



Social network stability is impacted by removing a dominant male in replicate dominance hierarchies of a cichlid fish

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Individuals with high social rank within a dominance hierarchy often have priority access to resources relative to subordinate individuals, but these rank-dependent fitness effects may depend on the stability of the social hierarchy. Here we studied temporal changes in network structure and social relationships in experimentally perturbed social hierarchies of the cichlid *Astatotilapia burtoni*. By removing a dominant male in replicate groups, we triggered more status changes compared to control, subordinate male removal groups. At the individual level, we found that dominant male removal resulted in dominant males significantly increasing the rate of chases ('chase strength'), but there was no significant increase in the rate of display ('display strength'). Dominant male removal also led to several changes at the group level: network reciprocity and network stability decreased in response to dominant male removal, while network density was not affected by our treatment. However, in contrast to the individual level network measures, these group level effects were present in display networks but not in chase networks. Display networks mostly connect dominant males within the network, making display networks more sensitive to changes in the hierarchy. Together, our results provide novel insights into complex social dynamics in experimentally altered social dominance networks.

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Dominance hierarchies exist when animals compete for access to reproductive and nonreproductive resources. In many vertebrates, low-ranking individuals behave submissively or avoid conflict and experience reduced reproductive opportunities, while high-ranking individuals show aggressive behaviours and monopolize resources and reproductive opportunities (Chase & Lindquist, 2016; Drews, 1993; Sapolsky, 2004). Being aware of the social environment and adhering to social rank reduces the need for constant conflict and risk of injury (Taborsky & Oliveira, 2012; Williamson, Lee, & Curley, 2016). The formation and maintenance of stable social hierarchies has been thoroughly studied across a wide range of taxa, including fish (Dey et al., 2015), reptiles (Wilczynski, Black, Salem, & Ezeoke, 2015), birds (Lukianchuk & Doucet, 2014) and mammals (Hodges et al., 2017; Sapolsky, 2005; Thavaraja, Fenkes, & Clutton-Brock, 2014). Social environment, however, can change due to many factors, including

birth, death or migration of group members. These changes in the social environment can lead to social mobility through the dominance hierarchy. For example, changes in the environment may offer subordinate males the opportunity to rise in the social hierarchy. During this process, subordinate males rapidly undergo behavioural and neuroendocrine changes to attain a higher dominance status (Huffman, Mitchell, O'Connell, & Hofmann, 2012; Sapolsky, 2004). Social rank changes may also result in behavioural changes in group members that do not undergo a change in rank. For example, in species where dominance is linked to territoriality, already existing dominant males need to re-establish territorial boundaries towards a previously subordinate male that becomes territorial. Studying the dynamics of social hierarchy formation and maintenance is key to understanding how the social environment influences the link between rank and fitness.

Social network analysis is one approach for examining social behaviour at the individual and group levels (Bierbach et al., 2014; Croft, Madden, Franks, & James, 2011; Wey, Blumstein, Shen, & Jordan, 2008). It can be used to understand how an

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organism's position within a group or group structure is associated with evolutionary fitness. For example, an individual's social connectivity has been linked to male reproductive success (Formica et al., 2012; Oh & Badyaev, 2010; Ryder, Parker, Blake, & Loiselle, 2009), and group level characteristics, such as network reciprocity, can impact the fitness of individuals within the group (Solomon-Lane, Pradhan, Willis, & Grober, 2015). While many studies have focused on static social networks of a single group of interacting individuals, more recent studies have begun to focus on temporal social networks in order to understand how relationships are formed, maintained and adjusted over time (Bierbach et al., 2014; Dakin & Ryder, 2020; Maldonado-Chaparro, Alarcón-Nieto, Klarevas-Irby, & Farine, 2018; Pinter-Wollman et al., 2014; Williamson et al., 2016). For example, Williamson et al. (2016) studied the formation and stabilization of social hierarchies over time in replicate social networks of mice and found that dominance hierarchies in this species are very linear and consistent over time. Network theory suggests that well-connected individuals or nodes have a disproportionate effect on network stability and structure (Albert, Jeong, & Barabási, 2000; Sih & Watters, 2005). 'Knock out' experiments, where an individual is removed from a social network to observe how that individual affects network functionality, provide a strong framework for analysing the influence of key individuals on social network structure and stability (Pinter-Wollman et al., 2014). For instance, removing a conflict manager from a group of pigtailed macaques, *Macaca nemestrina*, led to conflicts spreading more rapidly within the group (Flack, Krakauer, & de Waal, 2005). Studies on social stability have mainly focused on a single group (Flack, Girvan, de Waal, & Krakauer, 2006). However, studies where social stability is experimentally altered in several replicate groups are clearly warranted if we are to understand the causes and consequences of social rank and social relationships in animals (Farine & Whitehead, 2015).

In this study, we examined how social status, social behaviour and social network structure change in experimentally disrupted social hierarchies of the East African cichlid, *Astatotilapia burtoni*. This species lives in groups consisting of a small number of territorial dominant males along with nonterritorial subordinate males that school with the females. Dominant males are brightly coloured, vigorously defend territories and court females, while subordinate males have duller coloration and do not typically reproduce (Maruska & Fernald, 2018). Subordinate males grow faster than dominant males and may challenge dominant males such that the dominance hierarchy changes over time (Hofmann, Benson, & Fernald, 1999). In *A. burtoni*, males keep track of other members in the hierarchy, and changes in the physical and/or social environment can induce rapid changes in social status, altering an individual's physiology and behaviour (Desjardins, Hofmann, & Fernald, 2012; Fialkowski, Aufdemberge, Wright, & Dijkstra, 2021; Hofmann et al., 1999; Maruska & Fernald, 2010). We set up mixed-sex replicate groups, each consisting of three to four dominant males and eight to nine subordinate males. We quantified behavioural interactions at regular intervals to obtain snapshots of the social network over a 19-day period in these groups. On day 16, we experimentally created a vacant territory through the removal of a single dominant male in several experimental groups of *A. burtoni*, while a subordinate male was removed from control groups. In principle, dominant male removal would increase competition and social uncertainty as males are competing for the vacated territory. We predicted that the resulting social instability would be linked to more changes in the social networks and increased rates of aggression or social connectivity relative to control groups.

METHODS

Animals and Housing

For this experiment, adult *A. burtoni* were bred from a laboratory population originally derived from Lake Tanganyika, Africa (Fernald & Hirata, 1977). The fish were housed in 110-litre tanks, maintained at 28 °C on a 12:12 h light:dark cycle and fed cichlid flakes (Omega Sea Ltd, Painesville, OH, U.S.A.) every morning. All experimental tanks were set up with partial flowerpots placed in each corner to create four defendable territories per tank. A total of 16 groups were studied, each composed of 12 males ($N = 192$) and 14 females ($N = 224$). All fish were individually tagged through the dorsal musculature using a stainless-steel tagging gun and coloured beads. Experimental groups were given 4 weeks to settle and stabilize before observation began.

Experimental Design

The experimental design of this experiment has been described elsewhere in a study on the link between social rank and oxidative stress (Border, Brown, et al., 2019). In brief, groups were filmed over a 19-day period (on days 1, 3, 5, 7, 9, 11, 13, 14, 15, 16, 17, 18 and 19) for 10 min per session using a Canon EOS Rebel T5i for later quantification. All filming was performed at least 10 min after feeding in the morning. Groups were randomly assigned to either the dominant male removal treatment ($N = 9$) or the subordinate male removal treatment ($N = 7$). In the dominant male removal treatment, a dominant male was removed from an experimental group and a mock removal was performed on a randomly chosen subordinate male by netting and immediately releasing him. In the subordinate male removal treatment, a subordinate male was removed from the group and a mock removal was performed on a randomly chosen dominant male. The dominant removal treatment created a vacant territory over which males in the group competed for ownership, leading to more social instability in terms of more frequent status shifts (subordinate to dominant and dominant to subordinate) and an increased level of competition as the dominance hierarchy was re-established. The subordinate removal controlled for changes in fish density in the dominant removal groups. Male removals were performed immediately following filming on day 16. A schematic drawing of the timeline of the experiment can be found in Fig. 1.

Behavioural Observation

Using all-occurrence sampling for each 10 min video, the following behaviours were recorded among all members in each group as described previously (Fialkowski et al., 2021): fleeing, chasing, lateral displays and border displays (see also Fernald, 1977). Fish that are a target of a chase typically swim away (flee) from the aggressor. Display behaviours are usually not followed by a flee and are typically used in symmetrical interactions with two males engaging in border or lateral displays simultaneously towards one another. For both chase and display behaviours, we recorded the initiator of the behaviour as well as the target.

For each video, individual males were categorized as either dominant or subordinate. Dominant males were categorized as expressing brighter yellow or blue coloration, behaving more aggressively than subordinate males and using a flowerpot as the focal point of territorial defence (Desjardins et al., 2012). For each video, we also recorded which flowerpot the dominant male defended.

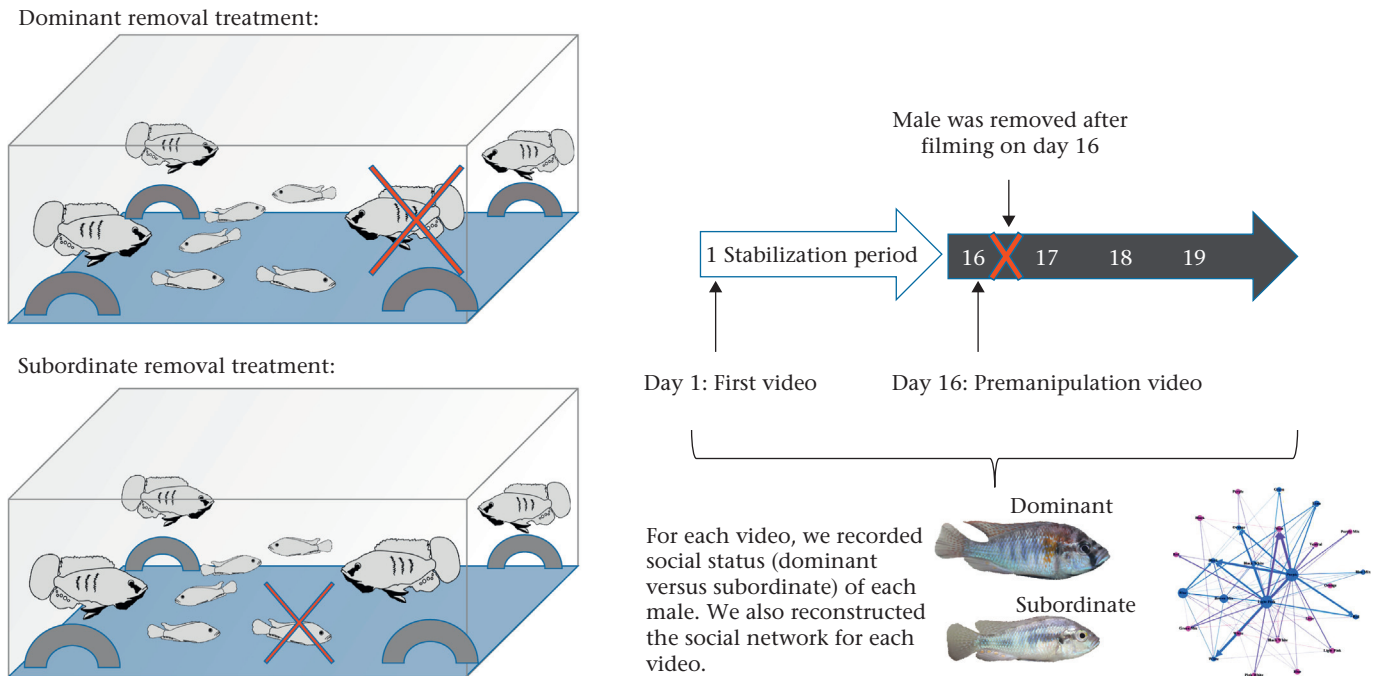


Figure 1. Experimental design. A dominant male was removed after filming on day 16 to induce social instability in nine experimental groups. A subordinate male was removed in seven control groups. Groups were filmed for 10 min either every other day from day 1 to day 13, or daily from day 13 to day 19. For each video, we recorded social status of each male and we reconstructed weighted, directed social networks. In the statistical analysis, day 16 is the pre-removal observation and days 17, 18 and 19 are the post-removal social networks. We only indicate a subset of subordinate males and females. Each group contained four flowerpots (partially broken) and each flowerpot was occupied by one male.

Ethical Note

Cichlids were studied under protocols approved by the Animal Care and Use Committees of Central Michigan University (IACUC protocol 15-22). This research adhered to the ASAB/ABS Guidelines for the use of animals in research. We attempted to reduce stress of the animals during the experiment by ensuring sufficient enrichment in the form of flowerpots. This also facilitated the establishment of multiple territories and social stability in the dominance hierarchy, avoiding excessive aggression by a single alpha male.

Social Network Analysis

From each 10 min video, we constructed and analysed two weighted, directed social networks. The chase aggression social network was based on chases, while the display aggression social network was based on lateral and border displays. The chase network is useful for characterizing outgoing relationships from one individual to another individual with a clear winner (the chaser) and loser (the fish fleeing from the aggressor). In contrast to chases, displays are typically used during symmetrical territorial interactions among dominant males trying to establish territorial boundaries. Hence, these two network types represent distinctly different agonistic networks that can provide a more comprehensive perspective on overall network structure and social change when observed separately. Statistical analyses were performed using R v.3.4.4 (<https://r-project.org>). Social networks were created for each video using the R package igraph v.1.1.2 (<https://igraph.org>). Using these networks, we calculated (node) weighted outdegree (referred to as 'strength') for each male for both the chase and display networks as measures of individual connectivity or prominence within the network. Chase strength and display strength are equivalent to the total number of chases

or displays a male performed during the 10 min video. We predicted that dominant male removal would induce social uncertainty and increase strength as the hierarchy was being re-established.

To determine network stability over time, we calculated the average correlation between sequential chase- or display-based matrices (networks) in each group using Pearson's r correlation. Correlations were based on a scale of -1 to 1, where -1 was total negative correlation, 0 meant no correlation and 1 was a total positive correlation between chase or display rates from one matrix to the next. We predicted that dominant male removal would increase social instability.

For each 10 min video, we calculated group level social network metrics before and after the male removal for both the chase and display social networks. Using the unweighted networks, we calculated 'network density' as the proportion of actual connections compared to all possible connections that could be made between individuals within a group (Wasserman & Faust, 1994). Males were considered connected if A directed behaviour to B and/or B directed behaviour to A. 'Network reciprocity' is the likelihood of vertices being mutually linked within a network and evaluates how often interactions occur in both directions between a target and its source (Schino & Aureli, 2010). Network reciprocity is measured from 0 (interactions always performed in one direction) to 1 (interactions always performed equally in both directions). We predicted that dominant male removal would cause more interactions between previously unconnected individuals while the dominance hierarchy was re-established, causing network density to increase. We further predicted that dominant male removal would increase uncertainty in the hierarchy, resulting in more unidirectional aggressive interactions, especially in display behaviour, since individuals may not yet have balanced in-out interactions as they would in a more stable hierarchy (Fulmer, Neumeister, & Preuss, 2017).

Statistical Analysis

To confirm that dominant male removal induces social instability, we first tested whether the number of groups where status shifts occurred were higher in the dominant removal groups than in the subordinate removal groups using chi-square tests. Status shifts included both social ascent (subordinate to dominant) and social descent (dominant to subordinate) between consecutive videos.

We implemented linear mixed models (LMM) and generalized linear mixed models (GLMM) using the R packages *lme4* (Bates, Mächler, Bolker, & Walker, 2015) and *glmmTMB* (Brooks et al., 2017). We examined LMM residuals to ascertain whether assumptions of normality and homogeneity of variances were met. All GLMMs were run using *glmmTMB*. Model assumptions of GLMMs were verified by plotting residuals against fitted values. For all effects we report estimates and standard error.

Individual level effect of dominant male removal

To test whether removing a dominant male would induce social instability and increase network connectivity, we examined treatment-dependent temporal changes in chase and display strength of each male in the analysis (as noted earlier, strength is based on the weighted outdegree, which is the frequency of chases or displays during a 10 min observation period). We included the data for all males in this analysis ($N = 108$ from dominant removal groups; $N = 84$ from subordinate removal groups). The data were categorized as either premanipulation period (day 16) or postmanipulation period (after male removal, days 17, 18 and 19). We used day 16 as the premanipulation period as our aim was to have a snapshot of the social network just prior to male removal. The postmanipulation period consisted of three days because a pilot study indicated that changes in the hierarchy due to male removal were still taking place after day 17. To test whether changes in chase-based or display-based strength between the pre- and the postmanipulation periods were dependent on male removal type, we first compared two different GLMMs using hurdle models with zero-truncated Poisson distribution to account for the large number of zeros. First, we built models with the two-way interaction term between male removal type (dominant removal or subordinate removal) and manipulation period (pre or post) and status as fixed effect. Second, we built models with the three-way interaction term between male removal type, manipulation period and status, because dominant and subordinate males are behaviourally distinct and are not necessarily expected to respond to the treatment in the same way. For both chase-based and display-based strength, the model with the three-way interaction had a significantly better fit than the model with the two-way interaction (based on AIC value). We then considered the male removal type and manipulation period interaction effect for subordinate and dominant males separately. In all models, we included ‘tank’ and ‘male identity (ID)’ as random variables. Random effects were fitted as random intercepts and ID was nested within tank.

Network level effect of dominant male removal

Using LMMs we tested whether male removal type influenced chase-based or display-based network stability (Pearson’s r correlation) by examining the interaction between manipulation period (premanipulation: days 15–16; postmanipulation: 17–18 and 18–19) and treatment. In this analysis, we excluded the day 16–17 network comparison because the two matrices differ in dimensions due to the male removal after the day 16 observation. In these models and in the network level analyses below, we used tank as a random variable.

We tested whether temporal changes in chase-based or display-based network density and network reciprocity were linked to male removal type by examining the interaction term between male removal type (dominant removal or subordinate removal) and manipulation period (pre or post). The premanipulation period was defined as day 16 and the postmanipulation period included days 17, 18 and 19. We assumed a beta distribution with logit link for network reciprocity and density using GLMMs.

We reported P values derived from the ‘lmerTest’ package (Kuznetsova, Brockhoff, & Christensen, 2017) in R, which utilizes Satterthwaite approximations for degrees of freedom. For the individual level analysis of chase strength and display strength, we also obtained P values by comparing the model estimates with estimates calculated from 1000 randomized sets of networks (P_{rand}) – obtained from edge permutations of the premanipulation (day 16) and postmanipulation (days 17, 18, 19) networks. In each permutation, we randomly swapped edge values (i.e. strength or the frequency of chases or displays during a 10 min observation period) among individuals, thus creating a new set of networks (each set consisting of daily networks from day 16–19) at each run. We allowed permutations of edge values across different days while restricting swaps to occur within each sex and status combination in relation to both the initiator of the behaviour and the receiver (for example, an edge value indicating the number of times a male chased a given female was swapped with another male–female combination in which the male was also the initiator of those chases). This restricted permutation procedure maintained the original distribution of degree and edge weight (strength) within each sex and status combination and avoided unrealistic edge values between certain dyad combinations. The permutation of edge values across different days and hence across the pre- and postmanipulation periods allowed us to test for temporal changes in chase and display strength in relation to male removal type. We extracted the model estimate as the test statistic from each permuted set of networks using the same statistical model that was used for the observed set of networks. Two-tailed P values were calculated as twice the proportion of random estimates that were larger or smaller than the observed estimate.

Note, however, that permutation approaches, although increasingly used in social network studies, can potentially inflate P values (Weiss et al., 2020). While one can account for network structure and node properties when creating random networks, it is difficult to accommodate all aspects of network structure (e.g. encounter probability, spatial structure) and node attributes (cognitive or motivational heterogeneity) when creating permuted networks. It is therefore very difficult to generate random networks that truly resemble the real or observed network setting. To increase the robustness of our findings, we report both conventional P values derived from our mixed models of the observed data and the permutation-based P values.

RESULTS

Does Dominant Male Removal Increase Individual Stability?

Each group was composed of three to four dominant males that defended one or two flowerpots as the focal point of their territories (there were 4 flowerpots available in each group; Fig. 2). Territories were nonoverlapping. We hypothesized that removing a dominant male would induce competition over a newly available territory, resulting in a previously subordinate male taking over the vacated pot and ascending to dominant status. We observed groups with multiple status transitions that could have been attributed to vacating a territory. To account for this dependence in the data, we counted the number of tanks with status transitions (ascent and

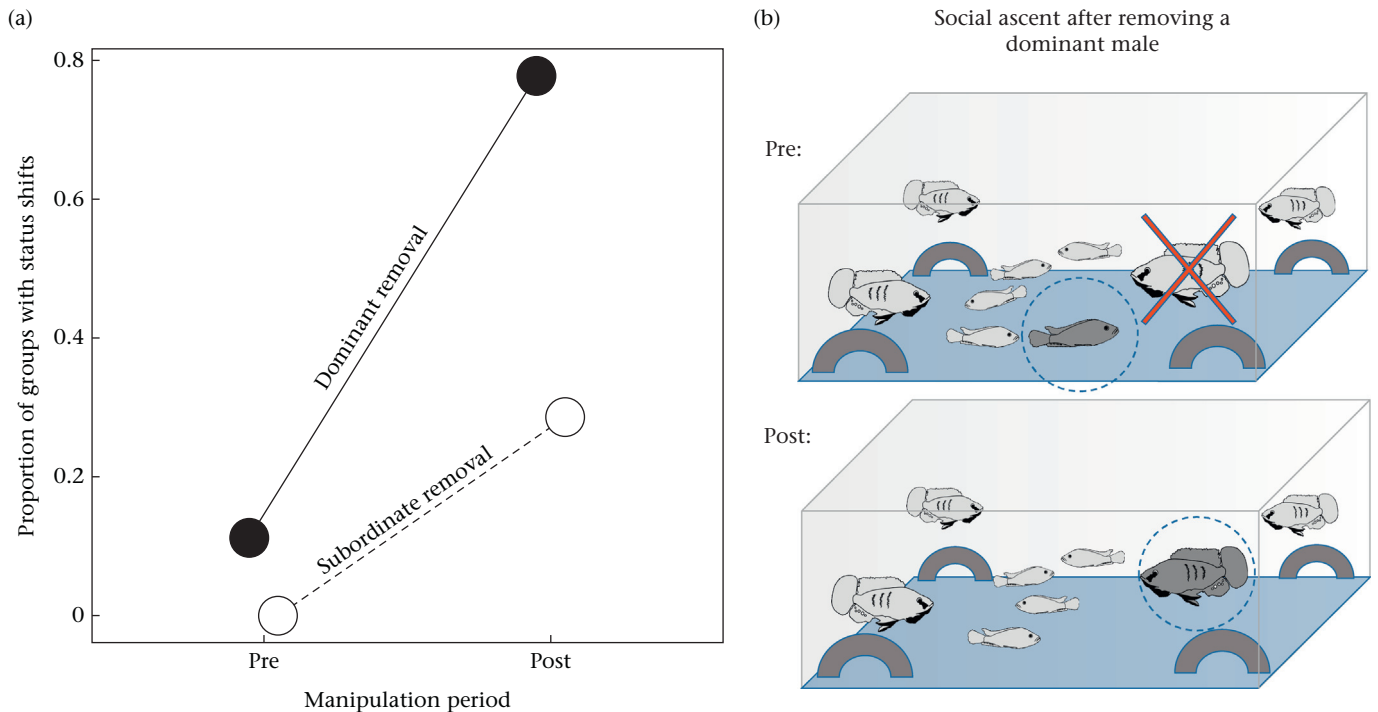


Figure 2. (a) Proportion of groups that underwent shifts in status between consecutive days before (pre) and after (post) a dominant or subordinate male was removed from a group. A status shift is defined as a male undergoing a shift from subordinate to dominant (ascent) or dominant to subordinate (descent) between consecutive observations. In the premanipulation period, we recorded changes in status between days 15 and 16. In the postmanipulation period, we recorded changes in status for days 16–17, days 17–18 and days 18–19. (b) Social ascent after removing a dominant male. The focal male is shown in dark grey and the dominant males are shown with erected fins. We only indicate a subset of subordinate males and females. Each group contained four flowerpots and each flowerpot was occupied by one male.

descent) between consecutive days (Fig. 2) in the premanipulation period (day 15–16) and the postmanipulation period (day 16–17, 17–18, 18–19). In the premanipulation period, there was no difference in status shifts between male removal types ($\chi^2_1 = 0.7438$, $P = 0.389$), with only one status shift occurring in a dominant removal group and none occurring in the subordinate removal groups. However, in the postmanipulation period, seven out of nine dominant removal groups experienced status shifts, while this was only the case for two out of seven subordinate removal groups. The difference was statistically significant ($\chi^2_1 = 3.874$, $P = 0.049$).

To our surprise, vacating a territory did not always lead to a subordinate male rising to dominance status and taking over the vacated territory. For example, on the first day after the dominant male removal (day 17), only five vacated flowerpots were taken over by a previously subordinate male, while in the remaining four cases an already existing dominant male expanded his territory by claiming the vacant flowerpot in addition to his original flowerpot. More details about the variability in responses in status shifts and space use can be found in the Appendix, Fig. A1. We hypothesized that the increased competition over the vacated territory would increase the individual connectivity within the social network (for representative social networks; see Fig. 3). We found that the best-fit models explaining variation in chase or display strength included the three-way interaction between male removal type, manipulation period and status (GLMM: treatment*pre–post*status; chase strength: -0.340 ± 0.096 , $P = 0.0004$, $P_{\text{rand}} = 0.082$; display strength: -0.302 ± 0.188 , $P = 0.108$, $P_{\text{rand}} = 0.434$). The results of these models suggest that the effect of male removal type on changes in chase strength and display strength could be status specific. We found that dominant males significantly increased chase strength after dominant male removal (GLMM: treatment*pre–post; chase strength: 0.205 ± 0.044 , $P = 0.00001$, $P_{\text{rand}} = 0.022$; Fig. 4) while this was not the case in

subordinate males (GLMM: treatment*pre–post; chase strength: -0.085 ± 0.090 , $P = 0.344$, $P_{\text{rand}} = 0.526$). There was no significant effect of removal type on changes in display strength in either dominant males (-0.109 ± 0.080 , $P = 0.171$, $P_{\text{rand}} = 0.972$) or subordinate males (-0.223 ± 0.181 , $P = 0.218$, $P_{\text{rand}} = 0.594$). These data suggest that across all individuals, removing a dominant male led to significant changes in social connectivity in chase networks but not in display networks.

Does Group Level Social Network Structure Change More after Removing a Dominant Male?

To test the effect of male removal type on network stability, we compared the Pearson's r correlation strength between days in the premanipulation period (days 15–16) to the correlation strength between days in the postmanipulation period (days 17–18, days 18–19). Consistent with a lower level of stability in dominant removal groups, there was a more pronounced drop in correlation strength between display networks after removing a dominant male compared to groups where we removed a subordinate male (LMM: treatment*pre–post: -0.2876 ± 0.12 , $P = 0.025$; Fig. 5). However, there was no significant effect of male removal treatment for the chase networks (LMM: treatment*pre–post: -0.1007 ± 0.09 , $P = 0.292$).

We were interested in testing the effect of male removal type on changes in two group level social network metrics: network density and network reciprocity. To this end, we compared each social network metric between the premanipulation period (day 16) and the postmanipulation period (day 17 through day 19). We predicted that dominant male removal would increase overall connectivity in the social network. However, network density was not influenced by male removal type for either the chased-based networks (GLMM: treatment*pre–post: -0.02267 ± 0.10 , $P = 0.824$; Fig. 6) or

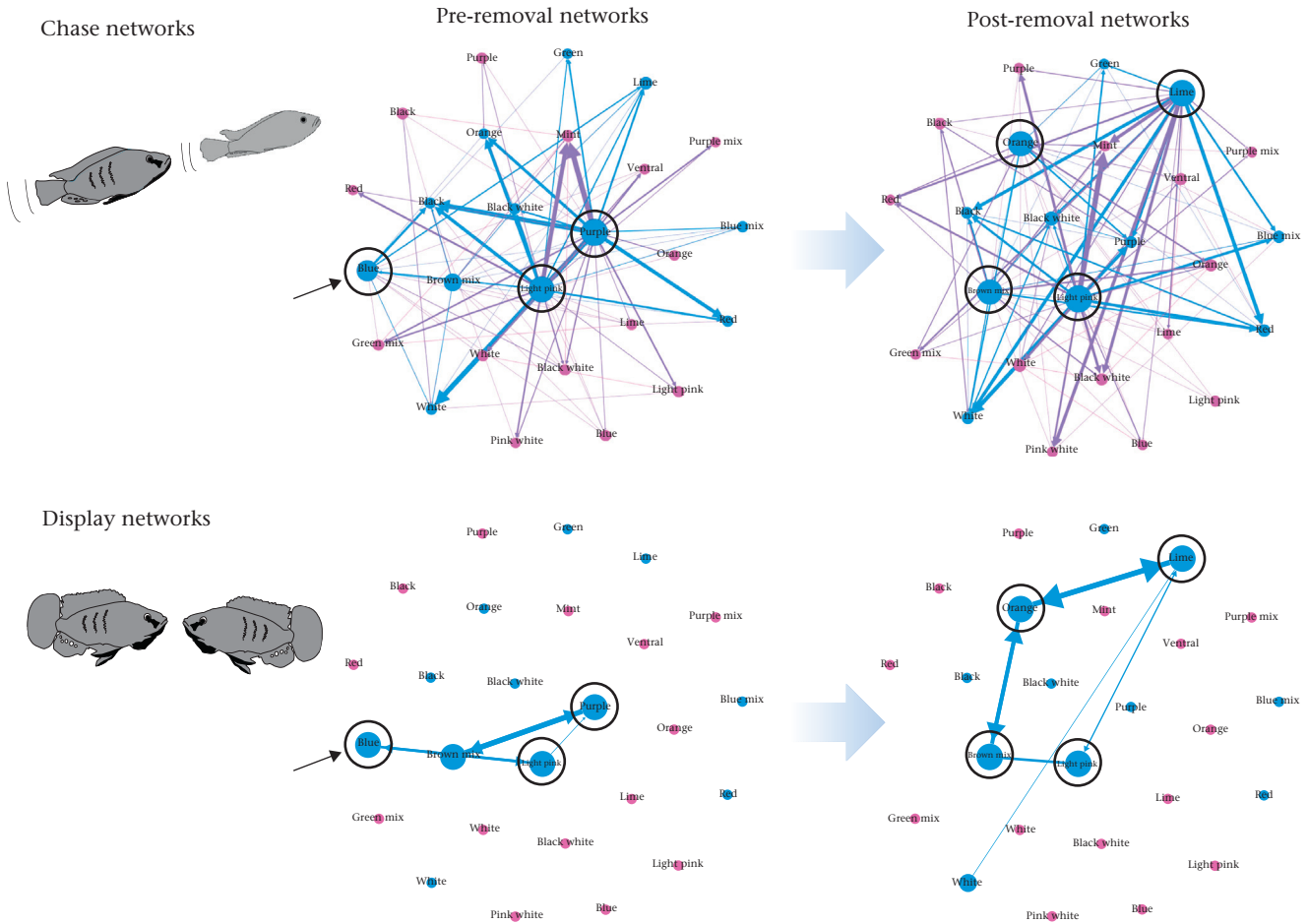


Figure 3. Chase and display networks of a representative group prior to and following removal of a dominant male. The pre-removal social network (left) is the social network based on a 10 min video recorded on day 16. The post-removal network (right) is based on a 10 min video on day 19. Males are shown in blue and females in pink. Individual fish are indicated by their tag colour. The dominant (territorial) males are indicated within a circle. The dominant male that was removed is indicated by a black arrow. In the post-removal network, three previously subordinate males became dominant, with one (Brown Mix) occupying the vacated flowerpot. All group members are shown in the same position in the two networks. Dominant males were more aggressive as indicated by higher weighted outdegree (strength) corresponding to thicker edges and larger nodes.

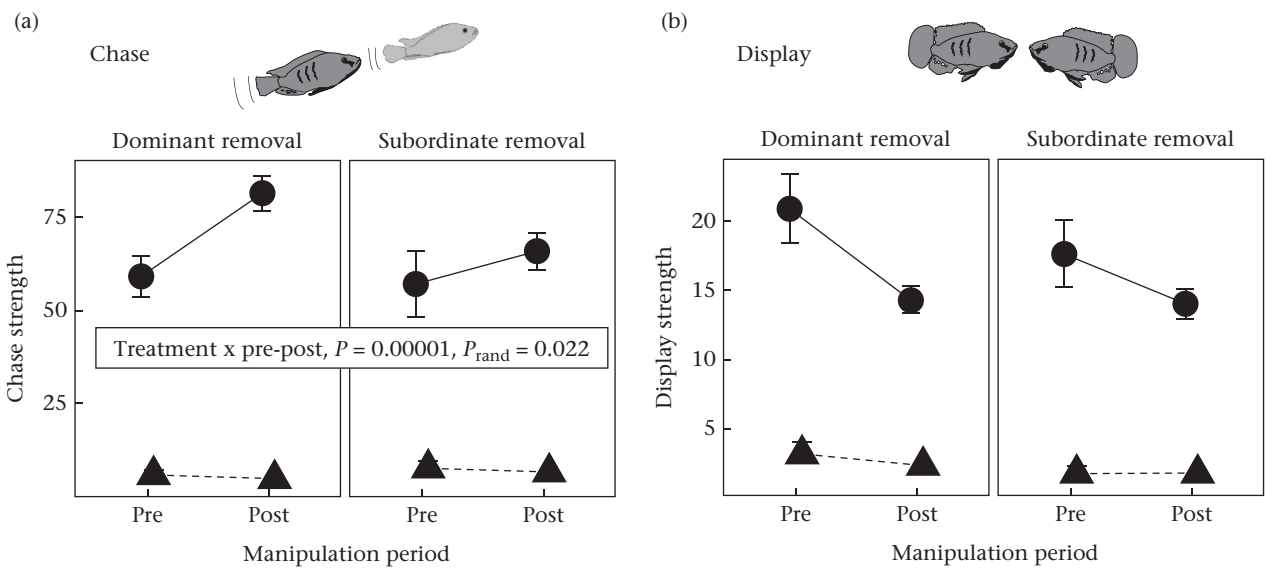


Figure 4. Change in (a) chase strength and (b) display strength of dominant and subordinate males prior to (day 16) and following (days 17, 18 and 19) removal of a dominant ($N = 9$) or subordinate ($N = 7$) male from the social group. Dominant males = circles; subordinate males = triangles. Chase and display strength are equivalent to the frequency of these behaviours during a 10 min video.

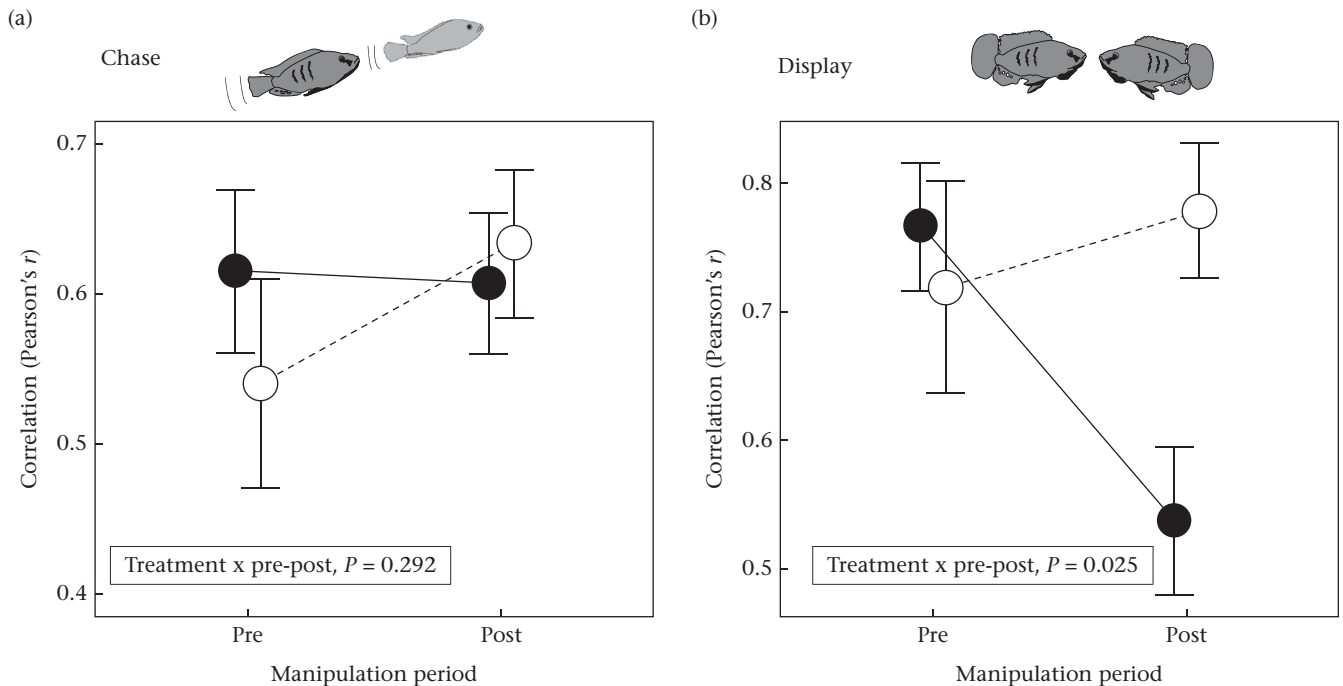


Figure 5. Daily (a) chase-based and (b) display-based (right) network correlations before (pre: days 15–16) and after (post: days 17–18, 18–19) removal of a dominant (solid circles, $N = 9$) or subordinate (open circles, $N = 7$) male on day 16 after filming. Correlations were calculated between adjacent matrices using Pearson's *r* correlation coefficients.

the display-based networks (GLMM: treatment*pre–post: -0.009726 ± 0.27 , $P = 0.971$; Fig. 6).

Male removal type did not influence the chase-based network reciprocity (GLMM: treatment*pre–post: -0.02506 ± 0.46 , $P = 0.957$; Fig. 6). However, we found that display-based network reciprocity changed significantly between the pre- and the post-manipulation period in a male removal-dependent manner (GLMM: treatment*pre–post: -1.563 ± 0.53 , $P = 0.003$; Fig. 6). This interaction effect was driven by a steeper drop in display-based network reciprocity after removing a dominant male compared to removing a subordinate male, suggesting that dominant male removal led to an increase in unidirectionality in display behaviour.

General Observations

Chase and display strength for subordinate and dominant males over the entire duration of the experiment (day 1–19) can be found in the Appendix, Fig. A2.

As can be seen in Fig. 6, network density was higher for the chase networks than the display networks, suggesting that fish were more connected in the chase networks than in the display networks. Display-based reciprocity was substantially higher than chase-based reciprocity (Fig. 6; see also Appendix, Fig. A4). Display behaviours, which included border display and lateral display, are often performed in unison with dominant males typically engaging simultaneously in reciprocal displays. Chase, on the other hand, is typically asymmetrical, with fish chasing lower-ranking individuals. The social stability and social network metric data for all observation days can be found in the Appendix, Figs A3 and A4.

DISCUSSION

In the current study we examined how social relationships and network structure change in response to dominant male removals in social hierarchies of *A. burtoni*. We found significantly more rank changes after the male removal in dominant removal groups

compared to subordinate removal groups, confirming that dominant male removal triggers social instability. We also found that this increased social instability was partly reflected by changes in both individual level and group level social network metrics. At the individual level, removing a dominant male resulted in significant changes in chase strength but not in display strength. At the group level, removing a dominant male resulted in significant changes in display-based network reciprocity and network stability, while chase-based social network metrics remained stable.

Does Removing a Dominant Male Increase Social Instability?

In all 16 groups that we studied, social hierarchies consisted of three to four dominant males with the rest being subordinate as is typically seen in experimental *A. burtoni* groups (Maruska & Fernald, 2018). We expected that creating an available territory in a group by removing a single dominant male would lead to more status shifts than removing a subordinate male. As predicted, in the dominant removal groups, more status shifts occurred after the male was removed and a territory was made available, compared to subordinate removal groups. We also observed far more instances of dominant males changing their space use, for instance by expanding their territory to include the vacated flowerpot in the dominant removal groups (see Appendix, Fig. A1). These data confirm that removing a dominant male resulted in social instability and increased competition for territories.

We hypothesized that chase and display strength would significantly increase after a dominant male was removed from a group due to the increased competition for the newly available territory. In line with this, dominant males increased chase strength after dominant male removal, but we did not observe this effect in display strength. In subordinate males, no effect of the manipulation was observed in chase strength or display strength. These findings suggest that creating a vacant territory led to an overall increase in overt aggressive behaviours in dominant males, which they most likely use to assert their dominance and territoriality

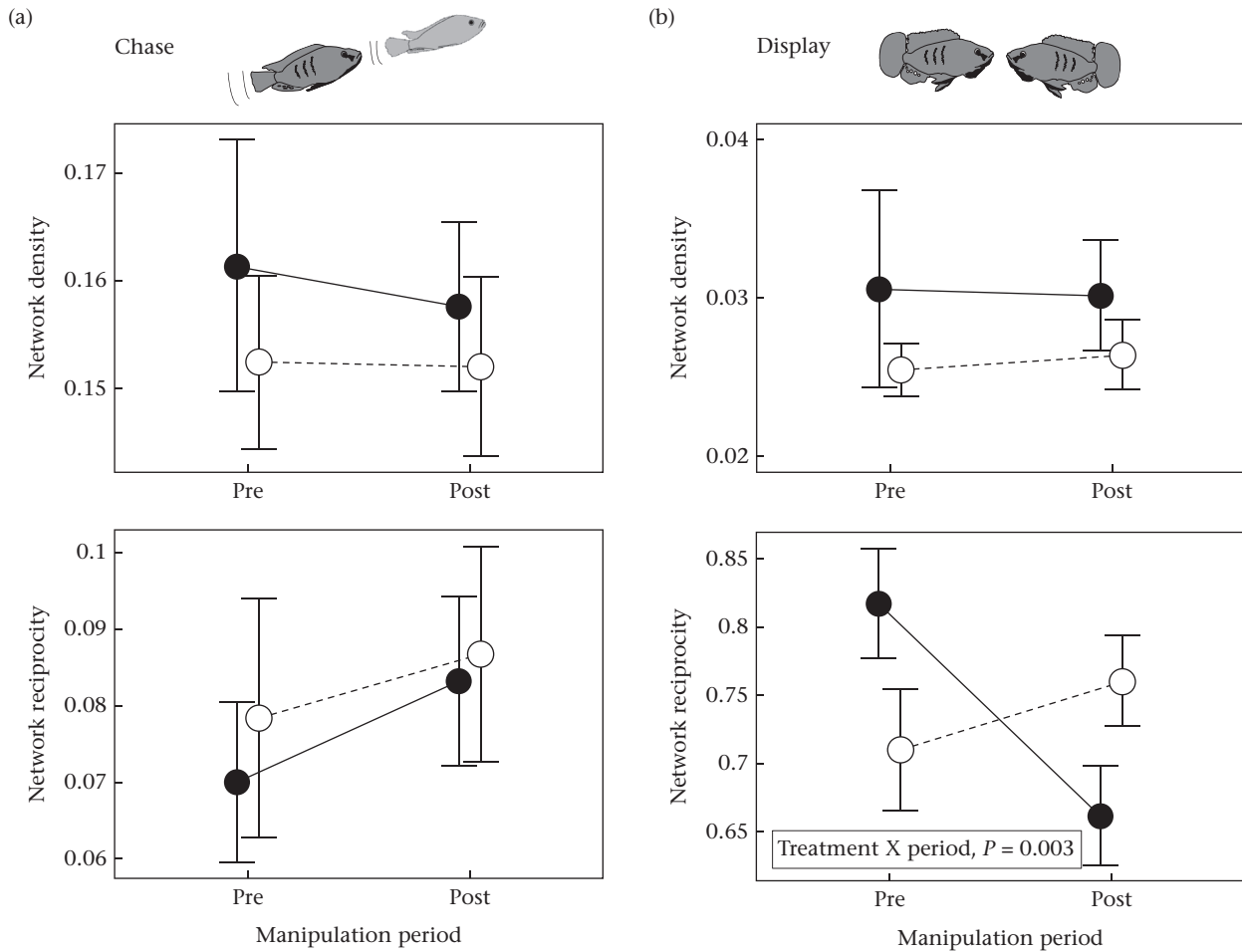


Figure 6. (a) Chase-based and (b) display-based social network metrics before (pre: day 16) and after (post: days 17–19) removal of a dominant (solid circles, $N = 9$) or subordinate (open circles, $N = 7$) male on day 16 after filming.

towards the rest of the group in response to more uncertainty in the hierarchy. Although this chase-specific effect of dominant male removal was not expected, in general more costly overt forms of aggression, such as chases, are expected in more unstable hierarchies that experience increased competition between group members (Sapolsky, 2005).

At the group level we found that dominant male removal influenced network stability and network reciprocity. The Pearson's r correlation strength analysis revealed significantly higher instability after male removal in dominant removal groups compared to subordinate removal groups. This was only significant in the display networks. Display behaviour is important for affirming dominant status and establishing territorial boundaries with neighbouring territorial males. Since display networks mostly reflect connections among dominant males, it is perhaps not surprising that display networks are sensitive to dominant male removal and changes in the social hierarchy.

Display-based network reciprocity, but not chase-based network reciprocity, decreased in dominant removal groups following the male removal. In contrast to chases, displays are often performed in unison, with dominant males typically engaging simultaneously in reciprocal displays. We hypothesize that this increase in asymmetrical display behaviour following dominant male removal results from males establishing new territorial boundaries. A recent simulation study suggests that a high degree of reciprocity can promote stability in cooperative networks of

wire-tailed manakins, *Pipra filicauda* (Dakin & Ryder, 2020). Although the situation in a competitive context might be different, our finding that experimentally perturbed network instability led to reduced network reciprocity provides a unique insight into the causal link between network instability and network reciprocity.

Collectively, we found that removing a dominant male led to changes in the social network: at the individual level we found these effects in chases only, while at the group level the effect was most apparent in the display networks. Each display network links mostly dominant males, making these networks more sensitive to altered behavioural dynamics among dominant males after the dominant male removal, at least at the group level.

The lack of an effect of dominant male removal on group level chase-based social network metrics was unexpected, but it could be explained by the fact that chase networks were denser than display networks. Other networks studies suggest that increased connectivity within a network can promote robustness of the system (Dunne, Williams, & Martinez, 2002). Our study suggests that the chase-based cichlid social networks stabilized rapidly despite changes in certain nodes that underwent dramatic changes in social roles after social ascent or territory expansion. The lack of an effect in the chase networks is somewhat consistent with previous work indicating that networks can be resilient to the loss of nodes and that individuals can quickly re-establish connectivity in response to perturbations owing to redundancy in the social network. For example, networks of the social wasp *Ropalidia*

marginata were resilient to randomly removing a few nodes because the remaining individuals quickly established new connections (Naug, 2009). However, in general, networks are highly vulnerable to loss of nodes vital in maintaining connectivity within the network (Albert et al., 2000). For example, removal of key individuals – such as alpha males in groups of rhesus macaques, *Macaca mulatta* – may lead to intense aggression and even the collapse of the social group (Beisner, Jin, Fushing, & McCowan, 2015). Note, however, that our cichlid groups differ greatly from more despotic social hierarchies with a single alpha male at the top of the hierarchy (Williamson et al., 2016). In our study, each experimental group contained multiple dominant males, each of whom defended their own territory. Even though removing a dominant male led to an increase in status shifts, none of the groups collapsed, with most dominant males retaining dominance status and their original flowerpot. This robustness in the social hierarchy could underlie the limited effect of dominant male removal on the chase network at the group level, despite a significant increase in the frequency of chases in dominant males.

One weakness of our study is that instability was imposed by permanently removing key individuals, which makes the quantification of social stability less straightforward. For example, the matrix correlation method requires that the same individuals are represented in all matrices (Hobson, Avery, & Wright, 2013), so we were not able to use this method to quantify stability between the premanipulation network and the first postmanipulation network. In a previous study, we used repeated flowerpot rearrangements to trigger sustained changes in the social hierarchy in cichlid groups (Border, Piefke, et al., 2019; Sawecki, Miros, Border, & Dijkstra, 2019). Similar manipulations, in which network properties are altered without removing individuals, have been done in other study systems (Jordan, Maguire, Hofmann, & Kohda, 2016; Maldonado-Chaparro et al., 2018). It would be interesting to see how habitat manipulations influence network structure and dynamics. The high frequency of agonistic interactions combined with the ability to record well-defined direct interactions between individuals make cichlid fish a promising model system to study social network dynamics in a controlled setting.

In conclusion, we found evidence that making a new territory available did not only lead to complex changes in individual rank and behaviour but also overall social network structure. More generally, the need for manipulating social networks has been strongly encouraged in several influential papers on social networks (Boogert, Farine, & Spencer, 2014; Croft, Darden, & Wey, 2016; Farine & Whitehead, 2015). Our work provides rare insight into the behavioural and social network responses in experimentally perturbed social networks.

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Appendix

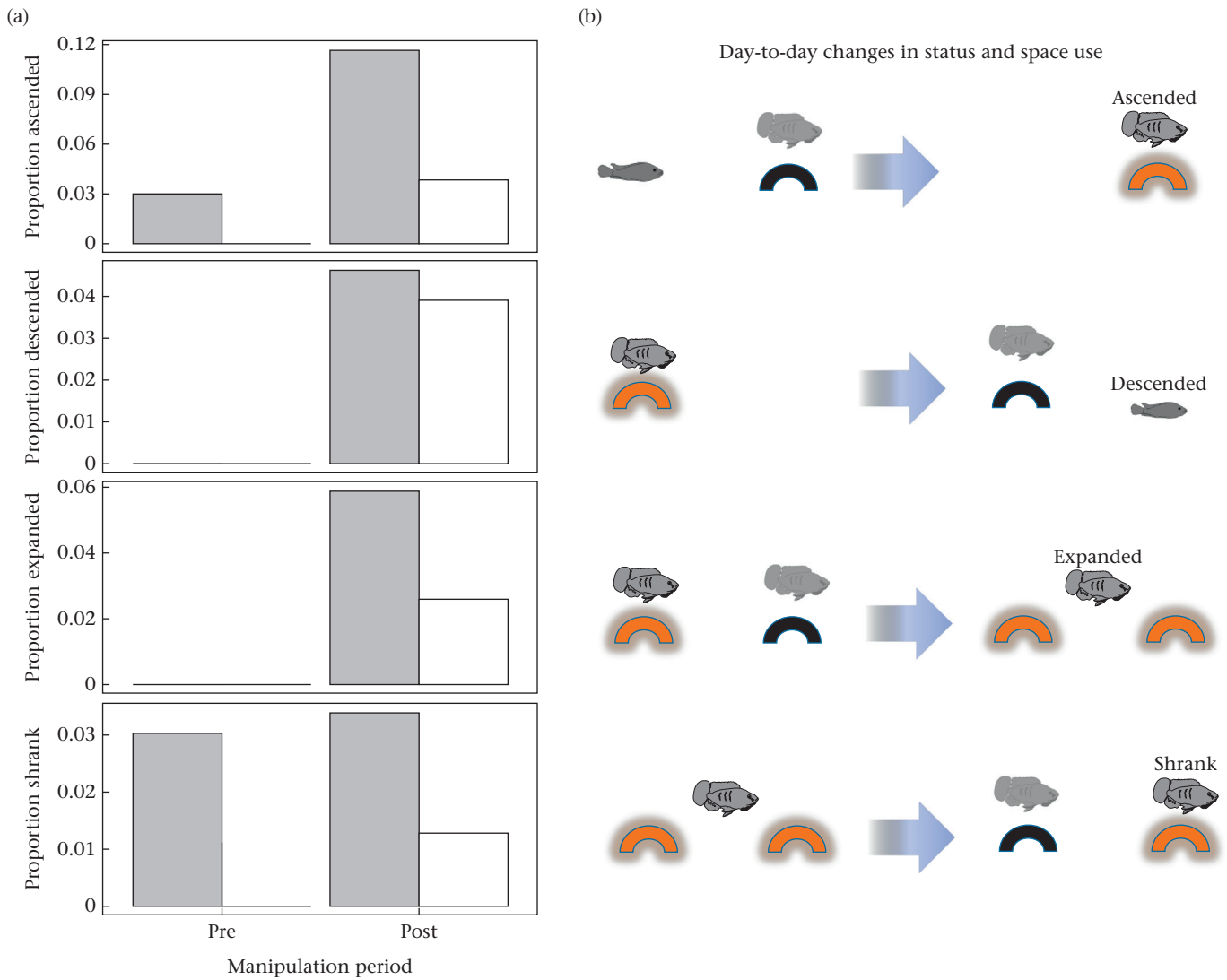


Figure A1. (a) Proportion of males relative to the total number of males that changed status or space use across all dominant (grey bars) and subordinate (open bars) removal groups before (pre) and after (post) a male was removed from a group. A status shift is defined as a male undergoing a shift from subordinate to dominant (ascended) or dominant to subordinate (descended) between consecutive days of observations. Males could also expand their territory by taking over a second flowerpot (expanded) or by losing their second flowerplot (shrank). In the premanipulation period, we recorded changes in status and space use on days 15–16. The daily changes for the postmanipulation period were the aggregate of the recorded status and space use changes for days 16–17, days 17–18 and days 18–19. (b) Illustration of changes in status and space use between two consecutive days. The focal male is shown in dark grey; shaded flowerpots denote flowerpots owned by the dominant focal male.

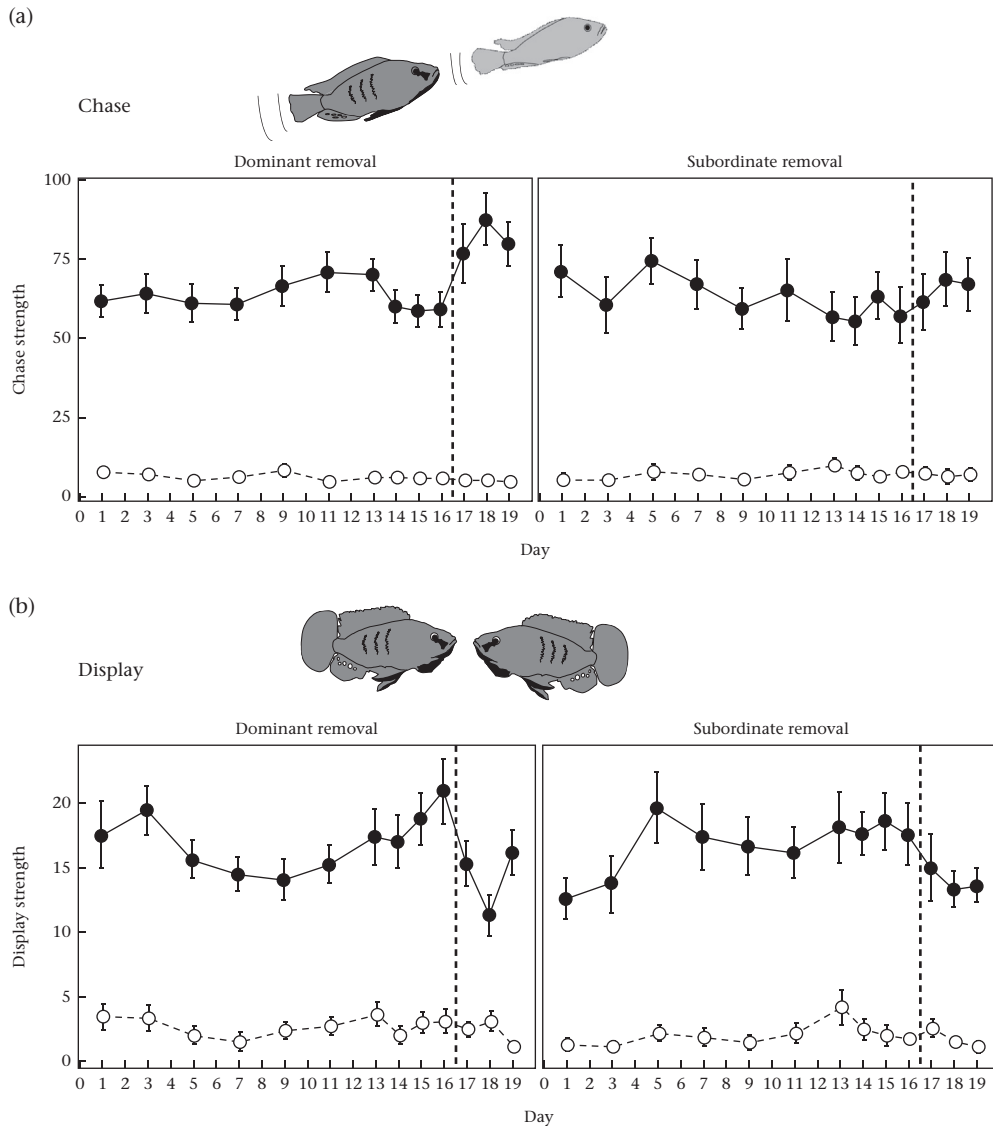


Figure A2. (a) Chase strength and (b) display strength for dominant (solid circles, $N = 740$ observations) and subordinate (open circles, $N = 1756$ observations) males across all days. The vertical dashed line indicates when male removal took place.

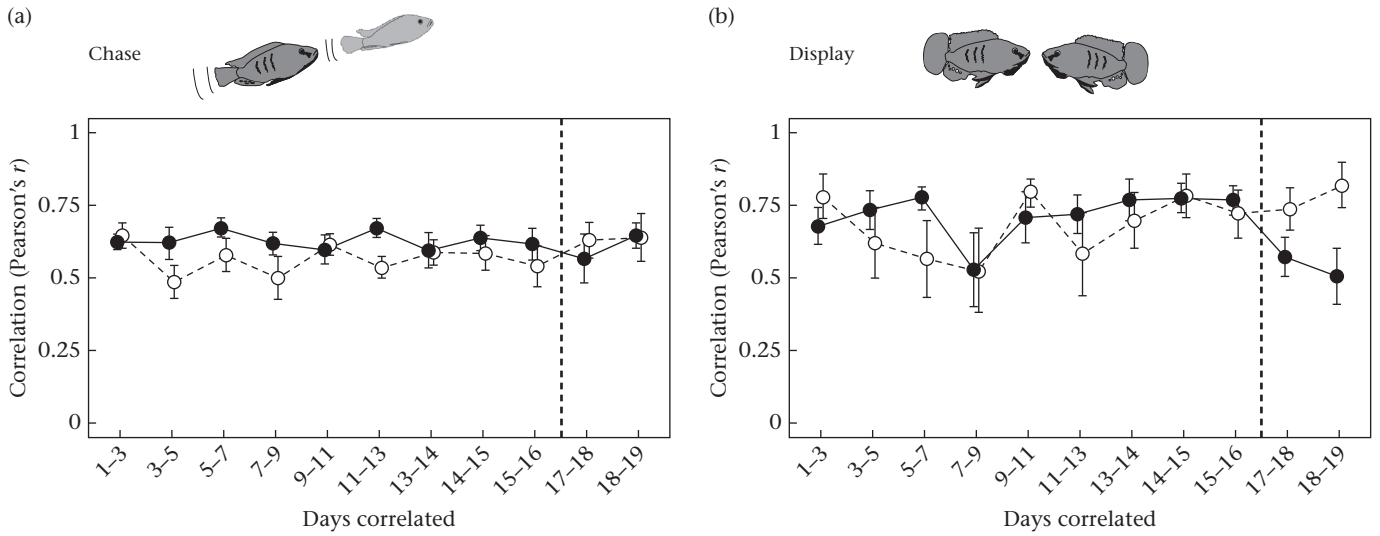


Figure A3. (a) Chase-based and (b) display-based daily matrix correlations following removal of a dominant (solid circles, $N = 9$) or subordinate (open circles, $N = 7$) male on day 16 after filming (dashed line). Correlations were calculated between adjacent days using Pearson's r correlation coefficients.

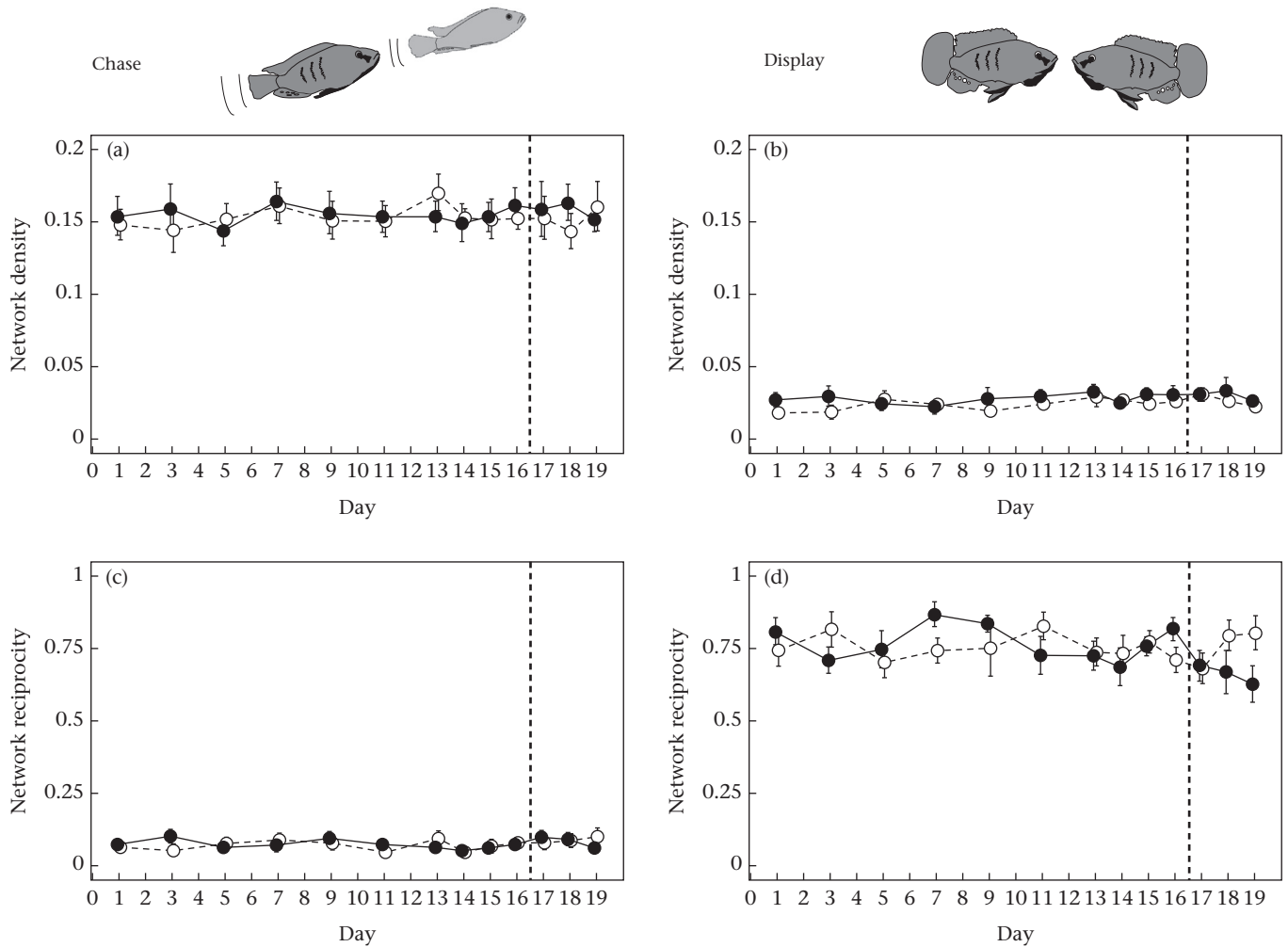


Figure A4. Group level network metrics for chase-based and display-based networks following removal of a dominant (solid circles, $N = 9$) or subordinate (open circles, $N = 7$) male. The vertical dashed line indicates when male removal took place. (a, b) Network density reflects the proportion of actual connections created compared to all possible connections that could be made between individuals within a group. Chase networks were denser than display networks. (c, d) Network reciprocity reflects the likelihood of vertices being mutually linked within a network and evaluates how often interactions occur in both directions between a target and its source. It ranges from 0 (aggression is never reciprocated) to 1 (aggression is always reciprocated). The level of reciprocity was higher in display networks compared to chase networks.