Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid

Dik Heg[1]*, Zina Bachar[1], Lyanne Brouwer[1,2] and Michael Taborsky[1]

[1]*Department of Behavioural Ecology, Zoological Institute, University of Bern, Wohlenstrasse 50a, CH-3032 Hinterkappelen, Switzerland*
[2]*Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, Kerklaan 30, 9750 AA Haren, The Netherlands*

Environmental conditions are thought to be responsible for the extent and benefits of cooperative breeding in many animal societies, but experimental tests are scarce. We manipulated predator pressure in the cooperatively breeding cichlid *Neolamprologus pulcher* in Lake Tanganyika, where predators have been suggested to influence helper and breeder survival, helper dispersal and group reproductive success. We varied the type and intensity of predation by releasing medium, large, or no predators inside large underwater cages enclosing two or three group territories. Helper and breeder survival, helper dispersal and group reproductive success decreased from the control, to the medium- and large-predator treatments. These effects were modified by helper body size and the number of adults protecting the group from predators, supporting the 'group augmentation hypothesis'. Predators forced helpers to stay closer to, and spend more time inside, protective shelters. The results demonstrate the importance of predators for group living in this species, and support the 'ecological constraints hypothesis' of cooperative breeding, in the sense that subordinates stay at home rather than leave and breed independently under the risk of predation.

Keywords: cooperative breeding; ecological constraints; helper dispersal; predation; group augmentation; cichlids

1. INTRODUCTION

Predator–prey interactions are one of the major ecological factors shaping the evolution of morphology, life histories and habitat selection in animals (Begon *et al.* 1990; Stearns 1992). Their effects have been widely studied, and some of the best studies have been performed in fishes (see, for example, Brönmark & Miner 1992; Reznick *et al.* 1997; Baker *et al.* 1998; Lankford *et al.* 2001). In general, predation risk is usually lower for individuals living in groups, high during dispersal, and high for individuals attempting to breed independently, hence selecting for individuals delaying dispersal under high predation pressure.

These arguments have also been applied to explain the ecology and evolution of delayed dispersal in cooperatively breeding species where, under predation risk, offspring may benefit from staying at home rather than to disperse and breed independently (Stacey & Koenig 1990; Solomon & French 1997; Choe & Crespi 1997). In many cases these offspring assist breeders in raising offspring, referred to as 'helpers', although notable exceptions occur where group members do not provide help (reviewed in Cockburn 1998). Dominant breeders may benefit from helpers both in terms of survival and offspring production, because in many species helpers engage in anti-predator defence (e.g. mammals: Rasa 1986, 1987; Clutton-Brock *et al.* 1999, 2003; birds: Dawson & Mannan 1991; Arnold 2000; Maklakov 2002; insects: Foster 1990; Kudo *et al.* 1995; fishes: Taborsky & Limberger 1981; Balshine-Earn *et al.* 1998). Under predation risk, breeders and helpers might both

benefit from group-living (e.g. 'group augmentation' Kokko *et al.* 2001) and groups might be 'safe havens' (Kokko & Ekman 2002) for helpers until a 'low-risk' nearby territory is available, or helpers may 'queue' for the breeding position in the group (Wiley & Rabenold 1984). Hence, it is likely that predation risk strongly affects helper dispersal, i.e. predation risk is an important component of the 'ecological constraints hypothesis' that has been proposed to explain the evolution of cooperative breeding (Emlen 1982; Koenig *et al.* 1992).

The 'ecological constraints hypothesis' predicts that helpers in cooperatively breeding species derive higher benefits from staying in the natal territory instead of dispersing and attempting to breed independently (Emlen 1982). Although ecological constraints have often been assumed to be an important factor in forcing helpers to stay at home, experimental evidence is scarce. Experiments have been confined to manipulating the availability of (high quality) breeding opportunities in the neighbourhood of helpers, and subsequently measuring helper dispersal and independent breeding (Pruett-Jones & Lewis 1990; Ligon *et al.* 1991; Du Plessis 1992; Komdeur 1992; Walters *et al.* 1992; Komdeur *et al.* 1995; Field *et al.* 1998). So far, no study has manipulated other environmental/ecological factors, for example predation risk, that may influence the occurrence and extent of cooperative breeding and whether helpers stay at home rather than disperse.

We experimentally tested the 'group augmentation' and 'ecological constraints' hypotheses by manipulating predation risk in the cooperatively breeding Lake Tanganyika cichlid *Neolamprologus pulcher* (subspecies or sister species of *N. brichardi*; see Grantner & Taborsky (1998)).

*Author for correspondence (dik.heg@esh.unibe.ch).

Predators have been proposed to influence the extent and benefits of group living in this species, because large group members defend the territory against piscivore lamprologine cichlids (*Lepidiolamprologus* spp., *Altolamprologus* spp., *Lamprologus* spp.) and mastacembelid eels preying on helpers and fry (Taborsky & Limberger 1981; Taborsky 1984; Balshine *et al.* 2001). At the southern tip of Lake Tanganyika (Zambia), predator density is high and almost all *N. pulcher* live in 'extended family groups', i.e. a dominant breeding pair with up to 14 helpers and additional offspring defending a territory (Balshine-Earn *et al.* 1998). Predators might affect the propensity of helpers to disperse for two reasons. First, helpers may delay dispersal because of the direct risk of being predated during the dispersal event. If this is the case, we expect helpers to stay closer to the protection of their home shelter and hide more. As a side effect, helpers might have fewer opportunities to check out potential sites for independent breeding in the neighbourhood. Second, helpers may delay dispersal because of the low benefits of independent breeding under predation risk; for example, because they have to defend their newly acquired breeding shelter against predators without, or with less assistance from, additional group members.

In a field experiment we introduced medium- or large-sized predators in large underwater cages encompassing two or three group territories. In control cages all predators were removed. We tested whether predators: (i) negatively influence breeder and helper survival, and whether this effect is mitigated by group size, which would support the 'group augmentation hypothesis'; (ii) decrease the tendency of helpers to disperse and breed independently, supporting the 'ecological constraints hypothesis'; (iii) force helpers to stay closer at their home shelters and hide more.

2. MATERIAL AND METHODS

We studied *N. pulcher* by scuba diving at the south tip of Lake Tanganyika, at Kasakalawe near Mpulungu, Zambia (8°46.849′ S, 31°04.882′ E) between 5 March and 27 May 2002 and 2 February and 21 April 2003. The study population consists of several, partly connected, colonies at 9.0–11.5 m depth in a sandy area with half submerged rocks (Balshine-Earn *et al.* 1998). The present experiments were conducted in a colony encompassing more than 200 groups. In this colony, *N. pulcher* groups breed in distinct patches of stones. Shelters are available and extended between and underneath the stones by digging away sand. Groups create a breeding shelter for the breeders (where eggs are laid on the stone surface) and hiding shelters for all group members (where individual group members hide from predator attacks; Balshine *et al.* 2001; Werner *et al.* 2003). Breeders, large and medium-sized helpers preferably forage in the water column (50–100 cm above the substrate), where zooplankton is most abundant, but retreat to the breeding and hiding shelters as soon as piscivore predators approach. All group territories were marked with numbered rocks and group composition was determined (number and size of breeding males, females, helpers and free swimming fry).

(a) Experimental set-up

Different groups were used in the 2002 and 2003 experiments, to prevent pseudoreplication and carry-over effects. Experimental units were created as follows. Two to three nearby groups, within a distance of 1–1.5 m were selected haphazardly and two to four helpers per group were captured, individually marked, and their

body measurements taken (standard length (SL) in millimetres, 0.5 mm accuracy). Marking involved injecting non-toxic acrylic paint into scale pouches and fin clips of the dorsal and anal fins. Other group members were recognizable from estimates of their size relative to the marked helpers, and natural body markings. SL was estimated by placing a millimetre board in the territory (0.5 mm classes), and was converted to true SL using a calibration regression line (i.e. estimated SL versus measured SL of marked individuals). Marking and measuring of all group members was not attempted, to avoid groups dissolving due to the disturbance (D. Heg and M. Taborsky, personal observation).

A 2 m × 2 m × 2 m cage was erected around these two or three groups, removing all medium and large predators, but including all other naturally occurring fishes. Cages consisted of a lightweight aluminium outer frame, all sides except the bottom covered with a sturdy plastic netting (Ø0.5 mm wire) with inner mesh size 2.5 mm × 2.5 mm (Lanz-Anliker AG, Rohrbach, Switzerland), allowing free flow of zooplankton, the main food of *N. pulcher*. To prevent escape from the cage, the bottom edges were covered with flexible nets and rocks, and no marked fish were seen to leave the cage. Fish inside the cages were showing no signs of stress and their feeding rate was comparable to fish outside the cages (200–400 bites per 15 min, similar to data in Balshine-Earn *et al.* (1998)). Per trial ($n = 7$), three cages were erected adjacently (between-cage distance 1–5 m), hence reducing the effects of local habitat quality, habitat structure (i.e. stone cover) and species composition (i.e. densities of intra- and interspecific competitors) on the outcome of the experiment. In each trial ($n = 7$ trials, includes 21 cages in total) one cage was selected at random for the control treatment (no predator added), one cage received the medium-predator treatment (medium-sized piscivore *Lepidiolamprologus elongatus* added of SL 11.9 ± 1.6 cm mean ± s.d., range = 9.9–14.2 cm, $n = 7$) and one cage received the large-predator treatment (large-sized *L. elongatus* or *Lamprologus lemairii* added of SL 14.7 ± 1.9 cm mean ± s.d., range = 13.0–17.7 cm, $n = 7$; in all trials large exceeded medium predators in size, there were no significant effects of predator species on the results). See § 2b for the number of groups and individuals involved in the experiment. Trials lasted four weeks, cages were removed and predators released after the end of each trial.

(b) Survival

For the survival analyses, all group members were counted and divided into five classes at the start of the trials: breeder males, breeder females, large helpers (estimated SL > 35 mm), medium helpers (estimated SL = 25.5–35 mm) and small helpers (estimated SL = 15.5–25 mm). Average group composition was as follows ($n = 67$): 0.90 breeding males, 0.97 breeding females, 3.73 large helpers, 2.01 medium helpers and 1.73 small helpers (total group size ± s.d. was 9.34 ± 4.87) and did not differ between the treatments (Kruskal–Wallis tests, one test for each class and one test for total group size with treatment as factor, all six tests: $\chi^2 = 0.08$–1.58, d.f. = 2, $0.45 < p < 0.96$). After four weeks, all surviving group members were counted and SL estimated, and divided according to the original five classes, taking into account: (i) the average daily growth rate (Skubic *et al.* 2004); (ii) assignment of dispersed helpers (see § 2c) and helpers replacing breeders to their appropriate original groups. All breeding males were larger than the breeding females and helpers, and most of the breeding females were larger than, or at least similar in size to, the largest helper in the group.

(c) *Dispersal and helper spacing*

Stiver *et al.* (2004) have shown that individuals preferentially disperse to neighbouring territories, but may disperse up to 12 m. To allow helper dispersal and independent breeding, at one unoccupied corner in each cage a dispersal shelter was constructed by removing sand from the underlying stones and placing extra stones and empty snail shells as protection. The within-cage distance from the territories to these shelters was between 0.5 and 1.5 m, which encompasses the average nearest-neighbour distance of 0.9 m in our study population. Every sixth day, occupancy of these dispersal shelters was determined during a 30 min observation. In the first weeks, some helpers swam back and forth between their home group and the dispersal shelter. At the end of each trial it was determined whether the dispersal shelter was permanently occupied and defended by the helpers, and their SL was estimated. Helper spacing behaviour was determined by 15 min focal observations. Two groups per cage were selected, and per group, a medium helper and a large helper were observed three times, using different individuals each time (*ca.* 5 days between observations, each group alternately). Observation of the medium helper was directly followed by observation of the large helper from the same group, or the reverse, in randomized order. To correct for time of day effects (Balshine-Earn *et al.* 1998) and allow for the best possible comparison between the treatments, three cages from one trial were subsequently observed in one dive, in a randomized order. The following two parameters were analysed: (i) estimated distance to the nearest shelter in centimetres (determined every minute, the 15 values averaged per observation before analyses); (ii) time inside the breeding shelter and hiding shelters in seconds. It has been suggested that the time inside the breeding shelter is a measure of helping behaviour (i.e. cleaning eggs, digging; see Balshine-Earn *et al.* (1998)). However, because helpers were seen to flee from the predators in both types of shelter, we prefer to use the sum of the two values as a measure of taking refuge. Restricting the analyses to the time in the breeding shelter, or the time in hiding shelters, gave essentially the same results (not included). The data of one observation were lost underwater, giving a total sample size of 251 observations (3 observations × 2 helper sizes × 2 groups × 21 cages = 252 − 1).

(d) *Reproduction*

Every 3–6 days and at the end of the trials all (new) free-swimming fry emerging were counted and SL was estimated (SL = 3–5 mm). Some groups had multiple breeding females using different breeding shelters, and these fry were counted separately. Based on the SL and the breeding shelter, fry could be assigned to the different broods. Per group, values for the different broods were summed to give (i) the total number of new fry produced and (ii) the total number of offspring reaching 10–15 mm of SL, henceforth called 'juveniles'. Fry and juveniles produced before the experiment started were discarded from all analyses. Eleven groups lost one or both breeders early in the experiment, which were not immediately replaced by new breeders, and the reproductive successes of these groups were discarded from the analyses (reducing the sample size from 67 to 56 groups).

(e) *Ethical note*

Predators occur at very high densities in this colony and hunt their fish prey by moving through the colony, often in groups (D. Heg, personal observation). The experiment comprised removing all medium and large predators from the system, hence reducing the impact of group hunting and the impact of certain predator species specializing on either offspring or adults. Most substrate-breeding cichlids, including our study species, seem adapted to the high level of predation pressure by showing a high reproductive rate: every 2–4 weeks, 100–200 eggs are laid. By reintroducing only one medium or large predator to the experimental treatments we created a moderate level of predation pressure. Additionally, all predators were fed fishes once every 3–5 days, and were released and replaced with a similar-sized predator when showing signs of unnatural behaviour (e.g. because of stress).

(f) *Statistical analyses*

Helper survival ($n = 501$) was analysed using weighted logistic general linear mixed models (GLMM) in R 1.0.8 (Crawley 2002). We used a logit-link to ensure linearity. R uses the restricted maximum-likelihood method (REML) to decompose the variances and derive parameter estimates. The significance of effects was tested using the maximum-likelihood fit, and we report the likelihood ratio LR with *p*-value (see Crawley 2002, pp. 706–707). Models with a significantly improved fit were retained until the final model with only significant effects was found. In case of significant interaction terms, the underlying main effects were also retained. Using REML GLMMs, we estimated the variance due to the two random effects: group nested within cage; and two fixed effects: treatment and helper size. Group size correlates positively with reproductive success (Balshine *et al.* 2001) and only breeders and large helpers defend the group against the medium and large predators, hence the group composition might modify the outcome of the experiment. To control for this, the number of adults (breeders plus helpers greater than 35 mm) was entered as a covariate.

Breeder survival ($n = 125$) was analysed using the same REML GLMM procedure as for helper survival, except that breeder sex was fitted as a fixed effect, instead of helper size. This GLMM gave significant effects of treatment, number of adults and their interaction, but large model-fit residuals (*ca.* 25%) for the breeders from the control group only, and the model was discarded. This was due to survival being 100% and constant in the control group (see § 3). Instead, breeder survival was analysed in three steps. First, survival of the males was similar to the females and were combined in the next two analyses. Second, breeder survival of the predator treatments was compared to the control treatment. Third, breeder survival for the two predator treatments was analysed with REML GLMM, with the two random effects, group nested within cage; the fixed effect, treatment; and the covariate, number of adults. This analysis allowed us to test for the modifying effects of the number of adults on breeder survival depending on treatment, and to test whether the two predation treatments differed from each other.

Helper dispersal was analysed on the cage level ($n = 21$), and because a negative linear association was expected from the control, to the medium-predator, to the large-predator treatment, it was analysed with a linear association χ^2 analysis and one-tailed hypothesis testing using SPSS 11.0. Helper spacing behaviour ($n = 251$) was normally distributed, and analysed using GLMM, controlling for random group within-cage effects in SPSS 11.0. This is a balanced design, so no REML procedure was necessary.

The number of fry and juveniles showed a bimodal distribution, and were analysed on the group level ($n = 56$) with non-parametric Kruskal–Wallis and Mann–Whitney *U*-tests in SPSS 11.0.

Note that the reference categories are indicated with a parameter estimate of zero. All significance levels were set at $\alpha = 0.05$ (two-tailed, except where otherwise stated).

3. RESULTS

(a) *Survival*

As expected, helper survival was significantly reduced in the two predation treatments, the effect being mitigated by the number of adults in the group (figure 1a). Moreover, helper survival was significantly related to helper size, but all helpers reacted similarly to the predation treatment (figure 1a,b). The result of the weighted logistic REML GLMM ($n = 501$ helpers) for treatment was as follows: LR = 19.0, d.f. = 2, $p = 0.0001$ (multiple comparisons by additional GLMMs: control versus medium: LR = 7.3, d.f. = 1, $p = 0.007$; control versus large: LR = 21.6, d.f. = 1, $p < 0.0001$; medium versus large: LR = 2.5, d.f. = 1, $p = 0.11$). The GLMM also showed a significant decrease in survival with helper size (LR = 104.5, d.f. = 2, $p < 0.0001$), but there was no significant interaction with the treatment. Finally, the GLMM showed a positive effect of the number of adults on helper survival (LR = 121.9, d.f. = 1, $p < 0.0001$), but contrary to expectation, not so for the small helpers (figure 1b, adults × helper size: LR = 75.9, d.f. = 2, $p < 0.0001$; s.d. of the random effects, cage: 0.3029, group: 0.0086 with residual 1.074). The fitted parameter estimates ± s.e.m. and significance from this GLMM were: overall intercept: -0.398 ± 0.376; treatment: control: 0, medium: -0.820 ± 0.270, $p = 0.007$, large: -1.311 ± 0.265, $p = 0.0001$; number of adults: 0.670 ± 0.053, $p < 0.0001$; helper size: large: 0, medium: 1.619 ± 0.472, $p = 0.0009$, small: 2.905 ± 0.493, $p < 0.0001$, and the interaction between number of adults and helper size: large: 0, medium: -0.459 ± 0.073, $p < 0.0001$, small: -0.717 ± 0.075, $p < 0.0001$. Note that the effect of the number of adults on the survival of small helpers is not significantly different from zero (slope is $0.670 - 0.717 = -0.047$, with the standard error including 0).

Also, breeders showed reduced survival in the two predator treatments (figure 1a). Owing to the constant 100% survival in the control treatment, breeder survival was analysed in three steps (see § 2b). First, breeder survival was independent of sex (analysed per treatment, control: both 100% survival; medium: LR $\chi^2 = 0.08$, d.f. = 1, $p = 0.78$; large: LR $\chi^2 = 0.09$, d.f. = 1, $p = 0.76$), and sexes were combined in subsequent analyses. Second, breeder survival was significantly lower in the medium and the large treatment, compared with the control treatment (LR $\chi^2 = 9.26$, d.f. = 1, $p = 0.002$ and LR $\chi^2 = 11.07$, d.f. = 1, $p = 0.001$, respectively). Third, the modifying effects of other factors were analysed for the two predation treatments only, using REML GLMM ($n = 89$). Breeder survival was significantly related to the number of adults (LR = 8.34, d.f. = 1, $p = 0.015$) and the interaction between the number of adults × treatment (LR = 5.58, d.f. = 1, $p < 0.02$), but marginally non-significantly related to treatment (LR = 5.63, d.f. = 1, $p = 0.06$; parameter estimates: overall intercept: -0.815 ± 0.175; treatment: medium: 0, large: -0.574 ± 0.282, $p = 0.065$; adults: -0.002 ± 0.024, $p = 0.93$; adults × treatment: medium: 0, large: 0.103 ± 0.043, $p = 0.02$; s.d. of the random effects, cage: 0.1037, group: 0.2933 with residual 0.266). The GLMM indicates that large predators caused more mortality in the breeders compared with medium
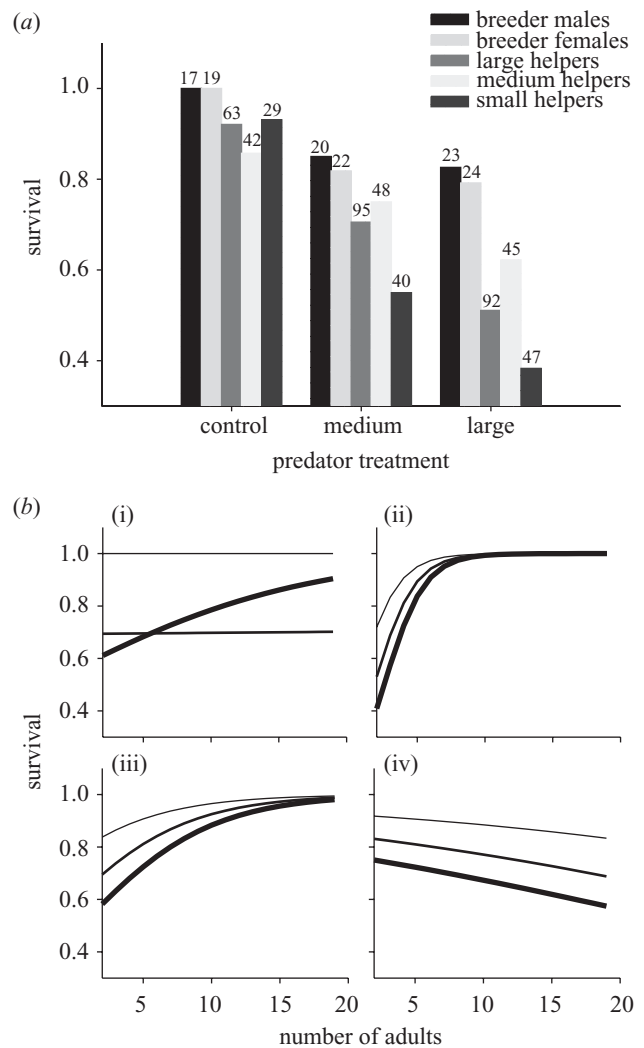

Figure 1. Survival of breeders and helpers was affected by the predator treatment: control without predator, medium or large predator. (a) Proportion surviving per treatment for breeder males, breeder females, large helpers, medium helpers and small helpers, respectively. Sample sizes are indicated at the top of the bars. (b)(i) Effects of treatment and the number of adults on breeder survival (from breeder survival REML GLMM without the control group, which had 100% survival throughout, see §§ 2b and 3a). Also shown are the effects of treatment and number of adults on helper survival per helper size class (from helper survival REML GLMM, see § 3a); (ii) large helpers; (iii) medium helpers; (iv) small helpers). Thin lines: control treatment; medium lines: medium-predator treatment; bold lines: large-predator treatment.

predators, but that this effect was cancelled out in groups with a large number of defending adults.

Finally, helpers had a significantly reduced survival compared to breeders (figure 1a, all data lumped, breeders versus: large helpers LR $\chi^2 = 16.31$, d.f. = 1, $p < 0.001$; medium helpers LR $\chi^2 = 7.26$, d.f. = 1, $p = 0.007$; small helpers LR $\chi^2 = 27.34$, d.f. = 1, $p < 0.001$).

(b) *Helper spacing and dispersal*

As expected, predators had a strong influence on helper spacing behaviour. Both medium and large helpers stayed significantly closer to the breeding shelter (GLMM $F_{2,49} = 24.4$, $p < 0.001$; figure 2a), and were hiding more (GLMM $F_{2,49} = 3.5$, $p = 0.037$; figure 2b), in both
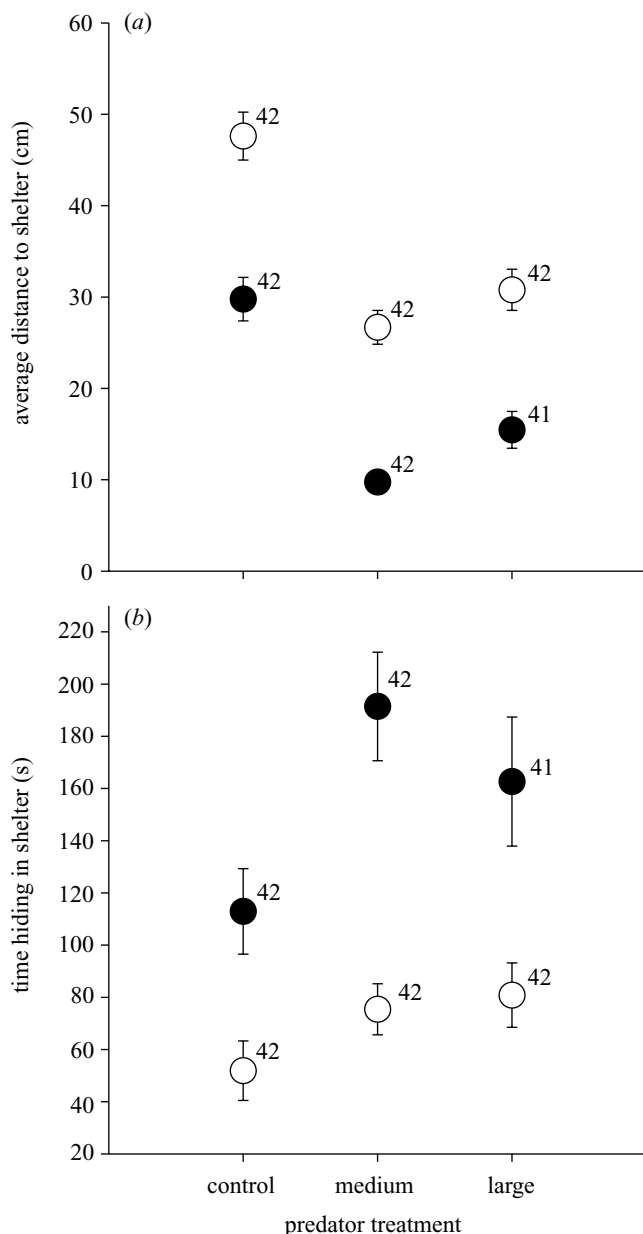
Figure 2. Helper spacing behaviour depended on helper size (medium: black circles; large: white circles) and the predator treatment (depicted are means ± s.e.m., with sample sizes): (*a*) average distance to the nearest shelter and (*b*) time hiding in shelter.

predator treatments compared with the control treatment. The differences between the two helper size classes were significant (for distance $F_{1, 198} = 117.2$, $p < 0.001$; and for hiding $F_{1, 198} = 45.0$, $p < 0.001$), whereas the interactions between treatment × helper size were not (for distance $F_{2, 198} = 0.8$, $p = 0.43$; and for hiding $F_{2, 198} = 2.6$, $p = 0.077$; all values corrected for random group within-cage effects). Multiple comparisons revealed significant differences between the control treatment and both predator treatments for distance (Dunnett T3, both $p < 0.001$), and partly for hiding (Dunnett T3, for medium helpers $p = 0.008$, for large helpers $p = 0.088$). By contrast, no significant differences between the two predator treatments were detected (Dunnett T3, $p = 0.076$ for distance and 0.896 for hiding).
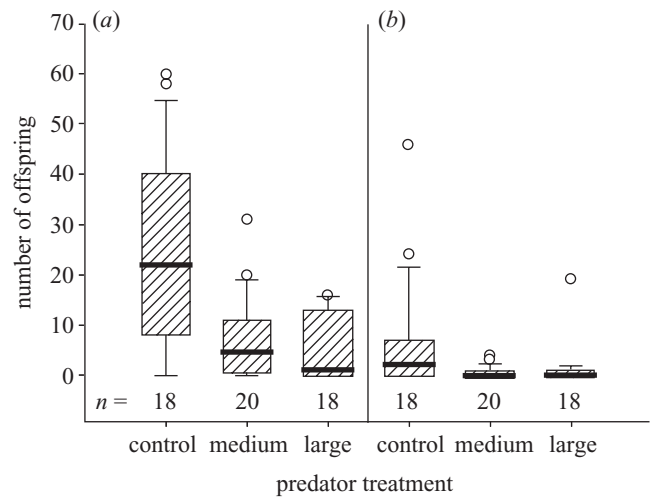
Figure 3. Group reproductive success was reduced in the predator treatments compared with the control situation in the total number of (*a*) free-swimming fry and (*b*) juveniles produced during the experiment. Box-plots show medians (horizontal lines), quartiles (boxes), 10% and 90% percentiles (vertical lines with caps), and outliers (circles), below the boxes are the sample sizes.

We expected helper dispersal to increase from the large-predator to the medium-predator and control treatments. Helpers moved to the experimental dispersal shelters in 7 out of 21 cages, and there was a significant linear association with the treatments in the direction predicted (four out of seven control cages, two out of seven medium-predator cages and one out of seven large-predator cages; linear association (LA) $\chi^2 = 2.76$, d.f. = 1, one-tailed $p = 0.049$). In one control cage and one medium-predator cage two new groups even settled, and if these were also taken into account, the association increased in significance (LA $\chi^2 = 3.41$, d.f. = 1, one-tailed $p = 0.033$). New groups consisted of one to five individuals ($n = 9$, mean ± s.d. $= 2.8 ± 1.4$), and five groups had already produced fry during the experiment. Mean standard length SL of the largest individuals in these groups was 49.3 mm (±4.7 s.d., range = 42–56 mm, $n = 9$ groups), average SL of all members was 42.1 mm (±9.1 s.d., range = 22–56 mm, $n = 26$ individuals).

(**c**) *Group reproductive success*

Predators had a negative impact on the total group reproductive output, both in terms of the number of free-swimming fry produced (figure 3*a*, Kruskal–Wallis $\chi^2 = 11.5$, d.f. = 2, $p = 0.003$) and the number of juveniles (figure 3*b*, Kruskal–Wallis $\chi^2 = 8.1$, d.f. = 2, $p = 0.017$). As expected, the control treatment differed significantly from both the medium-predator and the large-predator treatments (Mann–Whitney U-tests, for fry $z = -2.78$, $p = 0.006$, and $z = -3.04$, $p = 0.002$; for juveniles $z = -2.43$, $p = 0.015$ and $z = -2.37$, $p = 0.018$, respectively). No significant differences were detected between the two predator treatments (Mann–Whitney U-tests, for fry $z = -0.57$, $p = 0.59$, and for juveniles $z = -0.16$, $p = 0.88$).

4. DISCUSSION

To our knowledge, this is the first experimental evidence for the widespread assumption that predators may reduce

helper dispersal, supporting the 'ecological constraints hypothesis' (Emlen 1982). The results on spacing behaviour suggest this effect was mediated through more helpers hiding inside shelters, and moving less far away from them. Additionally, we found that breeder survival, helper survival and group reproductive success were reduced in the two predator treatments. Large- and medium-sized helpers experienced higher survival in groups with a greater number of adults protecting the group against predators, but unexpectedly this was not the case for small helpers. This suggests that 'group augmentation' effects provide substantial direct fitness benefits (survival) to all group members, including offspring (Balshine *et al.* 2001), except for the small helpers. Breeders also benefited from a larger number of adults in the group, although the effect was only pronounced in the large-predator treatment. Detailed analyses of predator attack rate/hunting success and the probability/level of defence of *N. pulcher* shown during such attacks, both depending on predator size, prey size and group size, should clarify several points: (i) why small helpers do not seem to benefit from more group members protecting them; (ii) why helper survival is related to helper size; and (iii) why breeders only benefit from additional group members in the presence of large predators.

Similar, albeit correlative, results have been obtained for two cooperatively breeding mammals. Rasa (1986) found a positive correlation between group size and juvenile survival in the dwarf mongoose (*Helogale undulata*). Small groups were more often attacked by raptors and lost more offspring than large groups (Rasa 1987). Clutton-Brock *et al.* (1999) compared the survival of meerkats (*Suricata suricata*) breeding either in a high predator density or a nearby low predator-density habitat. They found that juvenile mortality decreased in the low predator-density habitat, and juvenile mortality decreased with group size in the high density, but increased with group size in the low-density habitat.

Experimental evidence for the 'ecological constraints hypothesis' has now been accumulated for three types of ecological factor affecting the benefits and costs associated with helper dispersal and helper independent breeding: (i) the availability of independent breeding options ('habitat saturation'); (ii) habitat quality; and (iii) predation pressure. First, the experimental removal of breeders resulted in helpers inheriting the breeding position (Brown & Brown 1981; Hannon *et al.* 1985; Komdeur 1992; Walters *et al.* 1992; Komdeur *et al.* 1995; Balshine-Earn *et al.* 1998; Field *et al.* 1998; Shreeves & Field 2002) or nearby helpers dispersing and filling the vacancy (Pruett-Jones & Lewis 1990). These observations show that helpers are capable of independent breeding when given the opportunity, but are prevented from doing so by aggressive exclusion of competitors. Second, habitat quality effects were tested by translocation and removal experiments. In Seychelles warblers (*Acrocephalus sechellensis*) helpers modify their dispersal behaviour according to the territory quality of independent breeding options (Komdeur 1992; Komdeur *et al.* 1995). By contrast, subordinates in the hover wasp (*Liostenogaster flavolineata*) were unlikely to accept nearby experimental high-quality breeding sites, a free nest provided by the experimenters, which seems to relate to the high benefits of queuing for a breeding position in this species (Field *et al.*

1998, 1999, 2000). Third, this study demonstrates that predators affect helper dispersal.

It is important to note that the two major types of population regulation should influence dispersal propensity of helpers differently. If the population is regulated by density dependence and is stable, the constraints on independent breeding and predation risk during dispersal should not influence dispersal decisions (see Pen & Weissing 2000). Nevertheless, predation risk while staying at home and, for example, differences in predation risk depending on territory quality, group size or phenotype might still influence dispersal decisions (e.g. Kokko & Lundberg 2001; Kokko *et al.* 2001). By contrast, if the population is regulated without density dependence, predation risk, both during dispersal and while staying at home, might influence dispersal decisions (Pen & Weissing 2000). Because we created an extra breeding shelter for the helpers to disperse to and enclosed 'small populations' inside each of the cages, one could argue that we intrinsically created small populations that are regulated more or less without density dependence, at least during the experimental period. In these cages, the effects of predation risk might be more apparent than in populations regulated by density dependence.

We found the strongest effect of predators was on the survival of small helpers, whereas the effects on large- and medium-sized helpers were less pronounced. This effect might be responsible for the division of labour found in our study species: small helpers showing predominantly brood care (cleaning eggs and wrigglers) and breeding shelter maintenance (digging), with limited exposure to predators; and large helpers performing territory defence, which exposes them to attacks by piscivores (Taborsky & Limberger 1981; Taborsky 1984, 1985; Taborsky *et al.* 1986). Division of labour is a common phenomenon in cooperatively breeding societies, and in ants in particular it is often associated with morphological dimorphism (Bourke & Franks 1995). Although these strategies are fixed for life in ants, the type and frequency of helping behaviours in *N. pulcher* continuously change as they grow ('age-related polyethism'; see, for example, Clutton-Brock *et al.* (2003)).

We conclude that future ecological and behavioural studies of cooperatively breeding species should consider the potential effect of predation risk. We have shown that predators may critically affect many aspects of cooperative breeding societies, such as helper spacing, helper dispersal, reproductive success (see also Rabenold 1990), and survival, and through this perhaps also division of labour and group size. Variation in predation risk might account for the large variation seen in these aspects between groups living in different habitats, between populations, and between species (Stacey & Koenig 1990; Choe & Crespi 1997; Solomon & French 1997). Future studies should attempt to estimate the selection pressure exerted by predators in different habitats, populations and species, to elucidate this variation.

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