

2017-05-24

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<http://hdl.handle.net/10026.1/9765>

10.1098/rsbl.2017.0011

Biology Letters

The Royal Society

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How does environment influence fighting? The effects of tidal flow on resource value and fighting costs in sea anemones

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This is the author's accepted manuscript. The final published version of this work (the version of record) is published by Royal Society Publishing in Biology Letters 2017 available at: DOI: 10.1098/rsbl.2017.0011. This work is made available in accordance with the publisher's policies. Please refer to any applicable terms of use of the publisher.

Accepted: 1/5/17

Published: 13/5/17

Embargo release date: 13/5/18

ABSTRACT

An animal's decision to enter into a fight depends on the interaction between perceived resource value (V) and fighting costs (C). Both could be altered by predictable environmental fluctuations. For intertidal marine animals, such as the sea anemone *Actinia equina*, exposure to high flow during the tidal cycle may increase V by bringing more food. It may also increase C via energy expenditure needed to attach to the substrate. We asked whether simulated tidal cycles would alter decisions in fighting *A. equina*. We exposed some individuals to still water and others to simulated tidal cycles. To gain insights into V, we measured their startle responses before and after exposure to the treatments, before staging dyadic fights. Individuals exposed to flow present shorter startle responses, suggesting that flowing water indicates high-V compared to still water. A higher probability of winning against no-flow individuals and longer contests between flow individuals, suggests that increased V increases persistence. However, encounters between flow individuals were less likely to escalate, suggesting that C is not directly related to V. Therefore, predictable environmental cycles alter V and C, but in complex ways.

Keywords. Animal contests, contest costs, environmental cues, predictable cycles

1. INTRODUCTION

The initial Hawk-Dove model [1] and its subsequent developments (e.g. [2,3]) use two key variables to explain why animals might choose to fight: the value placed on the contested resource (V) and the cost of fighting (C). Thus, there has been substantial empirical focus on the effects of V and C on strategic decisions during contests. Most tests of contest theory involve staged encounters under stable laboratory environments. In contrast, information on how environmental conditions might influence V and C are lacking [4]. Given that fighting behaviour has evolved in fluctuating natural environments, rather than under stable laboratory conditions, this is an important omission. Therefore, studies focussing on the effects of fluctuating abiotic features of the environment could give new insights into the functions of agonistic behaviour.

There are several routes through which environmental conditions could influence animal contests by altering V and C. Weather stability may affect V by constraining or relaxing the reproductive period, thus affecting the value of territories [5]. Weather may also play a role in aerial contests: wind velocity can increase C through drag or convective cooling effects [6], and sunlight may increase territory V [7]. Furthermore, lunar cycles are known to alter mammal activity, and may thus affect C by making fighting individuals more conspicuous to predators [8]. On heterogeneous rocky shores, structural features may alter the strength of currents across the same shore. Strong currents could increase the risk of dislodgement such that animals need to allocate more energy to tenacity. This could increase the relative C if more energy is needed to maintain attachment to the rocky surface, both during routine activity and during fights. If animals have to pay more to stay attached, then less energy will be available for aggressive behaviours and, equally, any energy

expended on fighting will be lost to tenacity [9]. However, intertidal currents could also alter V . For sedentary animals, tidal currents bring food particles into their capture space. Fighting animals might therefore place greater value on territories that are associated with high flow and hence high rates of food supply. Thus, variation in tidal currents could influence both V and C , potentially in opposite directions.

The effects on V and C may alter motivational state [10] and contest dynamics [11,12]. If perceived V is higher for one opponent, this individual should have an increased chance of victory. When V is high for both opponents we should see lengthier and more aggressive contests. In contrast, high costs should have the opposite effect: reduce the chance of victory for the individual with higher C , while also reducing contest duration and escalation. V and C might thus interact. To disentangle this interaction, we need to manipulate the environmental variable of interest independently for each individual in the contest. Beadlet sea anemones, *Actinia equina*, show startle responses, which can be used to probe V , and readily fight for territories on the rocky intertidal [13]. They use specialised fighting tentacles, the acrorhagi, to damage opponents during fights (increasing C [14]), but some fights are resolved without stinging. Thus, *A. equina* is an ideal system for studying the influence of environmental fluctuations on fighting.

Here, we use an orthogonal design to investigate contests between pairs of focal and opponent anemones that have been exposed to either still or flowing seawater (with flow pattern arranged to mimic the tidal cycle). We first use startle response duration to assess the effect of flow rate on V . If high flow increases the perceived value of territory, anemones held under this condition should show shorter startle responses (prior to staged fights) than those held in still water. In subsequent fights, if high flow only increases V , individuals that experienced this should then be

more likely to defeat individuals held in still water, and contest duration and intensity should increase when both individuals have experienced flowing seawater. If high flow only increases C , individuals that experienced high flow should be less likely to defeat individuals held in still water, and contest duration and intensity should decrease when both individuals have experienced flowing seawater.

2. METHODS

(a) Collection, experimental treatment and startle responses

We collected 140 *A. equina* on the upper shore of Portwrinkle beach, Cornwall, U.K. and transported them to the lab. We housed all anemones within a large circular aquarium (diameter = 100 cm) filled with aerated seawater. Within this aquarium, we kept anemones individually in rectangular enclosures (36 cm²) made with mesh (0.325 cm²) to allow water flow during the entire experiment. We subdivided the circular aquarium (Fig. S1a) into two parts - an outer zone (N = 70) and an inner zone (N = 70) - by a solid PVC wall. This wall ensured that flow in the outer zone would not affect the water in the inner zone. We acclimated the anemones for three days before we started the experiment. Feeding schedules can be seen in Fig. S1b.

We simulated tidal cycles for the individuals in the outer zone only. We attached five reef pumps equidistantly to the side of the aquarium (flow speed: 7.8 ± 1.4 cm/s, Fig. S1a). We left the water flowing for 5 hours (incoming tide), turned them off for two hours (slack water), reversed the direction of the pumps and left the pumps on for five hours (outgoing tide; Fig. S1c). We repeated this process, simulating a full day of the tidal cycle. The individuals in the inner zone of the aquarium spent the same period in still water.

We then recorded the time the anemone took to recover from a startling stimulus as an index of perceived V [10]. These ‘startle response’ durations should, on average, reflect underlying motivational states driven by variation in V (longer startle responses reflect lower V [10]). We thus elicited the startle responses of all individuals before (control, or startle response 1) and after exposure to flowing or still seawater (startle response 2; Fig. S1b).

(b) Fighting and morphological measurements

We staged fights between pairs of anemones where one individual was designated as the ‘focal’ and the other as the ‘opponent’. We used four treatments determined by the prior experiences of flowing or still seawater: flow vs. flow (N-pair = 20), no-flow vs. no-flow (N-pair = 20), flow vs. no-flow (N-pair = 15) and no-flow vs. flow (N-pair = 15). We visually size-matched focals and opponents and we used a new rectangular aquarium (filled with seawater) for fights. After 1h of acclimation, we moved the two anemones to the centre of the aquarium until they had contact with each other’s tentacles (starting point of the fight). We considered the fight over when one anemone had moved one pedal disk diameter away (estimated visually) or retracted their tentacles for 10 min [14]. Following fights, we took samples from unused acrorhagi to measure nematocyst length (mm) [10] and then measured the dry weight of anemones (g) [14].

(c) Statistical methods

To test if flow individuals perceive V higher than no-flow individuals, we used an ANCOVA with startle response 2 as our response variable, startle response 1 was a covariate and treatment was a category (flow or no-flow). Since startle responses were not normal, we log-transformed them prior to analysis. Next, to test the effects of V and C on contest dynamics, first we used logistic regressions with (a) the outcome for

focal individuals (win or lose) and (b) whether fights escalated (stings or no stings) as our response variables, and treatment of focals and opponents as our categories. Startle responses, dry weight and nematocyst lengths are known to influence fighting ability in *A. equina* [14], and hence we used the absolute values of both focals and opponents as covariates in our model (Methods supplementary material). Since all continuous variables can scale exponentially with body size, we \log_{10} -transformed, centred and scaled them before adding to the model [15]. Second, we used a linear model to determine the effects of the predictors mentioned above on the contest duration (\log_{10} transformed). We considered all p-values below 0.05 significant. All analyses were made in R v. 3.3.1 [16].

3. RESULTS

Anemones that were exposed to flow had shorter startle responses than individuals exposed to still water ($F_{3,138} = 3.5$, $P = 0.017$, $R^2 = 0.07$, Fig. 1a). There was a non-significant trend for focals to be more likely to lose to opponents that had been exposed to flow ($\chi^2_{1,60} = 3.6$, $P = 0.058$; Fig. 1b). An interaction between focal and opponent treatment indicates that escalation was more likely when each anemone had experienced different pre-fight conditions and least likely when both had been exposed to flowing seawater before fights, with no-flow vs. no-flow in-between ($\chi^2_{1,60} = 6.27$, $P = 0.012$; Fig. 1c). Contests were longer when opponents had been exposed to flowing seawater ($F_{1,60} = 4.3$, $P = 0.042$). A near significant interaction effect indicates that the longest fights occurred when both opponents had been exposed to flowing seawater ($F_{1,60} = 3.9$, $P = 0.053$; Fig. 1d). For full details of the results including non-significant effects, see tables S1-S4.

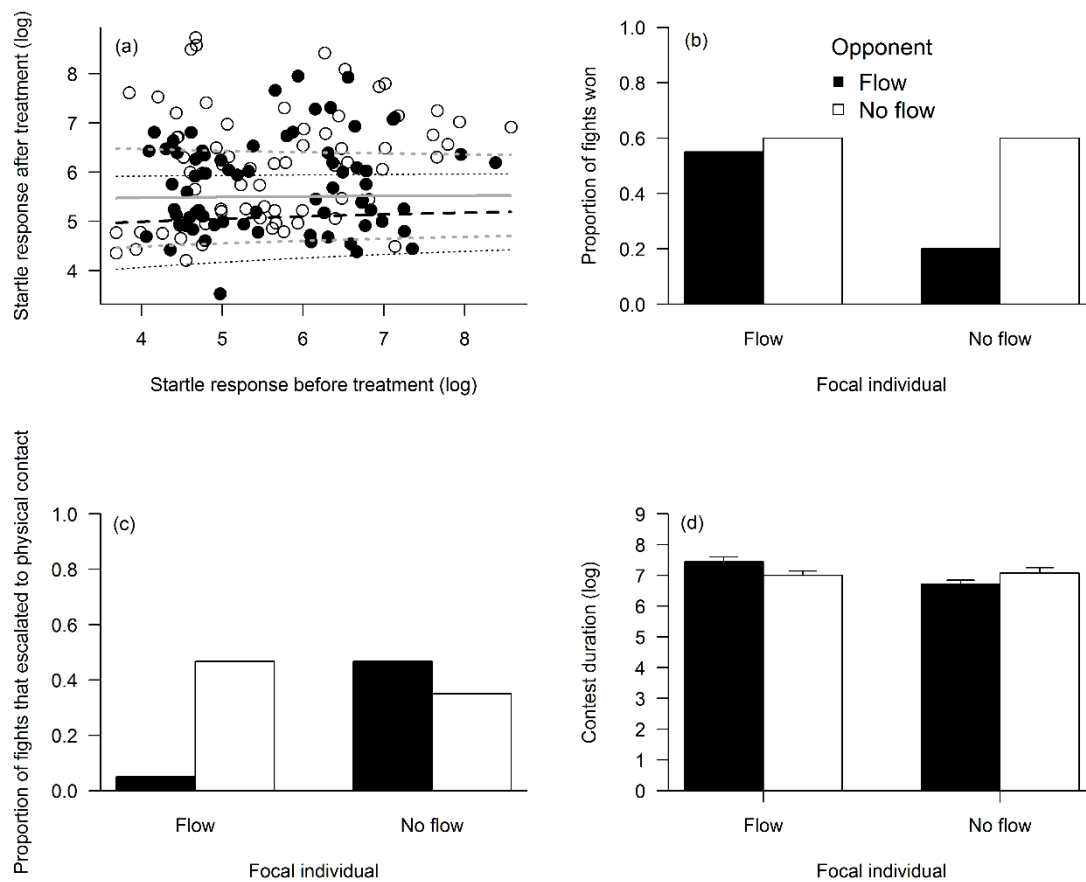


Fig 1. (a) Duration of the startle response of individuals that were exposed to a simulated tidal cycle (black dashed line) were on average lower than the duration of the startle response of individuals that were not exposed to flow (grey solid line), even when we consider the duration of startle responses before treatment was applied (x-axis). However, confidence intervals overlap (dotted lines). (b) Proportion of fights won by a randomly chosen focal against his opponent following a 2x2 factorial experimental design. (c) Proportion of fights that escalated to highly aggressive and costly behaviours (i.e. stinging) regardless of whom performed the behaviour. (d) Duration of contests between focals and opponents of *A. actina* anemones (mean + SE).

4. DISCUSSION

A small but significant difference in startle response durations between anemones held in simulated tidal flow and still water indicates that those held under a simulated tide were more motivated to return to their ongoing feeding behaviour, therefore perceiving their territory as being of greater value (V). The chance of victory decreased when no-flow individuals fought opponents exposed to flow: flow individuals won 21

out of 30 fights against no-flow individuals (70%). Therefore, flow rate appears to influence persistence in a fight and hence the chance of victory.

Nevertheless, for anemones that were in flowing seawater, contests were less intense than contests involving individuals that were exposed to still water. Fewer than 10% of fights involving stinging when both opponents had been exposed to flowing water. Individuals may be less willing to escalate when opponents show a similar high-V to avoid high levels of damage. Stinging results not only in damage to the opponent (e.g. skin necrosis, loss of feeding opportunities [14]), but in self-inflicted damage as well. The acrorhagial epithelium is 'peeled' away from the aggressor, which needs to be regenerated afterwards [17]. The focal individual with high-V might thus be more cautious, and less likely to sting their opponent, because if the opponent also has high-V it could significantly increase C. Thus, individuals with high-V may just respond to stinging, rather than initiating stinging. If true, this would decrease the chances of stinging behaviour in the flow vs. flow treatment. These results suggest that although more valuable resources might lead to greater persistence, high V does not necessarily lead to a high willingness to accept elevated chances of injury. Thus, V seems to be influencing decisions about persistence and injuries - both related to C - in different ways. If true, then longer fights between high V individuals are not unexpected. Since both opponents avoid costly behaviours, contests take longer to resolve because C accumulates only with persistence, and not with injuries.

Flowing seawater influences the perceived value of the territories but anemones only seem to accept greater persistence costs, and not greater injury costs, because of increases in V. What is clear is that decisions to escalate can be altered by the abiotic environment. The costs and benefits of animal contests are typically investigated under stable conditions but aggression has evolved under natural conditions that show

spatial and temporal variation. Thus, fighting behaviour may be considered a spatial-temporal mosaic in which the costs and benefits vary, sometimes due to predictable cycles (e.g. tidal, weather [6] stability [5]). Here we have shown how fighting animals can adjust the strategic (whether to give up) and tactical (whether to escalate) decisions frequently modelled by evolutionary theory based on fluctuating environmental cues.

Ethics. This work was performed on invertebrates not covered by the Use of Animals (Scientific Procedures) Act. Thus, there is no requirement for ethical approval within the university that deals with animals used in this experiment. However, experiments were conducted following the ASAB/ABS guidelines on the use of animals in education and research.

Data accessibility. Data used can be found in Dryad:

<http://dx.doi.org/10.5061/dryad.526g0> [18]

Authors' contributions. AVP and MB designed the study; AVP and MV performed the study; AVP, MV, SS and MB performed the statistical analyses; AVP, MB and SS wrote the manuscript; MV, SS and MB revised the manuscript. All authors approved the version to be published. All authors agree to be accountable for all aspects of the research.

Competing interests. We declare we have no competing interests.

Funding. Coordenação de Aperfeiçoamento de Pessoal de Nível Superiores (CAPES) for the Science Without Borders scholarship to AVP (99999.010227/2014-08) and MV. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the productivity grant to SS (311142/2014-1).

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SUPPLEMENT

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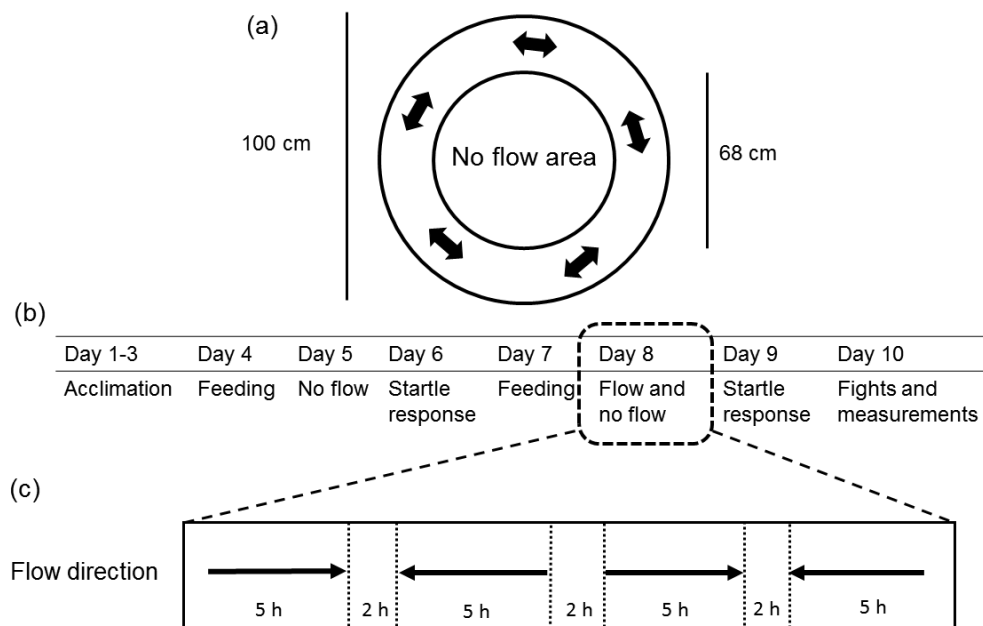


Fig S1. (a) Schematic representation of the experimental aquarium. Individuals were accommodated on both parts – the outer circle and the inner circle – but only individuals on the outer circle received flow on day 8. Arrows indicate where the reef pumps were positioned and that water flowed on both directions to simulate the tidal cycle. (b) Experimental design with and inset for the simulation of the tidal cycles, (c) in which arrows indicate the flow direction and the numbers indicate how many hours

in each stage. Dotted lines indicate resting periods in which the pumps were turned off and inverted the direction.

METHODS

Startle response duration, dry weight and nematocyst length presented an exponential distribution, rather than a Gaussian distribution that is required for F-tests. Thus, we log-transformed them prior to analyses. Additionally, we scaled startle response duration 2, dry weight and nematocyst length when using them as continuous covariables. We centered their means to zero and made their variances uniform by dividing the variable by one-time their standard deviation. Centering and scaling variables help in parameter estimation and make parameters easily interpretable and comparable when significant interactions are present (Schielzeth 2010, reference 16 in the manuscript). To test if individuals exposed to flowing seawater perceived a higher V than individuals that were not exposed to flow, we used an ANCOVA with the following model:

$$\text{Log(Startle response duration 2)} \sim \text{scale}(\text{log(Startle response duration 1)}) * \text{treatment}$$

Next, to test the effects of the treatment on contest outcome and contest escalation while controlling for RHP traits, we used the following GLMs:

$$\begin{aligned} \text{Contest outcome} \sim & \text{Focal_treatment} * \text{Opponent_treatment} + \text{Focal_scale}(\text{log(Startle} \\ & \text{response duration 2)}) + \text{Opponent_scale}(\text{log(Startle response duration 2)}) + \\ & \text{Focal_scale}(\text{log(dry weight + 1)}) + \text{Opponent_scale}(\text{log(dry_weight + 1)}) + \\ & \text{Focal_scale}(\text{log(nematocyst length + 1)}) + \text{Opponent_scale}(\text{log(nematocyst length + 1)}) \end{aligned}$$

$$\begin{aligned} \text{Contest escalation} \sim & \text{Focal_treatment} * \text{Opponent_treatment} + \text{Focal_scale}(\text{log(Startle} \\ & \text{response duration 2)}) + \text{Opponent_scale}(\text{log(Startle response duration 2)}) + \\ & \text{Focal_scale}(\text{log(dry weight + 1)}) + \text{Opponent_scale}(\text{log(dry_weight + 1)}) + \\ & \text{Focal_scale}(\text{log(nematocyst length + 1)}) + \text{Opponent_scale}(\text{log(nematocyst length + 1)}) \end{aligned}$$

Lastly, to test the effects of treatments on contest duration while controlling for RHP traits, we used the following linear regression:

$$\text{Log}(\text{contest duration}) \sim \text{Focal_treatment} * \text{Opponent_treatment} + \text{Focal_scale}(\text{log}(\text{Startle response duration 2})) + \text{Opponent_scale}(\text{log}(\text{Startle response duration 2})) + \text{Focal_scale}(\text{log}(\text{dry weight} + 1)) + \text{Opponent_scale}(\text{log}(\text{dry_weight} + 1)) + \text{Focal_scale}(\text{log}(\text{nematocyst length} + 1)) + \text{Opponent_scale}(\text{log}(\text{nematocyst length} + 1))$$

Table S1. Significance test from the logistic model to examine to effect of environmental cues and RHP-traits on contest outcome. All effects were log₁₀-transformed, centred and scaled prior to being used in the model. Marginally significant effects are printed in italics.

Effect	χ^2	df	P-value
Focal treatment	1.433	1	0.231
<i>Opponent treatment</i>	<i>3.603</i>	<i>1</i>	<i>0.058</i>
<i>Focal startle response 2</i>	<i>3.460</i>	<i>1</i>	<i>0.063</i>
Opponent startle response 2	1.030	1	0.310
Focal dry weight	1.175	1	0.278
Opponent dry weight	1.916	1	0.166
Focal nematocyst length	1.058	1	0.303
Opponent nematocyst length	0.526	1	0.468
Focal treatment * Opponent treatment	1.002	1	0.317
Residuals	81.836	60	-

df: degrees of freedom

Table S2. Significance test from the logistic model to examine to effect of environmental cues and RHP-traits on contest escalation. All effects were log₁₀-transformed, centred and scaled prior to being used in the model. Significant effects are printed in bold.

Effect	χ^2	df	P-value
Focal treatment	2.409	1	0.121
Opponent treatment	1.860	1	0.172

Focal startle response 2	0.407	1	0.523
Opponent startle response 2	0.879	1	0.348
Focal dry weight	2.770	1	0.096
Opponent dry weight	5.652	1	0.017
Focal nematocyst length	0.331	1	0.565
Opponent nematocyst length	0.090	1	0.763
Focal treatment * Opponent treatment	6.268	1	0.012
Residuals	66.479	60	-

df: degrees of freedom

Table S3. Significance test from the linear model built to examine to effect of environmental cues and RHP-traits on contest duration. All effects were log₁₀-transformed, centred and scaled prior to being used in the model. Significant effects are printed in bold and marginally significant are printed in italics.

Effect	Sum of squares	F-value	df	P-value
Focal treatment	0.124	0.284	1	0.596
Opponent treatment	1.877	4.299	1	0.042
Focal startle response 2	0.612	1.402	1	0.241
Opponent startle response 2	0.129	0.295	1	0.589
Focal dry weight	1.786	4.090	1	0.047
Opponent dry weight	0.103	0.236	1	0.629
Focal nematocyst length	0.032	0.072	1	0.788
<i>Opponent nematocyst length</i>	<i>1.725</i>	<i>3.952</i>	<i>1</i>	<i>0.051</i>
<i>Focal treatment * Opponent treatment</i>	<i>1.697</i>	<i>3.886</i>	<i>1</i>	<i>0.053</i>
Residuals	26.195	-	60	-

df: degrees of freedom

Table S4. Mean contest duration (log ± SE) of fighting sea anemones (*Actinia equina*) presented in Fig. 1d. Focal individuals are on columns and opponents on lines.

	Flow	No-flow
Flow	7.44 (± 0.16)	6.71 (± 0.13)
No-flow	7.00 (± 0.14)	7.07 (± 0.18)

