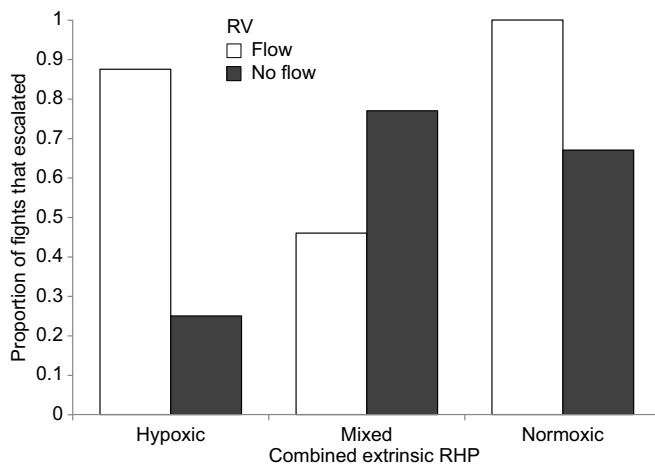
**Fig. 1. Effect of extrinsic resource holding potential and resource value on the occurrence of fights.** Effect of (A) the combined resource holding potential (RHP; dissolved oxygen level) of a pair of individuals ( $n=16$  hypoxic,  $n=31$  mixed,  $n=19$  normoxic) and (B) the resource value (RV; flow versus no flow) ( $n=30$  flow,  $n=36$  no flow) on the likelihood of a fight occurring.

further effect of RV on whether single or mutual attacks occurred ( $\chi^2=6.80$ ,  $P=0.009$ ). Mutual attacks occurred more often under high RV (flow) while single attacks occurred more often under low RV (no flow), suggesting that opponents were more likely to strike back when the contested resource was of high value (Fig. 3). Attack type (single or mutual attack), was not affected by combined extrinsic RHP ( $\chi^2=4.38$ ,  $P=0.11$ ) and there was no interaction between combined extrinsic RHP and RV ( $\chi^2=4.56$ ,  $P=0.10$ ). Contest duration was significantly affected by the combined RHP of pairs ( $\chi^2=8.43$ ,  $P=0.004$ ). *Post hoc* analyses revealed that fights between pairs of hypoxic individuals (H–H) were significantly shorter than fights between pairs of normoxic individuals (N–N;  $P=0.02$ ) and mixed pairs (H–N or N–H;  $P=0.02$ ) (Fig. 4), but duration was not affected by RV ( $\chi^2=0.04$ ,  $P=0.83$ ) and there was no interaction between combined RHP and RV ( $\chi^2=0.88$ ,  $P=0.58$ ). There was no effect of relative size difference or relative nematocyst length on any of the factors analysed (Table S2).

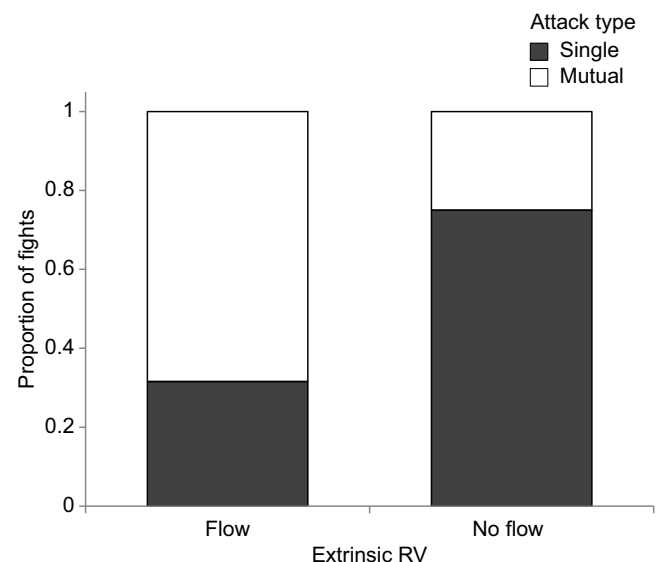
### Focal agonistic behaviour

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

(Table S3). Furthermore, RV and its interactions with focal and opponent RHP had no effect on the number of peels inflicted by focal individuals in escalated fights (Table S4). However, an interaction between focal RHP and opponent RHP ( $\chi^2=42.11$ ,  $P=0.01$ ) indicates that focal individuals inflicted more peels on opponents when both had received the same RHP treatment (H–H or N–N), compared with pairs that had received different treatments (H–N or N–H) (Fig. 5). Finally, contest outcome for focal individuals was significantly affected by a three-way interaction between focal RHP, opponent RHP and RV ( $\chi^2=4.25$ ,  $P=0.039$ ) (Fig. 6). However, this effect was lost when individuals that drew were removed from the dataset ( $\chi^2=1.31$ ,  $P=0.25$ ), indicating that this interaction was driven by differences in the distribution of fights that ended in draws across treatment combinations. Under flowing seawater conditions, every fight involved a clear outcome when both opponents were pre-treated with normoxic seawater. In contrast, under still seawater conditions, every combination of focal and opponent pre-treatment yielded a proportion of contests that resulted

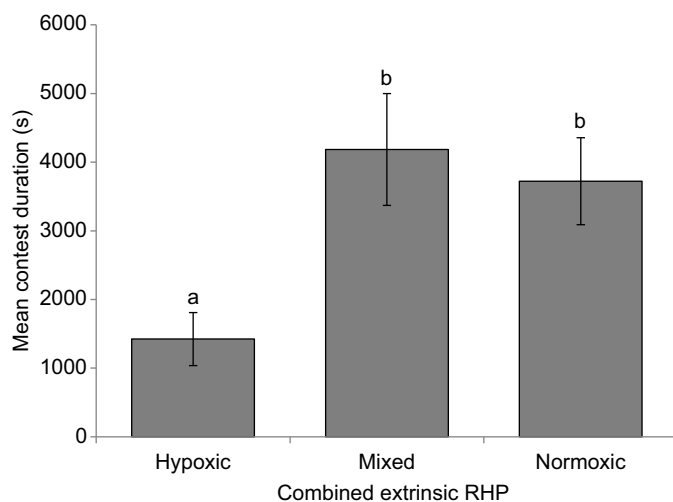


**Fig. 2. Interaction between combined extrinsic RHP and RV on the proportion of fights that escalated.** Hypoxic:  $n=8$  flow,  $n=8$  no flow; mixed:  $n=14$  flow,  $n=13$  no flow; normoxic:  $n=6$  flow,  $n=6$  no flow.



**Fig. 3. Effect of RV on the type of attack exhibited during escalated fights.**  $n=30$  flow,  $n=36$  no flow.





**Fig. 4. Effect of combined RHP on mean contest duration.** Different letters indicate significant differences at  $P < 0.05$  ( $n = 16$  hypoxic,  $n = 27$  mixed,  $n = 12$  normoxic).

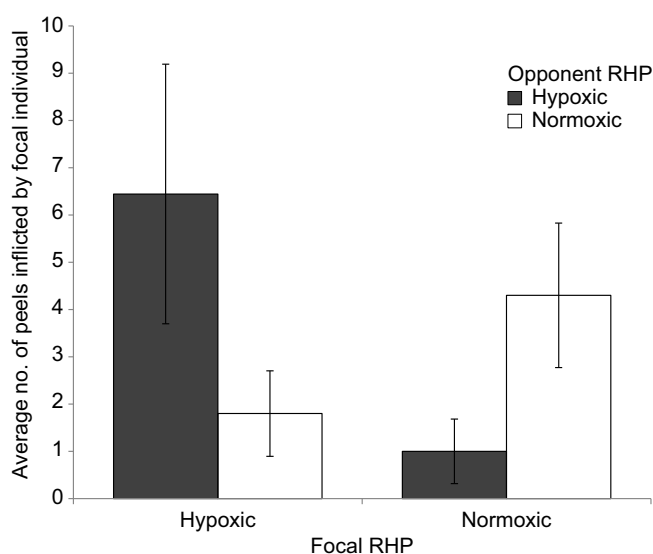
in a draw. There was no effect of relative size difference or relative nematocyst length on any of the factors analysed (Table S1).

#### Assessment rules

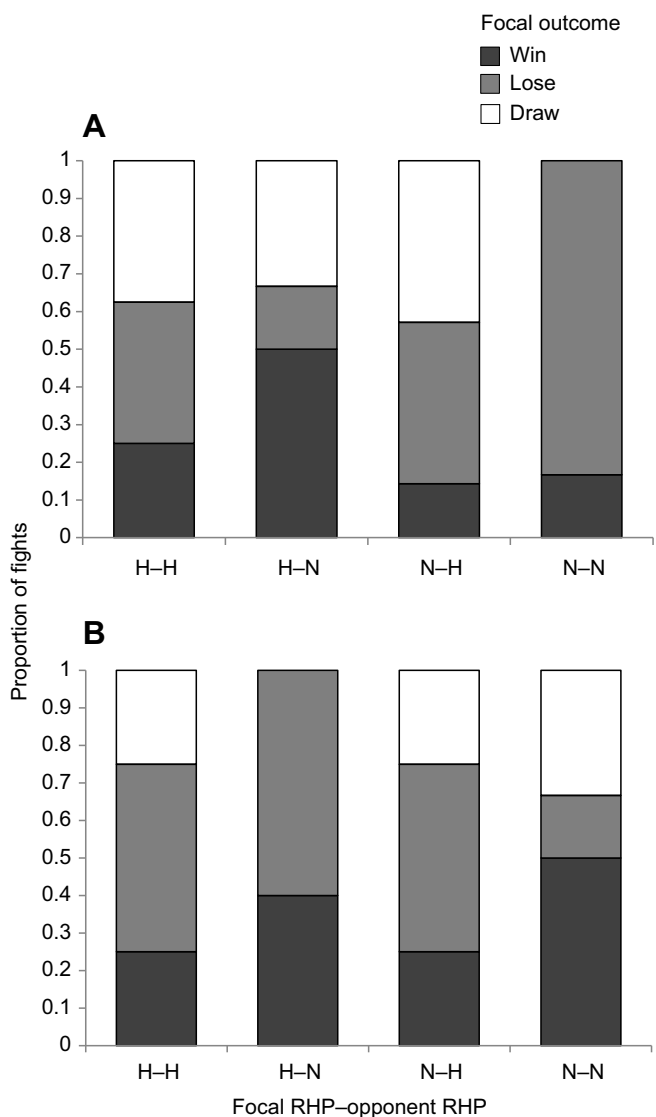
Contest duration was significantly affected by the extrinsic RHP of losers ( $t = 3.67$ ,  $P < 0.001$ ) such that fights were resolved more quickly when losers had been subject to the hypoxic treatment. In contrast, the treatment of winners had no effect on contest duration ( $t = 0.24$ ,  $P = 0.811$ ) (Fig. 7).

#### DISCUSSION

In this study, we have demonstrated that contest dynamics and decisions can be significantly impacted by extrinsic sources of variation in both RHP and RV. Furthermore, our results indicate that some aspects of fighting behaviour are affected by interactions between an individual's extrinsic RHP, the extrinsic RHP of its



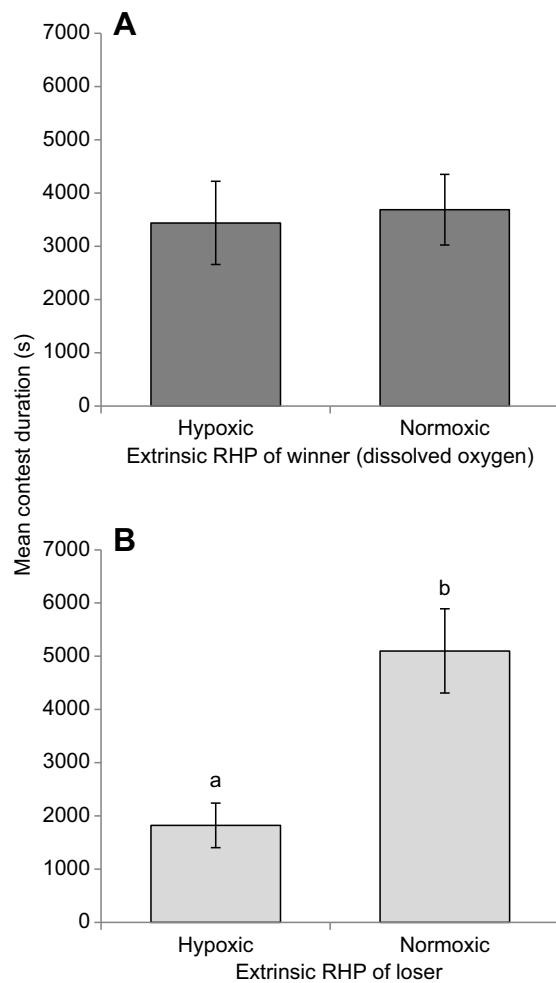
**Fig. 5. Interaction between focal and opponent extrinsic RHP on the mean number of peels inflicted by the focal individual.**  $n = 7$  hypoxic–hypoxic focal–opponent RHP;  $n = 6$  hypoxic–normoxic;  $n = 10$  normoxic–hypoxic;  $n = 7$  normoxic–normoxic.



**Fig. 6. Interaction between focal RHP, opponent RHP and RV on focal outcome.** Data were obtained under (A) high extrinsic RV (flow) [ $n = 8$  hypoxic–hypoxic (H–H) focal–opponent RHP;  $n = 7$  hypoxic–normoxic (H–N);  $n = 7$  normoxic–hypoxic (N–H);  $n = 6$  normoxic–normoxic (N–N)] and (B) low extrinsic RV (no flow) ( $n = 8$  H–H;  $n = 5$  H–N;  $n = 8$  N–H;  $n = 6$  N–N).

opponent and the value of the contested resource, while others are subject only to additive effects of RHP and RV.

Fights between hypoxic individuals, where the extrinsic component of RHP had been experimentally reduced, were significantly shorter than fights in which both individuals had been pre-treated with normoxic seawater and fights in which each individual had received a different pre-treatment (i.e. normoxic and hypoxic seawater). Similarly, fights in shore crabs, *Carcinus maenas*, have been shown to be shorter under hypoxic conditions (Sneddon et al., 1999) and hermit crabs, *P. bernhardus*, pre-exposed to hypoxic seawater fight less intensely than those exposed to normoxic seawater (Briffa and Elwood, 2000). As in the cases of these decapod crustaceans, it also appears that exposure to hypoxia caused a reduction in RHP in *A. equina*, as exposed individuals persisted for less time. However, despite this reduced fighting ability, the amount of damage (number of peels) inflicted by focal individuals was significantly higher in hypoxic pairs than in mixed pairs, indicating that hypoxic individuals fought more aggressively



**Fig. 7. Effect of winner and loser extrinsic RHP on contest duration.** (A) Winner RHP:  $n=23$  hypoxic,  $n=17$  normoxic. (B) Loser RHP:  $n=19$  hypoxic,  $n=21$  normoxic. Different letters indicate significant differences at  $P<0.05$ .

but only when matched with their opponents in terms of extrinsic RHP. Similarly, encounters involving at least one hypoxic individual were significantly more likely to result in a fight than those between two normoxic individuals. These findings suggest that, contrary to expectations, individuals with reduced fighting ability had an increased motivation to fight. Similar results were found in a study of shore crabs *C. maenas* in which starved and pyrene-exposed crabs fought with greater vigour and spent more time in possession of the contested resource than control individuals (Dissanayake et al., 2009). In contrast, previous studies investigating the effect of hypoxia on fighting behaviour have found that the reduction in RHP elicited by low oxygen levels leads to a decrease in competitive ability (Sneddon et al., 1999; Briffa and Elwood, 2000). Dissanayake et al. (2009) suggested that the discrepancy between these findings could be explained by the presence of a high value resource in their study that increased the motivation to fight, a possibility that also seems likely in the current study. Although 100% of interactions between hypoxic individuals resulted in a fight, the likelihood of these contests escalating to injurious fighting was dependent upon RV. Contests between hypoxic pairs were significantly more likely to escalate when RV was high (i.e. in the presence of flowing water). Thus, low extrinsic RHP appears to increase the motivation to escalate fights but only when the contested resource is of high value.

As we manipulated the extrinsic RHP independently for each opponent, we could also test for its effects on tactical (i.e. escalation) and strategic (i.e. giving up) decision making. Furthermore, we were able to ask whether these effects were modified by RV. During escalated fights, focal individuals inflicted a higher number of peels on their opponent when the two individuals had experienced the same pre-treatment prior to the contest. This result was expected as a general prediction of theory is that contests should be more intense, in terms of the agonistic tactics used, when opponents are matched in RHP (e.g. Enquist and Leimar, 1983). The outcome of a contest is expected to be driven by a similar interaction between contestant RHPs, such that (regardless of whether self- or mutual-assessment is being used) an individual's chance of winning should ultimately be driven by the difference between its own RHP and that of the opponent, but we did not find this result here. Rather, there was a three-way interaction between RV, the extrinsic RHP of focal individuals and the extrinsic RHP of their opponents. Furthermore, this interaction was driven primarily by the distribution of draws across the treatments, rather than by the distribution of victories and losses. Under conditions of high RV (flowing water), clear outcomes (fights in which there was a clear winner) were more likely when both individuals were of high extrinsic RHP (normoxic pre-treatment). In contrast, if RV was low (still seawater), draws were only recorded in pairs in which the focal individual had low extrinsic RHP (hypoxic pre-treatment) and the opponent had high extrinsic RHP (normoxic pre-treatment). In general, our ability to interpret contests that end in draws is limited by the fact that predictions from contest theory are based on the assumption of clear outcomes. Nevertheless, Jennings et al. (2005) make the point that draws may be common in nature, and might be under-represented in datasets obtained from fights staged under controlled conditions, often within a constrained space. The prevalence of draws in the current data, where fights were observed under conditions that simulated natural abiotic variation, support this view. Furthermore, in a previous study on *A. equina* (Lane and Briffa, 2017b) in which the same individuals fought twice, draws were more prevalent in the second fight than in the first. Again, repeated fights, within a short time frame, are likely in nature whereas in lab studies individuals often only engage in a single fight. In their study of fighting fallow deer, *Dama dama*, Jennings et al. (2005) concluded that drawn encounters were more likely when opponents were evenly matched in terms of RHP, which they inferred from the use of specific agonistic tactics. Here, we found a different pattern, where a clear outcome was more likely when both opponents had high RHP (i.e. both were pre-treated with normoxic seawater) and when fights took place under conditions of high RV (flowing seawater).

It appears then, that although extrinsic RHP contributes to the dynamics of fighting, it cannot fully explain fight outcomes. However, contest outcomes were also not explained by our intrinsic measures of RHP (body size and nematocyst length), which were shown to differ between winners and losers in previous studies (e.g. Rudin and Briffa, 2011, 2012). In those studies, extrinsic components of RHP and RV were not manipulated, so it is possible that in the current study the effects of these extrinsic factors have over-ridden the effects of intrinsic RHP. This still leaves the question of what might have differed between winners and losers in contests where the two opponents had been treated identically. One possibility is that winners and losers differed physiologically such that winners were best able to take advantage of the normoxic conditions. In giant freshwater prawns, *Macrobrachium rosenbergii* (Brown et al., 2003), and the ectoparasitoid wasp *Eupelmus vuilleti*

(Boisseau et al., 2017), for example, winners of fights had higher resting metabolic rates compared with those of losers. Similarly, in the damselfish, *Pomacentrus amboinensis*, winners had greater aerobic scope than that of losers (Killen et al., 2014). Although the idea that fighting can be energetically demanding is widely appreciated (Briffa and Sneddon, 2007) and links between metabolic rate and aggressiveness have been proposed (Reale et al., 2010), relatively few studies have directly measured the effects of variation in metabolic rate on fight outcomes (Earley and Hsu, 2013). Although we did not measure metabolic rate in the current study, our data suggest that the effect of variation in metabolism on fight outcomes might be dependent on external conditions. In *C. maenas*, for example, low oxygen leads to changes in the degree to which glycogen is mobilised during fights and the extent to which glycogen concentration differs between winners and losers (Sneddon et al., 1999). Thus, we suggest that extrinsic components of RHP, such as oxygen tension, might determine the relative importance of intrinsic RHP traits (e.g. body size, weapon size, energy reserves, metabolic rate, boldness).

What seems apparent is that, as in other marine species, dissolved oxygen (perhaps in conjunction with intrinsic physiological traits) represents an extrinsic source of variation in RHP for sea anemones. As we manipulated dissolved oxygen independently for each opponent, there is the potential to use the two RHP levels (normoxic=high RHP; hypoxic=low RHP) to probe the assessment rules used in the fights. In the case of losers, fights lasted longer when they had been pre-treated with normoxic seawater, whereas the pre-treatment of winners had no effect on contest duration. This pattern indicates that losers give up when they cross a threshold of persistence but that this decision is not influenced by the RHP of the opponent. In a previous study (Rudin and Briffa, 2011), we found an analogous result based on intrinsic RHP measures in *A. equina* that also indicated the use of self-assessment. In that case, however, the ability to identify an assessment rule was dependent on the choice of intrinsic RHP trait used in the correlative analysis. When nematocyst length was chosen as the measure of RHP, the data clearly indicated self-assessment, but when dry mass was used, there was no correlation between contest duration and either winner or loser RHP. An explanation for this discrepancy was that the importance of each intrinsic RHP trait depended on the level of escalation reached during the fight. Understanding how different RHP traits contribute across escalation levels in a contest is important but at the same time these differences in the importance of RHP traits can hinder our ability to probe assessment rules. Furthermore, by relying on correlative data there is the risk that additional unmeasured variables that co-vary with an assumed predictor (i.e. body size or weapon size) might drive or obscure the patterns of interest. The current data, where extrinsic RHP appears to over-ride the intrinsic traits that normally predict victory, show how manipulation of fighting ability offers an alternative approach that can potentially be used to clarify conclusions based on intrinsic RHP traits.

While many studies have investigated the effects of RHP and RV on animal contests, relatively few have directly tested the interactions between these factors. An exception is the study of Stockermans and Hardy (2013), who investigated the effects of subjective (i.e. intrinsic) RV, objective (i.e. extrinsic) RV and intrinsic RHP, revealing additive rather than interactive effects between the RV and RHP components studied. A potential difficulty in identifying interactions between RHP and RV is that intrinsic RHP components are difficult to manipulate. While extrinsic sources of RHP have been manipulated previously

(Sneddon et al., 1999; Briffa and Elwood, 2000), this is the first study to our knowledge to vary extrinsic RHP independently for each opponent in conjunction with manipulating extrinsic RV. In systems where it is feasible, manipulation of extrinsic RHP may be a useful step in probing or confirming contest assessment rules. Furthermore, differences in extrinsic RHP are likely to be important for animals living in heterogeneous environments, especially if they come into contact with individuals that have recently experienced different environmental conditions. Here, we have shown how the extrinsic RHP of both opponents can interact with extrinsic RV and, in sea anemones, over-ride the effects of the intrinsic RHP traits that are normally studied. Thus, it seems probable that fights in a natural setting are governed by a set of interactions between intrinsic and extrinsic components of RHP and RV. In order to fully understand the evolution of fighting behaviour, further experiments that investigate the interactions between these factors will be needed.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: S.M.L.; Methodology: S.M.L., M.B.; Formal analysis: S.M.L., M.B.; Investigation: S.M.L.; Writing - original draft: S.M.L.; Writing - review & editing: S.M.L., M.B.; Supervision: M.B.; Funding acquisition: M.B.

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#### Supplementary information

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