



Escalated fighting despite predictors of conflict outcome: solving the paradox in a South American cichlid fish

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Several studies have found behavioural differences between the interactions of opponents, early in a conflict. These differences consistently predict which individual will be the winner. Surprisingly, opponents appear not to use this information: they continue fighting and even proceed to highly damaging escalation. We attempted to resolve this paradox in a series of experiments on interactions between male cichlids. First, the cost of fighting and escalating in terms of energy expenditure (oxygen consumption: gill movements) and injuries was high. Second, previous social experience was important but could not explain the occurrence of predictors of conflict outcome. Third, a less artificial conflict set-up, in which territory ownership was asymmetrical and opponents were allowed to see each other before the conflict, resulted in shorter interactions with reduced escalation phases. This suggests that the occurrence of predictors of conflict outcome and the subsequent lack of response of the opponents is the result of unnatural, highly symmetrical designs, as used in many studies. We propose the following mechanism. Prospective winners postpone escalation as much as possible because of the high cost involved. At the same time, prospective losers continue the fight because they lack alternatives. We tested this 'desperado hypothesis' in a fourth experiment, in which losers were offered alternative options. These conflicts were shorter and both predictors of outcome and escalated fighting were absent.

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Competition over valuable resources is a key element in any organism's life history. Apart from overt aggression and attack, individuals often communicate during conflicts through threat displays. These have been studied by ethologists for decades, both experimentally and theoretically (for a review, see [Huntingford & Turner 1987](#)).

Although game theory has greatly improved our understanding of animal conflict, actual knowledge about the meaning of individual displays and the decision rules underlying their occurrence is limited. Whereas theoretical models predict that the behaviour of two opponents should not reveal reliable information about the chances of winning until shortly before the end of an interaction ([Maynard Smith 1982](#); [Enquist et al. 1990](#)), several experimental studies on aggressive interactions in fish have reported that eventual winners behave differently from their opponents, in the early stages of a conflict (e.g. [Harvey & Corbet 1986](#); [Popp et al. 1990](#); cichlids: [Barlow et al. 1986](#); [Turner & Huntingford 1986](#);

[Ribowski & Franck 1993](#)). Eventual winners perform certain behavioural patterns at higher frequencies, or earlier in the interactions, than eventual losers. These behavioural differences yield predictors of conflict outcome with reliabilities up to 80%. However, the animals do not seem to use this information in deciding the interactions quickly. Opponents are reported to continue fighting and even proceed through escalation phases that entail high injury risk and energy expenditure (e.g. [Barlow et al. 1986](#)).

Several hypotheses may account for these findings. First, it is generally assumed that aggression is a costly and dangerous activity. However, the display behaviours and escalated fighting as shown during aggressive encounters may not incur as much cost as investigators attribute to them. In that case, the selective advantage of an individual that accurately assesses and responds to predictors of conflict outcome, no matter how reliable, may be highly overrated.

Second, it is well established that social experience influences an individual's performance in a conflict, including its willingness to escalate ([Kruijt 1964](#); [Tooker & Miller 1980](#); [Turner & Huntingford 1986](#); [Beacham 1987](#); [Groothuis & Mullekom 1991](#)). Previous experience with social interactions may well increase the ability to perceive behavioural differences and to respond to them

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appropriately. However, few authors specify the level of social experience of their study animals.

Third, the contests staged in experiments may not be appropriate representations of the conflicts that occur in nature. One indication of the artificiality of set-ups is that escalated fighting, although not unnatural as such, is much more frequently observed in experiments than it is in nature (Tinbergen 1968; Maynard Smith & Price 1973; cichlids: Neil 1983; Barlow et al. 1986). These high levels of escalation may be the result of any one of several artificial aspects of experimental set-ups. First, staged encounters are often symmetrical: experimental animals are faced with an opponent that is often matched for size, sex, territoriality and payoff. It is reasonable to assume that these symmetric contests are rare in nature (Van Rhijn 1980). For example, territorial ownership (a) symmetry is a major factor determining the chances of winning (Hammerstein 1981; Enquist & Leimar 1987; Turner 1994). In a staged conflict, however, both opponents may consider themselves territory owners, expelling an intruder. Clearly, this constitutes an unnatural situation that yields long and escalated conflicts. Second, unlike interactions in the wild, losers in staged conflicts cannot escape from their opponents and may thus be exposed to continuous aggression and exclusion from valuable resources, such as shelter, food and mates. This perspective could force the losing individual into a desperado-like position, continuing the fight solely from lack of alternatives (Grafen 1987). Third, opponents in staged encounters are confronted with each other in a very sudden, involuntary manner (e.g. Enquist & Jakobsson 1986; Turner & Huntingford 1986) whereas in nature, individuals are likely to assess any potential adversary and the resources under its control before engaging in any aggressive interaction.

We attempted to analyse and explain the occurrence of long and escalated fighting despite early predictors of conflict outcome. With symmetrically designed conflicts, we investigated the aggressive and display behaviours of the South American cichlid *Aequidens rivulatus*. We looked for differences between opponents that revealed the chances of winning and then, to assess the relevance of these predictors, we investigated the cost associated with display behaviour and escalated fighting, in terms of energy expenditure (oxygen consumption: frequency of gill movements) and injury. We looked at the influence of social experience, by staging conflicts between both experienced and inexperienced fish. Then, we created a more natural set-up with an owner-intruder situation in which the fish could see each other before the conflict, and investigated how this influenced the course and escalation levels of the interactions. We investigated the cost of losing (injuries and weight loss) and thereby the probability of the desperado effect occurring, by making observations after the conflicts, when loser and winner were not yet separated. Finally, we tested the desperado hypothesis in a fourth experiment in which losers were offered alternative options: they could (1) withdraw into a safe area and (2) choose between different territory owners.

GENERAL METHODS

Animals and Housing

The cichlid *A. rivulatus* is a sexually dimorphic substrate breeder that occurs in the rivers of Ecuador and Peru (Stawikowski & Werner 1998). It has been found in a variety of habitats, including both fast- and slow-moving rivers, turbid and clear waters and over rocky, sandy and muddy bottoms with various amounts of vegetation. These fish are highly territorial, reproduce easily in captivity and, like most cichlids, possess a large display repertoire. We used fish bred in the Zoological Laboratory in Haren, with the exception of a few wild-caught individuals and some of their offspring. We avoided inbreeding by keeping large numbers of individuals and adding wild-caught fish to the laboratory stocks regularly. Wild-caught fish and their offspring showed similar behaviour to the fish bred in the laboratory. All fish were reared in sibling groups of 10–40 individuals and kept in tanks of 70–600 litres. Only males were used. All tanks were connected to a central biological filter system and water circulated continuously. Water temperature was kept at $25 \pm 2^\circ\text{C}$ and a 14:10 h light:dark cycle was maintained. The bottom of each tank was covered with 3 cm of gravel. Tanks contained several stones and flowerpots for shelter and a continuous air supply. Fish received food pellets four times a week and red mosquito larvae once a week.

Social Experience

We assigned the fish to two categories according to the level of their social experience. Experienced fish were those that had been kept in small groups containing both sexes, allowing territory settlement and pair formation. In addition, these males had been involved in at least three staged encounters, in which they had both won and lost. As a result, these fish had had repeated territorial and sexual interactions, the latter regularly resulting in reproduction. Inexperienced fish did not have such experience, since from hatching onwards they were kept in large schools in which territory establishment and reproduction did not take place. Age did not differ between experienced and inexperienced fish. In experiments 1 and 2 both experienced and inexperienced fish were used. In experiments 3 and 4 only experienced fish were used.

Observations

We observed and analysed the following behavioural patterns (Baerends & Baerends-van Roon 1950).

- (1) Fin display: dorsal, ventral and caudal fins were erected, while the fish often maintained a lateral position towards its opponent.
- (2) Tail beating: without moving forward, a fish gave a vigorous beat of the tail, pushing a wave of water towards the opponent.
- (3) Mouth fighting: the opponents grabbed each other's jaws and engaged in a pushing/pulling contest.

(4) Circling: both individuals swam head to tail and tried to bite each other ('carouselling'). Circling was considered the escalation phase of a fight (e.g. Turner & Huntingford 1986).

(5) Biting: this occurred mostly at the flank or fins of the opponent. Biting could result in the loss of scales or other minor injury to the victim such as fin cuts.

(6) Inferior: all fins were held close to the body, the fish maintained a head-down, tail-up position and avoided its opponent. This posture determined the end of a fight.

(7) Frontal orientation: all positions of an individual in which it was oriented towards its opponent.

In contrast to Fin display, Tail beating, Biting and Frontal orientation, Mouth fighting and Circling were strictly mutual activities.

The interactions were considered to be finished and observations stopped when one of the opponents persistently showed one or more of the following behaviours: maintenance of an inferior posture, fleeing from its adversary or hanging motionless in the upper water layer.

To assess the cost of fighting and losing, we measured respiration frequencies of both opponents throughout the conflicts by counting gill movements for 1 out of every 10 min (Van Rooij & Videler 1996; Grantner & Taborsky 1998). In addition, injuries were assessed after each interaction. We determined an injury score for each individual by attributing a value of 0, 1, 2 or 3 to each part of the body that was liable to injury: mouth (as a consequence of Mouth fighting), fins, tail and flanks. Summing these yielded an injury score for each individual that ranged from 0 to 12. In experiment 3 we also weighed the fish both before and 3 days after the interactions.

Like most cichlids, *A. rivulatus* shows a variety of colour patterns, which can change within seconds. The appearance of these patterns is considered to reflect the motivational status of the animal, and the characteristics of its surroundings. In particular, an elevated level of stress is thought to manifest itself by a darker coloration (Hulscher-Emeis 1992). Therefore, in experiments 1 and 2 we recorded, every 10 min, which of the two individuals was darker.

Statistics

Injury, coloration scores and chances of winning were tested nonparametrically (Wilcoxon signed-ranks test, Spearman rank correlation, Mann-Whitney *U* test, binomial test, Fisher's exact test). All percentages were arcsine transformed and tested parametrically, as were behavioural frequencies (*t* test, Pearson correlation, ANOVA). Visual inspection of data suggested distributions were normal. For small sample sizes, this conclusion was derived from parameter values for large sample sizes. All *P* values given are two tailed, unless specified otherwise. Power tests were carried out when tests with small sample sizes failed to show a significant, expected, effect.

Differences between winners and losers are expressed in two ways: a *P* value, originating from a paired test (*t* test or Wilcoxon signed-ranks test), and a percentage. The latter indicates the proportion of interactions (relative

to the total number of interactions in which the opponents differed) in which the expected winner did win. In other words, this percentage indicates the chances of winning.

Ethical Note

As staged contests may be stressful, we kept sample sizes small and observed the fish throughout the experimental procedures. The hypotheses under investigation required observations to continue until the conflicts were resolved. As soon as the experimental set-ups allowed, however, we separated opponents of interactions and placed them individually in large tanks to recover. The injuries that the fish sustained were relatively minor; the number of fin cuts or scales lost ranged from 0 to 12 and these healed completely within a week. From our experience with cichlid fish in both laboratory and field situations, we conclude that the experiments did not cause extreme stress or injury. As far as we were able to determine, no fish died as a result of our manipulations. All experiments were carried out under special licences from the ethical committee for animal experiments from Groningen University.

EXPERIMENT 1: FIRST SYMMETRICAL CONFLICT SERIES

Aim

In this experiment we investigated whether we could predict the eventual winner of an interaction, on the basis of the behaviour of the fish at the beginning of the fight. In addition, we assessed the structure of the conflicts, the level of escalated fighting and the cost of display and aggression. Both experienced and inexperienced fish were used, to determine the effect of previous social experience.

Design

We staged six conflicts between inexperienced fish and four between experienced fish. None of the fish had any previous experience with its opponent. Fish sizes ranged from 80 to 170 mm total length. Fish were matched for size, total length differences being smaller than 8%. The contents of the experimental aquaria ranged from 160 to 250 litres, except for the four largest individuals, which were tested in larger tanks (750 litres). In assigning individuals to tanks, we took care not to invoke any bias with regard to experimental groups. All pairs were placed in the experimental tanks a week before the observations. Males were placed at either end of the experimental tank, an opaque partition in the middle preventing them from seeing each other. We also placed one female in each compartment to enhance territoriality. These females were separated from the males by transparent partitions. Each of the four compartments contained one flowerpot and one flat stone for shelter.

One hour before the experiment, we removed the females and the transparent partitions, thereby preventing females interfering with the male–male interaction. Because the interactions were recorded on videotape, an additional fluorescent lamp was lit above the experimental tank to improve light conditions. The fish did not show any increase in fright behaviour as a result of the extra light. One hour later we removed the opaque partition between the fish. Video-recording started as soon as one of the males approached the other. We used a remote-control video camera (Panasonic wv-CL700) placed in front of the tank 24 h before the experiment. After conflicts had been settled, recording was stopped and the fish were separated immediately.

Analysis

Conflict durations varied considerably. To compare all fights, we performed the analysis as follows. We analysed the first and last 20 min of each interaction completely, since no interaction lasted less than 40 min. This analysis allowed us to analyse predictors of conflict outcome for the first 20 min and the level of escalated fighting in the last 20 min. The remaining period was divided into six periods of equal length. We included 5 min from each of these six periods in the analysis. The result is an analysis of 70 min, allowing comparison of all conflicts. Interactions that lasted less than 70 min were analysed completely. We used the Observer computer program, with a Video Interface and Time Code Generator (Noldus Information Technology, Wageningen, The Netherlands). Observations were registered with a precision of 0.01 s.

Results

Conflict structure

Fights lasted 45–378 min. The interactions between both experienced and inexperienced fish typically consisted of three phases (Fig. 1). In the first phase of the conflicts predominantly noncontact displays were used, such as Fin display and Tail beating. The second phase consisted of prolonged bouts of Mouth fighting. Fights were often completed with Circling. This phase entails the highest injury risk: the fish frequently attempted to bite each other. Of 10 interactions, eight showed this escalated fighting. Seven conflicts consisted of all three phases in the order described. One fight ended after the Tail-beating phase and another was decided without Circling. Only one interaction deviated from the average pattern, the fish proceeding to Circling without Mouth fighting. None of the 10 interactions showed an inverted sequence of phases. When present, Circling always constituted the final stage of an interaction. When no Circling was observed, Mouth fighting was the final phase.

Cost

The duration of Circling in an interaction was correlated with the number of injuries as assessed after the

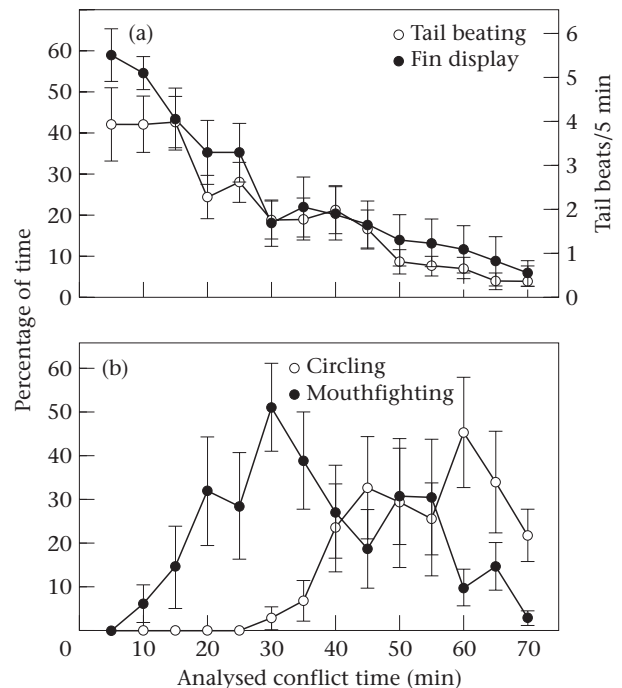


Figure 1. The structure of the conflicts of experiment 1, depicted as the amount of (a) noncontact displays and (b) contact displays per 5 min. The amounts of Fin display, Mouth fighting and Circling are expressed as percentages, Tail Beating is given as a frequency. Vertical bars indicate SE. Experienced and inexperienced fish are pooled.

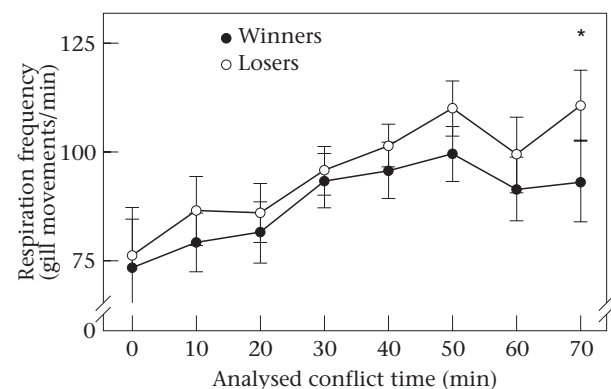


Figure 2. Respiration frequencies for winners and losers throughout the conflicts of experiment 1. Vertical bars indicate SE. * $P < 0.05$.

fight (Spearman rank correlation: $r_s = 0.83$, $N = 10$, $P < 0.01$). Winners and losers had similar injury scores (Wilcoxon: $T = 16$, $N = 10$, $P < 0.20$; median (and 95% confidence interval) for winners 7 (4.4–8.8) and losers 6 (3.5–7.7)).

Respiration frequencies increased in both winners and losers as the fights proceeded and a strong, although nonsignificant, trend suggests that losers had higher energy costs than winners: two-way ANOVA with on one factor the eight repeated measurements during the conflict ($F_{7,63} = 6.87$, $P < 0.01$) and on the other winner or loser ($F_{1,9} = 4.99$, $P = 0.052$; Fig. 2). The interaction effect between factors was not significant ($F_{7,63} = 0.95$, $P = 0.48$). However, since the energy costs were highest towards the

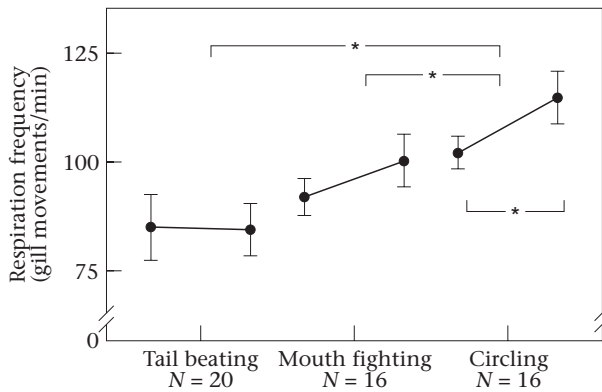


Figure 3. Respiration frequency during the Tail-Beating, Mouth-fighting and Circling phases of experiment 1. Vertical bars indicate SE. * $P < 0.05$.

end of the conflict (Circling, see below), the difference between opponents may not be apparent until that final stage. Indeed, a paired t test of respiration frequencies before the conflict showed no difference between winners and losers ($t_9 = 0.30$, $P > 0.7$), while at the end of the interactions it did ($t_9 = 3.01$, $P < 0.02$). To determine the costs of the different behaviours that occurred during conflicts, we looked at the respiration frequencies 5 min before and after the peak of that particular behaviour (Fig. 3). A MANOVA with on one factor the three behaviours as repeated measures (Tail beating, Mouth fighting and Circling), on the second the data before and after the behaviours as repeated measures, and on the third winner or loser showed significant differences in respiration frequency between the behaviours ($F_{2,9} = 10.31$, $P < 0.01$) and a significant increase over the behavioural bouts ($F_{1,9} = 6.39$, $P < 0.03$). There was no interaction with winning and losing ($P > 0.5$). The overall respiration frequency was highest during Circling (Circling versus Mouth fighting, paired t test: $t_{13} = 7.03$, $P = 0.02$), while the increase over the bouts of behaviour was significant only for Circling (paired t test: $t_{15} = 7.9$, $P < 0.02$).

Predictors of conflict outcome

Although length differences were small, larger fish won significantly more fights than smaller ones (binomial test: $P < 0.04$; 7 of 9 conflicts; in the remaining conflicts opponents were equal in length).

Behavioural differences between eventual winners and losers were found in the two main displays of the first conflict phase, Tail beating and Fin display. Winners performed more Tail beating than losers (Fig. 4a). After 20 min, which is less than half the total duration for all conflicts, the total difference between winners and losers was already significant (winners: $\bar{X} \pm \text{SE} = 17.6 \pm 2.81$ beats/5 min; losers: 10.8 ± 1.86 beats/5 min; paired t test: $t_9 = 2.72$, $P < 0.03$). The predictive value of this difference is 78%, winners performing more Tail beating than losers in seven of nine cases in which opponents differed. Winners also tended to perform more Fin display than losers, but the difference is not significant (winners: $\bar{X} \pm \text{SE} = 52.17 \pm 5.88\%$; losers: $43.52 \pm 6.15\%$; paired t test: $t_9 = 1.77$, $P = 0.13$; power = 0.28, necessary sample size

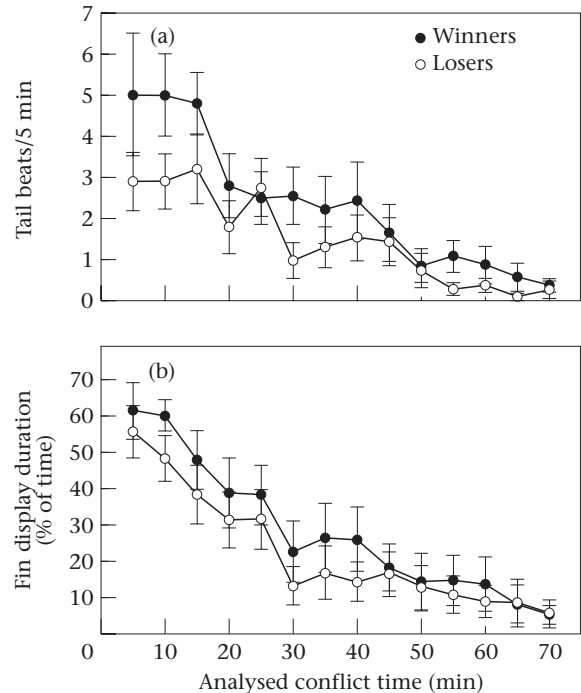


Figure 4. (a) Frequency of Tail Beating and (b) amount of time spent on Fin display for winners and losers during the conflicts of experiment 1. Vertical bars indicate SE.

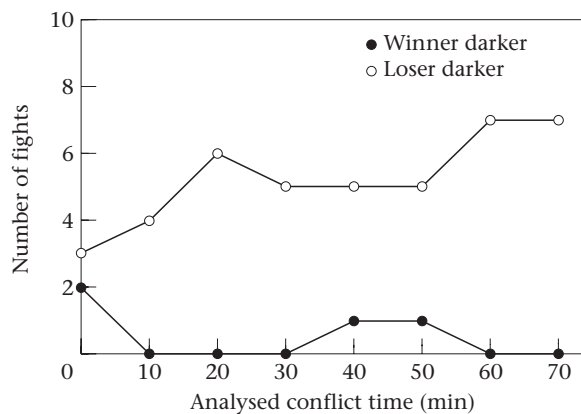


Figure 5. The difference in overall darkness between winners and losers during the interactions of experiment 1.

for 80% power = 31 in each group; reliability of predictor is 60%; Fig. 4b).

Winners and losers also differed in coloration (Fig. 5): losers almost always became darker than winners. After 20 min of observation, the difference was highly significant (binomial: $N = 6$, $P < 0.02$; reliability 100%, there were six interactions in which there was a difference between opponents at $t = 20$ min).

Apart from the winner–loser difference in coloration, the reliability with which individual behavioural differences predicted the chances of winning did not reach 100%. However, given the large variation in display patterns used, the opponents in an interaction had several sources of information at their disposal, and their decisions were not necessarily determined by a single

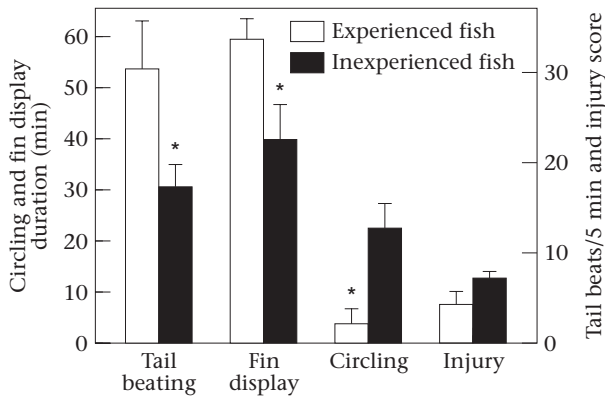


Figure 6. The differences in display and injuries between conflicts of experienced and inexperienced fish in experiment 1. Winners and losers are pooled. Vertical bars indicate SE. * $P < 0.05$.

behavioural parameter. Therefore we combined the performance of winners and losers for Tail beating and Fin display in one score, by log-transforming the data and summing them. This calculation yielded higher scores for all winners than their opponents, that is, a 100% predictor of conflict outcome.

We did not find a relationship between size asymmetry of opponents and the duration of a fight (Pearson correlation: $r_8 = -0.15$, NS). Similarly, the difference between winners' and losers' performance of Tail beating did not influence the duration of an interaction (Pearson correlation: $r_8 = -0.096$, NS), nor did it affect the time spent on Circling ($r_8 = -0.26$, NS). For Mouth fighting there was a nonsignificant trend ($r_8 = -0.58$, $P = 0.08$).

The influence of experience

We did not find any evidence that the occurrence of predictors of conflict outcome was influenced by an individual's level of social experience. Predictive differences between opponents in Tail beating and Fin display were found for both the experienced and inexperienced groups. The Tail-beating difference between opponents did not show any influence of experience (t test: $t_8 = 0.8$, $P = 0.42$). Fin display, however, did show a nonsignificant trend ($t_8 = 2.00$, $P = 0.08$), inexperienced opponents revealing larger differences than experienced ones. The structure of the interactions differed between experienced and inexperienced fish (Fig. 6). Compared with inexperienced fish, during the first 20 min experienced opponents spent more time on Fin display (t test: $t_8 = 2.42$, $P < 0.05$) and Tail beating (t test: $t_8 = 5.52$, $P < 0.01$). Over the whole conflict they spent less time Circling (t test: $t_8 = 3.31$, $P < 0.02$) and as a result tended to sustain fewer injuries (Mann-Whitney U test: $U = 3.0$, $N_1 = 4$, $N_2 = 6$, $P = 0.055$).

Conclusion

The confrontations observed showed a very distinct and consistent pattern of behavioural phases, with an assessment phase at the beginning and a costly escalation phase at the end. Larger fish had better chances of winning. Winners could be distinguished from losers by

their behaviour, early in the interactions during the assessment period. Nevertheless, the majority of fights continued for a long time and escalated fighting occurred in the majority of cases. Experienced fish showed more assessment display, less escalated fighting and sustained fewer injuries than inexperienced fish. The occurrence of predictors of outcome was not influenced by experience.

EXPERIMENT 2: SECOND SYMMETRICAL CONFLICT SERIES

Aim

The findings of the first experiment were the result of a post hoc analysis, without specific a priori hypotheses about which displays might predict conflict outcome. To test the reliability of these results, we therefore carried out a second experiment, in which we used exactly the same set-up and analysis method as before. We specifically tested the predictions that originated from experiment 1, namely that Tail beating, Fin display and overall darkness are predictive of the chances of winning.

Design

We staged seven conflicts between inexperienced fish and five between experienced fish. Owing to time constraints, only the first nine of these conflicts were analysed completely, in the same way as described for experiment 1 (four interactions between experienced fish and five between inexperienced fish). Of the remaining three only the first 20 min were analysed and conflict duration and outcome were registered. Fish sizes ranged from 81 to 154 mm total length; size differences between opponents were below 10%.

Results

Conflict structure

The structure of the nine conflicts that were analysed from beginning to end closely resembled that of the first series of interactions. Three consecutive phases in the expected order could be distinguished in eight of nine cases; in one interaction Mouth fighting did not take place. Circling was observed at the end of all fights. Conflicts lasted 35–210 min.

Predictors of conflict outcome

Again, we found differences between eventual winners and losers. As in the first experiment, the larger individual won the fight in the majority of cases (10 out of 12, binomial: $P < 0.02$). In addition, as in the first experiment, behavioural differences between opponents occurred in the first conflict phase. During the first 20 min of the interaction, eventual winners tended to perform more Tail beating, although not significantly so (winners: $\bar{X} \pm SE = 38.33 \pm 7.07$ beats/5 min; losers: 27.50 ± 4.49 beats/5 min; paired t test: $t_{11} = 1.86$, $P = 0.09$) and significantly more Fin display (winners: $\bar{X} \pm SE = 46.07 \pm 4.46\%$; losers: $35.23 \pm 3.62\%$; paired t test:

$t_{11}=2.37, P<0.04$). The reliabilities of the predictors are 66 and 83%. A combined value, based on the data for both Fin display and Tail beating, yields a 78% reliable prediction. As in the first series of conflicts, opponents differed in coloration. Again, losers tended to be darker than winners, although this was not significant (at $t=15$ min: 8 versus 2, binomial test: $P=0.055$; at $t=20$ min: 4 versus 1, $P=0.19$). Like the first experiment, the amount of Circling influenced the extent to which the fish sustained injuries (Spearman rank correlation: $r_s=0.84, N=9, P<0.02$).

Neither the occurrence nor the reliability of predictors of conflict outcome was influenced by experience: no significant differences between the experienced and inexperienced groups were found for the winner–loser difference in Tail beating (t test: $t_{10}=0.37, P=0.7$) and Fin display (t test: $t_{10}=0.84, P=0.4$).

Experiments 1 and 2 combined

Combining the results of both series of interactions yielded highly significant differences between winners and losers. After 20 min, Tail beating predicted conflict outcome with 71% reliability (paired t test: $t_{21}=2.70, P<0.02$) and Fin display with 73% reliability (paired t test: $t_{21}=2.92, P<0.01$). The combined value for all conflicts taken together predicted the eventual winner with 89% reliability (paired t test: $t_{21}=3.94, P<0.001$). Similarly, the winner–loser difference in coloration at 20 min was highly significant (binomial test: $N=22, P<0.01$; reliability 91%).

Conclusion

As in experiment 1, Tail beating, Fin display and coloration tended to differ between opponents, predicting the eventual winner. Again, this phenomenon occurred in both experienced and inexperienced fish. Furthermore, the structure of the interactions was consistent with the earlier findings and fierce escalation occurred regularly. Combination with the results of experiment 1 yielded highly reliable predictors of conflict outcome.

EXPERIMENT 3: OWNER–INTRUDER SET-UP

Aim

In the third series of conflicts we used a more natural set-up, to investigate whether the high levels of escalation after predictors of outcome, as observed in the previous experiments, were the result of the artificial set-up. We therefore (1) created an owner–intruder situation and (2) during the 7-day period preceding an interaction allowed the opponents to assess each other's territories through transparent windows. After the conflicts were decided, we assessed the cost associated with losing.

Design

We staged 14 conflicts in the following set-up. In tanks with contents ranging from 180 to 250 litres, one

of two males ('owner') was given a large territory (two-thirds of the tank), with a female behind a transparent partition. The other male ('intruder') was given one-third of the tank and no female. The territories were separated by a nontransparent partition with three small, transparent, windows (7×7 cm²). The windows allowed the intruder males to estimate the payoff of the fight in terms of territory quality and to get a rough estimate of the qualities of its opponent before the fight. Previous studies on the effect of pre-exposure have shown that cichlid fish are capable of estimating their relative size and perhaps strength by visual assessment alone (Enquist et al. 1987; Beeching 1992; Keeley & Grant 1993). Therefore we intentionally used windows that were smaller than the smallest fish, preventing the opponents from accurately assessing each other's size by lateral Fin display before the conflict. The windows also prevented assessment of the opponents' performance of Tail beating, by blocking the water current that is created in this display and which may be an important source of information. We wanted information transfer to be limited, because we were interested in the occurrence of behavioural predictors of outcome during the conflict itself, after removal of the windows. The males were held in this set-up for a week before the experiments started.

We started observations 1 hour after removal of the female and transparent partition. The windows were opened, enabling both males to swim freely across the tank. The fight was considered to begin as soon as the intruder entered the large territory. In the case of a territory owner swimming through a window at the onset of an interaction (which happened in a minority of cases) the observation was halted immediately and the fish were returned to their original compartments. Then, after at least 3 h, another trial was started. Fish ranged from 87 to 160 mm total length; size differences between opponents were below 6%. In seven of the interactions the owner was the larger fish, in the remaining seven the intruder was larger. Winners and losers were identified as described earlier. We did not separate the contestants immediately after the conflict was decided, but kept observing them for 4 days for 30 min a day, starting the day of the fight immediately after the conflict. In addition, we checked on the fish at least three times a day and separated them in cases of severe aggression. The period of 4 days was based on our experience that the period was sufficiently long to get an indication of the stability and consequences of the outcome of the conflict and sufficiently short to prevent long-term detrimental effects for the loser. Fish were weighed before the experiment and after the 4-day observation period.

Because of the more complicated set-up of the experiment, these conflicts were not video recorded but analysed directly on paper. As a result, Fin display and coloration could not be recorded. We used stopwatches to measure durations. For the whole conflict, the following behaviours were recorded for each individual: Tail beating (frequency), Mouth fighting (duration), Circling (duration) and Frontal orientation (duration).

Results

Owner and intruder roles

Owners won in nine of 14 conflicts (binomial test: $P=0.21$); smaller intruders were defeated in all but two conflicts. Throughout the conflicts, territory owners maintained a frontal position towards their opponents more often than intruders (total scores over the conflicts: owners: $\bar{X} \pm \text{SE}=86.71 \pm 20.70\%$; intruders: $27 \pm 5.71\%$; paired t test: $t_{13}=3.20$, $P<0.01$), indicating that they behaved more aggressively, and suggesting that the owner and intruder roles were perceived as such by the fish.

Comparison with symmetrical set-up

Some aspects of the interactions of this experiment differed from the previous series of conflicts. First, size no longer significantly influenced conflict outcome (in only 8 of 14 cases did the larger individual win; binomial test: $P=0.4$). However, this is not significantly different from the previous experiments (experiments 1 and 2 versus 3: $G_1=2.31$, $P>0.1$). Second, the escalation phase was considerably shorter than in the symmetrical conflicts (t test for Circling over the data of experiments 1 and 2 versus 3: $t_{31}=3.27$, $P<0.01$, $\bar{X} \pm \text{SE}=12.0 \pm 1.8\%$, $N=19$ versus $2.6 \pm 0.5\%$, $N=14$). The duration of the fights did not differ from previous observations (experiments 1 and 2 versus 3: $t_{31}=0.82$, $P=0.4$; $\bar{X} \pm \text{SE}=122.4 \pm 24.5$ min, $N=19$ versus 95.9 ± 16.9 min, $N=14$, power=0.35, necessary sample size for 80% power=50 in each group). Again, Tail beating differed significantly between winners and losers (paired t test: $t_{12}=2.19$, $P<0.05$, reliability 67%; winners: $\bar{X} \pm \text{SE}=10.5 \pm 4.1\%$; losers: $4.3 \pm 2.4\%$).

Cost of losing

After the interactions, winners continued to be very aggressive in two cases, in which we separated the fish immediately. In two other pairs new fights started a few hours after the conflict. One former loser defeated its opponent; in the other fight the original winner won again. In the remaining 10 dyads, winners appeared to tolerate their defeated opponents and hardly any aggression was observed. However, losers spent much time hiding themselves or maintaining an inferior posture. Furthermore, losers lost weight considerably, whereas winners slightly gained weight (paired t test: $t_{12}=-2.97$, $P<0.02$; original weights of winners: $\bar{X} \pm \text{SE}=33.77 \pm 5.71$ g; losers: 33.76 ± 5.45 g; Fig. 7). As in the previous experiments, fish recovered quickly from their injuries (<1 week) and weight loss (<2 weeks).

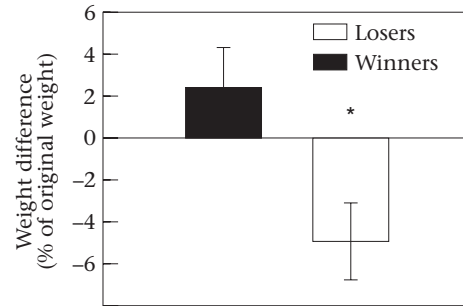


Figure 7. The weight of winners and losers after the conflicts of experiment 3; given as a percentage of the weight before the interaction. Vertical bars indicate SE. * $P<0.05$.

Conclusion

We conclude that the asymmetrical set-up was interpreted as such by the fish. The territory ownership asymmetry reduced the effect of size on the chances of winning that we found in the previous experiments. Tail beating performance still predicted conflict outcome, but there was less escalated fighting than in the symmetrical fights from experiments 1 and 2. The weight loss of losers indicates that the immediate cost of losing was high.

EXPERIMENT 4: TESTING THE DESPERADO HYPOTHESIS

Aim

Although the more natural set-up of experiment 3 influenced the structure of the conflicts considerably, predictors of outcome and subsequent escalation were still observed. We suggest that this is the result of the desperado-like position of the loser: losers continue the fight because alternatives are lacking. To test this hypothesis, we staged a series of conflicts in a set-up that was similar to that in experiment 3 but in which losers could (1) withdraw into a hiding area or (2) choose to attack another territory owner.

Design

We staged 14 conflicts in a 750-litre tank that was divided into six compartments (Fig. 8). Four of these contained male territory owners (A–D) and were each supplied with a flat stone for hiding and a female behind a transparent partition. The fifth fish (the test fish or

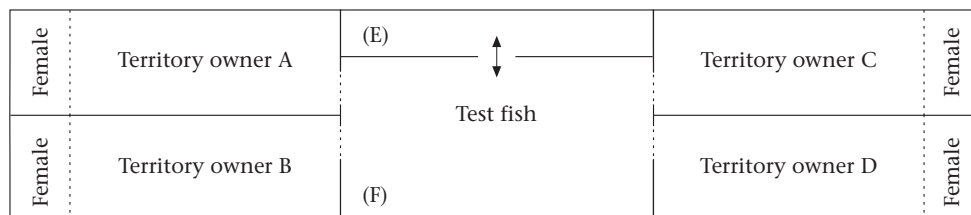


Figure 8. Schematic representation of the set-up in experiment 4. Dotted lines indicate transparent partitions, solid lines opaque ones. The arrow shows that the test fish could swim freely between the two compartments E and F. The four territories (A–D) were connected to F by windows that could be opened or closed, indicated by dotted lines (for details see text).

intruder) could freely swim across a hiding area (E) containing a flat stone for shelter and a central area (F) without any shelter. The four territories were connected to F by windows ($6 \times 8 \text{ cm}^2$) that could be open or closed with either opaque or transparent sheets.

In line with the previous experiments, the four territory owners and the test fish differed in length by less than 8%. There were always two smaller and two larger owners, which were randomly assigned to territories. Both test fish and owners were used more than once, both as owners and intruders (maximum 3 times), but the test fish was not familiar with any of the owners.

Fish were put in the tanks 5 days before the experiment and the windows were closed. The day before the experiment, the four windows were made transparent for seven periods of 15 min each, separated by 30 min. We recorded Tail-beating frequencies for all males, the time that the test fish spent near one of the windows (i.e. the distance between test fish and window was smaller than the test fish's body length) and the time spent on Frontal display. An eighth observation period was carried out immediately before the experiment.

We then removed the females and 15 min later opened the two windows of the largest territory owners. The largest owners were chosen because we wanted the test fish to be the loser in the resulting conflict, since the loser determines the end of the conflict and only the loser was offered alternatives. As soon as the test fish entered a territory, the other window was closed and observation started. For both opponents we recorded Tail-beating frequency, Mouth-fighting duration and Circling duration. The end of the conflict was determined as in the previous experiments.

Results

Pre-exposure observations

During the observations preceding the fights, the test fish spent most of their time near one of the windows ($\bar{X} \pm \text{SE} = 93.69 \pm 4.02 \text{ min}$, averaging 78% of the observation time), without showing consistent preferences for specific territory owners. There was a slight but non-significant trend for Tail-beating frequency to be higher for territory owners than for intruders (paired t test: $t_{13} = -1.76$, $P = 0.10$; owners: $\bar{X} \pm \text{SE} = 14.2 \pm 6.0 \text{ beats/5 min}$; intruders: $8.6 \pm 3.5 \text{ beats/5 min}$). As in experiment 3, owners tended to behave more aggressively (Frontal display: paired t test: $t_{13} = -1.99$, $P = 0.068$; owners: $\bar{X} \pm \text{SE} = 63.1 \pm 12.2\%$; intruders: $27.9 \pm 11.0\%$).

Conflicts

Owners won nine of 14 conflicts and only these are included in the analysis, for reasons explained above. Conflicts lasted $41.4 \pm 17.8 \text{ min}$, which is significantly shorter than those in both the asymmetrical (two-sample t test: $t_{21} = -2.14$, $P = 0.044$) and the symmetrical set-ups ($t_{26} = -2.14$, $P = 0.044$). The assessment phase, defined as the time until Mouth fighting occurred, was significantly shorter than in the previous experiments (2.7 ± 1.69 versus $19.29 \pm 4.38 \text{ min}$ in experiment 3: $t_{21} = 2.91$,

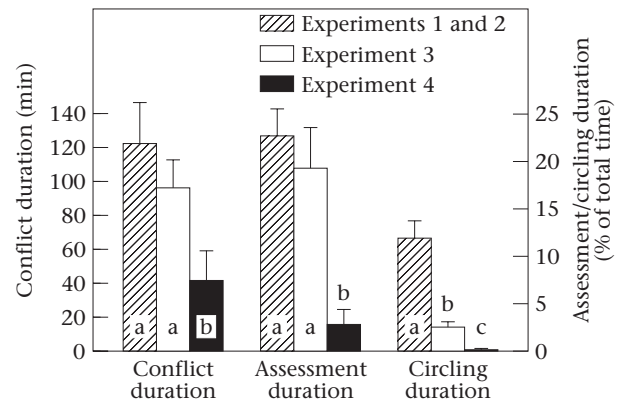


Figure 9. Overview of the results of experiment 4, in comparison with the previous symmetrical and asymmetrical experiments. Vertical bars indicate SE. Significant differences are represented by different characters in the bars. For statistics see text.

$P < 0.01$; and versus $22.78 \pm 2.89 \text{ min}$ in the symmetrical experiments: $t_{25} = 4.66$, $P < 0.001$). There was no difference in Tail-beating performance between opponents that could predict the chances of winning (winners versus losers: 43.11 ± 9.57 versus $33.11 \pm 7.76 \text{ beats/5 min}$; paired t test: $t_8 = -1.07$, $P = 0.32$).

Mouth fighting occurred in all fights. Circling was observed in one conflict only, which is significantly less than in both previous experiments (symmetrical set-up: 16/19; Fisher's exact test: $P < 0.001$; asymmetrical set-up: 8/14; Fisher's exact test: $P = 0.013$). Figure 9 shows an overview of the results in comparison with the previous experiments.

Conclusion

In this asymmetrical, several-options set-up, there were no predictors of conflict outcome. More importantly, escalation was nearly absent. The results thus confirm the hypothesis that fierce escalation, which follows after predictors of outcome have occurred, is caused by desperado losers.

DISCUSSION

Several studies of animal conflict have reported the occurrence of behavioural differences between opponents, predicting conflict outcome. Instead of terminating the interaction on the basis of this information, opponents are reported to engage in prolonged bouts of escalated fighting. We attempted to resolve this paradox by staging aggressive encounters between male cichlids and observing the behaviour of both opponents under different conditions.

In agreement with existing literature (e.g. Enquist et al. 1990; Keeley & Grant 1993; Neat et al. 1998), the structure of the interactions showed a very regular, consistent pattern of behavioural phases: conflicts started with low-risk, noncontact displays, then entered the more energy-consuming phase of Mouth fighting and were concluded with an escalation phase that entailed high injury risk.

During the first phase of the interactions, predictors of conflict outcome were found in two independent series of conflicts, experiments 1 and 2. In both experiments, Tail beating, Fin display and body coloration revealed the chances of winning with reliabilities up to 100%.

As in several other studies, the *A. rivulatus* males continued fighting for long periods and proceeded to escalated fighting even after predictive differences between opponents had been observed. The analysis of respiration frequencies and injuries shows that Circling in particular was very costly and it seems therefore advantageous for any individual to give up a contest as soon as its chances of winning become minimal. We observed the contrary, however. Furthermore, the magnitude of the difference in Tail-beating performance between opponents, that is, the strength of the predictor, did not influence the course of the remainder of the conflict: interactions in which the opponents' behaviour differed greatly during the first phase were not decided earlier, nor did they show less escalated fighting than other conflicts. Similarly, the magnitude of the size difference between opponents had no significant influence on the course of the conflicts.

One obvious explanation for this paradox is that *A. rivulatus* males do not assess the behavioural performance of their opponents relative to their own. One could argue that only with the aid of computer and statistical analysis can one detect the reported differences. However, since these differences reliably predict the chances of winning, selection should strongly favour those individuals that do perceive them. In fact, it seems unlikely that opponents cannot properly interpret the display behaviour in which they invest so much time and energy. Furthermore, prospective losers often showed such a clear and strong increase in overall darkness in contrast to winners that perceptual difficulties seem unlikely.

Predictors of conflict outcome were found for both experienced and inexperienced fish and the reliability of these predictors was not influenced by experience. Thus, a lack of social experience in the experimental animals cannot explain the occurrence of predictive behaviour, as hypothesized earlier. However, the structure and course of the interactions were markedly different for experienced and inexperienced opponents; experienced fish spent more time on low-cost displays and showed less escalated fighting. This suggests that social experience does play a role in aggressive interactions: deciding a conflict without high levels of escalated fighting and subsequent injury appears to be a skill that must be learnt. For the first two series of conflicts, the observed predictive differences between opponents may be explained by the artificial way in which opponents were confronted with each other. After acclimatizing to their territories and females for a week, the males were suddenly confronted with an opponent with exactly the same history. This experimental set-up (which is used in many studies) yields an extremely symmetrical conflict that hardly translates into any natural situation.

In the third experiment we therefore created a more realistic situation by imposing a territory ownership asymmetry and allowing the intruders to assess their

opponents and the resources being contested. As in the symmetrical experiments, winners and losers could be predicted by their behaviour. An important difference, however, was the considerable reduction of the escalation phase, whereas the total duration of the interactions was not influenced significantly.

This result suggests that prospective losers assess the difference between their own performance and the opponent's and react by giving up as soon as the opponent escalates. However, conflict durations remained unchanged. After predictive differences had arisen, fish continued the interaction for more than an hour, suggesting that losers could have economized much more than they did. Thus, the question why fights are not terminated earlier remains.

This issue should be considered separately from the winners' and the losers' perspectives. For prospective winners, we should ask why they do not escalate as soon as the chances of winning are clear. The answer may be that escalation is not the most efficient strategy for winning: we showed that escalation incurs high cost in terms of energy expenditure and injury for both winners and losers. Predictors of outcome did not predict costs, since winners and losers were injured equally severely. In addition, prolonged escalation may not be necessary for winning. Therefore, eventual winners should postpone escalation as much as necessary. Only when information exchange by low-risk display has reached its limit and the opponent still does not withdraw should the eventual winner proceed to escalating. (Of course in more natural contexts, time constraints or predation risk may shift the most effective strategy towards earlier escalation.)

For the loser, it seems obvious that it should withdraw as soon as it perceives the chances of winning to be zero. However, in the case of an incomplete predictability, as found here and in the studies cited, the losing individual must weigh the cost of escalation against the probability and the cost of losing. Losing can be considered costly in two ways. In the short term, the loser of a conflict may be subject to sustained aggression and harassment by the dominant individual. In experiment 3, although we observed very little aggression after the conflicts, losers lost weight whereas winners did not. This suggests that, at least in a confined situation, losing is costly in the short term. Although it is most likely that this weight loss is due to stress and exclusion caused by the winner, it may be caused by a higher cost of fighting for the weaker individual, that is, the loser. In the long term, losing may result in the loss of a territory and thereby exclusion from valuable resources such as food and mates. For instance, if breeding opportunities are at stake and losing signifies failing to breed, even a slight chance of winning may be enough to engage in escalated fighting. If valuable resources are contested, prospective losers may become desperadoes (Grafen 1987). The losers in our owner-intruder experiments may have perceived their situations as desperate, their only chance of reproduction being the take-over of the opponent's territory. In that case, they will not surrender even when they perceive their chances of winning to be minimal, thus behaving like desperadoes sensu Grafen (1987).

To test this 'desperado hypothesis', we staged asymmetrical conflicts in which the intruder had alternative options: losing intruders could withdraw into a safe area and/or choose to intrude into another territory later. If fierce escalation following the occurrence of predictors of outcome, as observed earlier, is caused by desperate losers, then the conflicts in this final experiment should have lacked those characteristics.

Indeed, escalation was nearly absent and conflict duration was significantly reduced. The assessment phase was considerably reduced as well, and predictive behavioural differences were not observed. The assessment phase might have been reduced because it served primarily to communicate ownership, which is a major cue to determine whether to engage in a conflict. Mouth fighting then quickly follows as a more costly and more informative check of the information gained during Tail beating. In the previous experiments, ownership asymmetry was either absent (1 and 2) or not used by the desperado loser as a cue to withdraw, resulting in prolonged assessment phases.

All cichlid species show elaborate display repertoires, including ritualized behaviours such as Tail beating, Fin display, Mouth fighting and Circling. We know that they occur in the field as well (Neil 1983; personal observation) and they must have evolved under natural selection. Escalated fighting does not occur solely as a result of the desperado effect. The cost of escalated fighting must be weighed against the value of winning and as soon as the payoff is high, escalation becomes a successful strategy. Furthermore, it is not the occurrence of escalated fighting per se that results from the desperado effect. It is the prolonged escalation that follows consistent behavioural predictors of outcome that we think is the result of extreme symmetrical experimental set-ups in which losers have no alternatives but to continue the fight.

To conclude, our result should have consequences for the interpretation of staged encounters in the laboratory. The desperado mechanism must be ruled out before laboratory results can be understood in an evolutionary framework. Furthermore, this study indicates that, at least for *A. rivulatus* males, perception of behavioural signals is very accurate. The decision rules that rely on this perception are shown to be complex, taking into account not only the chances of winning and the immediate costs of fighting, but also future perspectives of territory settlement and reproduction.

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