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Out-group threat promotes within-group affiliation in a cooperative fish

Running title: Consequences of out-group threats

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Abstract

In social species, conflict with outsiders is predicted to affect within-group interactions, and thus influence group dynamics and the evolution and maintenance of sociality. While empirical evidence exists for a relationship between out-group conflict and intragroup behaviour in humans, experimental tests in other animals are rare. In a model fish system, we show that simulated out-group intrusions cause post-conflict increases in intragroup affiliation, but no changes in post-conflict intragroup aggression. Post-conflict affiliation was greater following intrusions by neighbouring compared to non-neighbouring individuals; neighbours represent greater threats to the dominance rank and breeding success of residents, and are visible in the aftermath of the intrusion. By providing strong evidence of a link between out-group conflict and post-conflict intragroup behaviour, and demonstrating that intragroup affiliation is affected by the nature of the out-group intrusion, our study shows the importance of considering post-conflict behaviour for our understanding of cooperation and social structure.

Background

In many social species, stable persistent groups of individuals defend collective territories (Kitchen and Beehner 2007; McComb et al. 1994; Radford 2003) and face a variety of threats from outsiders. Conspecific groups or coalitions may invade in an attempt to annex the territory or acquire critical resources contained within it (Radford and Du Plessis 2004; Ridley 2012), while individual intruders might indicate the imminent attack of other groups (Herbinger et al. 2009) or pose a challenge to particular group members in terms of their position or reproductive success (Kleiber et al. 2007; Mares et al. 2012). Studies on a wide range of taxa have considered the immediate defensive responses to such out-group threats and the factors determining the outcome of interactions with outsiders (Desjardins et al. 2008; Kitchen and Beehner 2007; Kleiber et al. 2007). However, there has been far less investigation of the impact of out-group conflicts, after intrusions and associated defence have ceased, despite the likely influence on group dynamics, individual fitness and social evolution (Bowles 2009; Radford 2008a; Radford and Fawcett 2014; van Schaik 1989).

Theoretical work has long predicted that conflict with outsiders should affect subsequent behaviour among group-mates (Alexander and Borgia 1978; Hamilton 1975). For instance, an increased out-group threat should favour higher levels of cooperation, especially if cohesion between group members is important for success (Reeve and Hoelldobler 2007); greater within-group affiliation or redirected aggression may result if conflict with out-group rivals generates tension or stress between group-mates (Von Holst 1998). Considerable human research using economic games has shown that cooperation between subjects, and punishment of non-cooperators, increases when current pay-offs are directly affected by competition from other groups (Erev et al. 1993; Gneezy and Fessler 2012; West et al. 2006). However, to understand more fully the evolutionary roots of sociality, experiments investigating post-conflict behaviour are required in other species, because species-specific differences in sociality and cooperation could affect intragroup behaviour (Polizzi di Sorrentino et al. 2012; Radford 2008b) and out-group conflict is expected to be a major selective force through its potential impacts on both survival and reproduction (Mares et al. 2012; Polizzi di Sorrentino et al. 2012; Wilson and Wrangham 2003). To our knowledge, there have been only two experimental studies on non-human animals examining the impact of

simulated threats from out-group rivals on post-conflict within-group behaviour. Radford (2008b) found that playback of vocal choruses from rival groups led to an increase in within-group affiliative behaviour in a wild population of green woodhoopoes (*Phoeniculus purpureus*), a cooperatively breeding bird. Polizzi di Sorrentino et al. (2012) found no change in within-group affiliation, but increased within-group aggression in a single captive group of tufted capuchin monkeys (*Cebus apella*) when visual aggressive interactions with a rival group were allowed.

The extensive literature on conflict between members of the same group (intragroup conflict) suggests that subsequent behaviour is affected not just by the occurrence of the interaction, but by the characteristics of the conflict, the identity of those involved and the relationship between them (Aureli et al. 2002; Schino et al. 1998). Different out-group rivals can represent different levels of threat to the group or to particular group members (Mueller and Manser 2007; Radford 2005), conflicts with outsiders can vary greatly in their duration and intensity (Radford and Du Plessis 2004; Wich and Sterck 2007), and individuals of different dominance status, sex and age may not contribute equally to interactions with intruders (Bruintjes and Taborsky 2008; Heinsohn and Packer 1995). Yet, little is known about the importance of these differences in out-group conflict characteristics and threat levels for post-conflict behaviour; Radford (2008a) provides the only experimental test, finding a greater increase in post-conflict within-group affiliation in green woodhoopoes following playback of stranger groups compared to neighbours.

Here we investigate how interactions with out-group rivals affect post-conflict intragroup behaviour in the cooperatively breeding cichlid fish *Neolamprologus pulcher*. Groups of this species are territorial and all group members, including the dominant breeding pair and subordinates of both sexes, contribute to defence against conspecifics (Bruintjes and Taborsky 2008; Desjardins et al. 2008; Taborsky and Limberger 1981). Intrusions by out-group individuals can represent a threat to the position of existing, similarly sized group members (Balshine-Earn et al. 1998). Discrimination of conspecifics is possible from visual cues alone (Balshine-Earn and Lotem 1998; Hert 1985). Clear and easily quantifiable behavioural acts (e.g. affiliation and aggression) are common between group-mates (Mileva et al. 2009; Reddon et al. 2012; Taborsky 1984). Small experimental groups of unrelated individuals are representative of natural situations where there is high predation pressure and rapid turnover rates of breeders (Dierkes et al. 2005; Heg et al. 2004).

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Numerous previous studies have used captive populations to answer a range of evolutionary, developmental and behavioural questions (Bruintjes and Radford 2014; Taborsky 1984; Zöttl et al. 2013).

We conducted experimental territorial intrusions to answer two main questions. First, how does conflict with out-group individuals affect subsequent intragroup behaviour? We predicted that intragroup affiliation would increase during the post-conflict period (see Radford 2008b). Post-conflict intragroup aggression might be expected to decrease if group members are enhancing group cohesion (Cords 2002) or to increase if there is redirected aggression (Polizzi di Sorrentino et al. 2012). Second, does the nature of the out-group conflict influence subsequent intragroup behaviour? We compared two biologically relevant scenarios: (1) intrusions by non-neighbouring individuals (who were not visible before or after the intrusion); and (2) intrusions by neighbouring individuals (who were visible in their own neighbouring territory before and after the intrusion). In *N. pulcher*, resident group members show strong defensive responses to intrusions by unfamiliar individuals (Desjardins et al. 2008), but neighbours potentially represent the greater overall threat to the position in the group and breeding success of individual residents (Bruintjes et al. 2011; Dierkes et al. 2005; Stiver et al. 2004). Thus, if post-conflict behaviour is related to aggression levels against intruders, we predicted greater changes in intragroup behaviour following intrusions by non-neighbouring individuals, whereas if post-conflict behaviour relates to out-group threat level, we predicted stronger intragroup behaviour following neighbour intrusions.

Methods

Study Species and Husbandry

Neolamprologus pulcher is found in Lake Tanganyika in groups consisting of a dominant pair and 0–16 subordinate helpers (Balshine et al. 2001; Taborsky and Limberger 1981). All group members defend the territory against conspecific and heterospecific intruders (Bruintjes and Taborsky 2011; Taborsky and Limberger 1981). Groups typically consist of unrelated and related individuals with a low overall relatedness (Stiver et al. 2005) and subordinates trade cooperative behaviour to be allowed to stay in the territory (Bergmüller et al. 2005b; Fischer et al. 2014). See Appendix for details of study population and husbandry, as well as full methodological details.

Experimental Set-up

Two experiments were conducted, in July–August 2012 and June–July 2013. For each experiment, 14 new groups of three fish comprising a dominant male, a dominant female and a female subordinate were formed in 80-L transparent aquaria following standard methods (Bruintjes and Taborsky 2008). Female subordinates were used because they do not impose reproductive costs compared to subordinate males (Heg et al. 2008). Dominant males were >5 mm larger than dominant females, which were >5 mm larger than subordinates. Each aquarium had two clay flower-pot halves in the middle for hiding and breeding.

Pairs of aquaria were placed with their short sides circa 3 mm apart, to form neighbouring groups (Fig. A1 Appendix). Neighbouring groups were arranged so that they were not visible by fish in other aquaria and neighbouring individuals were carefully size-matched. Stable groups (those where all individuals were free to move throughout the territory without being harassed by fellow group members, cf. Bruintjes and Radford 2013) were given >7 days to become accustomed to their neighbours before an experiment. In Experiment 1, we were unable to stabilise two groups and therefore no data were collected from these (resulting in n=12 groups); all groups were available for data collection in Experiment 2 (n=14).

Both experiments involved controlled intrusions into resident territories. To standardize those intrusions, we used three clear Plexiglas presentation cylinders that were either empty (control) or contained one conspecific fish each. Conspecific fish were either the three neighbouring individuals or size- and sex-matched individuals from a non-neighbouring group. The presentation cylinders were placed 5 cm apart, circa 5 cm from the entrance of the breeding shelters, for 10 min per intrusion.

In Experiment 1, the 12 resident groups received two treatments: (1) presentation of empty cylinders (control), with neighbours visible before, during and after the presentation; and (2) territorial intrusions by neighbouring individuals, who were visible in the adjacent tank before and directly after the intrusion. In Experiment 2, 14 different resident groups also received two treatments: (1) territorial intrusions by neighbouring individuals, who were again visible in the adjacent tank before and after the conflict; and (2) territorial intrusions by non-neighbouring individuals (from an aquarium was not visible to the residents before or after the intrusion), with neighbours present in the adjacent tank before, during and after the

intrusion. Resident groups received one intrusion per day, with the two treatments separated by 1–4 days, but conducted within the same 2 h window; treatment order was counterbalanced in both experiments.

Data Collection and Analysis

All behavioural definitions followed previous work on the study species. During each experimental intrusion, we recorded frequencies of overt attacks (ramming, biting and tail beats) and aggressive displays (fast frontal approach, fin and opercula spreading, head-down display and S-shaped bending) directed by each of the three resident group members towards the presentation cylinders (Bruintjes et al. 2010); individuals were identified by size. In Experiment 2, we also recorded activity levels of the intruders on a scale ranging from 0 (no movement) to 5 (very active) (Bruintjes and Taborsky 2011).

To examine the influence of out-group intrusions on post-conflict behaviour, we recorded frequencies of two types of intragroup behaviour during the 10 min before and the 10 min immediately after an intrusion (i.e. when the cylinders were removed from the focal tank): (a) affiliation (soft touches [also called 'bumps'], parallel swimming and following: (Mileva et al. 2009; Reddon et al. 2012)); and (b) aggression (overt attacks and aggressive displays; see before). For the two types of behaviour, we recorded both which individual displayed or initiated the act and to which group member it was directed.

Paired-sample t-tests and Wilcoxon matched-pairs signed-ranks tests were used to compare total occurrences of a given behaviour exhibited by all group members in the two treatments in a given experiment. We then used linear mixed-effects models (LMMs) to explore whether group member types (dominant male, dominant female, subordinate) were differentially involved in the different behaviours per treatment. Separate paired-sample t-tests or Wilcoxon tests were performed to test treatment differences per group member type. For aggression (directed at out-group intruders and between members of the same group), separate tests were conducted on the frequency of overt attacks and aggressive displays and on the combined total. When considering intragroup behaviour, separate analyses were conducted for the initiation/donation of an act and for its receipt. If a particular type of group member never initiated or received a particular behaviour in any of the groups, they were excluded from that analysis. In all mixed models, the random effect individual was included to correct for the repeated measures design. For all

intragroup behaviours, we analysed the differences between the pre- and post-intrusion periods. All behavioural data are deposited in the Dryad digital depository: http://dx.doi.org/10.5061/dryad.3r3v3 (Bruintjes et al. 2015).

Results

Experiment 1: Effect of out-group intrusions

Simulated intrusions into a neighbouring territory had the expected effect on the immediate defensive behaviour of residents. Significantly more total acts of direct aggression (overt attacks and aggressive displays combined) were performed towards cylinders containing out-group individuals than towards empty cylinders (control condition) (LMM, treatment: $F_{1,33}$=110.66, p<0.001). However the treatment-based difference was significantly affected by group member type (treatment*group member: $F_{2,33}$=27.90, p<0.001; group member: $F_{2,33}$=31.21, p<0.001; Fig. 1a). While all three group member types attacked out-group individuals more than empty cylinders (paired t-tests on treatment differences, dominant males: t_{11}=7.60, p<0.001; dominant females: t_{11}=7.21, p<0.001; subordinates: t_{11}=2.24, p=0.046), dominant males exhibited a greater increase in defence than both dominant females (independent-samples t-test: t_{22}=2.16, p=0.042) and subordinates (Mann Whitney U test: W=78.0, n_1=n_2=12, p<0.001), and dominant females increased their defence more than subordinates (W=80.0, n_1=n_2=12, p<0.001). Qualitatively similar results were found when considering overt attacks and aggressive displays separately (see Appendix).

There was a significantly greater increase in total post-conflict intragroup affiliation following intrusion of out-group individuals compared to the presentation of empty cylinders (paired t-test: t_{11}=5.12, p<0.001; Fig. 2a). Affiliation donation increased significantly more following out-group intrusion compared to the control condition (LMM, treatment: $F_{1,33}$=17.41, p<0.001), with all three group member types showing a similar treatment-based difference (no significant treatment*group member interaction: $F_{2,33}$=0.21, p=0.813; group member: $F_{2,33}$=2.82, p=0.074; Fig. 2b). Received affiliation also increased significantly more following intrusions of out-group individuals compared to the control condition (treatment: $F_{1,33}$=21.74, p<0.001). However, the treatment-based difference was significantly affected by group member type (treatment*group member: $F_{2,33}$=7.15, p=0.003; group member: $F_{2,33}$=6.46, p=0.004;

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Fig. 2c). Dominant males (paired t-test: t_{11}=3.45, p=0.005) and dominant females (t_{11}=3.89, p=0.003) received more affiliation following intrusions of out-group individuals compared to the control condition, but subordinates received similar levels of affiliation in both treatments (Wilcoxon test: Z=-0.11, n=12, p=0.914).

No significant differences in post-conflict intragroup aggression were detected between treatments (out-group intruders versus empty cylinders) when considering either overall responses (Wilcoxon test, overt attacks: Z=-0.36, n=12, p=0.720; aggressive displays: Z=-0.15, n=12, p=0.878; all aggressive acts combined: Z=-0.24, n=12, p=0.812) or those at an individual level (Appendix).

Experiment 2: Effect of intrusions by neighbouring versus non-neighbouring individuals
Simulated intrusions of non-neighbouring individuals led to higher total levels of defence behaviour by residents than intrusions from neighbours (LMM: treatment: $F_{1,39}$=4.39, p=0.043). While group member types differed significantly in their overall contributions to defence (group member: $F_{2,39}$=11.42, p<0.001), with subordinates contributing significantly less than both dominant males (independent-sample t-test: t_{26}=4.41, p<0.001) and females (t_{26}=26, p<0.001) who did not differ significantly (t_{26}=0.66, p=0.515), all three types showed qualitatively the same greater defensive response to non-neighbour (no significant treatment*group member interaction: $F_{2,39}$=0.95, p=0.397; Fig. 1b). Qualitatively similar results (higher levels of defence against non-neighbours by all group member types) were found when considering only overt attacks, whereas there was no significant difference in the frequency of aggressive displays between treatments (see Appendix). The greater levels of aggression exhibited towards non-neighbours compared to neighbouring individuals was not a consequence of any significant difference in activity levels of the two intruder types (Mann-Whitney test: Z=-1.65, n=28, p=0.100).

Post-conflict intragroup affiliation increased following both treatments, but was significantly greater after intrusions of neighbours compared to non-neighbouring fish (paired t-test: t_{13}=2.58, p=0.023; Fig. 3a). Affiliation donation increased significantly more following intrusions by neighbours compared to non-neighbours (LMM: $F_{1,39}$=4.41, p=0.042), with all three group member types showing a similar treatment-based difference (no significant treatment*group member interaction term: $F_{2,39}$=1.33, p=0.276; group

member: $F_{2,39}$=0.58, p=0.565; Fig. 3b). Similarly, affiliation received increased significantly more following intrusions by neighbours compared to non-neighbours ($F_{1,39}$=6.75, p=0.013), with all three group member types showing a similar treatment-based difference (no significant treatment*group member interaction term: $F_{2,39}$=0.13, p=0.877; group member: $F_{2,39}$=1.04, p=0.362; Fig. 3c).

Overall levels of post-conflict intragroup aggression did not differ significantly depending on whether the intruders were neighbouring or non-neighbouring individuals (overt attacks, Wilcoxon test: Z=-0.14, n=14, p=0.888; aggressive displays, paired t-test: t_{13}=1.65, p=0.122; all aggressive acts combined: t_{13}=1.34, p=0.205). At an individual level, there was also no significant effect of intruder treatment on intragroup aggression; there was a non-significant trend for dominant females and subordinates to receive more aggressive displays from group-mates following the intrusion of neighbours compared to that of non-neighbouring individuals (Appendix).

Discussion

Simulated territorial intrusion of out-group individuals, which generated the expected defence behaviour (see also Desjardins et al. 2008), resulted in post-conflict increases in intragroup affiliation but no significant changes in post-conflict intragroup aggressive behaviour. Evidence was found that dominant individuals, who defended more than subordinates, subsequently received more affiliation from their group-mates. Moreover, the level of post-conflict affiliation was affected by the intrusion scenario: there was greater affiliation in the aftermath of intrusions by neighbouring compared to non-neighbouring individuals, even though aggression levels were higher against the latter. Our study therefore provides novel empirical support for a direct link between out-group conflict and post-conflict intragroup behaviour in non-human animals, and indicates that the nature of the intrusion can influence the amount of post-conflict affiliation shown among group-mates.

Our finding that out-group intrusions lead to increased intragroup affiliation (more soft touches, follows and parallel swimming by *N. pulcher* group members) matches that from a study of birds (Radford 2008b), but contrasts experimental work on a single monkey group (Polizzi di Sorrentino et al. 2012). At a proximate level, increases in post-conflict affiliation may be the result of greater social stress or tension

induced by conflict (Von Holst 1998). Participation in allogrooming (a commonly recorded affiliative behaviour) reduces indicators of stress in both mammals and birds (Aureli and Yates 2010; Radford 2012; Schino et al. 1988), and increased time in close proximity is also a recognised means of reducing post-conflict tension in primates (e.g. Mallavarapu et al. 2006; Verbeek and deWaal 1997). Whether post-conflict affiliative behaviour also has this effect in fish has yet to be determined, although it is known that the receipt of tactile stimulation (e.g. soft touches) can lower stress levels (Soares et al. 2011), while follows and parallel swimming result in prolonged close proximity of group members. Ultimately, such affiliative behaviour might be used to strengthen bonds between group-mates and thus enhance group cohesion (Dunbar 1991).

All three types of group member increased the donation of post-conflict affiliative behaviour following out-group intrusions, but it was the dominant pair who received the most in Experiment 1. Since dominants performed more aggressive acts than subordinates towards out-group intruders, this finding is in line with previous studies showing that green woodhoopoe group members contributing the most to out-group conflict received the most allogrooming, both in the immediate aftermath (Radford 2008b) and in border areas where such conflicts were most likely (Radford 2011). One theoretical possibility is that affiliative behaviour is traded for participation in out-group conflicts (as is true for some birds and mammals (e.g. Barrett et al. 1999; Radford 2008b; Seyfarth and Cheney 1984)), but whether this is also the case in fish remains to be tested. More generally, considering links between defence contributions and subsequent intragroup interactions might provide insight into the variation often found in the helping efforts of different group members (Bergmüller and Taborsky 2005; Radford 2008b).

As predicted, the amount of post-conflict affiliation shown by *N. pulcher* group members was affected by the intrusion scenario: the greatest increase in affiliative behaviour was seen following neighbour intrusions, to which the residents had exhibited lower levels of aggression than when non-neighbouring individuals intruded. Unlike in green woodhoopoes (Radford 2008b), higher levels of out-group aggression were therefore not followed by greater levels of intragroup affiliation, and so immediate and subsequent responses to territorial intrusions are not necessarily always tightly coupled. One possibility for the lower level of intragroup affiliation following intrusions of non-neighbours is that the

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residents are energetically constrained by the greater defence effort, as aggression is costly in *N. pulcher* (Grantner and Taborsky 1998). Alternatively, higher levels of post-conflict affiliative behaviour could signal social cohesion to neighbours that were still visible in the aftermath in Experiment 1 and 2 (see also Cords 2002); non-neighbouring intruders were out of sight during the post-conflict period. Most plausibly, perhaps, the greater affiliation might be because neighbouring individuals represent the bigger threat to individual resident group members (Bruintjes et al. 2011; Dierkes et al. 2005; Stiver et al. 2004), as subordinate group members can sporadically change groups (Bergmüller et al. 2005a). Resident individuals might therefore be more stressed, which could lead directly to more affiliative behaviour (Von Holst 1998).

We found no clear-cut effects of out-group intrusions on post-conflict aggression in either experiment. Our results contrast with work performed on a single group of captive tufted capuchin monkeys that showed an increase in intragroup aggression following visual exposure to another group (Polizzi di Sorrentino et al. 2012). Those authors argued that the aggression increase might be caused by the high male sex ratio in the adjacent group, as group composition can influence conflict outcome (e.g. Mueller and Manser 2007; Radford and Du Plessis 2004). The individuals used for our experimental intrusions were carefully size- and sex- matched to rule out this possibility. In our experiments, all group members contributed to defence against intruders. Since social monitoring of groupmates is common in group-living species, including *N. pulcher* (Hellmann and Hamilton 2014), future work might profitably explore the possibility of punishment if individuals, especially subordinates, fail to contribute when responding to out-group threats (Gneezy and Fessler 2012).

In conclusion, our experimental work has demonstrated that territorial intrusions by out-group individuals can influence subsequent levels of affiliation between *N. pulcher* group-mates, and thus provides evidence of a link between out-group conflict and post-conflict intragroup behaviour in a fish. Moreover, we show that different out-group intruder scenarios not only induce differences in aggression levels during the conflict, but also affect intragroup behaviour in the aftermath; immediate and subsequent responses to intrusions may not, however, be tightly coupled. As it becomes clearer that the relationship between out-group conflict and intragroup behaviour is not uniquely human, future work on tractable

study systems such as the model fish species used here will allow a deeper understanding of both the functional and mechanistic underpinnings of social evolution.

Acknowledgments

Additional Methods

Ethics

All experiments were conducted according to the guidelines of the Association for the Study of Animal Behaviour. All procedures were approved by the University of Bristol Ethical Committee (UB/10/034).

Husbandry

Fish used for this study were descendants of individuals caught at the southern end of Lake Tanganyika, Zambia in 2006 and reared at the University of Bern, Switzerland (details of the transfer to Bristol University, UK in 2011 are provided in (Bruintjes and Radford 2013)). After transport, all fish were initially kept in an 800-L aggregation tank (size: 500 x 58 x cm, height: 33 cm) equipped with a Vecton 600 ultraviolet water steriliser, a biological filter and two heaters (Rena SmartHeater 200). Water temperature was kept constant at 27±0.5°C with a light:dark regime of 13:11h. Fish were fed five times per week with TertraMin food flakes, once per week with bloodworms and once with ZM-300 food (zmsystems.co.uk).

Experimental Set-up

The 80-L transparent aquaria measured 71 by 38 cm with a height of 30 cm. Each aquarium was equipped with a water filter (Eheim ecco 2032) and a heater (as above), had 3 cm of sand at the bottom, and included an opaque tube near the surface (to allow possibility for escape in response to intragroup aggression, but this was hardly ever used). Water conditions and feeding were as for husbandry (see above).

Standard lengths (mean±SE) of the fish in Experiment 1 were: 58.9±0.9 mm for dominant males, 51.3±1.3 mm for dominant females and 38.4±2.2 mm for subordinates; in Experiment 2 standard lengths were: 50.8±1.1 mm for dominant males, 45.5±0.9 mm for dominant females and 37.5±1.1 for subordinates. Experiments were conducted in paired aquaria, placed 3 mm apart (Fig. A1). The neighbouring groups were arranged so that they were not visible by fish in other aquaria and neighbouring individuals were carefully size-matched; i.e. the same group member types (dominant males, dominant females and subordinates) in neighbouring groups differed only 3.9±1.1 mm (mean±SE) in length in Experiment 1 and 1.7±0.6 mm in length in Experiment 2. Groups were checked daily for indications of non-acceptance and stress (e.g. head-up displays), with individuals replaced where necessary (n=2). Daily checks also allowed egg detection; clutches were removed and no experiments were conducted until >24 h had elapsed. Groups were not used as intruders during the period that they were receiving treatments as the resident group (mean±SE separation of roles: 10.8±2.4 days).

Plexiglass presentation cylinders were 15 x 8 cm in size, with 0.3 mm wall thickness and mesh on one side. Which cylinder position contained which group member (dominant male, dominant female, subordinate) was randomised for each experimental intrusion.

Data Collection and Analysis

All observations were conducted using Observer (Version XT 10, Noldus, Wageningen, the Netherlands). The very low (but non zero) number of attacks directed at the empty cylinders in Experiment 1 (Fig. 1a; Fig. A2a,b Appendix) may be due to the addition of a novel object to the tank, and supports the need to use this as a control treatment.

All datasets were checked for normal distributions and homogeneity of variance, with parametric tests used when these assumptions were met and non-parametric tests used in other cases. Linear mixed-effect models (LMMs) were used when assessing responses of individual group members, to control for repeated measures from the same individual (as all fish were observed in both treatments in each experiment) and the same group. As fixed effects, we used treatment (degrees of freedom (df) = 1), group member type (df = 2) and the treatment*group member interaction effect (df = 2), while as a random effect we used individual nested within group (df = number of individuals minus 3 already included in the intercept); this model was used to analyse total defence, received and donated affiliation in both experiments (see Results main paper). In several analyses shown in the Appendix, one group member type did not perform a certain behaviour, and therefore only two group member types were used (Experiment 1, Received intragroup aggression: All aggression; Donated intragroup aggression: All aggression and Aggressive displays, Table A1; Experiment 2, Received intragroup aggression and Donated intragroup aggression: All aggression and Aggressive displays, Table A2). In cases where the individual effect was redundant, it was removed from the analysis to allow the convergence criteria to be satisfied. However, to account for the potential group effect on such occasions, group was added to the model as a random effect (Experiment 1: Received intragroup aggression: Overt aggression and Aggressive displays, Table A1). If the group effect also gave redundancy, this was removed from the model too (Experiment 1: Donated intragroup aggression: Overt attacks, Table A1).

Running the analyses with different versions of the model (e.g. without the individual effects, but with the random group effect), gave similar results. All tests were two-tailed with $p<0.05$ for significance and conducted with IBM SPSS Statistics (v. 19.0, Armonk NY, USA).

Additional Results

Experiment 1: Effect of out-group intrusions

Experiment 1 involved two intruder treatments: the three presentation cylinders placed in the resident territory were either empty (control) or contained the three size-matched neighbouring individuals.

Overt attacks and aggressive displays during intrusions.

Resident group member performed significantly more overt attacks towards the cylinders when they contained intruders rather than being empty (LMM, treatment: $F_{1,33}=59.64$, $p<0.001$; group member: $F_{2,33}=24.49$, $p<0.001$; treatment*group member: $F_{2,33}=19.21$, $p<0.001$; Fig. A2a). Qualitatively the same results were found for aggressive displays (treatment: $F_{1,33}=99.22$, $p<0.001$; group member: $F_{2,33}=19.43$, $p<0.001$; treatment*group member: $F_{2,33}=18.53$, $p<0.001$; Fig. A2b).

Post-conflict intragroup aggression.

There were no significant effects of treatment, group member type or their interaction on the change in total received intragroup aggression, received overt attacks or received aggressive displays (Table A1); dominant males did not receive sufficient aggression for analysis. Similarly, there were no significant effects on the change in total donated intragroup aggression, donated overt attacks and donated aggressive displays by dominant males and females (Table A1); subordinates did not donate sufficient aggression for analysis.

Table A1. Factors affecting levels of received and donated intragroup aggression.

Received intragroup aggression	*All aggression*		*Overt attacks*		*Aggressive displays*	
	F	*p*	F	*p*	F	*p*
Treatment	$F_{1,22}=0.35$	0.561	$F_{1,33}=0.63$	0.433	$F_{1,33}=0.05$	0.830
Group member	$F_{1,22}=0.03$	0.867	$F_{1,33}=0.28$	0.600	$F_{1,33}=0.09$	0.772
Treatment*group member	$F_{1,22}=0.04$	0.846	$F_{1,33}=0.07$	0.793	$F_{1,33}=0.01$	0.943
Donated intragroup aggression	*All aggression*		*Overt attacks*		*Aggressive displays*	
	F	*p*	F	*p*	F	*p*
Treatment	$F_{1,22}=0.28$	0.599	$F_{1,44}=0.42$	0.521	$F_{1,22}=0.04$	0.845
Group member	$F_{1,22}=2.08$	0.164	$F_{1,44}=1.67$	0.202	$F_{1,22}=1.76$	0.199
Treatment*group member	$F_{1,22}=0.32$	0.861	$F_{1,44}=2.28$	0.138	$F_{1,22}=0.52$	0.477

Experiment 2: Effect of intrusions by neighbouring versus non-neighbouring individuals

Experiment 2 involved two intruder treatments: the three presentation cylinders placed in the resident territory contained either the three size-matched neighbouring individuals or three size-matched individuals from a non-neighbouring group.

Overt attacks and aggressive displays during intrusions

Resident group member tended to exhibit more out-group overt attacks during intrusions by neighbours than by non-neighbouring individuals (LMM, treatment: $F_{1,39}=3.77$, $p=0.059$; group member: $F_{2,39}=9.03$, $p=0.001$; treatment*group member: $F_{2,39}=1.73$, $p=0.191$; Fig. A3a). No significant difference in out-group aggressive display levels was found between treatments (treatment: $F_{1,39}=0.84$, $p=0.774$; group member: $F_{2,39}=7.04$, $p=0.002$; treatment*group member: $F_{2,39}=1.72$, $p=0.192$; Fig. A3b).

Individual post-conflict intragroup aggression.

There were no significant effects of treatment or interaction between treatment and group member on the change in total received intragroup aggression, received overt attacks and received aggressive displays by dominant females and subordinates (Table A2); dominant males did not receive intragroup aggression. Dominant females and subordinates tended to receive higher intragroup aggressive displays following intrusions of neighbours, whereas no such effect was found in the amount of overt attacks (Table A2). There were no significant effects of treatment, group member type or the interaction between the two on the change in donated intragroup aggression by dominant males and females (Table A2); subordinates never exhibited aggression towards dominant group members.

Table A2. Factors affecting levels of received and donated intragroup aggression.

Received intragroup aggression	*All aggression*		*Overt attacks*		*Aggressive displays*	
	F	p	F	p	F	p
Treatment	$F_{1,26}=2.49$	0.127	$F_{1,39}=0.01$	0.925	$F_{1,26}=3.84$	0.061
Group member	$F_{1,26}=8.94$	0.006	$F_{1,39}=1.08$	0.305	$F_{1,26}=9.23$	0.005
Treatment*group member	$F_{1,26}=0.09$	0.766	$F_{1,39}=3.22$	0.081	$F_{1,26}=2.52$	0.620
Donated intragroup aggression	*All aggression*		*Overt attacks*		*Aggressive displays*	
	F	p	F	p	F	p
Treatment	$F_{1,26}=1.80$	0.191	$F_{1,52}=0.01$	0.939	$F_{1,26}=3.00$	0.095
Group member	$F_{1,26}=1.73$	0.200	$F_{1,52}=1.69$	0.199	$F_{1,26}=1.19$	0.286
Treatment*group member	$F_{1,26}=0.20$	0.658	$F_{1,52}=0.01$	0.939	$F_{1,26}=0.37$	0.551

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Figure Legends

Fig. 1. Total out-group aggressive acts (sum of overt attacks and aggressive displays; shown are means ± SE) by dominant males (dm), dominant females (df) and subordinates (sub) towards (a) intrusions of neighbours (Rivals) and empty cylinders (Control), and (b) intrusions of neighbouring and non-neighbouring individuals.
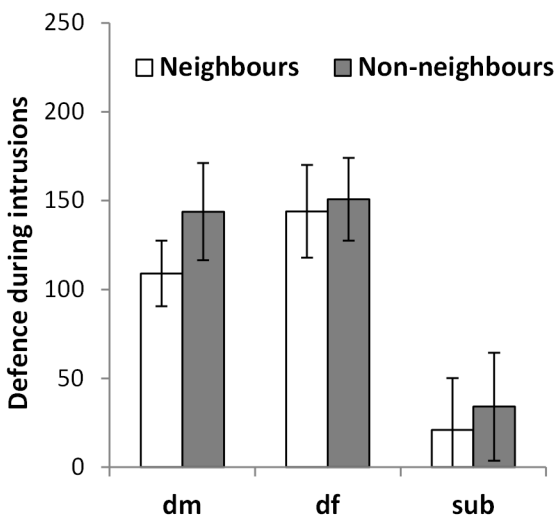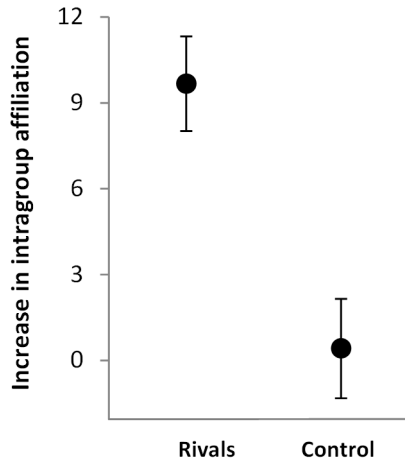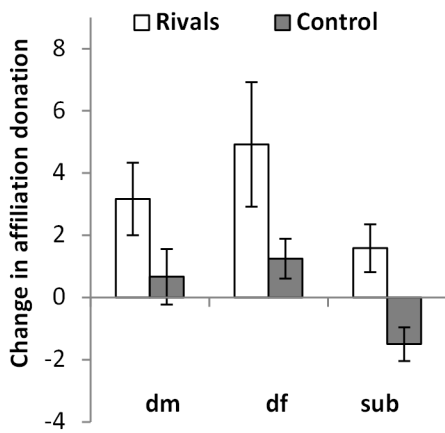
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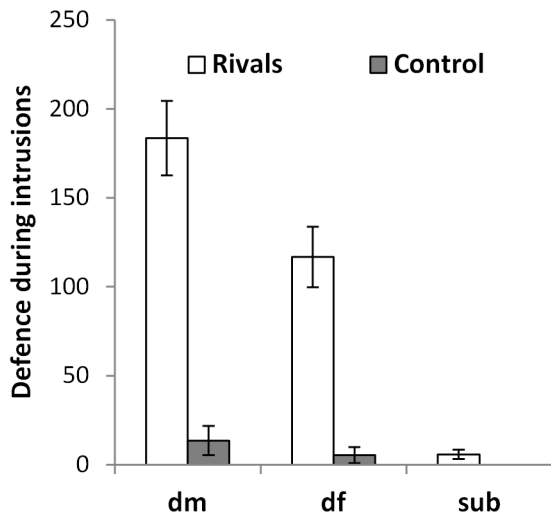
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Fig. 2. Post-conflict intragroup affiliation following intrusions of neighbours (Rivals) and empty cylinders (Control); shown are mean ± SE differences in the number of affiliative behaviours observed, post-intrusion minus pre-intrusion. (a) Total intragroup affiliation levels, (b) individual affiliation levels donated and (c) individual affiliation levels received by dominant males (dm), dominant females (df) and subordinates (sub). N = 12 groups.
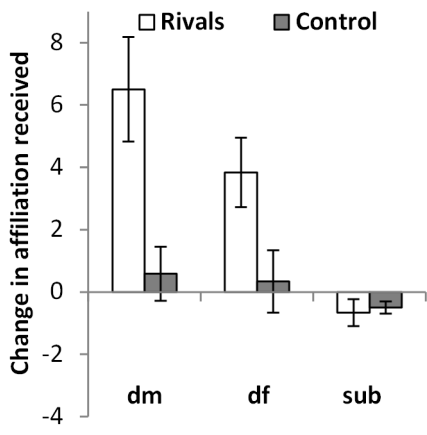
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C

Fig. 3. Post-conflict intragroup affiliation following intrusions of neighbouring and non-neighbouring individuals; shown are mean ± SE differences in the number of affiliative behaviours observed, post-intrusion minus pre-intrusion. (a) Total intragroup affiliation levels, (b) individual affiliation levels donated and (c) individual affiliation levels received by dominant males (dm), dominant females (df) and subordinates (sub). N = 14 groups.
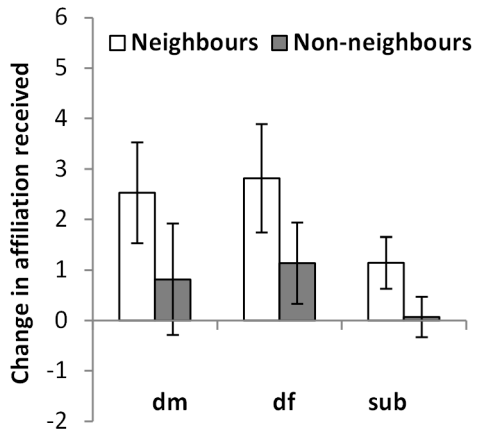
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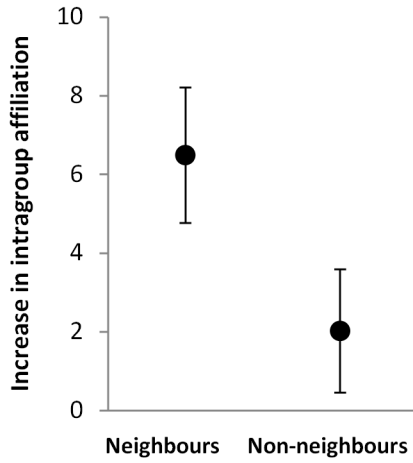
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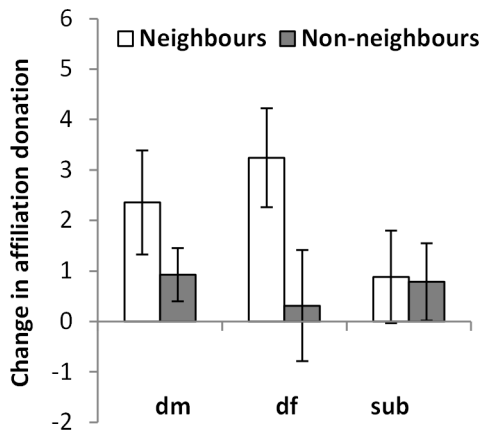
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Fig. A1. Overhead view of the aquaria set-up. The cone-shaped forms represent flower pot halves (two per aquarium), dashed circles with letters represent the positions of intruders/empty cylinders, the rectangular forms represent opaque hiding tubes and the small circles in the corner of the aquaria represent the heaters.

Fig. A2. Mean ± SE numbers of (a) overt attacks and (b) aggressive displays directed by dominant males (dm), dominant females (df) and subordinates (sub) towards presentation cylinders containing either out-group individuals (rivals) or no fish (control). N = 12 resident groups.

Fig. A3. Mean ± SE numbers of (a) overt attacks and (b) aggressive displays directed by dominant males (dm), dominant females (df) and subordinates (sub) towards presentation cylinders containing either neighbouring or non-neighbouring individuals. N = 14 resident groups.