

Size, more than colour, drives dyadic interactions in sub-adults of a colour polymorphic cichlid

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Social and aggressive behaviours often affect the fitness of multiple interacting individuals simultaneously. Here, we assessed dyadic interactions in a colour-polymorphic cichlid fish, the red devil, *Amphilophus labiatus*. We found that sub-adult red devil pairs of smaller body size interacted more, and were more aggressive towards each other, than pairs comprising larger individuals. Interactions did not significantly differ between colour morphs, i.e. between dark, gold and heterotypic pairs. Interestingly, within a broad range of parameters, an automated measure of time that the two fish spent in close proximity was an accurate proxy for their level of aggression, as measured by an observer from video recordings. These results show that, between sub-adult red devils, (aggressive) interactions significantly depend on body size, but not colour morph, of the interacting individuals. In addition, the results support the use of automated data-gathering approaches as an appropriate tool for studies of aggression.

ADDITIONAL KEYWORDS: aggression – *Amphilophus* – automated tracking – behavioural interaction – body size – cichlid fish – colour polymorphism – social behaviour.

INTRODUCTION

A myriad of factors can influence social interactions between individuals. For example, predation and foraging contexts are known to modulate social behaviour in many taxa [e.g. arthropods: [Detrain et al. \(1999\)](#); fish: [Pavlov & Kasumyan \(2000\)](#); reptiles: [Chapple \(2003\)](#); birds and mammals: [Elgar \(1989\)](#)]. In the colonial web-building spider, *Metepeira incrassata*, larger individuals aggressively vie for protected positions in the centre of the colony ([Rayor & Uetz, 1990](#)), while, in Lake Malawi cichlids, less aggressive species form shoals to access areas defended by those that are more aggressive ([Marsh & Ribbink, 1986](#)). Social and aggressive tendencies are commonly context-dependent and can change over an individual's lifetime ([Pavlov & Kasumyan, 2000](#)). For example, in the jewelfish, *Hemichromis bimaculatus*, juveniles shoal until the age of ~100 days, after which the shoal disbands, and individuals become more solitary and

aggressive ([Chen et al., 1983](#)). More generally, such behaviours are often influenced by reproductive or social statuses ([Parker, 1974](#); [Lehtonen et al., 2010](#); [Roth & Sterck, 2020](#)) or phenotypes of the interacting individuals [e.g. body size: [Morrell et al. \(2005\)](#); [Rodgers et al. \(2015\)](#) or colour: [Pryke \(2009\)](#); [Kraft et al. \(2018\)](#)].

Depending on context, individuals that are alike may be more inclined to associate with ([Breden et al., 1982](#); [Wright et al., 2006](#)), or respond more aggressively towards ([Genner et al., 1999](#); [Pauers et al., 2008](#); [Scali et al., 2021](#)), each other than those that are dissimilar, while certain colours, especially red and orange, may also be linked to aggressive behaviour ([Pryke, 2009](#)). More generally, body coloration is known to affect social interactions in the contexts of mate choice ([Hill, 1991](#); [Weaver et al., 2017](#)), group formation ([Green & Leberg, 2005](#); [Rodgers et al., 2010](#)) and aggression ([Pryke, 2009](#); [Lehtonen, 2014](#)). Such colour biases in social interactions may have significant ecological and evolutionary consequences with regard to competition ([Tynkkynen et al., 2004](#); [Andersson & Grether, 2010](#)), reproduction ([Price & Rodd, 2006](#); [Tyers et al., 2021](#)),

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and even speciation potential (Kocher, 2004; Seehausen & Schluter, 2004; Dijkstra *et al.*, 2007).

The red devil, *Amphilophus labiatus*, is a member of the Midas cichlid species complex, in which both sexes, when guarding their breeding territory, display high levels of aggression towards intruders (Lehtonen *et al.*, 2016; Lehtonen, 2017; Sowersby *et al.*, 2017). By contrast, sub-adult individuals, both in the wild and in captivity, commonly associate with conspecifics in loose shoals (comprising two to tens of individuals) to forage (Oldfield *et al.*, 2006; T. Lehtonen, pers. obs.), but can also display aggression (Barlow & Ballin, 1976; Barlow & Siri, 1987; Barlow & Francis, 1988; Holder *et al.*, 1991), albeit less so than reproductively active individuals. In the wild, such social groupings are typically assorted by size, but not sex (Oldfield *et al.*, 2006). Both male and female red devils are polychromatic, being either 'dark' (greyish or brownish) or 'gold' (from yellow to red, usually orange), with the colour polymorphism being determined by an inverted repeat in a single gene (Kratochwil *et al.*, 2022). Such phenotypic differences can be highly relevant to social interactions. Indeed, earlier findings suggest that, in breeding *Amphilophus* cichlids, mating is positively colour assortative (McKaye, 1980; Lehtonen, 2017), heterochromatic pairs show less coordinated parental behaviours (Lehtonen, 2017), mature males tend to be larger than females (Lehtonen, 2017; Sowersby *et al.*, 2017), and aggression is biased towards the colour morph of the aggressor (Lehtonen, 2014), while breeding territory holders of different colour morphs exhibit similar overall levels of aggression (Lehtonen, 2017; Sowersby *et al.*, 2017). In juvenile and sub-adult *Amphilophus citrinellus*, gold individuals were found to be socially dominant over dark ones (Barlow & Ballin, 1976; Barlow, 1983). Furthermore, over a range of size classes, individuals of the two colour morphs differ in their background colour matching abilities (Sowersby *et al.*, 2015). Such differences might be particularly relevant if colour plays an important communication function (Barlow *et al.*, 1977; Lehtonen, 2017) or if it affects an individual's conspicuousness to predators (in the study population, cormorants and other piscivorous birds). Yet, much of what we know about the roles of colour and size in mediating social interactions have come from studies investigating their role in mate choice, and territorial acquisition and defence. By contrast, much less is known about their effects on behavioural interactions in other contexts.

Accordingly, we used sub-adult red devils as our model to assess dyadic interactions, with a particular focus on aggression. The study had the following main aims. First, we tested whether dyadic interactions are influenced by either the body size or colour morph of the interacting individuals. Second, we assessed the

extent to which interactions in dyadic encounters (pairs) of sub-adult red devils are driven by aggression. Here, we investigated both the output of an automated tracking system (see Material and Methods for details) and video recordings, and compared the results of the two approaches. Automated tracking is increasingly being employed in behavioural research, including in fish. However, apart from a few model organisms [e.g. zebrafish, *Danio rerio*: Green *et al.* (2012)], researchers very rarely cross validate automated tracking approaches with more conventional manual scoring of behaviour (either live or from video recordings). This is surprising, particularly when proximity between test subjects could be motivated by a variety of reasons (i.e. not just aggression), thus making it critical to compare and validate the different approaches.

MATERIAL AND METHODS

Red devils for the study came from Hazelwood Pondage in south-eastern Australia (lat. 38° 17.87' S, long. 146° 21.54' E). In the lake, the fish were exposed to warm ($\geq 25^\circ\text{C}$) and relatively murky water, similar to many parts of their native range in Nicaragua (Sowersby *et al.*, 2020). More than half of the individuals of this feral Australian population are of the gold colour morph (Sowersby *et al.*, 2015; B. Wong *et al.*, unpublished capture data), which contrasts with the much lower proportion of gold individuals found in their native range (Elmer *et al.*, 2010), while the sex ratio is relatively even (not significantly different from 1:1; T. Lehtonen & B. Wong, unpublished data, 2013). Red devils were collected using handlines in October 2015. Fish were transported to the Clayton campus of Monash University and housed in multiple stock tanks [max. 20 individuals per tank; $N = 2$: 95 cm (length) \times 45 cm (width) \times 43 cm (water level), each with a separate 95 cm \times 45 cm \times 25 cm filter compartment \approx 290 L; $N = 3$: 60 cm \times 45 cm \times 36 cm \approx 97 L] that had sand and gravel as a substrate and several rocks and sections of PVC pipe for shelter. Tanks were maintained at a temperature of $\sim 25^\circ\text{C}$ on a 12 h:12 h light:dark cycle. Fish were fed daily on commercial fish food pellets. The proportion of gold individuals in each tank was always more than 50% but less than 100%, reflecting the higher proportion of the gold morph in the lake (Sowersby *et al.*, 2015). At the time of the experiments, the fish were sub-adults (immature, possibly close to maturation) and between 105 and 150 mm in total length (TL). Because sexing sub-adult individuals would have necessitated killing the fish, their sex remained unknown.

The trials were conducted in April 2016. To specifically investigate the effects of fish size and colour morph on dyadic interactions, and to evaluate

the utility of an automated tracking system (see below) for measuring them, we ran social interaction assays on six ‘batches’ of four fish. In each experimental batch, we had two dark and two gold individuals, with a total of four dyadic encounters staged between them (see below for details). Hence, in total, the study included 12 dark (mean total length \pm SE: 125 ± 4 mm) and 12 gold (124 ± 3 mm) red devils. This sample size was defined by logistic limitations related to catching the fish, keeping them at reasonable densities in the laboratory, the availability of the less numerous dark morph individuals, and the need to retain some of the stock fish for other, unrelated studies. The four fish within a batch had been stocked in different tanks prior to experimentation to eliminate the possibility of prior social interactions or pre-established dominance hierarchies from influencing the results of the dyadic encounters. For each batch, we ran in total four trials, two at a time, with interactions between two fish being tested in each trial. The two trials in one of the two rounds were made up of homotypic pairings (i.e. one dark \times dark pair and one gold \times gold pair), while, in the other round, we ran two trials of heterotypic pairings (i.e. dark \times gold). Thus, each individual was tested twice, once with a homotypic pair and once with a heterotypic pair. In both rounds, the pairs were formed blind to the behaviour of the fish, which was only investigated later. For each batch, we alternated whether the two homotypic pairings or two heterotypic pairs were tested first. The four individuals within a batch were visually matched for size, within the limitations of fish availability (mean \pm SE difference in total length: 5 ± 0.6 mm, $N = 24$ assessed pairs). Size matching was done because sub-adults tend to group with individuals of the same size (Oldfield *et al.*, 2006; T. Lehtonen, pers. obs. in Australia and Nicaragua) and the availability of fish for the current study did not allow for studying the effects over a range of size differences.

At the beginning of each trial, two fish were placed into a test arena, consisting of a 50 cm \times 49 cm \times 30 cm glass tank filled with water to a depth of approximately 26 cm. After 30 sec, we initiated the recording of behaviours, with each trial running for 30 min. After the completion of two simultaneous trials in the first round, each of the four fish were caught and placed into individual containers. Approximately 10 min later, we carried out a second round of two simultaneous trials, the procedure for which was identical to the one used in the first round of trials, except that each fish was paired with a different individual. After the assays, each fish was quickly digitally photographed (not anesthetized) against a sheet marked with 1 mm grid lines, which was later used as a scale for assessing the TLs of the fish, using ImageJ 1.51k software (NIH, USA, <http://imagej.net/ImageJ>). After photography,

each fish was returned to a new stock tank to ensure that individuals were only used in one batch and, hence, were not reused in later trials.

The trials were run using the Zebcube automated behavioural monitoring system (Viewpoint Inc., France), comprising two enclosed units (hereafter referred as ‘cubes’) that were connected to a single computer that processed the behavioural data. One test arena was placed within each cube, and during the trials, the doors of the cubes were closed, insulating the test arenas from external disturbances, such as light and sound. Each cube had a wide-angle lens, encircled by an adjustable white LED light source (peak wavelength: 453 nm), mounted in the ceiling. The lens captured monochrome videos of the fish within the arena. The two cubes were individually connected via a high-speed data cable to a custom-built video card attached to a computer which was equipped with Zebrelab software (Viewpoint Inc, France) programmed to analyse video input from the cubes via pre-set analytical modules. Since we were interested in assaying dyadic interactions, we analysed the video files in the ‘social-contact’ module, which enabled us to define a distance between the pair of fish within the arena (in a two-dimensional XY plane), as a proxy for social contacts. Specifically, whenever the fish were within a pre-specified ‘threshold distance’ (see below), Zebrelab considered them to be interacting. The cubes were housed in a constant temperature (26 °C) room with ambient humidity. We set the brightness within the cubes at 500 lux for the fish to see their surroundings and manually adjusted the ‘detection threshold’ (unit-less metric to differentiate signal from noise in the arena) to ensure that the software tracked the fish rather than visual distractions, such as large air bubbles or reflections.

With this set-up, we extracted the following measures of interactions between the focal pair of fish. First, ‘contact duration’ (denoted as ‘fusion duration’ in the software) was defined as the cumulative time the pair of fish spent so close to each other that the software considered them to be engaged in full contact (i.e. fused together). Second, ‘proximity duration’ (denoted as ‘contact duration’ in the software) gave the cumulative time spent by the pair in the pre-determined proximity zone in the XY plane over the 30-min assay period. We defined ‘proximity’ by setting four different threshold distances: 2 cm, 5 cm, 8 cm and 11 cm between the two fish. These thresholds covered the full range of distances that we, based on earlier observations, considered relevant for interacting sub-adult fish in an aquarium setting. This approach also allowed us to assess the effect of the parameter settings on the assessment of social interactions.

Finally, after having observed that interactions between fish of all phenotypes were often aggressive,

we assessed the extent to which the above automated interaction measures correlated with manually assessed aggressive encounters. For this purpose, we watched the video recordings provided by Zebracube and tallied the number of occasions the two fish came within approximately 5 cm of each other. Each of these events was scored in the following fashion: 0 when neither fish displayed aggression, 1 when only one of the two fish was behaving aggressively, with the other being inactive or retreating away from the aggressive individual, or 2 when both fish were behaving aggressively. Typically, aggressive behaviours consisted of displays, such as flared out gill covers, tail lashing (with the fish sometimes circling each other), body shakes or quivers. Infrequently, one-sided aggression (score: 1) escalated into bites, and mutual aggression (score: 2) escalated into jaw locks. The sum of these scores per pair is hereafter referred to as 'aggression score'. A similar score, the 'total rate of aggression', was used by some previous studies that assessed aggression displayed by a single focal aggressor (e.g. Lehtonen, 2017; Sowersby *et al.*, 2017). When the fish were not aggressive, they either rested at various distances from (or even on top of) each other or swam around the arena. Due to a technical failure, no video recordings from two out of the 24 pairs were available for the analysis of manual aggression.

EFFECTS OF SIZE AND COLOUR MORPH ON SOCIAL INTERACTIONS

All analyses were performed in R 3.6.2 software (R Development Core Team, 2019). To analyse whether colour morph combination (three levels: dark \times dark vs. gold \times gold vs. heterotypic) or the average size (TL) of the two interacting fish influenced interactions within the dryad, we ran a separate linear mixed model ('nlme' package) for each of our interaction measures: contact duration, proximity duration (with the four different thresholds) and aggression score. In each case, the assumptions of normality and homogeneity of variances were met [assessed with the Shapiro–Wilk test (function: shapiro.test) and the Bartlett test (function: bartlett.test)], with contact duration being log-transformed for the analysis. In each model, the interaction measure was denoted as the response variable, and fixed effects included colour morph combination, average size and morph combination \times average size interaction. In addition, the size difference between the two fish in each pair (because of imperfect visual size matching) and the test order (i.e. whether the two focal fish were being tested for the first or second time) were added as covariates in all models. Batch ID was added as a random effect. The fixed effects were interpreted using the F-tests generated by the 'anova' command.

RELATIONSHIPS BETWEEN THE DIFFERENT INTERACTION MEASURES

To assess relationships between the different aggression and interaction proxies, we ran Pearson's correlation tests between the different behavioural measures (aggression score, contact duration, and proximity duration with the different settings). As above, contact duration was log-transformed to ensure it conformed to a normal distribution.

ANIMAL ETHICS STATEMENT

All applicable national and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the study was conducted (Animal Ethics Committee of Monash University, Australia: BSCI/2012/23 and BSCI/2016/10).

RESULTS

EFFECTS OF COLOUR MORPH AND SIZE ON SOCIAL INTERACTIONS

The full results tables are provided as supplementary material (Supporting Information, Tables S1–S6). These show that the average size (TL) of the two interacting fish had a significant negative effect on aggression score (linear mixed model, $F_{1,9} = 5.283$, $P = 0.047$, Supporting Information, Table S1), contact duration ($F_{1,11} = 19.61$, $P = 0.001$, Supporting Information, Table S2) and proximity duration with the 5 cm threshold ($F_{1,11} = 5.191$, $P = 0.044$, Supporting Information, Table S3). In addition, there was a marginally non-significant trend towards the same direction with the 8 cm threshold ($F_{1,11} = 4.141$, $P = 0.067$, Supporting Information, Table S4) but not with 2 cm ($F_{1,11} = 3.114$, $P = 0.11$, Supporting Information, Table S5) or 11 cm thresholds ($F_{1,11} = 2.421$, $P = 0.15$, Supporting Information, Table S6). In particular, pairs made up of small fish interacted more than those comprising larger fish (Fig. 1). Colour morph combination of the interacting pair did not have a significant effect on aggression score ($F_{2,9} = 1.739$, $P = 0.23$, Supporting Information, Table S1; Fig. 1), contact duration ($F_{2,11} = 0.918$, $P = 0.43$, Supporting Information, Table S2) or proximity duration with any of the spatial thresholds examined ($F_{2,11} \leq 0.2014$, $P \geq 0.82$, Supporting Information, Table S3–S6). Similarly, the colour morph combination \times size difference interaction ($F_{2,11} \leq 1.034$, $P \geq 0.39$, Supporting Information, Table S1–S6), size difference between the two interacting fish ($F_{1,11} \leq 0.5808$, $P \geq 0.46$, Supporting Information, Table S1–S6), and whether the fish were being tested

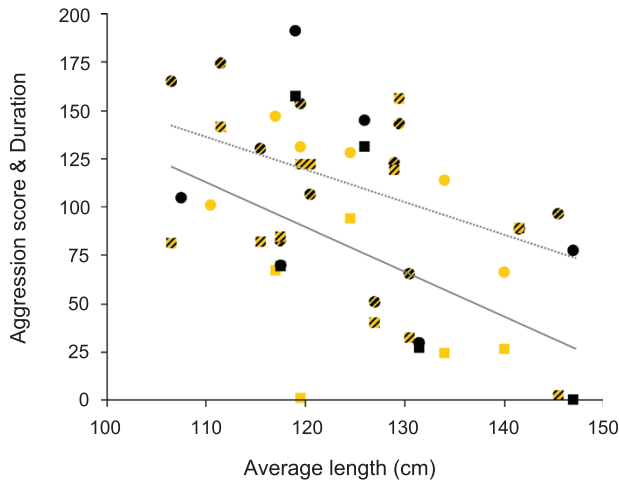


Figure 1. The average total length of each red devil cichlid pair in relation to aggression score (squares and solid trend line, $r = -0.49$, $N = 22$) and proximity duration (with 5 cm threshold, circles and dashed trend line, $r = -0.47$, $N = 24$). Pairs with two dark individuals, two gold individuals, and one dark and one gold are marked with solid black, solid gold and diagonally striped black&gold markers, respectively. Aggression score and duration are both on the same scale on the y-axis with the latter presented as the number of 10 sec (1/6 min) time periods.

for the first or second time ($F_{1,11} \leq 1.666$, $P \geq 0.22$, Supporting Information, Table S1, S3–S6) did not have a significant effect on any of the response variables, with one exception. In particular, contact duration was lower when the fish were being tested for the first time ($F_{1,11} = 11.12$, $P = 0.0067$, Supporting Information, Table S2).

RELATIONSHIPS BETWEEN THE DIFFERENT INTERACTION MEASURES

The measures generated by the Zebracube set-up—contact duration and proximity duration—correlated with the manually assessed aggression score (Pearson's correlation, contact duration: $r = 0.428$, d.f. = 20, $P = 0.047$; proximity duration, 2–11 cm: $r \geq 0.524$, d.f. = 20, $P \leq 0.012$; Fig. 2), with the different thresholds yielding similar information (Fig. 2).

DISCUSSION

We found that levels of interaction and aggressiveness within pairs of sub-adult red devils are linked to their size; pairs comprising smaller fish spent more time interacting with each other, and being aggressive, than larger pairs. Such a result may seem surprising in the sense that larger individuals often dominate contests in fish (e.g. Turner, 1994; Chellappa *et al.*, 1999;

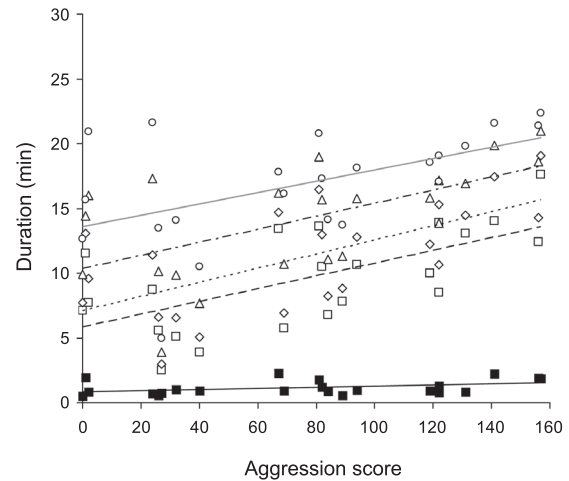


Figure 2. The relationship between aggression score and contact duration [black squares, solid trend line, raw data (shown): $r = 0.39$, $N = 22$; log-transformed: $r = 0.43$, $N = 22$], proximity duration with 2 cm threshold (empty squares, the lowest level dashed line, $r = 0.65$, $N = 22$), 5 cm threshold (diamonds, the second lowest dashed line, $r = 0.65$, $N = 22$), 8 cm threshold (triangles, the highest dashed line, $r = 0.60$, $N = 22$) and 11 cm threshold (circles, the solid highest line, $r = 0.52$, $N = 22$).

Takahashi *et al.*, 2001; Prenter *et al.*, 2008), including *Amphilophus* cichlids (Barlow, 1983). Moreover, the rate of aggression has, in some cases, been found to be associated with the body size of territory owners or intruders (e.g. Beeching, 1992; Wacker *et al.*, 2012; Lehtonen, 2014). The current study, however, suggests that, in sub-adult fish, smaller individuals use a more aggressive interaction strategy than larger ones. This pattern can be adaptive over a range of size classes when, for example, aggression is less costly between two small, as opposed to large, individuals, with the latter being more capable of inflicting injuries if the contest escalates (see Barlow, 1983). It remains possible, however, that this strategy only applies in sub-adult individuals, and that mature individuals might interact differently. The current result is also in accordance with the previously suggested scenario that smaller fish may behave aggressively to compensate for their size and to leave less opportunity for their opponents to make an accurate assessment of their competitive phenotype (Moretz, 2003; Morrell *et al.*, 2005; Svensson *et al.*, 2012). Here, we mimicked a typical natural social setting (Oldfield *et al.*, 2006; T. Lehtonen, pers. obs.) by measuring interactions among individuals of approximately the same size. Other aspects of the social, as well as physical, environment may also affect the patterns of aggression observed in cichlid fish. For example, the question of whether background colour or light intensity affects aggression

provides an interesting avenue for future research (see [Dijkstra et al., 2005](#); [Sowersby et al., 2015](#)). Moreover, similar to other laboratory-based experiments, the confined space in our study potentially intensified aggression compared to conditions experienced in the wild.

Small differences in TLs of interacting individuals, due to our imperfect size matching, did not significantly affect any of the assessed behavioural variables. We assume that this was due to factors other than such small body size differences being important drivers of individual—and between pair—differences in aggression and other interactions ([Dall et al., 2004](#); [Kortet & Hedrick, 2007](#); [McGhee & Travis, 2010](#); [Lichtenstein & Pruitt, 2015](#)). However, had we allowed larger size differences between the interacting fish, we may well have observed less interaction with increasing size difference ([Shaw, 1978](#); [Pavlov & Kasumyan, 2000](#)).

In contrast to body size, we did not find any significant effect of colour on aggression levels in our study. Colours and colour patterns are relevant in the context of aggressive and other interactions in many animals ([Pryke, 2009](#)), including cichlids ([Dijkstra et al., 2006](#); [Pauers et al., 2008](#); [Anderson et al., 2016](#); [Tyers et al., 2021](#)) and other fish ([Tinghitella et al., 2015](#); [Kraft et al., 2018](#)). Within the Midas cichlid species complex, *Amphilophus sagittae* individuals that defend their breeding territories/offspring in the wild bias aggression towards intruders of their own colour type ([Lehtonen, 2014](#)). Similar aggression biases have also been observed in African cichlids ([Pauers et al., 2008](#); [Tyers et al., 2021](#)). Differently coloured individuals may also differ in their abilities to adjust their coloration and, hence, effectively respond to one another based on visual signals and cues ([Barlow et al., 1977](#); [Sowersby et al., 2015](#); [Lehtonen, 2017](#)). The reasons for why we, nevertheless, did not find differences between the behaviour of dark, gold and heterotypic pairs, could include the relatively young age of sub-adult individuals, the non-breeding context of our dyadic encounters, and behavioural differences between species or populations within colour polymorphic *Amphilophus* cichlids. Finally, our sample size may not have been large enough to detect more subtle colour biases in behaviour (if they exist).

The aggression score we manually assessed from the video recordings correlated with both contact duration and proximity duration, independent of the cut-off setting of the latter (2–11 cm). These findings are comparable to those of [Scherer et al. \(2017\)](#), who found that the average distance to a perceived intruder was a good proxy measure for male aggressiveness in an African riverine cichlid, the kribensis, *Pelvicachromis pulcher*. Hence, taken together, the results show that both the average distance between fish and time they spent in close proximity (here: ‘proximity duration’) can be reliable

measures of aggression levels in cichlids. In this regard, the automated data-tracking approach provides a promising tool for future studies to test for the intensity of social interactions. It is worth noting, however, that the tightness of the correlation between proximity duration and average body size of the interacting fish varied depending on the distance threshold used, with the 5 cm setting providing the strongest correlation. This result could be explained by different behaviours being performed at slightly different distances, with the 5 cm threshold being particularly effective at capturing a range of aggressive displays. Similarly, only contact duration was affected by whether the fish were being tested for the first or second time, and this measure also had the lowest correlation with aggression score, indicating that it was, to a larger extent, defined by behavioural patterns other than aggression.

To conclude, we found that, in dyadic encounters, smaller pairs of red devils were more aggressive and interacted more with each other than did larger pairs. This indicates that the association strategy of red devils depends on body size, as also seen in some other species of fish ([Moretz, 2003](#); [Morrell et al., 2005](#); [Svensson et al., 2012](#)). In contrast, independent of the measure used, association patterns did not significantly differ depending on the colour of the interacting individuals within the pair. We also found ‘proximity duration’ (e.g. with 5 cm threshold) to be a good proxy for assessing aggression in these cichlids. This finding should be applicable to other comparable set-ups that score behavioural interactions.

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DATA AVAILABILITY

The data are available as supplementary material ([Supporting Information, Table S7](#)).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. GLMM result table showing the effects of the assessed variables on aggression score, measured for pairs of red devils. For all GLMMs, we used the 'nlme' package and the 'lme' function.

Table S2. GLMM result table showing the effects of the assessed variables on contact duration in pairs of red devils.

Table S3. GLMM result table showing the effects of the assessed variables on proximity duration in pairs of red devils when a 5 cm proximity threshold was used.

Table S4. GLMM result table showing the effects of the assessed variables on proximity duration in pairs of red devils when an 8 cm proximity threshold was used.

Table S5. GLMM result table showing the effects of the assessed variables on proximity duration in pairs of red devils when a 2 cm proximity threshold was used.

Table S6. GLMM result table showing the effects of the assessed variables on proximity duration in pairs of red devils when a 11 cm proximity threshold was used.

Table S7. The data set that the results (Supporting Information, Tables S1-S6) are based on.