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First- and second-order sociality determine survival and reproduction in cooperative cichlids

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Cooperative breeders serve as a model to study the evolution of cooperation, where costs and benefits of helping are typically scrutinized at the level of group membership. However, cooperation is often observed in multi-level social organizations involving interactions among individuals at various levels. Here, we argue that a full understanding of the adaptive value of cooperation and the evolution of complex social organization requires identifying the effect of different levels of social organization on direct and indirect fitness components. Our long-term field data show that in the cooperatively breeding, colonial cichlid fish *Neolamprologus pulcher*, both large group size and high colony density significantly raised group persistence. Neither group size nor density affected survival at the individual level, but they had interactive effects on reproductive output; large group size raised productivity when local population density was low, whereas in contrast, small groups were more productive at high densities. Fitness estimates of individually marked fish revealed indirect fitness benefits associated with staying in large groups. Inclusive fitness, however, was not significantly affected by group size, because the direct fitness component was not increased in larger groups. Together, our findings highlight that the reproductive output of groups may be affected in opposite directions by different levels of sociality, and that complex forms of sociality and costly cooperation may evolve in the absence of large indirect fitness benefits and the influence of kin selection.

1. Introduction

In cooperatively breeding species, subordinate individuals typically delay dispersal and reproduction to instead help raising the offspring of others [1]. This observation does not conform with the fundamental prediction of evolutionary theory that individuals should maximize the production of own offspring [2]. For this reason, cooperative breeders have become model organisms for the study of cooperation and altruism [3]. Two alternative, non-mutually exclusive hypotheses to explain the evolution and maintenance of cooperative breeding have been proposed, which also apply to the evolution of cooperation in general [1,2]. First, cooperation may be explained by indirect fitness effects, where the enhancement of reproduction by closely related individuals increases the number of own genes in the next generation [4]. Second, cooperators may gain direct fitness benefits by enhancing the production of own offspring produced over their lifetime (e.g. due to improved survival chances when delaying reproduction) [1,5]. Several mechanisms by which direct fitness benefits may be reaped from cooperation have been proposed, including reciprocity [6], commodity trading [7], benefits from queuing for high-quality resources [8] and group augmentation [9]. A key aspect of direct benefits of cooperation in group-living animals is that group membership yields higher fitness than solitary life [9].

The benefits of group membership may depend on certain group traits. Group size, for instance, can affect a group's success and the fitness of group members

[10,11]. Larger groups have been shown to yield higher survival [12,13], higher reproductive output [14,15] and better foraging success [16,17] to group members in a wide range of animals. In cooperatively breeding species, increased cooperation levels are expected to enhance group performance [10], and indeed greater numbers of helpers often increase the reproductive output of groups [18–20]. Thus, cooperating individuals can gain from large group size in at least two ways: first, dominant breeders may enjoy greater reproductive output, increasing their own inclusive fitness and that of related group members. Second, both dominants and subordinates may obtain survival benefits from large group size, making cooperation mutually beneficial [9,21].

Individual fitness in cooperative breeders is usually scrutinized at the level of group membership (i.e. at the first or lowest level of social organization). However, the benefits of cooperation among group members are likely to vary also with factors determined by interactions between groups (i.e. at the second level of social organization). Indeed, social relations and interactions between groups significantly affect the fitness of group members in many species [22–25]. To date, however, the existence of multi-level social organization and its implications for social evolution have been recognized primarily in species that do not breed in cooperative groups [26–29]. The implications of higher-order sociality for within-group cooperation and possible downstream effects on individual fitness have rarely been quantified in cooperative breeders. This is unfortunate given that cooperatively breeding groups are often embedded in larger social units (e.g. large communal nests [30], communal territories [31], social networks [32] or colonies [33]), which probably influence within-group cooperation. For instance, groups may benefit from a larger size of the respective higher social unit (e.g. colonies [33]). Thus, to understand the evolution of sociality in cooperative breeders, it seems necessary to investigate the influence of higher-order social organization on the organization and performance of cooperatively breeding groups, and the potential effects on their members' individual fitness.

Here, we report on a long-term study of the cooperatively breeding cichlid fish *Neolamprologus pulcher*. Over the course of 3 years, we investigated the influence of group size and local density on the survival and productivity of individuals and groups in a natural population. This enables us to determine and compare the direct and indirect fitness effects of cooperative breeding in nature. In these cichlids, group sizes and local population densities vary considerably on small geographical scales [11,34], allowing us to investigate these factors in an otherwise similar environment. Larger groups of *N. pulcher* have been shown to persist longer [11] and to have higher reproductive output [20,34]. Fish prefer to settle at higher colony densities [33], and this entails benefits from communal anti-predator behaviour [35]. However, the influence of local density, in particular the role of between-group sociality and colonial breeding, on the productivity and survival of individuals and groups is currently unknown (see electronic supplementary material, appendix S1, for a more detailed description of the study species).

Based on the demonstrated benefits of large group size for group persistence and productivity in *N. pulcher*, we hypothesized that individuals in large groups would survive better. Further, we hypothesized that higher local population density would positively affect survival and productivity of individuals and groups, due to benefits of increased

communal predator deterrence. This would imply that individuals may accrue benefits of group living at different levels of social organization, which might be a fundamental component of the evolution of multi-level social organization in highly social organisms. Due to the low relatedness between large, sexually mature subordinates and dominants [36], we further hypothesized that the effects of group living on the indirect component of subordinates' inclusive fitness would be low compared with their direct fitness gains.

2. Material and methods

(a) Study colony and general methods

This study reports on demographic changes in a natural colony of *N. pulcher* over the course of 3 years, from 2011 to 2013. The focal colony was located off the Zambian coast of Lake Tanganyika, near Kasakalawe Point west of Mpulungu (8°46.849' S, 31°04.882' E). It covered an area of approximately 30 by 30 m at 10–12 m depth, and consisted of 166 group territories. Using scuba diving, we surveyed this colony in three consecutive years for approximately three months each, generally extending from end of August to end of November. We recorded the exact location and group size of all territories belonging to the colony in each year and marked each territory individually. Due to the high site fidelity of group members in this species [37], which was also revealed by our observation that most individually marked fish that were recaptured between years were found in the same groups where they had been marked (see [38], and results of this study), we assumed that territories continuously populated by *N. pulcher* groups in different years were inhabited by the same group (albeit not necessarily including all previous group members; cf. [11]). This allowed us to track group productivity and group survival, and to link these data to group size and local colony density. In 2011 and 2012, we caught and marked (see below) a total of 263 individuals in groups belonging to the focal colony. Based on the long-term surveys of these individuals, we determined individual survival and estimated individual fitness, and we linked both to group size and colony density.

(b) Local population density and group size

All territories were observed each year for a minimum of 5 min, during which we assessed their size and the respective group's size and structure. Repeated checks of the same groups within the same year revealed that this provided reliable estimates of group size and composition. We determined each territory's geometric centre by observing the respective group's behaviour and estimating the area it defended [34]. All distances (in metres) between territories were estimated from centre to centre. For every territory, we counted the number of territories located within 2 m, which corresponds to the typical ranging distance of these fish [38]. We also measured each territory's nearest neighbour distance. Group sizes were determined by including all *N. pulcher* tolerated in the respective territory. As juvenile fish below 1.5 cm standard length (SL) [39] typically do not participate in territory defence or maintenance [40], we calculated group size as the number of all fish larger than 1.5 cm (i.e. both dominant breeders and all subordinates above 1.5 cm SL). In addition, we counted the number of large, potentially sexually mature subordinates greater than 3.5 cm SL for our fitness estimates (see below). We determined the number of juveniles between 0.5 and 1.5 cm SL as a proxy of group reproductive output. We did not include free-swimming fry (i.e. fish less than 0.5 cm) in our count, as fry numbers are difficult to assess reliably without considerable disturbance of the group.

(c) Identifying and following individuals

In 2011 and 2012, individual *N. pulcher* were caught in their respective home territories using hand nets and Plexiglas tubes. Upon capture, we recorded each fish's sex and size (SL to the nearest mm), took a small fin-clip for DNA extraction and marked the fish with an individual subcutaneous elastomer tag (visible implant elastomer; Northwest Marine Technologies). Each individual was classified as dominant male (DM; $n = 52$), dominant female (DF; $n = 94$), male helper (subordinate males greater than 1.5 cm SL; SM; $n = 59$), or female helper (subordinate female greater than 1.5 cm SL; SF; $n = 58$) [41]. Afterwards, fish were released into their respective home territory, and their continued group membership was checked 24 h later. No fish processed in this way was lost or evicted from its territory during this time. In the subsequent year, we re-captured all fish that had elastomer marks and again took a small fin-clip. Individual identification between years was solely based on DNA fingerprinting; using alleles from 13 microsatellites, we unambiguously confirmed the identity of 137 individuals marked in one year and recaptured in the next. We considered the 126 individuals that had disappeared from the colony as being dead for the following reasons: (i) long distance dispersal is rare in this species [42] (A. Jungwirth, M. Zöttl, D. Bonfils and M. Taborsky 2015, unpublished data), (ii) we checked several neighbouring colonies for marked individuals, and (iii) in 2012 and 2013, we caught more than 200 unmarked fish spread over the entire focal colony and processed them as described above (in only one case had a fish been considered unmarked, but genetic analyses revealed it had completely lost its tags). Thus, while a few marked fish may have dispersed outside of our working range or may have remained undetected due to a loss of elastomer tags, we are confident that this does not apply to a large proportion of the fish considered in this study.

(d) Estimating fitness

To estimate direct and indirect components of inclusive fitness, we combined the information obtained in this study (i.e. counts of juveniles and large subordinates) with results from previous work. It has been shown that (on average) in *N. pulcher*, mature male subordinates sire 4.5% and mature female subordinates produce 14.5% of juveniles in a territory [43]. The mean degree of relatedness, r , between mature subordinates and DMs is 0.05, whereas it is 0.2 between mature subordinates and DFs [36]. The mean degree of relatedness between sexually mature subordinates is 0.2 [36]. Due to the brood care help provided by large subordinates, groups produce on average approximately 18% more offspring for every large subordinate present [20,21]. Combining these estimates with our measures of the number of juveniles produced in a group and the number of large (mature) subordinates, we estimated each marked individual's direct and indirect fitness components for the entire observation period (see electronic supplementary material, appendix S2, for details of the procedure).

It is important to note that these estimates do not reflect an individual's actual reproductive success or fitness. However, they allow for a comparison of the relative importance of direct and indirect fitness gains for dominant and subordinate fish. Notably, by using the same equations and the same parameter estimates (for relatedness, extra-pair reproduction and enhanced reproduction due to subordinates' help) for all fish of identical sex and status, while inserting the measured values of reproductive output and number of large subordinates in an individual's actual group, we obtain a relative measure of fitness costs and benefits of group membership for all marked individuals. This allows us to compare the estimated fitness benefits for dominants and subordinates derived from membership in groups of different size, composition and location within the colony.

(e) Statistical analyses

All analyses were carried out using R v. 3.1.1 [44]. Normally distributed data were analysed using linear mixed-effects models (LMEs). Non-normally distributed data were analysed using either Mann-Whitney U -tests (U -test), Wilcoxon matched-pairs signed-ranks tests (Wilcoxon test), generalized linear models (GLMs) or generalized linear mixed-effects models (GLMMs). GLMs and GLMMs were either fitted with logarithmic link function (log link; Poisson and quasi-Poisson distributed data) or with logistic link function (logit link; binomially distributed data). For detailed comparisons of different levels of single factors within GLMs and GLMMs, Tukey multiple comparison post hoc tests (Tukey test) were performed. GLMMs were fitted with the R package lme4 v. 1.1–7 [45]. Tukey tests were performed with the R package multcomp v. 1.3–7 [46]. See electronic supplementary material, appendices S3–S11, for detailed descriptions of the models.

3. Results

(a) Influence of group size and local density on group persistence

During a period of 3 years, we identified a total of 166 *N. pulcher* territories in the study area. In 2011, 135 territories were occupied. In 2012, seven of these groups had gone extinct, but 18 new territories had been established. In 2013, another five groups had gone extinct, but 16 territories had been newly established (three of which had been occupied by other groups in 2011 already). Thus, the colony increased in size from 2011 (135 groups) to 2012 (146 groups) and 2013 (157 groups) by 11 groups per year (approx. 8%).

Group size and nearest neighbour distance were highly negatively correlated with each other in any given year (LME: d.f. = 5, $p < 0.001$; electronic supplementary material, appendix S3). The average nearest neighbour distance was highly negatively correlated with the mean number of territories located within 2 m of a group across all years, revealing nearest neighbour distance as a good proxy of local colony density (GLM log link: $p < 0.001$; electronic supplementary material, appendix S4).

Groups that went extinct during our observation period were significantly smaller than groups that persisted (values averaged for groups observed in multiple years; groups that went extinct: mean size = 3.1 members, range = 1–8; persisting groups: mean group size = 5.8 members, range = 1–14.3; U -test: $p < 0.001$; figure 1a). Groups that went extinct had greater nearest neighbour distances than groups that persisted between years (values averaged for groups observed in multiple years; groups that went extinct: mean nearest neighbour distance = 1.04 m, range = 0.36–1.96; persisting groups: mean nearest neighbour distance = 0.71 m, range = 0.16–2.78; U -test: $p = 0.008$; figure 1b).

(b) Influence of group size and local density on group performance

A group's size in one year was correlated with its size in the subsequent year (groups that went extinct were excluded from this analysis; GLMM log link: $p < 0.001$; electronic supplementary material, appendix S5). Group size and nearest neighbour distance interactively influenced a group's productivity (GLMM log link: interaction between group size and nearest neighbour distance: $p < 0.001$; figure 2; electronic

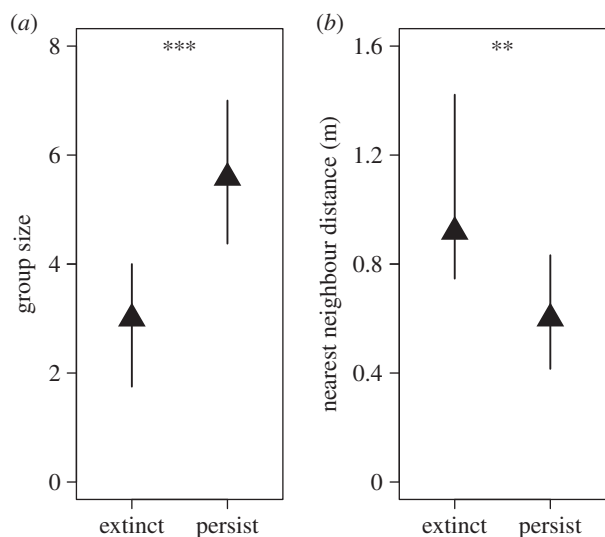


Figure 1. The (a) sizes and (b) nearest neighbour distances of groups that persisted throughout the observation period ($n = 154$) and those that went extinct ($n = 12$). Triangles represent medians and lines represent the interquartile range. Asterisks indicate significance levels based on U -tests (see results; $**p < 0.01$; $***p < 0.001$).

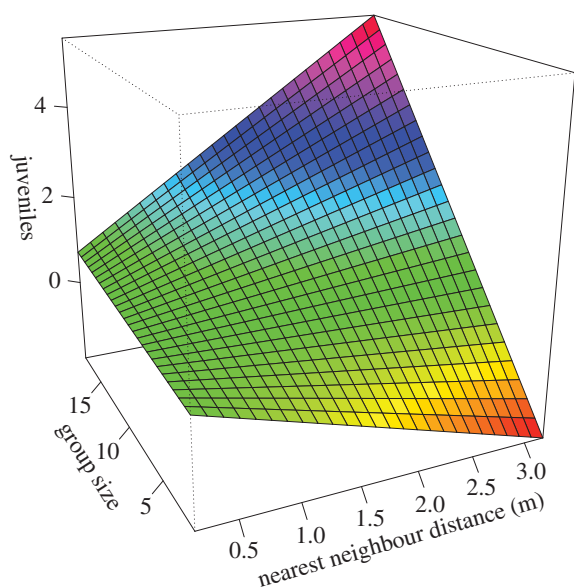


Figure 2. A group's reproductive output (i.e. the number of young between 0.5 and 1.5 cm SL) in relation to its size and nearest neighbour distance. The regression plane is based on values predicted by the link function of the respective GLMM (see results). Group size and nearest neighbour distance had a significant interactive influence on reproductive output: Larger groups had higher reproductive output at lower densities (i.e. at greater nearest neighbour distances), while the opposite was true for smaller groups. (Online version in colour.)

supplementary material, appendix S6): larger groups produced more offspring when local density was low (i.e. when having greater nearest neighbour distances), while the opposite was true for smaller groups. A similar effect was observed when investigating the influence of group size and nearest neighbour distance in a given year on the probability to reproduce in the next year (GLMM logit link: interaction between group size and nearest neighbour distance: $p = 0.011$; electronic supplementary material, appendix S6): larger groups were more likely to reproduce in the subsequent year when they were located in less dense areas (i.e. had greater

nearest neighbour distances). The opposite was true for smaller groups, which were more likely to reproduce in the next year when having nearer neighbours.

(c) Individual survival

Of the 52 DMs and 94 DFs marked in a given year, 21 (approx. 40%) and 60 (approx. 64%), respectively, survived to the next year. Of the 59 marked SMs and 58 marked SFs, 26 each (approx. 44% and approx. 45%, respectively) survived to the next year. DF survival was significantly more likely than DM (Tukey test: $p = 0.01$; electronic supplementary material, appendix S7). There were no differences in survival between any other two classes of individuals. Group size did not differentially influence individual survival between the different classes of individuals (GLMM logit link: interaction between group size and class: $p = 0.59$; electronic supplementary material, appendix S8), but there was a general trend for lower individual survival in larger groups (GLMM logit link: $p = 0.06$; electronic supplementary material, appendix S8, figure). Nearest neighbour distance did not differentially influence individual survival between the different classes of individuals (GLMM logit link: interaction between nearest neighbour distance and class: $p = 0.28$; electronic supplementary material, appendix S8), and nearest neighbour distance had no general effect on individual survival (GLMM logit link: $p = 0.9$; electronic supplementary material, appendix S8, figure).

(d) Estimated fitness

Inclusive fitness estimates diverged between different classes of individuals (GLM log link: class: $p < 0.001$; electronic supplementary material, appendix S9). DFs had higher inclusive fitness estimates than SMs (Tukey test: $p < 0.001$) and SFs (Tukey test: $p = 0.006$), but did not differ from DMs (Tukey test: $p = 0.5$). DMs had higher inclusive fitness estimates than SMs (Tukey test: $p = 0.007$), but not than SFs (Tukey test: $p = 0.29$). Inclusive fitness estimates of SMs and SFs did not differ from each other (Tukey test: $p = 0.36$). There was a trend for group size and individual class to interactively influence inclusive fitness (GLMM log link: interaction between group size and class: $p = 0.074$; electronic supplementary material, appendix S10). DMs and SMs had higher inclusive fitness in larger groups, while DFs and SFs had lower inclusive fitness in larger groups (electronic supplementary material, appendix S10, figure). Nearest neighbour distance did not influence individual inclusive fitness estimates in any way (GLMM log link: interaction between nearest neighbour distance and class: $p = 0.96$; GLMM log link: nearest neighbour distance: $p = 0.86$; electronic supplementary material, appendix S10), and there was no interactive effect of group size and nearest neighbour distance on inclusive fitness (GLM log link: $p = 0.3$).

When considering only the direct fitness component of individual inclusive fitness, a similar pattern was observed: class influenced individual direct fitness (GLM log link: class: $p < 0.001$; electronic supplementary material, appendix S9) in a way that DFs had higher direct fitness estimates than SMs (Tukey test: $p < 0.001$) and SFs (Tukey test: $p = 0.006$), and DMs had higher estimated direct fitness than SMs (Tukey test: $p = 0.004$), but not than SFs (Tukey test: $p = 0.17$). DMs did not have higher estimated direct fitness than DFs (Tukey test: $p = 0.66$), and direct fitness estimates of

SMs and SFs did not differ (Tukey test: $p = 0.4$). There was a trend for group size and class to interactively influence individual direct fitness (GLMM log link: interaction between group size and class: $p = 0.065$; electronic supplementary material, appendix S10). DMs and SMs had higher direct fitness estimates in larger groups, while DFs and SFs had lower estimated direct fitness in larger groups. Nearest neighbour distance did not influence individual direct fitness in any way (GLMM log link: interaction between nearest neighbour distance and class: $p = 0.96$; GLMM log link: nearest neighbour distance: $p = 0.86$; electronic supplementary material, appendix S10), and there was no interactive effect of group size and nearest neighbour distance on direct fitness (GLM log link: $p = 0.29$).

Indirect fitness effects also differed significantly between some classes of individuals (GLM log link: class $p < 0.001$; electronic supplementary material, appendix S9). Here, however, DMs had significantly lower estimated indirect fitness than DFs (Tukey test: $p < 0.001$), SMs (Tukey test: $p = 0.02$) and SFs (Tukey test: $p < 0.001$). No other two classes of individuals differed with regard to their estimated indirect fitness. Group size did not interactively influence the estimated indirect fitness of the different classes of individuals (GLM log link: interaction between group size and class: $p = 0.2$; electronic supplementary material, appendix S10). However, larger group size generally increased indirect fitness of individuals (GLM log link: group size: $p < 0.001$; figure 3). Nearest neighbour distance did not influence indirect fitness estimates in any way (GLMM log link: interaction between nearest neighbour distance and class: $p = 0.89$; GLMM log link: nearest neighbour distance: $p = 0.93$; electronic supplementary material, appendix S10), and there was no interactive effect of group size and nearest neighbour distance on inclusive fitness (GLM log link: $p = 0.74$).

Estimated direct fitness significantly exceeded the indirect component of fitness for all classes of individuals (figure 4; electronic supplementary material, appendix S12).

4. Discussion

Both group size and local population density appear to determine the persistence of cooperative groups in *N. pulcher*: small groups and groups located in sparsely populated areas went extinct with greater likelihood than larger groups and groups in denser areas (figure 1). Remarkably, group productivity was interactively influenced by group size and local population density, with larger groups being more productive in less densely populated areas, and smaller groups being more productive in denser areas of the colony (figure 2). The same pattern was observed for a group's probability of reproducing in the subsequent year: larger groups were more likely to reproduce in the next year when they were located in less dense areas. Smaller groups, on the other hand, had higher chances of reproduction in the next year when being located in denser areas. Thus, while within-group and between-group levels of sociality both had a significant positive influence on group survival, their influence on group productivity was not straightforward. This is remarkable, because group size and local density were highly correlated with each other, suggesting positive feedbacks. Their interactive influence on productivity, however, highlights that it is important to scrutinize potential fitness effects of both parameters separately.

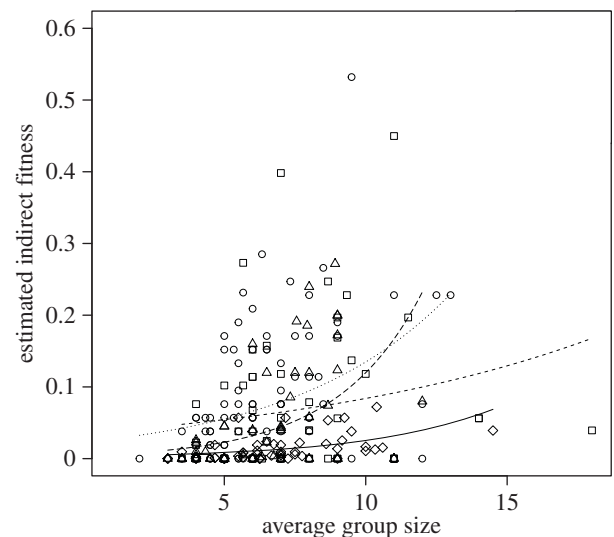


Figure 3. The estimated indirect fitness of each marked individual over the whole observation period in relation to its average group size. Fish classified as DFs in their first year of marking are represented by circles, SFs by rectangles, SMs by triangles and DMs by diamonds. Trend lines are based on the respective GLMs and represent significant increases in estimated indirect fitness with increasing average group size for all classes (see results).

Interestingly, neither group size nor local population density affected individual survival or inclusive fitness in a similar way as they affected group persistence and productivity. In fact, individual survival tended to be lower in larger groups. This discrepancy between the influence of group size and density on group persistence and on individual survival suggests significant competition effects among groups and individuals of *N. pulcher*. First, within-group conflict over rank apparently increases with group size. Group members indeed frequently exchange aggressive behaviours among each other [47,48], and a field experiment showed that lazy subordinates receive significantly more aggression from their peers in larger than in smaller groups [39]. Second, access to shelters may be more limited in larger groups; Field experiments revealed that a reduction of available shelters caused group sizes to shrink [34]. Third, increased between-group competition may offset potential benefits of high density. Groups of *N. pulcher* often engage in aggressive interactions at common territory borders [49], (A. Jungwirth and M. Taborsky 2015, personal observation). Fourth, individuals appear to benefit from living at high densities mainly by communally defending against predators. This allows for the reduction of individual anti-predator investment, while the combined anti-predator effort is similar across different local population densities [35]. Such a reduction in individual anti-predator investment can have long-term effects on fitness that are hard to detect, while causing similar instantaneous survival rates across different densities [13].

The described competition effects may also explain why we did not find a positive influence of larger group size on individual fitness estimates: An individual's estimated indirect fitness increased with increasing group size for all classes of individuals (figure 3), but direct fitness estimates were not affected by group size. Because indirect fitness was much lower than direct fitness (figure 4), inclusive fitness was not significantly related to group size. Fitness estimates of individuals initially caught and marked as subordinates were clearly lower than those of individuals that were dominant

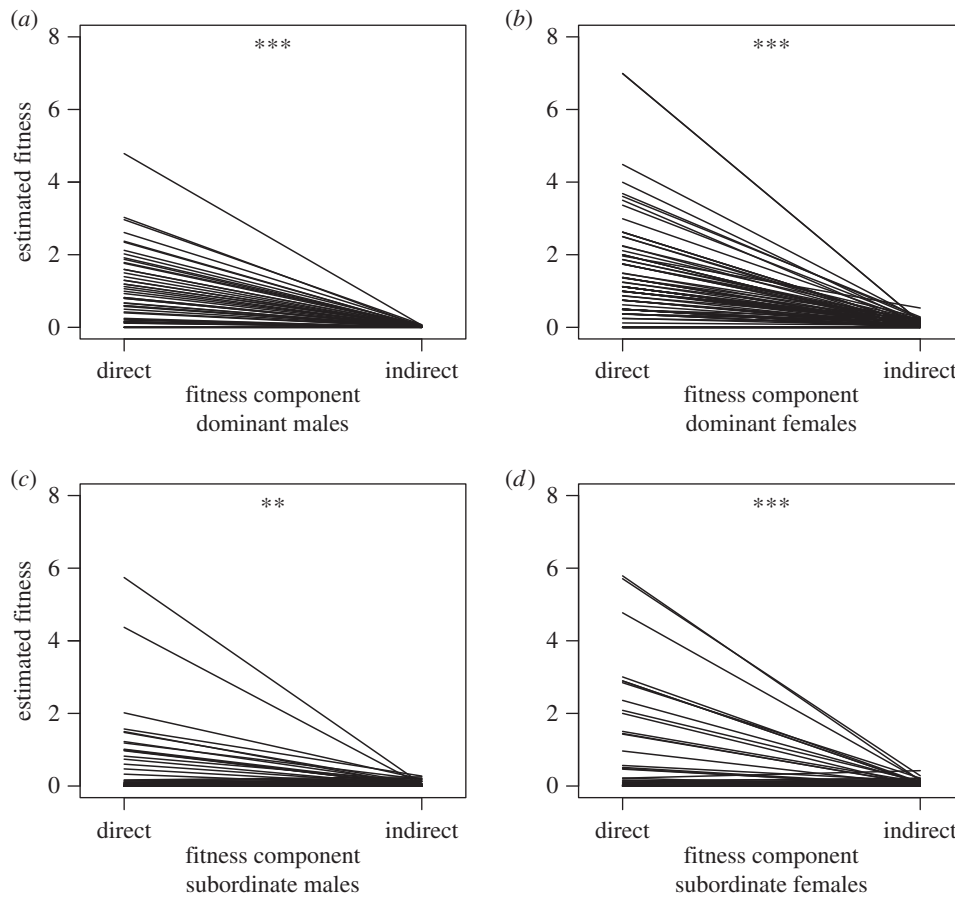


Figure 4. The estimated direct and indirect fitness components for all marked individuals. Each line represents an individual fish and connects the estimate of its direct fitness with the estimate of its indirect fitness. Values for (a) DMs, (b) DFs, (c) SMs and (d) SFs. Asterisks indicate significance levels from Wilcoxon tests (cf. electronic supplementary material, appendix S12; $**p < 0.01$; $***p < 0.001$).

throughout the observation period. This was caused by very low levels of direct and indirect fitness of subordinates while being helpers in a territory of dominants, because of their low relatedness to the dominants and the dominants' offspring, and due to their small share in reproduction. Subordinates that survived to become dominants themselves, however, achieved fitness estimates similar to those of fish that had been dominant throughout (cf. figure 4; electronic supplementary material, appendix S12). Thus, it appears that in *N. pulcher*, the major function of subordinates' cooperation with dominants is the enhancement of their chances to survive and reproduce later, rather than direct or indirect fitness gains during their helper stage. A crucial component of this pattern is the pay-to-stay mechanism [50,51], by which subordinates trade help for access to shelters and protection provided by dominants [7,52]. An increased cost of membership in larger groups may thus explain the absence of positive fitness effects of large group size [39].

It is important to note that all data presented here were collected with the strict objective to minimize disturbance to the groups and study colony, because we aimed at assessing fitness correlates under natural, undisturbed conditions. Thus we refrained from collecting offspring to assign actual paternity and maternity, and we confined catching and marking of individuals to relatively large fish, which are much easier to catch than small group members, and we did not repeatedly sample groups within a given year, except for the purpose of calibrating our group size and composition estimates. Further, we calculated fitness estimates based on established values of within-group relatedness, helper effects and extra-pair

reproduction, rather than measuring these parameters in our colony. While this implies that our results do not reflect actual fitness or reproductive output of individuals and groups, the analysis employed a standardized procedure, allowing us to compare the relative influence of group size and density on the performance of groups and individuals. Further, the data allow us to compare the relative importance of direct and indirect fitness components derived from group membership in groups of varying size and structure for fish of different sex and social status. There is theoretical evidence that class-specific means provide suitable tools to estimate fitness in class structured populations [53].

A positive effect of group size on group persistence and productivity had already been suggested by previous work on *N. pulcher* [11,34], but local population density had not been considered in these studies, nor had individual fitness been estimated. This neglect led to the erroneous conclusion that membership in larger groups would generally yield higher fitness, partly by increased survival and reduced effort for all group members, and partly by enhanced productivity of dominants [11,34]. Our findings show that individual *N. pulcher* do not necessarily gain from larger group size: at high densities, groups show a similar reproductive output, irrespective of their size. This is different at lower densities, where larger groups produce many more offspring (figure 2). In larger groups, however, individual survival seems to be compromised, perhaps resulting from increased competition among group members, as outlined above. This cost of membership in larger groups may be counterbalanced by the increased reproductive output observed at low densities, but at high

densities membership in large groups appears to be maladaptive. Consequently, members of the same group may have divergent fitness interests with regard to group size, depending on their status and the group's position in a colony: at high densities, all group members appear to be better off in small groups. This is because here reproduction is not significantly improved by large group size, but survival may be negatively affected in larger groups. At low densities, however, dominants may seek to increase group size, because they are the main beneficiaries of increased reproductive output (cf. our results on fitness estimates). Subordinates, however, may benefit from smaller group size, because they gain relatively little from increased reproduction, but their chances of survival and inheritance of the territory may be low in larger groups.

Group size positively relates to survival of individuals and groups also in other highly social animals (e.g. in cooperatively breeding dwarf mongooses [54] and meerkats [12,55], and in social spiders [42,56]). However, a lack of survival benefits from large group size (e.g. scrubwrens [57]), or even a negative impact of large group size on survival (e.g. Seychelles warblers [58]), have also been documented. Positive effects of large group size on productivity have been reported in carrion crows [15], Florida scrub jays [18], meerkats [19], dwarf mongooses [54] and social wasps [59,60], but the opposite effect has been observed in social spiders [56] and scrubwrens [57]. Thus, there is currently equivocal evidence for a potential general directional effect of group size on group persistence, productivity and individual survival. Our data may clarify some of the underlying mechanisms involved in inconsistent group size effects by showing that the positive impact of group size on group persistence and productivity does not necessarily affect individual survival, probably because of within-group competition as outlined above. Furthermore, we show that the effects of group size can be strongly influenced by local population density. This highlights that the costs and benefits of cooperation are dependent on factors observed at different levels of social organization. Within the same species, selection may favour larger or smaller group size, contingent on the broader social environment and an individual's social status. Thus, the fact that larger groups are favoured in some species but are penalized in others may be due to effects of the wider social system in the respective species. Additional studies investigating the interplay of different forms of social organization within the same species are needed for a better understanding of social evolution.

There is general agreement that high relatedness favours the evolution of cooperation and complex societies, because of

the importance of indirect fitness and kin selection [61–63]. However, higher direct than indirect fitness components for helpers in cooperative breeders have been determined in Seychelles warblers [64] and in paper wasps [65]. In these species, subordinates apparently cooperate to gain direct fitness either via reproduction as subordinates, or via inheritance of the dominant breeder position. Our study reveals that similar effects may have selected for the evolution of cooperative breeding also in cichlid fish. This suggests that the general importance of direct fitness benefits for the evolution of cooperative breeding is currently underestimated [66].

We demonstrate that the benefits of cooperation in a highly social species depend interactively on more than one level of social organization, and that these benefits may predominantly be derived from gains in direct fitness. This suggests that the current focus on first-order sociality (i.e. group membership) and indirect fitness benefits of cooperation in natural systems may be too narrow. Especially in species in which individuals are embedded in multi-level social organization, and where relatedness among interacting individuals is variable or generally low, the effects of inter-group relationships and the relative importance of direct and indirect fitness components should be considered in future studies.

Ethics. We successfully minimized disturbance of individuals and groups, as indicated by no observable negative impact on fish behaviour and survival within a field season and the growth of the colony between field seasons. All work reported here complied with Zambian laws and was carried out in agreement with local authorities under the Memorandum of Understanding issued by the Department of Fisheries: Ministry of Agriculture and Cooperatives, Zambia, dated 20 March 2009.

Data accessibility. All data presented here can be found in the Dryad repository: <http://dx.doi.org/10.5061/dryad.qh7k8>.

Authors' contributions. A.J. and M.T. designed the study and collected the data. A.J. wrote the first draft of the manuscript and performed the analyses. A.J. and M.T. contributed to revisions of the manuscript.

Competing interests. We declare we have no competing interests.

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