

# Experimental evidence for helper effects in a cooperatively breeding cichlid

Lyanne Brouwer,<sup>a,b</sup> Dik Heg,<sup>a</sup> and Michael Taborsky<sup>a</sup>

<sup>a</sup>Department of Behavioural Ecology, Zoological Institute, University of Bern, CH-3032 Hinterkappelen, Switzerland, and <sup>b</sup>Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14 Haren, The Netherlands

*Neolamprologus pulcher* is a cooperatively breeding cichlid fish, in which helpers stay in their natal territory and help with brood care, territory defense, and maintenance. In this study we investigated helper effects by an experimental group size reduction in the field. After this manipulation, focal helpers in reduced groups tended to feed less, and small helpers visited the breeding shelter significantly more often than same-sized helpers in control groups. No evidence was found that remaining helpers compensated for the removed helpers by increasing territory defense and maintenance behavior. Breeders, however, did show a lower defense rate, possibly caused by an increase in brood care effort. Survival of fry was significantly lower in removal than control groups, which provides the first experimental proof in a natural population of fish that brood care helpers do effectively help. The data suggest that in small, generally younger, helpers, kin selection may be an important evolutionary cause of cooperation. Large helpers, however, who are generally older and less related to the breeders than small helpers are suggested to pay to be allowed to stay in the territory by helping. All group members benefit from group augmentation. **Key words:** cooperative breeding, group size reduction, helping behavior, Lake Tanganyika cichlids, reproductive success. [*Behav Ecol* 16:667–673 (2005)]

In at least 3% of bird and mammal species and in some fishes, offspring remain with their parents and help to rear kin, often even after they are sexually mature (Brown, 1987; Taborsky, 1994). This is an intriguing behavior because of the apparent paradox of individuals helping to rear offspring of conspecifics instead of breeding on their own. Numerous studies have aimed to explain why individuals stay and why they help (Emlen, 1982; Griffin and West, 2003; Maccoll and Hatchwell, 2004; Richardson et al., 2002; Taborsky, 1984, 1985; for review see Brown, 1987; Clutton-Brock, 2002; Cockburn, 1998; Emlen, 1991; Koenig et al., 1992). Several studies have focused on the role of ecology in the evolution of cooperative breeding (Arnold and Owens, 1999; Davies et al., 1995; Koenig and Mumme, 1987; Rabenold, 1990; Reyer, 1980, 1984). Individuals might be limited to disperse or breed independently because of ecological constraints like habitat saturation or high risk of dispersal (Brown, 1974; Emlen, 1982; Heg et al., 2004; for review see: Emlen, 1997; Koenig et al., 1992). Furthermore, individuals might stay because they gain benefits by staying in the natal territory, like gaining protection, feeding benefits, or inheriting the territory (Balshine-Earn et al., 1998; Ligon and Ligon, 1978; Reyer, 1980, 1984; Taborsky, 1984). In contrast to the emphasis on ecological constraints, the life-history hypothesis emphasizes the role of life-history traits in the evolution of cooperative breeding (i.e., the combination of low mortality, low fecundity, and low dispersal promotes the likelihood of the species showing cooperative breeding; see Arnold and Owens, 1998; Poiani and Jermini, 1994; and for review see: Hatchwell and Komdeur, 2000).

Helping may be a consequence of staying in the natal territory, when individuals pay by helping for being allowed to stay while waiting for future breeding opportunities (Balshine-Earn et al., 1998; Bergmüller and Taborsky, 2005; Emlen, 1982; Gaston, 1978; Mulder and Langmore, 1993; Taborsky,

1984). On the other hand, helping may be beneficial because of the production of kin (Brown, 1987; Griffin and West, 2003; Komdeur, 1994; Stacey and Koenig, 1990). Alternatively, if individuals survive or reproduce better in larger groups, cooperative behavior can be explained by group augmentation (Brown, 1987; Clutton-Brock, 2002; Kokko et al., 2001; Woolfenden, 1975). In this case individuals benefit from raising new group members even if these are unrelated (Kokko et al., 2001).

*Neolamprologus pulcher* is a cooperatively breeding cichlid, a subspecies or sister species of *Neolamprologus brichardi*, which is commonly known as the “Princess of Burundi” (see Grantner and Taborsky, 1998, for a discussion of the taxonomic status of the species). Groups consist of a breeding pair and 1–17 helpers of both sexes, and their territories are widely distributed along the shores of Lake Tanganyika, Africa, at depths ranging from 3 to 45 m (Balshine et al., 2001; Taborsky, 1984; Taborsky and Limberger, 1981; Heg D, Taborsky M, Brouwer L, personal observations). Helpers assist breeders by defending the territory against space competitors and predators and by maintaining the territory by digging and carrying sand and debris away from the shelters. Helpers also provide direct brood care by cleaning and fanning eggs and larvae and defending them from egg stealers. *N. pulcher* feed in loose aggregations on zooplankton floating in the water column above the territories but usually do not actively compete over food.

Although these fish have been studied in Lake Tanganyika for many years, it is still not completely clear why individuals stay in the natal territory and help until well after sexual maturity (Balshine-Earn et al., 1998; Taborsky, 1984, 1985; Taborsky and Limberger, 1981). Several populations of *N. pulcher* have been studied. In the northern part of Lake Tanganyika (Burundi) shelters are scarce, and fish live in large aggregations after leaving the natal territory (Taborsky, 1984; Taborsky and Limberger, 1981). However, in the southern population (Zambia) where this study was conducted, *N. pulcher* groups breed in rather tight colonies within vast areas of apparently suitable, but largely unoccupied

Address correspondence to D. Heg. E-mail: dik.heg@esh.unibe.ch.

Received 14 March 2004; revised 22 February 2005; accepted 25 February 2005.

© The Author 2005. Published by Oxford University Press on behalf of the International Society for Behavioral Ecology. All rights reserved. For permissions, please e-mail: journals.permissions@oupjournals.org

habitat, and large aggregations of nonterritorial fish were never observed (Taborsky M, Heg D, Brouwer L, personal observations). It is therefore unlikely that in this population, delayed dispersal of *N. pulcher* is a result of habitat saturation. Helping is energetically costly, and helpers incur fitness costs by growing slower (and thereby slowing down sexual maturity) than same-sized nonhelpers (Grantner and Taborsky, 1998; Taborsky, 1984; Taborsky and Grantner, 1998). These costs can be outweighed by kin selection (Balshine et al., 2001; Taborsky, 1984), protection due to antipredator defense by larger group members (Heg et al., 2004; Taborsky, 1984), successful participation in the reproduction of territory owners (Dierkes et al., 1999), or by inheritance of a territory and partner (Balshine-Earn et al., 1998). Kin selection seems an obvious explanation for helping behavior. However, helpers of *N. pulcher* may not be related to the breeders as they usually stay in the territory and continue helping when one or both of the breeders are exchanged (Balshine-Earn et al., 1998; Stiver et al., 2004; Taborsky, 1984). This is especially the case for large helpers: the older a helper gets, the more likely that one or both of its parents have been exchanged already (Taborsky and Limberger, 1981; Dierkes P, Heg D, Taborsky M, Skubic E, Achmann R, personal communication).

Experiments showed that after temporarily removing a helper from its group, the returning helpers were attacked by other group members and were eventually evicted in 29% of the cases, suggesting that helpers have to pay for being allowed to stay (Balshine-Earn et al., 1998; see also Bergmüller and Taborsky, 2005; Bergmüller et al., 2005). In contrast to predictions derived from the pay-to-stay hypothesis, however, breeders did not respond aggressively towards the removed helper (Balshine-Earn et al., 1998; Bergmüller and Taborsky, 2005). In *N. pulcher*, group size is positively correlated with reproductive success and feeding rate (Balshine et al., 2001). Survival is positively correlated with group size for the breeders and the larger helpers under the risk of predation (Heg et al., 2004). Furthermore, breeders from larger groups have a lower workload (Balshine et al., 2001). This suggests that the cooperative behavior can be explained here at least partly by the direct benefits individuals derive from group augmentation, like dilution effects or a deterrent effect on potential predators or intruding competitors (Heg et al., 2004; Taborsky, 1984; see Kokko et al., 2001). However, increasing group size also has disadvantages as the number of available shelters is a limiting resource (Balshine et al., 2001). Furthermore, the described correlations could be a consequence of variation in territory quality as group size is also correlated with territory quality (Balshine et al., 2001).

Apparently several different mechanisms work in conjunction in the evolution of cooperative breeding in this species. The aim of this study was to test experimentally whether kin selection, pay-to-stay, and group augmentation may be involved in the evolution of cooperative behavior in *N. pulcher*. We used a group size reduction experiment and observed the behavior of the remaining helpers in the group. In addition, we measured the effect of group size and helping on reproductive success. Previous removal experiments in cooperative breeders showed that helpers increase reproductive success, at least under some circumstances (Brown et al., 1982; Komdeur, 1994; Mumme, 1992; Shreeves and Field, 2002; but see Leonard et al., 1989). A failure to find an effect might be due to other group members compensating for the removed helpers, so in birds, for example, the total amount of food delivered may remain the same compared to groups with no helpers removed (Hatchwell and Russell, 1996; Legge, 2000; Wright and Dingemanse, 1999). Hence, it is paramount to study compensatory effects by other group members in such experiments, that is, changes in parental investment

of the breeders and helping behavior of the remaining helpers.

If helpers help primarily because of the benefits of kin selection, one would expect that reduced group size would lead to a higher workload for the remaining helpers because the same number of offspring and the same territory have to be defended and maintained. So group size reduction should lead to compensation of helping behavior by the remaining helpers, especially the smaller, more related helpers. If helpers pay for being allowed to stay, one would expect that reduced group size should lead to less competition between the helpers. Furthermore, each helper becomes more valuable for the breeders, consequently group size reduction should not lead to compensation of the removed helpers, but it might even lead to a decrease in helping behavior. If *N. pulcher* survives and/or reproduces better in large groups, that is, due to group augmentation, group size reduction would have negative effects on survival, feeding rate, or reproduction.

## METHODS

### Study area and data collection

The study was done in a *N. pulcher* colony at Kasakalawe Point, Mpulungu, Lake Tanganyika, Zambia (colony 2, 8°46.849' S, 31°04.882' E, between 5 March and 27 May 2002). This colony consisted of a cluster of >100 groups (average group size: 6.5, range = 3–18, including all individuals larger than 19.5 mm SL; see also Balshine et al., 2001) at a depth of 9–11 m, approximately 200 m from the shore. Observations were recorded on PVC slates while scuba diving. A 3–5 min habituation period was allowed before each observation. Preliminary analysis of underwater videos suggested that the fish are not strongly influenced by the presence of observers (Balshine et al., 2001). All observations were carried out between 0600 and 1300 h.

### Removal experiment

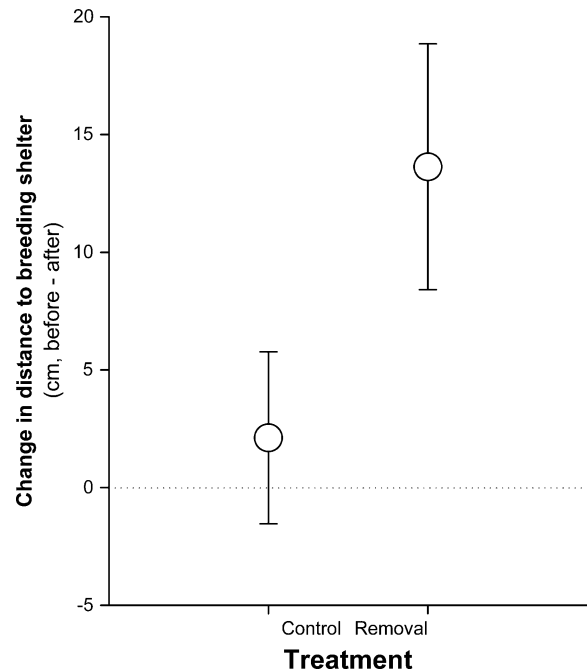
The effect of group size reduction on helping behavior and reproductive success was tested with the help of a removal experiment in which only groups with offspring were included. Offspring are defined as small fish (<15 mm SL), including fry that do not help. By this selection criterion the effect of reduced group size on the survival of young could be investigated while the experimental groups were matched for the phase in the breeding cycle. Group composition and reproductive status were assessed by repeated visits to each territory. Pairs of groups matched for group size and similar numbers of large, medium, and small helpers were selected ( $n = 10$  removal and  $n = 10$  control groups). From each of the two matched groups two focal helpers were selected, again matched for size, one potentially sexually mature (>37 mm SL) and one probably immature (25–37 mm SL; Taborsky, 1984). This was done in such a way that each helper in a group was matched for size with a helper in the corresponding group. The selected individuals were observed for 15 min in sequence, the order of the two observations (control fish or experimental fish) randomized, during which we recorded territory defense (attacks on neighbors, competitors and predators), the number and time of visits to the breeding shelter and other shelters ("hiding shelters," used for hiding from predator attacks), territory maintenance (digging and carrying of snails and sand), feeding frequency (number of bites), and interactions with group members (submissive behavior and aggressive interactions). Furthermore, once every minute the distance of the focal fish to the breeding

shelter was estimated, and averaged per observation before analyses. As it was not possible to observe behavior in the breeding shelter, the time and visits to the breeding shelter were recorded to obtain a potential correlate of brood care (Balshine-Earn et al., 1998; Balshine et al., 2001). In addition, the whole group was observed for 10 min during which territory defense and territory maintenance of all group members were recorded by all occurrence sampling. To control for temporal variation, each observation was followed or preceded by its control. After these observations of unmanipulated groups, each group was randomly assigned to the control or removal treatment.

On the same day, fish were captured in the selected groups by guiding them into transparent PVC tubes with hand nets. The fish were measured, sexed (by examination of the genital papilla), and individually marked underwater. Marking involved fin clipping and injection of nontoxic acrylic paint into 1–3 scale pouches in 18 possible locations on the body (Balshine et al., 2001). These marks fade after a few weeks and do not affect the fish behavior (Balshine et al., 2001). Unmarked fish could be individually identified based on their territory affiliation, body size, and unique natural body markings. Sexing and marking were done directly after the unmanipulated observations. After processing, the caught helpers designated for removal from the experimental groups were put in a cage outside the colony, while the other helpers (including all helpers from the control groups and the focal helpers of both types of groups) were released back into their territory. From each removal group 30–50% of the helpers were removed ( $40 \pm 3\%$ , mean  $\pm$  SE). The behavioral observations were repeated 1, 2, and 7 days after removal to determine if there were any effects of reduced group size on helping behavior and reproductive success. After completion of the experiment, all removed helpers, which all survived, were released back into their respective territories. No observations were made after this.

### Data analyses

Nine pairs of groups consisting of one control and one removal group each were matched in original size, as originally planned. Unfortunately in the tenth pair the focal marked helpers in the control group were disturbed due to our catching effort and therefore had to be discarded from the behavioral analyses. The remaining removal group could be matched to another pair of groups, giving a trio of one control and two removal groups. This left us with 10 removal and 9 control groups, matched into 9 pairs (8 pairs and 1 trio). Most of the data were not normally distributed (territory maintenance behavior, breeding shelter visits, aggressive interactions, submissive behavior), and therefore these data were normalized using the adjusted square-root transformation:  $\sqrt{x + 3/8}$  (Zar, 1996). However, breeding shelter visits and submissive behavior remained nonnormally distributed; therefore, these data were analyzed for large and small helpers separately using nonparametric tests with help of the program SPSS 12.0. To correct for any group effects, behavioral data obtained after the removal of helpers were averaged and subtracted with the value from the observation done before the removal of the helpers. As in each family a large and a small helper were observed, these data cannot be considered independent data points. To take the hierarchical structure and nonindependence of data into account, multilevel modeling was used in MlwiN 2.0 (Rasbash et al., 2004). MlwiN uses a general linear mixed-modeling approach with a hierarchy of nested effects. We created the following levels (from highest to lowest level): (1) pair number (nine pairs, random effect), (2) group number (19 groups, random



**Figure 1**

Helpers stayed closer to breeding shelters after the manipulation in the removal but not in the control treatment. Depicted are the mean changes in the distance to the breeding shelter ( $\pm$ SE), by subtracting the manipulation after measurement from that before, for removal and control groups, respectively.

effect, with fixed effect “treatment”), and (3) helper size (two sizes).

Both time and frequency of visits to the breeding and hiding shelters were recorded, but as times and frequencies correlated strongly with each other (Spearman rank correlation, breeding shelters:  $r_s = .989$ ,  $n = 255$ ,  $p < .001$ ; hiding shelters:  $r_s = .989$ ,  $n = 255$ ,  $p < .001$ ) only the frequency of visits to these shelters were analyzed.

The number of offspring, all fish smaller than 15 mm SL, was counted on day 0 and day 7 of the experiment in the 10 control and 10 experimental groups. To test for the effect of helpers on offspring, the percentage offspring survival between day 0 and 7 was calculated. This parameter followed a normal distribution (Kolmogorov-Smirnov tests, control groups:  $z = 0.87$ ,  $n = 10$ ,  $p = .44$ ; experimental groups:  $z = 0.69$ ,  $n = 10$ ,  $p = .73$ ) and was compared between the two treatments with a paired-samples  $t$  test. All significance tests were two-tailed, and means are expressed with standard errors if not mentioned otherwise. Due to the matched-pairs design, most confounding variables were corrected for.

## RESULTS

### Effects of helper removal on behavior

Estimates of the distance to the breeding shelter show that after removal, helpers from reduced groups stayed closer to the breeding shelter than helpers from control groups (Figure 1; Table 1). Furthermore, helpers from reduced groups visited the breeding shelters more often than helpers from control groups; however, this was only the case for small helpers (Wilcoxon tests, small:  $T = -2.52$ ,  $n = 9$ ,  $p = .008$ ; large:  $T = -0.68$ ,  $n = 9$ ,  $p = .56$ ; Figure 2a). Group size reduction did not influence the rate of other helping behaviors as helpers from reduced groups did not maintain or defend their territory more than helpers from control

Table 1

Results of the hierarchical general linear mixed models, testing for effects of treatment (removal or control), helper size (small or large), and their interaction (all three  $df = 1$ ), on each of the different behaviors of the focal helpers ( $n = 38$ ) separately

| Dependent variable           | Treatment |      | Helper size |     | Helper size $\times$ treatment |     |
|------------------------------|-----------|------|-------------|-----|--------------------------------|-----|
|                              | $\chi^2$  | $p$  | $\chi^2$    | $p$ | $\chi^2$                       | $p$ |
| Distance to breeding shelter | 4.79      | .03  | 0.09        | .76 | 0.03                           | .86 |
| Territory defense            | 0.54      | .46  | 0.03        | .86 | 1.65                           | .20 |
| Territory maintenance        | 0.001     | .97  | 0.68        | .41 | 1.18                           | .28 |
| Feeding rate                 | 3.72      | .054 | 0.85        | .36 | 0.35                           | .55 |
| Hiding shelter visits        | 0.001     | .97  | 3.46        | .06 | 0.30                           | .58 |
| Aggressive interactions      | 0.08      | .78  | 0.12        | .73 | 0.26                           | .61 |

Statistics derived from the normal response mixed-modeling procedure in MlwiN, correcting for random variation due the "group number" (medium level) within "pair number" (highest level, matched control, and removal groups) effects.

groups (Table 1). Helpers from reduced groups had lower feeding rates than helpers from control groups; however, this was just not significant ( $p = .054$ , Figure 2b). Group size reduction did not affect the number of visits to the hiding shelters (Table 1).

Helpers from reduced groups did not show more or less submissive behavior or have more or less aggressive interactions with their group members than helpers in control groups (Table 1; submissive behavior: Wilcoxon tests, small helpers:  $T = -0.35$ ,  $n = 9$ ,  $p = .79$ ; large helpers:  $T = -0.84$ ,  $n = 9$ ,  $p = .44$ ).

Although helpers did not change their defense behavior when group members were removed and the average territory maintenance frequencies per group member did not differ between control and reduced groups (paired  $t$  test:  $t_8 = -0.66$ ,  $p = .53$ ), the observations of all group members together showed that the average territory defense frequencies per group member were lower for reduced than for control groups (paired  $t$  test:  $t_8 = -3.24$ ,  $p = .012$ ). This indicates that breeders responded to helper removal by decreasing their defense effort.

In each group, a small (mean  $\pm$  SE =  $33.8 \pm 0.50$  mm, range: 30.0–37.0 mm) and a large (mean  $\pm$  SE =  $46.8 \pm 1.0$  mm, range: 38.0–58.0 mm) focal helper were observed. Apart from the differences in breeding shelter visits, small helpers tended to visit the hiding shelters more often than large helpers, which was independent of group size reduction, however, and just not significant ( $p = .06$ ). All other behaviors did not differ as well between large and small helpers (Table 1).

The focal helpers were not matched for sex. The overall sex ratio of the observed marked helpers was male biased, though not significantly (67% male; binomial test,  $n = 30$ ,  $p = .10$ ). The sex ratio was not skewed within the reduced or control group or within the large and small helper groups ( $\chi^2 = 1.20$ ,  $n = 30$ ,  $p = .27$ ;  $\chi^2 = 1.09$ ,  $n = 30$ ,  $p = .30$ , both  $df = 1$ ). Due to small sample sizes no effects of sex on helping behavior could be tested.

### Effects on reproductive success

Group size reduction affected offspring survival. The percentage of offspring per group surviving the week after removal was higher in controls than in reduced groups (paired  $t$  test,  $t_9 = -2.66$ ,  $p = .026$ ; Figure 3). From five pairs of groups that

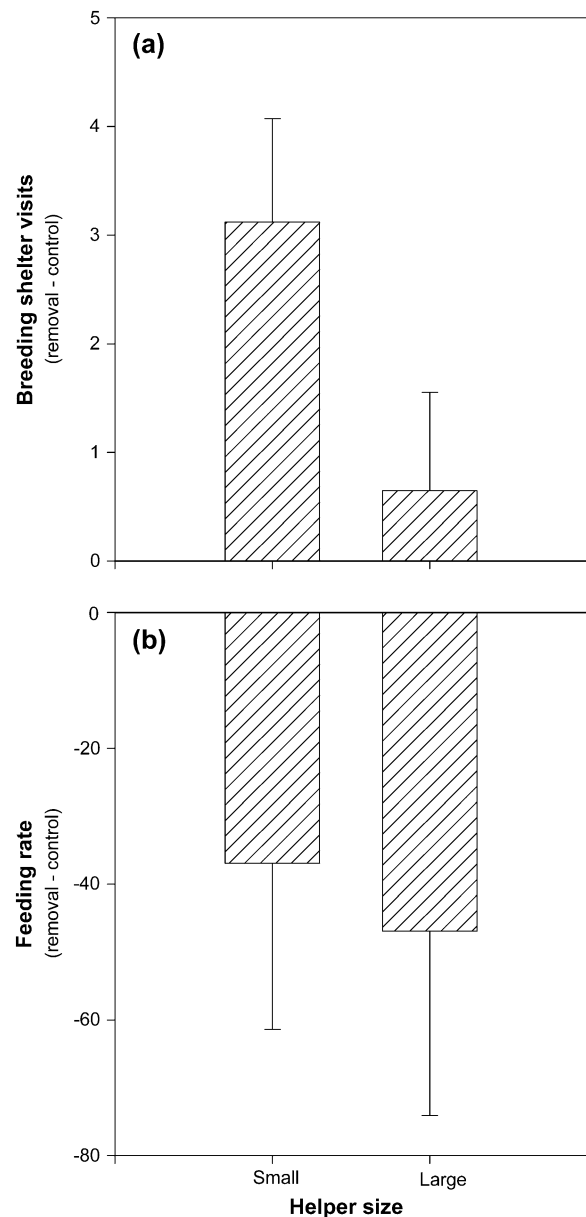


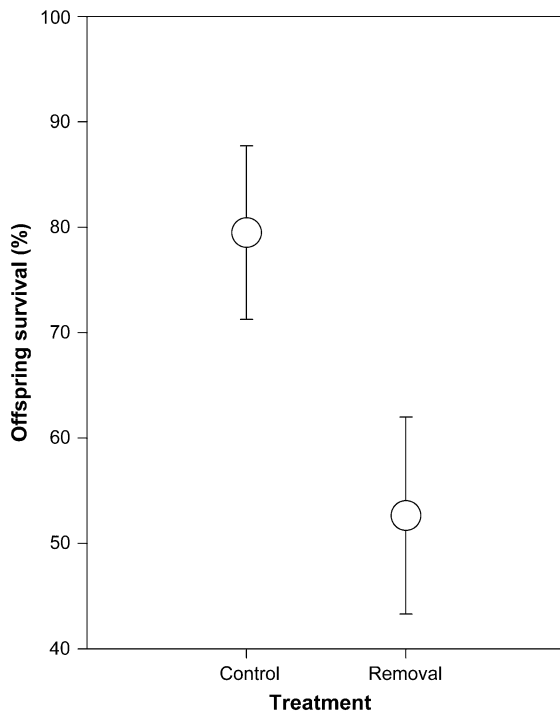
Figure 2

Difference in helper (a) breeding shelter visits and (b) feeding rate between removal and control groups for small ( $<37$  mm) and large ( $>37$  mm) helpers.

were checked after the last observations, one control and three reduced groups produced new fry between 1 and 2 weeks after removal, and one control and one reduced group produced new fry between 2 and 3 weeks after removal.

### Effects of group size

Group stability was not influenced by the treatment: only 3 out of 167 observed helpers in total went missing, presumably due to predation. Before removal, groups contained on average  $8.0 \pm 0.8$  helpers ( $\pm$ SE, range: 4–17). The effect of removal on feeding rate, breeding shelter visit, or survival of offspring was not stronger in small than in large groups (Spearman rank correlations, all three parameters  $r_s < .35$ ,  $n = 10$ ,  $p > .32$ ). On average,  $40 \pm 3\%$  ( $\pm$ SE, range: 30–50%) of the helpers were removed. Removing a larger proportion of



**Figure 3**  
The mean percentage survival of offspring was higher in control than in removal groups, measured after 7 days from start of the experiment. Means  $\pm$  SE are shown.

helpers did not have a stronger effect on the difference in feeding rate, breeding shelter visits, or survival of offspring between control and reduced groups (for all comparisons  $r_s < .15$ ,  $n = 10$ ,  $p > .67$ ).

## DISCUSSION

Our experiment showed that helpers raise the reproductive success of breeders as the survival rate of offspring was lower in removal than in control groups. This proves that helpers do really help, so their behavior may be subject to kin selection or to selection based on reciprocity (pay-to-stay; see Taborsky, 1984). We should be cautious when interpreting our results as evidence for the importance of kin selection in causing delayed dispersal and helping behavior in *N. pulcher*, as many large helpers are unrelated to the breeding pair in this population of *N. pulcher* (average genetic relatedness of large helpers to the breeding male is 0 and to the breeding female is 0.25; Dierkes P, Heg D, Taborsky M, Skubic E, Achmann R, personal communication), so they help raising unrelated beneficiaries. The relative importance of kin selection and pay-to-stay mechanisms may be distinguished by analyzing the behavioral reaction of remaining helpers to the removal of group members. Helpers should be expected to increase their workload if kin selection is the major evolutionary cause of cooperative breeding in this species. According to the pay-to-stay hypothesis, helpers should not pay more if group size declined, especially if there is social competition among helpers (Ragsdale, 1999). As helpers increase the breeders' reproductive success (Taborsky, 1984; this study), a group size reduction might render each helper more valuable to breeders, that is, helpers may even have to pay *less* to remain tolerated.

We found evidence that the remaining small helpers compensated for the removed group members, supporting the kin selection hypothesis. Small helpers visited the breed-

ing shelter more often after manipulation, and in general the remaining helpers fed less. Helpers did not visit other hiding shelters more often due to the group size reduction, suggesting that increased breeding shelter visits were not selfish behavior (e.g., to seek protection from predators). As small helpers are specialized to participate in brood shelter maintenance and in egg and larvae care (Taborsky, 1984; Taborsky et al., 1986) and as they are on average more closely related to beneficiaries than large helpers are (Taborsky and Limberger, 1981; Dierkes P, Heg D, Taborsky M, Skubic E, Achmann R, personal communication), these results meet the predictions derived from the kin selection hypothesis. However, we cannot rule out the possibility that after manipulation small helpers visited breeding shelters more often because these had been monopolized by larger group members before the manipulation (Werner et al., 2003; see also Bergmüller and Taborsky, 2005; Bergmüller et al., 2005) as there is competition between helpers for shelters in *N. pulcher* territories (Balshine et al., 2001; Werner et al., 2003).

In birds, increased survival of offspring was found to result from helping behavior such as feeding young or territory defense (Brown et al., 1982; Komdeur, 1994; Mumme, 1992). In our study, behavior of helpers conspicuously related to offspring protection, such as defense, did not increase after helper removal. Rather, the overall defense rate of the group decreased. Apparently, breeders reduced their defense effort after helper removal; they may have compensated for the removed helpers by increasing other behaviors like brood care, which was not recorded. The higher survival rate of offspring in control groups may have resulted from the greater attendance and guarding by helpers, which may have deterred potential predators or warned offspring of predator attacks. The reduced numbers of potential protectors in removal groups may have been the reason why helpers of the reduced groups stayed closer to the breeding shelter (see also Heg et al., 2004).

Large helpers did not show any significant changes in their helping or social behaviors in response to group size reduction. This is in accordance with expectations from both the kin selection and pay-to-stay hypotheses: large helpers are often unrelated to beneficiaries, and dominant group members should not demand more help from fewer helpers because the reduction in number makes each of them more valuable (see above; Hamilton and Taborsky, 2005). Independent of the helpers' behavior, some of our results can be explained also by the benefits of group augmentation (Kokko et al., 2001) as the group size reduction resulted in a lower juvenile survival and an initial reduction of feeding rates of helpers.

An alternative explanation for our results may be provided by the social disruption hypothesis (Mumme, 1992). Reduced feeding rates, increased breeding shelter visits, and reduced survival of offspring could all result from the social disruption caused by removal of group members. However, we did not find evidence for the social disruption hypothesis because the removal of helpers did not affect the frequency of aggressive interactions between group members or the territory size or group stability. Moreover, reduced groups even produced new fry in the first 2 or 3 weeks after manipulation.

Variation in the fraction of helpers removed (30–50%) did not affect the behavior of the remaining group helpers. In addition, group size was not correlated with feeding rates as found in a previous study (Balshine et al., 2001). This discrepancy might be due to differences in the time of day of observations. In our study all observations were done in the morning, while Balshine et al. (2001) observed the fish at all times of the day. Due to the matched-pairs design, any

potential influence of daytime is corrected for in our experiment. The discrepancy between the results of the two studies may also be due to the different approaches as the Balshine study was correlative while this one was experimental.

In conclusion, despite the experimental approach, our data and small sample sizes do not allow us to separate clearly between kin selection, pay-to-stay, and group augmentation mechanisms as driving forces of helping behavior in *N. pulcher*. However, these mechanisms are not mutually exclusive. Our results suggest that the behavior of small helpers may be influenced by kin selection, while large helpers rather pay to stay. In addition, group augmentation results in higher success rates of breeders, and it appears to benefit all group members by allowing higher feeding rates. Pay-to-stay and group augmentation both can explain the existence of unrelated helpers (Kokko et al., 2001, 2002), which is an intriguing characteristic of this social system.

We thank the Zambia Ministry of Agriculture, Food, and Fisheries, in particular Kapasa, for permission to conduct this study. We want to express our gratitude to the people of the Fisheries Department in Mpulungu, particularly Harris Phiri, Rueben Shapola, and Lorenz Makasa, for their continuous support of our project. Many thanks also to our coworkers of the Lake Tanganyika Diving Expedition 2002 for their support. We thank three anonymous reviewers for their constructive comments on the manuscript. This project was funded by the Swiss National Science Foundation (SNSF grant 3100-064396 to M.T.). L.B. received financial support from the Marco Polo Fund from the University of Groningen. The experiment described in this paper complies with the current laws of Zambia, the country in which the study was conducted.

## REFERENCES

- Arnold KE, Owens IPF, 1998. Cooperative breeding in birds: a comparative analysis of the life history hypothesis. *Proc R Soc Lond B* 265:739–745.
- Arnold KE, Owens IPF, 1999. Cooperative breeding in birds: the role of ecology. *Behav Ecol* 10:465–471.
- Balshine S, Leach B, Neat F, Reid H, Taborsky M, Werner N, 2001. Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behav Ecol Sociobiol* 50:134–140.
- Balshine-Earn S, Neat FC, Reid H, Taborsky M, 1998. Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behav Ecol* 9:432–438.
- Bergmüller R, Heg D, Taborsky M, 2005. Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. *Proc R Soc Lond B* 272:325–331.
- Bergmüller R, Taborsky M, 2005. Experimental manipulation of helping in a cooperative breeder: helpers 'pay to stay' by pre-emptive appeasement. *Anim Behav* 69:19–28.
- Brown JL, 1974. Alternative routes to sociality in jays—with a theory for the evolution of altruism and communal breeding. *Am Zool* 14: 63–80.
- Brown JL, 1987. *Helping and communal breeding in birds*. Princeton: Princeton University Press.
- Brown JL, Brown ER, Brown SD, Dow DD, 1982. Helpers: effects of experimental removal on reproductive success. *Science* 215: 421–422.
- Clutton-Brock TH, 2002. Breeding together: kin selection and mutualism in cooperative vertebrates. *Science* 296:69–72.
- Cockburn A, 1998. Evolution of helping behavior in cooperatively breeding birds. *Annu Rev Ecol Syst* 29:141–177.
- Davies NB, Hartley IR, Hatchwell BJ, Desrochers A, Skeer J, Nebel D, 1995. The polygynandrous mating system of the alpine accentor, *Prunella collaris*. I. Ecological causes and reproductive conflicts. *Anim Behav* 49:769–788.
- Dierker P, Taborsky M, Kohler U, 1999. Reproductive parasitism of broodcare helpers in a cooperatively breeding fish. *Behav Ecol* 10: 510–515.
- Emlen ST, 1982. The evolution of helping. I. An ecological constraints model. *Am Nat* 119:29–39.
- Emlen ST, 1991. Evolution of cooperative breeding in birds and mammals. In: *Behavioural ecology: an evolutionary approach*, 3rd ed (Krebs JR, Davies NB, eds). Oxford: Blackwell; 301–337.
- Emlen ST, 1997. Predicting family dynamics in social vertebrates. In: *Behavioural ecology: an evolutionary approach*, 4th ed (Krebs JR, Davies NB, eds). Oxford: Blackwell; 228–253.
- Gaston AJ, 1978. The evolution of group territorial behavior and cooperative breeding. *Am Nat* 112:1091–1100.
- Grantner A, Taborsky M, 1998. The metabolic rates associated with resting, and with the performance of agonistic, submissive, and digging behaviours in the cichlid fish *Neolamprologus pulcher* (Pisces: Cichlidae). *J Comp Physiol B* 168:427–433.
- Griffin AS, West SA, 2003. Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science* 302:634–636.
- Hamilton IM, Taborsky M, 2005. Unrelated helpers will not fully compensate for costs imposed on breeders when they pay to stay. *Proc R Soc Lond B* 272:445–454.
- Hatchwell BJ, Komdeur J, 2000. Ecological constraints, life history traits and the evolution of cooperative breeding. *Anim Behav* 59: 1079–1086.
- Hatchwell BJ, Russell AF, 1996. Provisioning rules in cooperatively breeding long-tailed tits *Aegithalos caudatus*: an experimental study. *Proc R Soc Lond B* 263:83–88.
- Heg D, Bachar Z, Brouwer L, Taborsky M, 2004. Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proc Roy Soc Lond B* 271:2367–2374.
- Koenig WD, Mumme RL, 1987. *Population ecology of the co-operatively breeding acorn woodpecker*. Princeton: Princeton University Press.
- Koenig WD, Pitelka FA, Carmen W, Mumme RL, Stanback MT, 1992. The evolution of delayed dispersal in cooperative breeders. *Q Rev Biol* 67:111–150.
- Kokko H, Johnstone RA, Clutton-Brock T, 2001. The evolution of cooperative breeding through group augmentation. *Proc R Soc Lond B* 268:187–196.
- Kokko H, Johnstone RA, Wright J, 2002. The evolution of parental and alloparental effort in cooperatively breeding groups: when should helpers pay to stay? *Behav Ecol* 13:291–300.
- Komdeur J, 1994. The effect of kinship on helping in the cooperative breeding Seychelles warbler (*Acrocephalus sechellensis*). *Proc R Soc Lond B* 256:47–52.
- Legge S, 2000. The effect of helpers on reproductive success in laughing kookaburra. *J Anim Ecol* 69:712–724.
- Leonard ML, Horn AG, Eden SF, 1989. Does juvenile helping enhance breeder reproductive success? *Behav Ecol Sociobiol* 25: 357–361.
- Ligon JD, Ligon SH, 1978. Communal breeding in green wood-hoopoes as a case for reciprocity. *Nature* 276:496–498.
- Maccoll ADC, Hatchwell BJ, 2004. Determinants of lifetime fitness in a cooperative breeder, the long-tailed tit *Aegithalos caudatus*. *J Anim Ecol* 73:1137–1148.
- Mulder RA, Langmore NE, 1993. Dominant males punish helpers for temporary defection in superb fairy-wrens. *Anim Behav* 45:830–833.
- Mumme RL, 1992. Do helpers increase reproductive success? An experimental analysis in the Florida scrub jay. *Behav Ecol Sociobiol* 31:319–328.
- Poiani A, Jermy LS, 1994. A comparative analysis of some life-history traits between cooperatively and non-cooperatively breeding Australian passerines. *Evol Ecol* 8:471–488.
- Rabenold KN, 1990. *Campylorhynchus* wrens: the ecology of delayed dispersal and cooperation in the Venezuelan savanna. In: *Co-operative breeding in birds: long-term studies of ecology and behavior* (Stacey PB, Koenig WD, eds). Cambridge: Cambridge University Press; 157–196.
- Ragsdale JE, 1999. Reproductive skew theory extended: the effect of resource inheritance on social organization. *Evol Ecol Res* 1:859–874.
- Rasbash J, Steele F, Browne W, Prosser B, 2004. *A user's guide to MLwiN version 2.0*. London, Institute of Education.
- Reyer HU, 1980. Flexible helper structure as an ecological adaptation in the pied kingfisher (*Ceryle rudis rudis* L.). *Behav Ecol Sociobiol* 6:219–227.

- Reyer HU, 1984. Investment and relatedness: a cost-benefit analysis of breeding and helping in the pied kingfisher (*Ceryle rudis*). Anim Behav 32:1163–1178.
- Richardson DS, Burke T, Komdeur J, 2002. Direct benefits and the evolution of female-biased cooperative breeding in Seychelles warblers. Evolution 56:2313–2321.
- Shreeves G, Field J, 2002. Group size and direct fitness in social queues. Am Nat 159:81–95.
- Stacey PB, Koenig WD, 1990. Cooperative breeding in birds: long-term studies of ecology and behaviour. Cambridge: Cambridge University Press.
- Stiver K, Dierkes P, Taborsky M, Balshine S, 2004. Dispersal patterns and status change in a cooperatively breeding fish; evidence from microsatellite analyses and behavioural observations. J Fish Biol 65: 91–105.
- Taborsky M, 1984. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: their costs and benefits. Anim Behav 32:1236–1252.
- Taborsky M, 1985. Breeder-helper conflict in a cichlid fish with brood care helpers: an experimental analysis. Behaviour 95:45–75.
- Taborsky M, 1994. Sneakers, satellites and helpers: parasitic and cooperative behaviour in fish reproduction. Adv Study Behav 23:1–100.
- Taborsky M, Grantner A, 1998. Behavioural time-energy budgets of cooperatively *Neolamprologus pulcher* (Pisces: Cichlidae). Anim Behav 56:1375–1382.
- Taborsky M, Hert E, von Siemens M, Stoerig P, 1986. Social behaviour of *Lamprologus* species: functions and mechanisms. Ann Mus R Afr Cent Sci Zool 251:7–12.
- Taborsky M, Limberger D, 1981. Helpers in fish. Behav Ecol Sociobiol 8:143–145.
- Werner NY, Balshine S, Leach B, Lotem A, 2003. Helping opportunities and space segregation in cooperatively breeding cichlids. Behav Ecol 14:749–756.
- Woelfenden GE, 1975. Florida scrub jay helpers at the nest. Auk 92: 1–15.
- Wright J, Dingemanse NJ, 1999. Parents and helpers compensate for experimental changes in provisioning effort of others in the Arabian babbler. Anim Behav 58:345–350.
- Zar JH, 1996. Biostatistical analysis, 3rd ed. London: Prentice hall International.