



Helpers in a cooperative breeder pay a high price to stay: effects of demand, helper size and sex

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'Pay to stay' is a mechanism generating cooperation in some highly social vertebrates. However, it is unknown which behaviours subordinate helpers preferentially use to pay for the commodities provided by resource owners. Helpers could either provide 'cheap' service to save costs or trade expensive actions for the benefit of being tolerated. In the cichlid *Neolamprologus pulcher*, unrelated helpers pay to stay in a safe territory of dominant breeders by performing a range of behaviours including direct brood care, territory maintenance and defence. We investigated which behaviours helpers differing in size and sex showed in response to increased demands. By presenting high or low perceived intruder pressure we tested (1) whether helpers adjust their level of payment to the intensity of competition, (2) which behaviours are used for payment, and (3) whether the response to the treatment differs between helper size and sex. All helpers performed more defence behaviour in the high-density situation and large helpers put significantly more effort into digging and defence than small helpers did in both treatments. In the low-density treatment, large helpers reacted to breeder aggression by increased submissiveness, whereas in the high-density treatment they responded by increased territory defence, as predicted by the pay-to-stay hypothesis. Helper sex did not influence their behavioural responses. Our results suggest that helpers respond to demand and large helpers pay generally more than small ones to remain tolerated in the breeders' territory.

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Cooperation and seemingly altruistic behaviour have been a major focus of evolutionary biology for several decades (e.g. Hamilton 1963, 1964; Trivers 1971; reviewed in Lehmann & Keller 2006). At the empirical level, evolutionary explanations for helping others have been studied primarily in cooperatively breeding species (Taborsky 1984, 1994; Choe & Crespi 1997; Solomon & French 1997; Cockburn 1998; Clutton-Brock et al. 2001, 2002; Russell et al. 2003; Koenig & Dickinson 2004). In cooperative breeders, subordinates may stay in the territory of dominants because ecological constraints make dispersal unrewarding or risky (Emlen 1982; Koenig et al. 1992; Heg et al. 2004) and because of benefits that subordinates gain at home (e.g. Taborsky 1984; Wiley & Rabenold 1984; Stacey & Ligon 1991; Komdeur 1992; Balshine-Earn et al.

1998; Kokko et al. 2001). In an alternative approach, the role of life history traits such as longevity and intrinsic dispersal tendency has been emphasized to explain the evolution of cooperative breeding (Arnold & Owens 1998; Hatchwell & Komdeur 2000; Covas & Griesser 2007). All three proposed explanations emphasize different aspects of the same concept (Koenig et al. 1992; Kokko & Ekman 2002). Regardless of the ultimate causes for delayed dispersal, the question remaining is why subordinate group members delaying dispersal show helping behaviour (Komdeur 2006), especially if they are unrelated to the breeders (Dierkes et al. 2005). Helpers may gain direct fitness benefits by recruiting helpers for their own subsequent reproduction (Ligon 1983), through participation in reproduction (Taborsky 1985; Dierkes et al. 1999; Cant & Reeve 2002; Clutton-Brock et al. 2006; Heg et al. 2006) or group augmentation (Kokko et al. 2001; Shreeves & Field 2002; Heg et al. 2005). Alternatively, helpers may trade their investment for being tolerated in the territory

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of dominant breeders ('pay to stay'; Gaston 1978; Taborsky 1984, 1985; Balshine-Earn et al. 1998; Kokko et al. 2002; Bergmüller & Taborsky 2005; Bergmüller et al. 2005b).

If pay to stay applies, breeders should demand help according to need. However, if it is costly to evict subordinates, help will not fully compensate for the costs that breeders incur from the helpers' presence (Hamilton & Taborsky 2005). Punishment is one mechanism that might ensure that subordinates will pay their share (Kokko et al. 2002; Hamilton 2004). However, empirical evidence for punishment in animal societies is scarce (Clutton-Brock & Parker 1995; but see Boyd et al. 2003 for examples in humans). Punishment is less obvious, however, if it is anticipated by 'pre-emptive appeasement', by which subordinates reduce the probability of being punished by increasing cooperation and submissive behaviour in situations where this is demanded by dominants (Bergmüller & Taborsky 2005).

We tested, in *Neolamprologus pulcher*, whether helpers increase payment in response to rising demands and, if so, whether they use rather cheap or more expensive behaviours to do so. This cooperative breeder is an endemic cichlid in Lake Tanganyika (for species descriptions of *N. pulcher* and *N. brichardi* see Grantner & Taborsky 1998; Duftner et al. 2007). A dominant pair has on average five to eight helpers plus additional offspring in their territory (Taborsky & Limberger 1981; Taborsky 1994; Balshine et al. 2001). *Neolamprologus pulcher* is a substrate breeder that uses holes and crevices for shelter and reproduction. Large helpers are usually unrelated to the present breeding pair, because predation results in high turnover rates of breeders and helpers stay when breeders are replaced (Taborsky & Limberger 1981; Dierkes et al. 2005). As a consequence, helpers frequently help to rear nonkin broods. Therefore, kin benefits alone are not sufficient to explain the cooperative behaviour in this species (Taborsky 1984, 1985; Brouwer et al. 2005; Stiver et al. 2005).

By confronting mature large and small subordinates with high and low perceived intruder pressure, we tested experimentally how helpers respond to varying demands. The pay-to-stay hypothesis predicts that in a high perceived density situation (1) helpers should increase the level of expensive payment; that is, they should show risky defence behaviour rather than territory maintenance such as digging sand away from the breeding shelter; (2) alternatively, helpers might increase submissive behaviour towards dominant individuals; (3) large helpers should show more helping behaviour than small helpers because large helpers are a bigger threat for the breeders with regard to territory take-over (Taborsky 1987; Balshine-Earn et al. 1998), they are more likely to parasitize the breeders' reproduction (Dierkes et al. 1999; Skubic et al. 2004; Heg et al. 2006) and they should be more efficient in defending the territory than small helpers; (4) helpers should receive more aggression from the breeding pair if helping behaviour is not increased and (5) male helpers should provide more help than female helpers because of the higher potential costs they may inflict (Taborsky 1985).

METHODS

Experimental Groups

We conducted the experiment at the Ethologische Station Hasli, Institute of Zoology of the University of Bern, Switzerland, from 2 December 2004 to 3 March 2005 under licence of the Veterinary Office of Kanton Bern. We used laboratory-reared offspring of *N. pulcher* caught at the southern end of Lake Tanganyika at Kasakalawe Bay near Mpulungu, Zambia. The fish were kept in 200-litre tanks with a water temperature of $27 \pm 1^\circ\text{C}$. Water quality was kept constant, close to the values found in Lake Tanganyika (see Taborsky 1984 for details) and the light regime was 13:11 h light:dark. The tank bottom was covered with sand (30 mm, about 1 mm grain size) and the fish were fed once a day, twice a week with frozen food (consisting of daphnia, *Artemia salina* nauplia and chironomid larvae) and four times per week with commercial dry food (Tetramin). The experimental groups were composed of two breeders and two sexually mature helpers ($\bar{X} \pm \text{SD}$: small helpers: 39.8 ± 1.1 mm standard length [SL]; large helpers: 49.5 ± 2.0 mm SL). The two helper fish were of opposite sex in 11 of the 12 groups. Five groups consisted of a large helper male and a small helper female, six groups of a large helper female and a small helper male and one group consisted of large and small male helpers. All focal groups were combined haphazardly of unrelated individuals to prevent a potential influence of relatedness and familiarity (Taborsky 1984). This resembles the natural situation since unrelated focal groups are common because of a high breeder turnover rate (Dierkes et al. 2005). Groups were established following standard procedures. We introduced the two helper fish first. When these fish were accustomed to the new environment (after 1–4 days), two fish of breeder size were added. All breeders were >60.0 mm SL with females being at least 5.0 mm smaller than males, to resemble the natural situation (Balshine et al. 2001).

Experimental Procedure

We divided the experimental 200-litre tanks into a central 100-litre compartment and two lateral 50-litre compartments on either side by two transparent Plexiglas partitions (Fig. 1). The focal group was housed in the central compartment and was provided with four flowerpot halves positioned close together to act as breeding and hiding shelters. We waited for a minimum of 2 days before the experiments started, to let the fish habituate to the set-up. Every focal group received the high- and the low-density treatment in random order. All fish quickly habituated to the new environment, and the first focal group produced a clutch 4 days after it was established. When a breeding pair spawned, eggs were removed and used for further analyses (Taborsky et al. 2007). In total, six groups produced a clutch in the high-density treatment and seven in the low-density treatment, which indicates that the groups were not stressed by the treatments.

Both lateral compartments held eight mixed-sex aggregation fish in the high-density treatment (range 40.4 ± 5.1 – 66.3 ± 4.0 mm SL) or two mixed-sex aggregation

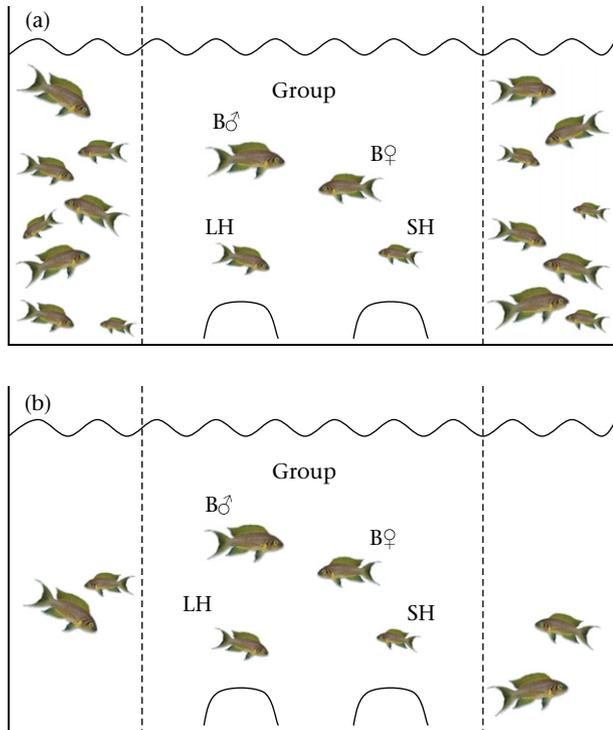


Figure 1. Experimental set-up in a 200-litre tank, viewed from the front. The dotted lines indicate clear Plexiglas partitions and the n-shaped forms at the bottom represent flowerpot halves, which were used as hiding and breeding shelters. The focal groups consisted of a breeder male ($B\delta$), a breeder female ($B\eta$), a large helper (LH) and a small helper (SH). (a) Set-up during the high perceived density, where eight *N. pulcher* were presented on the left and right sides of the focal group. (b) Set-up during the low perceived density, where two fish were presented on the left and right sides of the focal group.

fish in the low-density treatment (small fish, i.e. always male: 52.9 ± 2.8 mm SL; large fish, i.e. always female: 68.3 ± 4.0 mm SL). No significant differences in aggregation fish sizes per treatment were found (independent t test: $t_{193} = 0.363$, $P = 0.717$). The large fish in the low-density treatment was always a female to reduce the chance that the two fish would pair up and defend a breeding territory. No clutches were found in the lateral compartments in both treatments, suggesting that no pair formation occurred. Mixed-sex aggregations occur naturally in Lake Tanganyika at certain locations and usually consist of mature individuals that do not reproduce because of a lack of breeding shelters (Taborsky & Limberger 1981; Taborsky 1984).

In both treatments, experimental periods lasted for approximately 8 days, with an occasional addition of 1–2 days because of (1) egg laying ($N =$ two cases), after which no observations were made for 1 day as breeders in the egg-laying phase are more aggressive towards their helpers (Taborsky 1985), or (2) temporary helper expulsion ($N =$ four cases), after which the main aggressor was put in a small net cage for 2–4 h, which normally led to reacceptance of the helper after the main aggressor had been released again.

To induce helping behaviour, in this case digging, we completely covered all flowerpot halves with sand four

times per test period in both treatments, but not more often than once per day. We waited 20 min before starting the behavioural recordings, because the latency to dig of helpers and the breeding pair was usually 15–20 min (personal observation). Afterwards, we recorded the behaviour of both helper fish for 10 min in random order with the software The Observer, version 3.0 (Noldus, Wageningen, The Netherlands). We recorded data randomly between 0900 and 1800 hours. Recorded behaviours (described in Taborsky 1984, 1985) included overt attacks (biting, mouth fighting, ramming, tail beating), restrained aggressive displays (fin spreading, fast frontal approach, head-down display, head jerking, opercula spreading and S-shaped bending), submissive behaviours (escaping, hook displays and tail quivering), territory maintenance (digging) and total activity (sum of all described behaviours). Overt attacks and restrained aggressive displays towards the aggregation fish were regarded as defence behaviours. In addition, every minute we scored the position of the two focal helpers and the breeders, that is, whether they were within 5 cm of the lateral compartments, in the breeding shelters or in the remaining part of the aquarium.

Ethical Note

Group densities in Lake Tanganyika are often very high and groups can consist of one male breeder with up to four breeding females (Dierkes et al. 2005; personal observation) and on average five helpers per group (Balshine et al. 2001). The natural territory area of a group is on average 0.315 m² (range 0.078 – 1.010 m²; Balshine et al. 2001). The high perceived density used in this experiment (8 fish in 0.250 m²) thus corresponds to high natural density conditions. Almost all recorded aggressive behaviours were directed by the focal group towards the fish in the aggregation compartments, which were separated by Plexiglas partitions. Therefore, no injuries were observed, which was confirmed by daily observations of the group.

Aggregation fish might have been stressed by the attacks of the members of the focal group. However, they showed normal activity levels and engaged in aggressive displays to each other, which suggests that stress levels were comparable to those experienced in natural environments. Because group members can potentially injure each other, we provided opaque plastic tubes near the water surface to serve as shelters. Two aggregation members did not show natural behaviour, for example by carrying out continuous head-up display which may reveal increased levels of stress in this social species, and we replaced them by a conspecific of the same size and sex.

Statistical Analysis

We analysed the frequencies of all recorded behaviours and the duration of digging with repeated measures ANOVAs with treatment, sex and helper size as independent variables. When the three-way interaction effects

were not significant they were removed from the models. The residuals of the models were tested for normality with one-sample Kolmogorov–Smirnov tests for goodness of fit against a normal distribution and for homogeneity of variances Levene's tests. When necessary, data were normalized by logarithmic or square-root transformations. Data on defence behaviour were subdivided into restrained, overt and all defence and analysed multiple times. A false discovery rate control (Verhoeven et al. 2005) was applied for the defence data. Overall effects, without taking the treatment into account, were analysed with paired *t* tests when the data were normally distributed. When the above-mentioned transformations did not normalize the data we used Wilcoxon signed-ranks tests. All figures are presented with untransformed data. Correlations were calculated with the Pearson correlation coefficient when data were normally distributed and the variances were homogeneous; otherwise we used the Spearman rank correlation analysis. For all tests we used SPSS version 11.5 (SPSS Inc., Chicago, IL, U.S.A.). All tests are two tailed.

RESULTS

Defence Behaviour

Both small and large helpers showed significantly more defence behaviour in the high-density treatment than in the low-density treatment (repeated measures ANOVA: treatment: $F_{1,21} = 7.929$, $P = 0.010$; Fig. 2a) and no significant interaction effects were present. Overall, large helpers carried out more defence than small helpers (helper size effect), but no effect of helper sex was found (see Table 1 for statistical details).

When analysing overt and restrained defence separately, we found no difference in the frequency of overt defence between the two density treatments and there were no significant interaction effects. A clear between-subjects effect was found with regard to helper size, but not with regard to helper sex. Large helpers showed distinctly more overt defence than small helpers (Table 1).

More restrained defence was shown in the high- than in the low-density treatment by both small and large helpers, but no interaction effects were observed between treatment and helper size. Large helpers participated more frequently in restrained defence than small helpers and there was no sex difference in restrained defence frequencies.

Breeder males and females did not differ in the time spent close to the side compartments and they also showed no difference in this respect between the density treatments (Table 1). Breeders of both sexes spent significantly more time close to the aggregation fish than small and large helpers in the low-density set-up. In the high-density set-up, breeder males and females also spent more time close to the aggregation fish than small helpers did, but there was no difference between the breeders and large helpers (Table 1).

Digging

The duration of digging was not affected by the density treatment (Table 2) and there were no significant interaction effects with sex and helper size. No overall

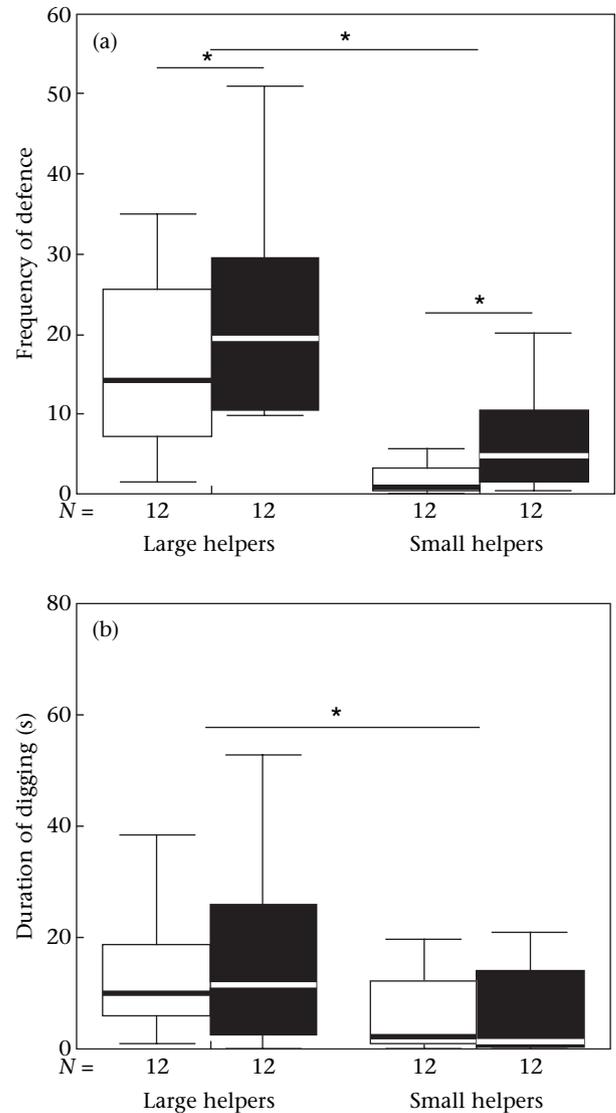


Figure 2. (a) Frequency of territorial defence (number/10 min) and (b) duration of digging (5/10 min) of small and large helpers in the low- (□) and high-density (■) treatments. Horizontal lines are medians, boxes are upper and lower quartiles and whiskers indicate the highest and lowest values excluding extreme values and outliers. * $P < 0.05$.

effect of sex was seen in relation to the duration of digging. Disregarding treatment, large helpers spent more time digging than small helpers (Fig. 2b, Table 3).

Submissive Behaviour

No treatment differences and interaction effects were found in the frequencies of submissive behaviour shown towards all group members (Table 2). Overall, small helpers showed more submissive behaviours towards all group members than large helpers did in both density treatments, and no sex effect was observed (Table 2).

If solely the frequency of submissive behaviours towards the breeders was considered, no within- and between-subject treatment effects were found (Table 2). The

Table 1. Results of repeated measures ANOVAs testing for the amount of defence (overall, overt and restrained) with treatment, sex and helper size as independent variables

	$F_{1,21}/t_{11}$	P
Defence by helpers		
Density	7.929	0.010*
Density*sex	<0.001	0.987
Density*helper size	1.107	0.305
Sex	0.962	0.338
Helper size	42.431	<0.001*
Overt defence by helpers		
Density	1.666	0.211
Density*sex	0.819	0.376
Density*helper size	0.198	0.661
Sex	1.336	0.261
Helper size	17.445	<0.001*
Restrained defence by helpers		
Density	9.386	0.006*
Density*sex	0.017	0.898
Density*helper size	0.050	0.825
Sex	0.569	0.459
Helper size	41.094	<0.001*
Time breeders spent near side compartments		
Density B♂	-0.356	0.729
Density B♀	-1.403	0.188
Comparisons of time spent near side compartments		
B♀ versus SH Low density	5.237	<0.001*
B♀ versus LH Low density	3.776	0.003*
B♂ versus SH Low density	5.028	<0.001*
B♂ versus LH Low density	3.189	0.009*
B♀ versus SH High density	2.945	0.013*
B♀ versus LH High density	1.716	0.114
B♂ versus SH High density	2.635	0.023*
B♂ versus LH High density	1.764	0.105
B♀ versus B♂ Low density	-1.371	0.198
B♀ versus B♂ High density	-0.608	0.556

Time spent near side compartments containing aggregation fish was analysed with paired t tests. B♂ = breeder male; B♀ = breeder female; LH = large helper, SH = small helper.

After the false discovery rate control (Verhoeven et al. 2005) was implemented all P values marked with an asterisk were still significant.

frequency of submissive behaviours directed towards large helpers by small helpers was not affected by the treatment (Wilcoxon signed-ranks test: $Z = -5.10$, $N = 12$, $P = 0.610$).

Received Aggression

Breeders did not change aggression frequencies towards helpers in accordance with the difference in perceived density (Table 2) and no overall effects of sex and helper size were found (Table 2). When densities were considered separately, large helpers received more aggression than small helpers in the low-density treatment (paired t test: $t_{11} = 2.482$, $P = 0.030$). In the high-density treatment no difference in received aggression was found (Wilcoxon signed-ranks test: $Z = -0.593$, $N = 12$, $P = 0.553$).

Defence per Received Aggression

The frequency of helper defence per received aggressive act by a breeder was significantly higher in the high-density treatment than in the low-density treatment

Table 2. Results of repeated measures ANOVAs testing for the duration of digging, frequencies of submissive behaviour and received aggression from group members with treatment, sex and helper size as independent variables

	$F_{1,21}$	P
Digging		
Density	0.874	0.360
Density*sex	0.009	0.926
Density*helper size	0.055	0.816
Sex	1.131	0.300
Helper size	3.135	0.091
Submissive behaviour towards all group members		
Density	2.758	0.112
Density*sex	0.969	0.336
Density*helper size	0.104	0.750
Sex	0.672	0.422
Helper size	5.423	0.030
Submissive behaviour directed towards B♀ & B♂		
Density	1.002	0.328
Density*sex	1.636	0.215
Density*helper size	0.001	0.979
Sex	0.060	0.809
Helper size	0.049	0.827
Received aggression from B♀ & B♂		
Density	0.422	0.523
Density*sex	0.013	0.909
Density*helper size	0.826	0.374
Sex	2.429	0.134
Helper size	0.390	0.539

B♂ = breeder male; B♀ = breeder female.

when helper sizes were pooled (Wilcoxon signed-ranks test: $Z = -3.053$, $N = 17$, $P = 0.002$). Large helpers showed a significantly higher frequency of defence per received aggressive act in the high-density treatment than in the low-density situation, whereas this was not the case for small helpers (large: $Z = -2.666$, $N = 8$, $P = 0.008$; small: $Z = -1.260$, $N = 8$, $P = 0.208$).

Submissiveness in Response to Received Aggression

The frequency of submissive behaviour shown by small helpers towards large helpers was positively correlated

Table 3. Tests for overall helper size and sex effects on the duration of digging, frequencies of submissive behaviour and received aggression (paired t tests)

	t_{11}	P
Digging per		
Helper sex	-1.319	0.217
Helper size	2.862	0.015
Submissive behaviour towards B♀ & B♂ per		
Helper sex	0.438	0.670
Helper size	-0.602	0.524
Received aggression from B♀ & B♂ per		
Helper sex	0.898	0.390
Helper size	1.686	0.120

Treatment effects are not considered in these analyses. B♂ = breeder male; B♀ = breeder female.

with the frequency of aggression received from large helpers, but only in the high-density treatment (Pearson correlation: high density: $r_{10} = 0.754$, $P = 0.005$; low density: $r = 0.348$, $P = 0.267$). The frequency of submissive behaviour shown by small helpers towards breeders was positively correlated with the frequency of received aggression in the low-and high-density situations (Spearman rank correlation: high density: $r_s = 0.592$, $N = 12$, $P = 0.043$; low density: $r_s = 0.710$, $N = 12$, $P = 0.010$). For large helpers a positive correlation was found between the frequency of submissive behaviour and the frequency of aggression received from breeders in the low-density situation only (high density: $r_s = 0.025$, $N = 12$, $P = 0.939$; low density: $r_s = 0.631$, $N = 12$, $P = 0.028$).

Total Activity

The total level of activity did not differ between the treatments and there was no significant interaction (repeated measure ANOVA: density: $F_{1,21} = 1.354$, $P = 0.258$; density*sex: $F_{1,21} = 0.162$, $P = 0.692$; density*helper size: $F_{1,21} < 0.001$, $P = 0.987$). The sex of the helpers did not relate to the overall activity level, but large helpers were more active than small ones (sex: $F_{1,21} = 2.297$, $P = 0.145$; helper size: $F_{1,21} = 5.842$, $P = 0.025$).

DISCUSSION

Our results show that all helpers carried out more defence behaviour when confronted with a high perceived density situation, whereas the frequency of submissive behaviour directed towards the breeders remained unaltered by the treatments. In addition, large helpers carried out more defence behaviour and dug for longer than small helpers, but no sex differences were found in any of the observed behaviours. These results are in accordance with predictions of the pay-to-stay hypothesis (Gaston 1978; Kokko et al. 2002), because large helpers inflict higher costs on breeders than small ones (Taborsky 1985; Dierkes et al. 1999; Heg et al. 2006). In addition, only large helpers increased territory defence in response to being attacked more by breeders in the high-density treatment, suggesting that larger helpers pay a higher price for being allowed to stay.

Can these results also be explained by purely selfish behaviour, that is, do we need to invoke the pay-to-stay concept to explain the observed pattern? If the behaviour of helpers is entirely selfish, no size-dependent differences in territory defence and digging would be predicted, but large helpers did show higher levels of these behaviours than small ones. In addition, aggression by dominant group members might cause higher submissiveness levels, but it should not raise territory defence effort. Particularly large helpers responded to the breeders' aggression by increased defence levels when demands for territory defence were high. This is in accordance with the pay-to-stay hypothesis, which predicts that if constraints and demands increase, the level of payment for the commodities the breeders provide should also increase (Kokko et al. 2002; Hamilton & Taborsky 2005). Large helpers should pay more than small helpers, because large helpers are a greater threat for breeders with regard to territory

take-over (Taborsky 1987; Balshine-Earn et al. 1998) and they engage more in parasitic reproduction (Dierkes et al. 1999; Heg et al. 2006). By increasing defence, helpers pay with costly behaviour, which risks injury, reduces antipredator vigilance, takes time and thereby poses opportunity costs. In addition, defence uses on average 3.6 times more energy than being inactive (Taborsky & Grantner 1998). Digging, on which large helpers spent 1.7 times more time than small helpers, raises resting metabolic rate more than sixfold in *N. pulcher* (Grantner & Taborsky 1998; Taborsky & Grantner 1998).

It is unlikely that our results can be explained by size-dependent helper benefits, since it is improbable that their continued presence in a territory defended by the breeders is more beneficial to large than to small helpers. Small helpers in particular are threatened by predators and have little chance of surviving outside a territory defended by larger group members (Taborsky & Limberger 1981), which has been shown experimentally in both the field and laboratory (Taborsky 1984; Heg et al. 2004). This also corresponds with the observation that the propensity to leave the territory deliberately increases with helper size, dependent on environmental conditions (Heg et al. 2004; Bergmüller et al. 2005a, b). In addition, the energetic costs of helping behaviours (digging and defence) apparently do not differ between helpers and breeders of different sizes (Grantner & Taborsky 1998; Taborsky & Grantner 1998). The potential to inherit the territory is greater for large than for small helpers (Balshine-Earn et al. 1998), but under natural conditions this is apparently only an option for female helpers (Dierkes et al. 2005). Therefore, even if the payoff of staying in a territory as a helper may be size dependent, it is probably the smaller helpers that benefit more, particularly because of the significant protection bonus.

Subordinate group members in cooperative breeders can appease dominants in two ways: they may show submissive behaviour or increase helping levels (Bergmüller & Taborsky 2005). In our experiments, small helpers increased submissive behaviour when attacked. Against breeders, this was true in both densities, whereas against large helpers it occurred only in the high-density treatment. This makes sense because in high densities small helpers are exposed to many potential competitors for their group position. Stiver et al. (2004) and Bergmüller et al. (2005a) showed that helpers do immigrate into groups in this species. By increasing submissiveness against aggressive large helpers, small helpers might reduce the probability of being expelled when alternative candidates are available. In large helpers, submissiveness increased with the frequency of aggression received from breeders only in the low-density situation, whereas in the high-density situation they responded with more territory defence. This confirms the results of Bergmüller & Taborsky (2005), who found a negative relation in helpers between submissive and helping behaviours after helpers had been experimentally prevented from participating in territory defence. Bergmüller & Taborsky (2005) suggested that helping behaviour serves a pre-emptive appeasement function to diminish punishment from breeders for previous abstaining from cooperation.

The relation between breeders' aggression and territory defence of helpers in our study suggests a similar functional relation. Breeders were more aggressive towards large helpers than towards small helpers in the low-density treatment, whereas in the high-density treatment large helpers increased their defence behaviour and breeders decreased their aggression to the same level as shown towards small helpers. Apparently, increased helping behaviour did not have to be enforced by the breeders through high aggression levels, which supports both the pay-to-stay and pre-emptive appeasement hypotheses.

In cooperatively breeding meerkats, *Suricata suricatta*, subordinates receive more aggression as they get older (Clutton-Brock et al. 2005; Kutsukake & Clutton-Brock 2006), which is in agreement with our findings that in *N. pulcher* larger (older) helpers receive more aggression than smaller ones in the low-density treatment. However, in meerkats female helpers receive more aggression than male helpers, whereas we found no sex differences in the level of received aggression from dominants. Male meerkats that prospect more contribute less to pup feeding (Young et al. 2005), which is similar to *N. pulcher* where helpers preparing to disperse reduce help (Bergmüller et al. 2005b). Only male helpers in meerkats are punished for being lazy or showing 'false feeding' (i.e. pretending to feed pups while eating the collected food themselves; Clutton-Brock et al. 2005). This suggests that pay to stay may partly explain helping behaviour in male but not in female meerkat helpers. In the eusocial wasp *Liostenogaster flavolineata*, older and more dominant individuals that are more likely to become the major reproductive female work less hard than individuals that are further away from the dominant position (Field et al. 2006). This is exactly opposite to our results, since in *N. pulcher* large helpers, which are closer to obtaining a breeding position, are paying more than small helpers (Balshine-Earn et al. 1998). The main reason for this difference may be that female helpers in *L. flavolineata* are not paying for being allowed to stay, as is the case in *N. pulcher*. In conclusion, we have shown that large subordinates pay generally more than small ones and that subordinate helpers are prepared to increase payment for being allowed to stay when demands rise by increasing defence behaviour, which bears severe costs.

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