



## Individual variation in helping in a cooperative breeder: relatedness versus behavioural type

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Helpers in cooperatively breeding groups can vary hugely in the variety and level of care they provide. Several studies suggest that kin selection alone cannot be invoked to explain variation in helping for many species, but there have been few explicit tests of this under controlled conditions. Here, we investigated whether relatedness to the breeding pair or consistent individual differences in behaviours explained variation in helping by the cooperatively breeding cichlid *Neolamprologus pulcher*. We established standardized social groups consisting of a breeding pair and one related ( $r = 0.5$ ) and one unrelated ( $r = 0$ ) helper. Two forms of helping, territory maintenance and territory defence, were measured repeatedly under controlled conditions: helping was variable between, but consistent within, individuals. Furthermore, there was some evidence that helpers that carried out more maintenance also performed more defence. Contrary to the kin selection hypothesis, relatedness did not predict the amount or variety of helping executed. Risk responsiveness, activity levels and aggressiveness were repeatable within individuals, so constituted 'behavioural types' (or personality traits), but were uncorrelated with each other. More aggressive, risk-prone or more active helpers participated in more territory defence than submissive, risk-averse or inactive helpers. Risk-prone individuals contributed more to territory maintenance than risk-averse helpers. Overall, differences in behavioural type, rather than relatedness, explained most variation in helping behaviour in *N. pulcher*. This study highlights the importance of considering consistent individual differences in behaviour for predicting participation and performance in complex social interactions.

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Helpers in cooperatively breeding groups can assist breeders by, for example, provisioning young, defending against predators and conspecific intruders, maintaining nests and caring for eggs (Taborsky & Limberger 1981; Stacey & Koenig 1990; Emlen 1991). Individuals can vary in terms of who they help, and also in the variety and level of care provided (Heinsohn & Legge 1999; Arnold 2000). The presence of helpers within a group has been found to boost the reproductive success and reduce the workloads of the breeders, and improve survival rates of both breeders and offspring (Taborsky 1984; Emlen 1991; Balshine-Earn et al. 2001; Brouwer et al. 2005). Helpers suffer costs, including lost mating opportunities, energy expenditure and injury risk (Taborsky 1984; Grantner & Taborsky 1998; Heinsohn & Legge 1999).

The accrual of indirect fitness benefits may offset costs if helpers are related to recipients (Hamilton 1964). Indeed, some studies

(Clarke 1984; Reyer 1984; Emlen & Wrege 1988; Komdeur 1994; Stiver et al. 2005; Wright et al. 2010) have found that individuals adjust their helping based on relatedness to the breeders, but others have not (Wright et al. 1999; Clutton-Brock et al. 2001; Canestrari et al. 2005). If most or all individuals within a group are relatives of some degree, then a blanket rule of helping any group member might ensure kin-selected benefits accrue without the need for kin discrimination mechanisms. In many species unrelated helpers are found in groups (Reyer 1984; Magrath & Whittingham 1997; Van Horn et al. 2004; Dierkes et al. 2005; Wright et al. 2010), so direct fitness benefits, for example reduced predation risk and improved foraging opportunities (Hamilton 1964; Taborsky 1984; Heg et al. 2004), may be sufficient to drive selection on helping (Clutton-Brock 2002; Griffin & West 2002). As related helpers receive both direct and kin-selected fitness benefits, they may be expected to help more than nonkin. Similarly, related helpers are predicted to perform more costly or risky tasks, such as intruder defence, than nonkin (Balshine-Earn et al. 2001; Arnold et al. 2005). By contrast, nonkin might focus on low-risk helping such as territory maintenance and brood care. Conversely, if helpers essentially

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have to 'pay to stay' on the territory (Gaston 1978; Balshine-Earn et al. 1998), nonkin should help more than relatives (Kokko et al. 2002), as they are less tolerated by breeders than relatives. Thus, the predictions concerning relatedness and helping are complex.

Factors other than relatedness may also influence the amount and form of help an individual is prepared to, or able to provide: group size, body condition, sex, social status, helper experience and more recently 'behavioural type' (Heinsohn & Legge 1999; Schürch & Heg 2010a). Faced with the same environment or behavioural stimuli, and measured under standardized conditions, individuals of the same species often show 'consistent individual differences' in behaviour (Sih et al. 2004; Bell & Sih 2007). Commonly measured behaviours include: aggression (aggressive–submissive), activity (active–inactive), exploration (fast–slow explorers) and risk responsiveness (risk prone–risk averse, bold–shy or neophobic–neophilic) (Sih et al. 2004; Bell & Sih 2007). When behaviours are consistent within, but vary between, individuals across a range of situations or contexts, they can be defined as 'personality traits' or 'behavioural types' (the latter being the term we use in this paper). As behavioural types appear to have a heritable component (Dingemanse et al. 2002) and may also be programmed by early life experiences, such as social, hormonal, nutritional or maternal effects (Arnold et al. 2007; Arnold & Taborsky 2010; Schürch & Heg 2010a; reviewed by Sih et al. 2004), individuals may become specialized in behaviours that then affect their fitness, such as foraging (Herborn et al. 2010), dispersal (Schürch & Heg 2010a) or helping (Arnold 2000; Bergmüller & Taborsky 2007; Bergmüller & Taborsky 2010; Schürch & Heg 2010b). The definition of behavioural syndromes emphasizes the existence of 'suites of correlated behaviours': that is, correlations between different behavioural types across contexts or situations (Sih et al. 2004).

*Neolamprologus pulcher*, a cooperatively breeding African cichlid endemic to Lake Tanganyika, lives in social groups consisting of a dominant breeding pair and 1–14 helpers, which vary in size, sex and relatedness (Taborsky & Limberger 1981; Balshine-Earn et al. 2001; Dierkes et al. 2005). Helpers aid breeders by cleaning and fanning eggs, keeping the breeding shelter free of sand and debris, and defending the territory and other group members against predators and intruding conspecifics (Taborsky & Limberger 1981; Taborsky 1984). Helpers may specialize in certain tasks depending on their body size (Bruitjes & Taborsky 2011). As in the wild larger helpers also tend to be less related to the breeding pair than smaller helpers, relatedness can appear to predict the type of helping performed (Dierkes et al. 2005; Stiver et al. 2005). Helping is costly, for example in terms of energy expenditure and growth (Taborsky & Limberger 1981; Taborsky 1984; Grantner & Taborsky 1998; Taborsky & Grantner 1998), but helpers can receive direct fitness benefits through group living, such as improved survival (Taborsky 1984; Heg et al. 2004), enhanced reproductive success via parasitism of the reproduction of the breeders (Dierkes et al. 1999; Heg et al. 2006, 2008) and/or inheritance of the breeding territory (Dierkes et al. 2005; Stiver et al. 2006). Additionally, related helpers are predicted to accrue indirect fitness benefits (Taborsky 1984; Brouwer et al. 2005). Previously, it has been established that juvenile *N. pulcher* can recognize kin via phenotype matching based on chemical rather than visual cues. These full siblings had been reared apart from focal fish, so were unfamiliar to them before the two-way choice trials (Le Vin et al. 2010). So, helpers should be able to assess relatedness to breeders and accordingly adjust their care. However, a previous study of *N. pulcher* uncovered mixed results on the effects of relatedness on helping effort in captivity versus the wild (Stiver et al. 2005). In the field, helpers related to the breeding female and unrelated to the breeding male took part in the most territory defence. In the laboratory, by contrast, helpers unrelated to both breeders carried

out the most territory defence and territory maintenance, compared with helpers related to both breeders (Stiver et al. 2005). As that study did not carry out manipulations to standardize helping effort, there was likely to have been variation between groups in the amount of helping required. Recent studies on different populations have also provided evidence for consistent individual differences in exploratory tendency, risk responsiveness and aggression in *N. pulcher* (e.g. Riebli et al. 2010), which can predict helper dispersal, group stability and reproductive decisions (Schürch & Heg 2010a, b). Patterns of correlations between behavioural types and helping differed between studies of the same species (Bergmüller & Taborsky 2007; Schürch & Heg 2010a, b; Witsenburg et al. 2010), which warrants further investigation. Importantly, these studies did not simultaneously assess helper relatedness and behavioural types in predicting helping.

The aim of our experiment was to simultaneously consider the effects of relatedness and consistent individual differences in behaviour on individual helping in *N. pulcher*. We tested *N. pulcher* in a controlled laboratory set-up within standardized social groups that accounted for body size, familiarity and relatedness between individuals. We assessed two helping behaviours in *N. pulcher*: the amount of digging helpers carried out when the breeding shelter was filled in experimentally with sand (territory maintenance), and the level of defence shown against a size-matched conspecific intruder (territory defence). We addressed the following specific questions via experimental manipulations: (1) Is there variability between and temporal consistency within individuals in helping effort? (2) Are territory defence and maintenance correlated within individuals (i.e. are some individuals generally more helpful than others)? (3) Does relatedness to the dominant breeding pair affect the amount or form of helping shown? (4) Do aggressiveness, activity and risk responsiveness show variability among and consistency within individuals? (5) Are different behavioural types correlated, constituting a behavioural syndrome? (6) While controlling for relatedness, does an individual's behavioural type predict the amount or form of helping effort it performs?

## METHODS

Adult *N. pulcher* were transported from the University of Bern, Switzerland to the University of Glasgow in February 2007. The fish were a mixture of wild caught ( $N = 10$  pairs) and captive bred ( $N = 10$  pairs). The wild-caught adults originated in Nkumbula Island, near Mpulungu, Zambia in 2006. The captive-bred adults were the offspring of wild fish caught at Kasakalawe, near Mpulungu, Zambia, in 1996. Captive-bred and wild-caught fish can behave differently, but we found that focal offspring from the two populations did not differ significantly in any of the behaviours analysed below ( $P > 0.2$  in all cases; see Appendix). Moreover, microsatellite analyses have shown that the breeding stock fish used in this study were not inbred, with 90% of pairs having a relatedness score of less than 0.125 and 73% being completely unrelated (see Appendix).

Fish were kept in mixed-sex tanks until breeding began in early June 2007. These holding tanks ranged in size from 50 to 250 litres and stocking densities ranged from 3 to 27 individuals. Tanks were provisioned with 1–1.5 cm of coral sand on the base, an airstone, foam filter and several clear plastic tubes suspended at the top of the tank to act as refuges. The water temperature was kept in the range of  $26.8 \pm 1$  °C, pH in the range 8–8.4 and a 13:11 h light:dark cycle. Adult fish were fed once daily with either a commercial dry cichlid food, frozen bloodworm or *Daphnia*.

For breeding, one male and one female were placed into a  $80 \times 40$  cm and 50 cm high 140 litre breeding tank provisioned with 1–1.5 cm of coral sand on the base, an airstone, foam filter, two pieces of plastic guttering pipe and two terracotta flowerpot halves

to serve as breeding shelters and refuges (see above for water parameters and feeding regime). Shelters were then checked for eggs in the morning and evening. When eggs were found they were removed 24 h later and reared in family-specific tanks isolated from their parents and the F1s from other families (for more details see Le Vin et al. 2010). Thus, in the helping experiments the breeders were unfamiliar with the helpers, even their own offspring, and the helpers were unfamiliar with each other.

#### *Establishing Standardized Social Groups*

To investigate helping effort, social groups of *N. pulcher*, consisting of a pair of adults that had bred together previously (see above) and two juvenile F1 helpers were set up. One helper was an unfamiliar offspring of the breeders (related helper;  $r = 0.5$ ) and the other helper was unrelated and unfamiliar to the breeders (unrelated helper;  $r = 0$ ). Helpers were also unfamiliar with each other. Helpers were approximately of a sexually mature size, >35 mm standard length (SL) and were matched for SL (mean related =  $39.12 \text{ mm} \pm 0.46$ ; mean unrelated =  $38.94 \text{ mm} \pm 0.49$ ; paired  $t$  test:  $t_{16} = 0.41$ ,  $P = 0.69$ ), mass (mean related =  $1.59 \text{ g} \pm 0.07$ ; mean unrelated =  $1.55 \text{ g} \pm 0.07$ ; paired  $t$  test:  $t_{16} = 1.05$ ,  $P = 0.31$ ) and age (mean related =  $305.94 \text{ days} \pm 14.98$ ; mean unrelated =  $317 \text{ days} \pm 13.56$ ; paired  $t$  test:  $t_{16} = -1.45$ ,  $P = 0.17$ ). Thus, within social groups helpers should not have varied in their condition or experience and, therefore, their physical ability to help. Unfortunately, a number of helpers could not be reliably sexed, so sex was not considered in this study, the consequences of which are discussed later.

The breeding tanks and conditions were identical to those described above except that two clear plastic tubes were also suspended at the top of the tanks, as refuges for fish receiving aggression from group members. Breeding shelters were checked daily for eggs, which were counted and removed. The shelter was then replaced with a new shelter without eggs, as breeders have been observed to be more aggressive towards helpers when a new clutch is present (Taborsky 1985). Also, we wished to control for differences in breeding stage between experimental groups. Only four pairs produced eggs once and the rest never laid a clutch.

Before being introduced to the experimental tank, both helpers and breeders were anaesthetized in a benzocaine solution and SL and mass were recorded. For anaesthesia, we put tank water at the correct temperature and pH etc. into a beaker and added 10 ml of benzocaine per litre of water. At this concentration animals were suitably anaesthetized within 1 min, no fish suffered any adverse reactions and they recovered within 1–2 min in a tank containing fresh water. After 20 min recovery on their own, they were then introduced to their new experimental tank. No aggressive encounters between helpers were observed when they were introduced together to the experimental tank or over the following 24 h (they were checked every hour during daylight). Breeders were anaesthetized and added to the experimental tank the next day, following the same protocol.

For identification, each fish was uniquely marked by fin clipping. Less than 5 mm was taken from either the ventral or caudal fin; this was randomly chosen with respect to relatedness. This was found to be the most effective and least invasive method of marking these small fish (compared, for example, with passive integrated transponder (PIT) tags and injected dyes under the scales) that could be seen from a distance and all angles. No fish died or developed infected wounds after the procedure. To minimize breeder aggression, the two helpers were first introduced to the tank. After 24 h, the breeders were then introduced and the group allowed to acclimate for a further 24 h. During this period, we observed the group for 5 min every 2 h from 0900 hours to 1900 hours to ensure

that the helpers were not being harassed and had no injuries. If any individual had been subject to sustained attack resulting in injury, we would have terminated the experiment and removed the victim, but this never occurred. Groups were then left overnight and checked again the following morning.

In total, 24 groups were established, but in 17 of these breeders did not initially accept helpers, instead forcing them out of the breeding shelter and/or to hide in the refuge tubes. Using a technique used by previous studies, we tried to encourage acceptance of helpers by restraining the breeders for up to 24 h in mesh cages within the experimental tank. The mesh cage measured approximately  $20 \times 15 \text{ cm}$  and 16 cm high. Fish were able to turn around and move freely in these mesh cages and none acquired any injuries while in these tanks. Breeders were then released from the cage and the group was reassessed. Helpers were perceived to be accepted into the group when they had free access to swim around the tank, including in and around the breeding shelters ( $N = 10$ ). Some direct aggression towards the helpers, which included chasing, biting, approaching with opercular spread and slow approaches that led to the other fish retreating, was observed, but mainly breeders prevented helpers from entering the breeding shelters. The refuges at the top of the tank worked in that breeders did not follow helpers inside them. Again, if we had observed any injuries, we would have terminated the experiment and split up the group, but this never happened.

In seven of the 24 original groups, helpers were accepted straight away ('quick'), and in ten they were accepted after the breeders had been restrained ('slow') (see Appendix). Among helpers, helper acceptance (quick or slow) did not explain variation in helper territory defence, territory maintenance, aggressiveness (mean across nine observation periods), activity levels or risk responsiveness (see Appendix). At the group level, the mean number of aggressive interactions between group members over the nine observation periods, total mean territory defence and territory maintenance by the group did not differ between groups that were quick or slow to accept helpers (see Appendix). The time between groups being established and the start of experiments was on average 4.65 days.

#### *Assessment of Helping Effort*

The amount of territory maintenance and the amount of territory defence shown by helpers were quantified in separate experiments, each consisting of three trials. Territory maintenance and defence trials were carried out in a random order and there was at least 48 h between subsequent trials. Before any observations, fish were allowed a 3 min acclimation period, as has been used in other similar experiments (Bergmüller et al. 2005), from the point when the observer either entered the room and sat behind the screen, filled in a shelter with sand or introduced an intruder. Then, before each trial, the focal group was observed for 10 min to ensure that helpers were accepted and to assess helper aggressiveness.

#### *Territory maintenance*

To standardize the group's helping requirements, we manually filled in one of the two breeding shelters with sand to the top of the breeding shelter (Bruintjes & Taborsky 2011). The group's behaviour was then recorded on a video camera for 50 min. On the video, the observer (who was blind to the identities of the helpers) identified the time when helpers began digging sand away from the shelter. For the next 10 min, the number of times each helper dug using either the body or mouth to move sand was counted (Grantner & Taborsky 1998). Mean territory maintenance (number of digging acts per helper per 10 min observation) was then calculated across the three trials.

### Territory defence

Each trial consisted of an intruder phase and a control phase presented in a random order. At the start of each phase a glass jar (1.2 litre) was introduced into the centre of the experimental tank between the two breeding shelters, and the group was observed for 10 min. In the intruder phase, the jar contained a conspecific, and in the control phase it was empty. At least 1 h before the trial the intruder (an unfamiliar, unrelated, size-matched conspecific) was allowed to acclimate in a glass jar, which prevented direct interactions between individuals and thus fight injuries. The perforated lid of the jar allowed the exchange of chemical cues with the experimental tank. The number of defensive behaviours shown towards the empty jar or jar plus intruder was recorded: approaching the jar with opercular spread, biting the jar, swimming at the jar in a head down position and fast swimming at the jar, equivalent to ramming. During the trials, the conspecific intruder generally oriented itself to the fish 'attacking' it and did not try to escape or avoid it. The helpers were more aggressive towards the jar plus intruder (mean number of defensive behaviours =  $9.08 \pm 1.52$ ) than the jar alone (mean =  $1.20 \pm 1.57$ ). Territory defence was scored as the number of defensive behaviours shown in the intruder phase minus the number of defensive behaviours shown in the control phase. This controlled for levels of aggression shown towards an object (the jar) introduced into the territory, as opposed to a conspecific. Mean territory defence was calculated per helper across the three trials.

### Assessment of Behavioural Types

Three behaviours were measured in *N. pulcher* and assessed by an observer blind to the relatedness of the helpers to the breeders.

### Aggressiveness

In total, nine (10 min) aggressiveness trials were recorded in three contexts: three general observation periods, after which no helping trials occurred; three before territory maintenance trials; and three before territory defence trials. Aggressive acts recorded included biting, chasing and approaches with opercular spread (Bergmüller et al. 2005).

After all helping trials finished, the breeders were removed from the experimental tank and the helpers allowed 24 h to acclimate. Breeders were removed before assessing activity levels and risk responsiveness, as they might have interfered with the movements of helpers and/or influenced helper reactions. Individuals could not be assessed on their own, as we discovered that solitary individuals did not behave normally; thus we did not have activity and risk responsiveness for the first three groups tested. The experimental tank was divided into five vertical zones along the length of the tank (each 16 cm wide by 50 cm high) and numbered one to five from left to right. Zones were marked out with 0.5 cm wide waterproof tape. The two refuges were located in zones one and five and the two breeding shelters were located in zones two and four.

### Activity levels

Following a 3 min settling period after the observer moved behind the screen, the number of movements between the five zones was recorded for one helper for 10 min and then for the second helper. The order in which helpers were observed was randomized. Activity levels in this non-novel environment were re-measured the next day.

### Risk responsiveness

Helpers were exposed to one of two novel objects: a purple plastic half sphere (2.5 cm high, 5 cm in diameter) or a red Buddha figurine (5 cm high, 3.5 cm wide). A trial started when the observer placed a novel object in the centre of the tank between the

breeding shelters and immediately sat behind the screen. The latency to first approach the novel object within two body lengths was our measure of risk responsiveness. If fish failed to approach within 10 min they were scored as 600 s. The next day the second novel object was presented, with the object order randomized.

### Ethical Note

All work was carried out under licence from the U.K. Home Office and approved by the University of Glasgow Ethics Committee. In November 2006, adult *N. pulcher* were caught in Lake Tanganyika, in Zambia, by members of the Bern Diving Expedition, and transported to the University of Bern, Switzerland, by air, under licence from the Ministry of Agriculture and Co-operatives in Zambia. In March 2007, 68 adult *N. pulcher* were transported by air, from the University of Bern, to the University of Glasgow, under licence from the Scottish Executive Environment and Rural Affairs Department. Fish were transported in two insulated polystyrene crates (59 × 40 cm and 33 cm high), with each crate containing six thick plastic bags, stocked with four to six fish each. The fish were in transit for less than 12 h, and water temperature was maintained above 20 °C. During this period the fish were not fed, to prevent degradation of water quality. As the fish are normally fed once every 24 h, this was not an unduly long period without food. During transit to Glasgow no mortality occurred. However, eight fish died in the subsequent 2 weeks after their arrival. The causes of these deaths were not obvious, but they were not due to physical injury.

Fish were kept within their group in mixed-sex tanks until breeding began in early June 2007. These holding tanks ranged in size from 50 to 250 litres, and stocking densities ranged from 3 to 27 individuals. At the end of the experiments, all fish, including the breeding adults, were maintained under the same tank conditions as previously described.

### Data Analysis

Data were checked for normality and homogeneity of variance. Where these assumptions were violated, nonparametric tests were carried out. We assessed that territory maintenance, territory defence, aggressiveness, activity and risk responsiveness were repeatable, by carrying out single-factor ANOVAs (Lessells & Boag 1987). Where behaviours were found to be repeatable using these analyses, then mean values per individual were calculated and used in later analyses.

We investigated the relationships between risk responsiveness, aggressiveness and activity level versus the amount of helping shown, by carrying out generalized linear models (GLMs) that also controlled for the relatedness of the helpers. We removed nonsignificant interactions and then nonsignificant main effects from the GLMs in a backwards stepwise procedure ( $P > 0.05$ ). When investigating whether aggressiveness of helpers affected the amount of territory maintenance shown, we used the aggressiveness scores from the trials directly before the territory maintenance trials; likewise for aggressiveness before territory defence trials. Where the assumptions of sphericity were violated, we made corrections using the corresponding significance value (using the Greenhouse–Geisser, Huynh–Feldt or lower bound significance values; Field 2005). Means ± SEs are presented. All tests were two tailed. All data were analysed using SPSS versions 15.0 and 18.0 (SPSS Inc., Chicago, IL, U.S.A.).

## RESULTS

### Variability and Repeatability in Helping Efforts

Individuals varied widely in the amount of helping carried out (Table 1; GLM  $F_{1,67} = 2.57$ ,  $P = 0.004$ ). Territory maintenance



**Table 1**  
Interindividual variation in behaviours by helpers

Trait	Range	Mean±SE
Territory maintenance*	0–60.67	11.04±2.26
Territory defence†	0–28.67	7.88±1.41
Aggressiveness (Gen Obs)‡	0–5.50	1.28±0.24
Aggressiveness (TM)‡	0–7.67	1.19±0.26
Aggressiveness (TD)‡	0–8.00	1.11±0.28
Activity level§	2–70.50	35.68±3.20
Risk responsiveness**	15.50–600.00	281.89±36.44

The range, mean and SE of behaviour over the three territory maintenance, territory defence and aggressiveness trials and the two activity and risk responsiveness trials.

\* Mean number of acts of digging per 10 min.

† Mean number of aggressive acts per 10 min towards the conspecific intruder in the jar minus mean number towards the jar alone.

‡ Mean number of within-group aggressive acts in general observations with no helping trial afterwards (Gen Obs), before a territory maintenance trial (TM) and before a territory defence trial (TD) per 10 min.

§ Mean number of moves between zones per 10 min.

\*\* Mean latency to approach a novel object (s).

(ANOVA:  $F_{29,60} = 3.01$ ,  $P < 0.001$ ,  $r = 0.40$ ) and territory defence (ANOVA:  $F_{29,60} = 5.31$ ,  $P < 0.001$ ,  $r = 0.59$ ) were repeatable within individuals across trials. So, there was variability between individuals and consistency within individuals in their helping efforts.

#### Correlations Between Territory Defence and Maintenance

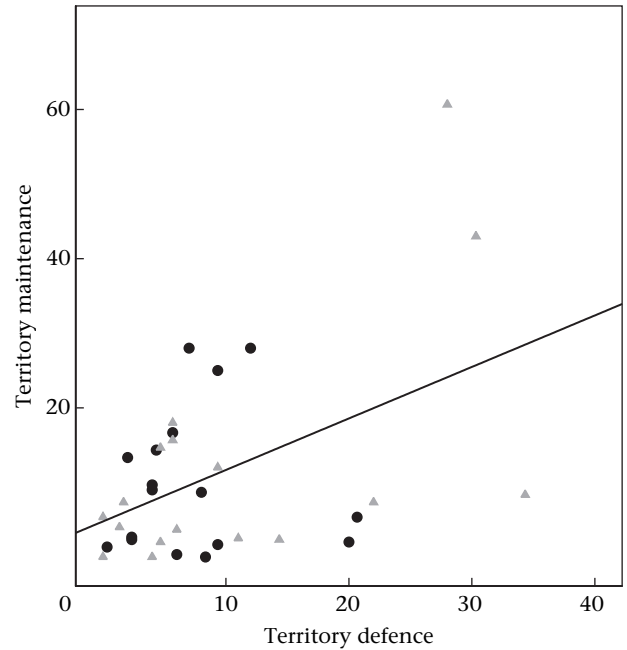
The amount of territory defence an individual performed was correlated with its territory maintenance (Spearman rank correlation:  $r_s = 0.34$ ,  $N = 34$ ,  $P = 0.05$ ; Fig. 1). Thus, some individuals were generally more helpful than others. Body condition (standardized residuals of mass against SL) was not related to territory maintenance ( $r_s = 0.23$ ,  $N = 34$ ,  $P = 0.19$ ) or mean territory defence by helpers ( $r_s = 0.11$ ,  $N = 34$ ,  $P = 0.55$ ). Similarly, SL did not relate to territory maintenance ( $r_s = 0.24$ ,  $N = 34$ ,  $P = 0.19$ ) or territory defence ( $r_s = 0.16$ ,  $N = 34$ ,  $P = 0.38$ ), but all helpers in this project were similar in size and were size matched within groups.

#### Effects of Relatedness on Helping

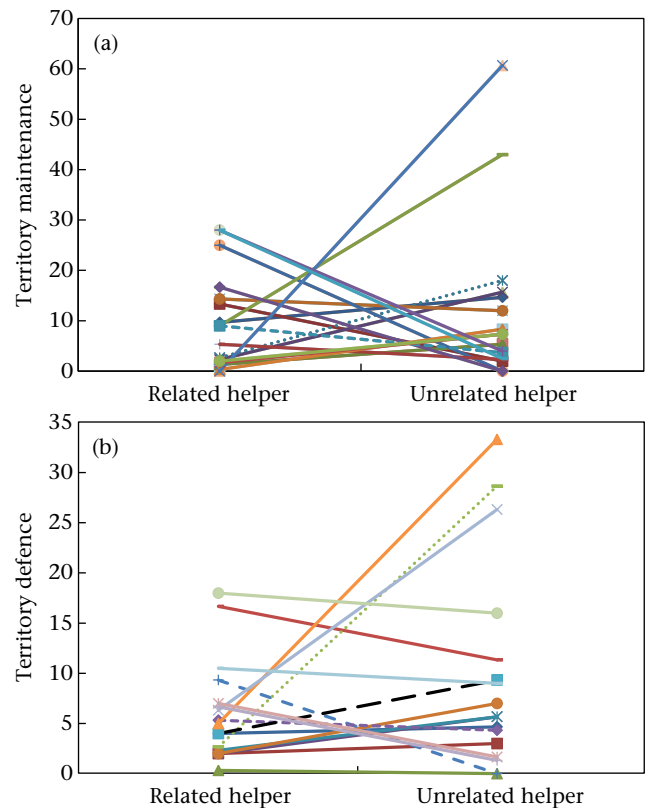
Helpers related to the breeders did not perform more territory maintenance (Wilcoxon signed-ranks exact test;  $Z = -0.24$ ,  $N = 17$ ,  $P = 0.81$ ; Fig. 2a) or territory defence (paired  $t$  test;  $t_{16} = -1.32$ ,  $P = 0.21$ ; Fig. 2b) than nonkin helpers. Furthermore, the related and unrelated helpers did not differ in their total amount of help (territory maintenance and defence combined; Wilcoxon signed-ranks exact test:  $Z = -0.09$ ,  $N = 17$ ,  $P = 0.94$ ). Finally, the difference in body size between the related and unrelated helper did not correlate with the difference in either territory maintenance (Kendall rank correlation:  $\tau = -0.015$ ,  $P = 0.93$ ) or territory defence ( $\tau = 0.074$ ,  $P = 0.68$ ). Therefore, potential differences between helpers in dominance based on body size were unlikely to explain differences in propensity to provide care.

#### Variability and Repeatability in Nonhelping Behaviours

Three nonhelping behaviours were assessed: aggressiveness ( $N = 17$  pairs of helpers), activity level ( $N = 14$  pairs of helpers) and risk responsiveness ( $N = 14$  pairs of helpers). We found that there was variability between helpers in their aggressiveness, activity levels and risk responsiveness (Table 1; ANOVA:  $F_{1,32} = 4.23$ ,  $P = 0.048$ ). Within individuals, all behaviours were repeatable across trials: aggressiveness during general observations after which no helping trial occurred ( $F_{27,56} = 2.63$ ,  $P = 0.001$ ,  $r = 0.36$ ), aggressiveness by helpers before maintenance trials ( $F_{29,60} = 2.70$ ,



**Figure 1.** Relationship between territory maintenance (mean number of digging acts per 10 min) and territory defence (mean number of aggressive acts towards a conspecific intruder per 10 min) by helpers. Circles: related helpers; triangles: unrelated helpers. See text for statistics.



**Figure 2.** Mean amount of (a) territory maintenance (mean number of digging acts per 10 min) and (b) territory defence (mean number of aggressive acts towards a conspecific intruder per 10 min) by helpers. Each line represents one pair of helpers within a group. See text for statistics.

**Table 2**  
Lack of correlations between personality traits

Traits	N	Correlation	P
Aggressiveness (Gen Obs)*–Activity level†	28	0.26	0.19
Aggressiveness (TM)‡–Activity level†	28	0.28	0.15
Aggressiveness (TD)§–Activity level†	28	0.36	0.06
Aggressiveness (Gen Obs)*–Risk responsiveness**	28	0.02	0.92
Aggressiveness (TM)‡–Risk responsiveness**	28	0.13	0.52
Aggressiveness (TD)§–Risk responsiveness**	28	–0.09	0.66
Activity level†–Risk responsiveness**	28	–0.25	0.19

Correlation coefficients shown are all Spearman rank correlations except for a Pearson correlation between activity level and risk responsiveness.

\* Mean number of within-group aggressive acts (in general observations with no helping trial afterwards (Gen Obs)).

† Mean number of moves between zones per 10 min.

‡ Mean no. of aggressive acts per 10 min before a territory maintenance trial (TM).

§ Mean number of aggressive acts per 10 min before a territory defence trial (TD).

\*\* Mean latency to approach a novel object.

$P < 0.001$ ,  $r = 0.36$ ), aggressiveness before intruder trials ( $F_{29,60} = 4.44$ ,  $P < 0.001$ ,  $r = 0.53$ ), activity levels ( $F_{27,28} = 8.36$ ,  $P < 0.001$ ,  $r = 0.78$ ) and risk responsiveness ( $F_{27,28} = 2.19$ ,  $P = 0.02$ ,  $r = 0.37$ ). Moreover, aggressiveness was repeatable across all nine observation periods using a repeated measures ANOVA with relatedness as a between subject factor and fish identity as a random factor (within subjects effect:  $F_{1,23} = 0.729$ ,  $P = 0.40$ ). As individuals were repeatable in their behaviours, we concluded that aggressiveness, activity and risk responsiveness constitute behavioural types (sometimes also called personality traits) in *N. pulcher*. Therefore, we calculated a mean behavioural value per individual for later analysis.

Related helpers were not more aggressive (Wilcoxon signed-ranks exact test:  $Z = -0.23$ ,  $N = 17$ ,  $P = 0.83$ ), active ( $Z = -0.22$ ,  $N = 17$ ,  $P = 0.86$ ) or risk prone ( $Z = -0.66$ ,  $N = 17$ ,  $P = 0.54$ ) than unrelated helpers.

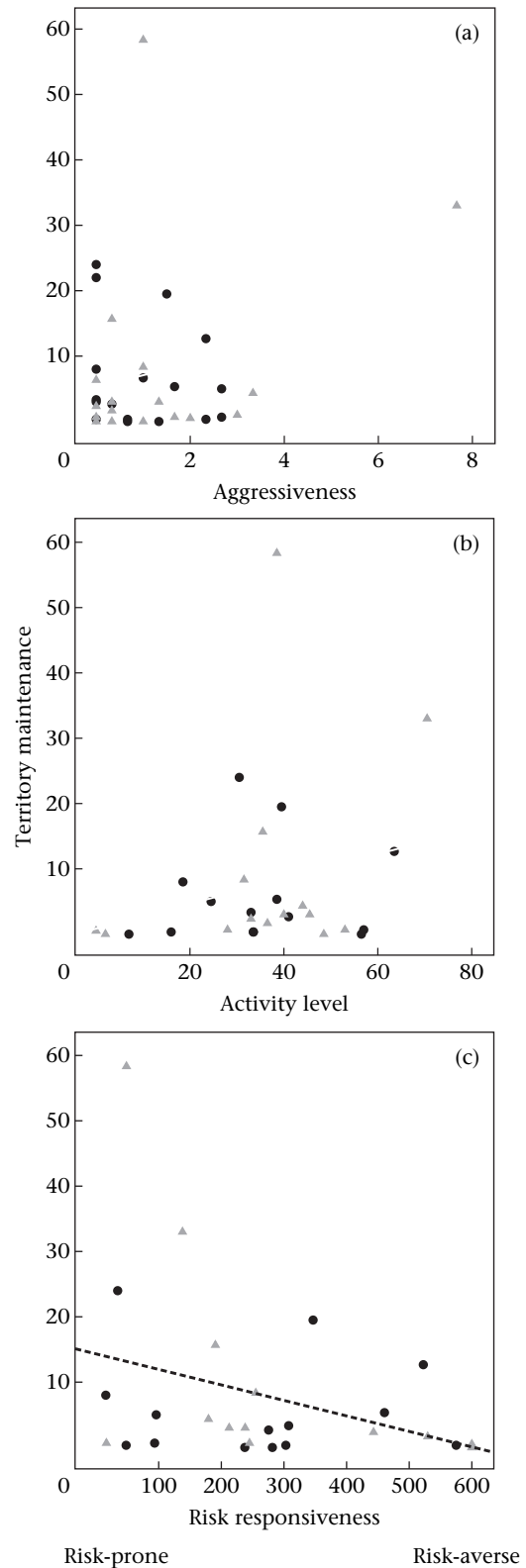
#### Relationships Between Behavioural Types

Within-group aggression, risk responsiveness and activity did not correlate, so did not form a behavioural syndrome in this population (Table 2). Although there was a nonsignificant trend for the mean aggressiveness before an intruder trial to correlate with activity levels ( $P = 0.06$ ), we did not find this trend in any other analyses (Table 2).

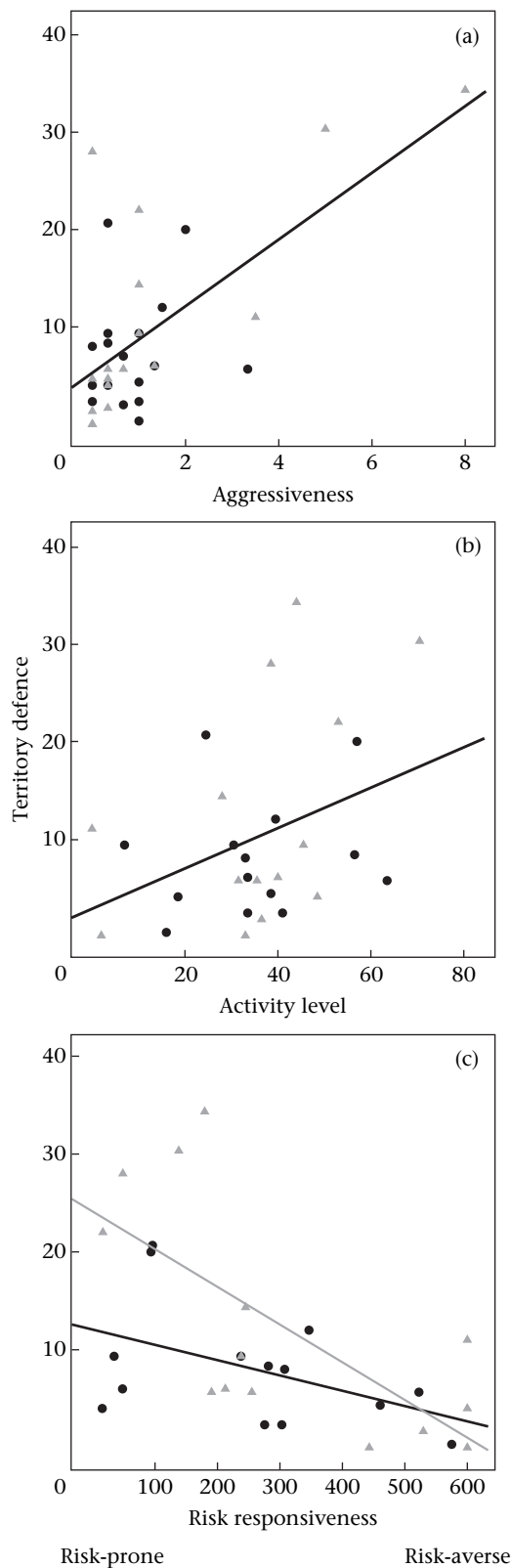
#### Relationships Between Behavioural Types and Helping

The amount of territory maintenance was neither related to helper aggressiveness (GLM:  $F_{1,32} = 0.95$ ,  $P = 0.34$ ; Fig. 3a) nor activity levels (GLM;  $F_{1,26} = 0.99$ ,  $P = 0.33$ ; Fig. 3b). Helpers that were faster to approach a novel object performed more territory maintenance (GLM;  $F_{1,27} = 6.74$ ,  $P = 0.016$ ; Fig. 3c) than more risk-averse helpers, even when two particularly vigorous diggers were removed from the data set (Spearman rank correlation:  $r_s = -0.38$ ,  $N = 28$ ,  $P = 0.046$ ). Relatedness dropped out of all the models as a nonsignificant factor.

Helpers' aggressiveness before a territory intruder trial was positively correlated with their intruder defence contribution (GLM:  $F_{1,32} = 27.16$ ,  $P < 0.001$ ; Fig. 4a). More active helpers defended more against a conspecific intruder than less active helpers ( $F_{1,26} = 4.44$ ,  $P = 0.04$ ; Fig. 4b). Finally, risk responsiveness was also found to be positively correlated with territory defence ( $F_{1,25} = 13.12$ ,  $P = 0.001$ ; Fig. 4c). In addition, we found that relatedness was a significant factor in this model ( $F_{1,25} = 4.58$ ,  $P = 0.04$ ), with a stronger relationship between risk responsiveness and territory defence in unrelated helpers than in related helpers. However, contrary to this,



**Figure 3.** Territory maintenance by helpers (mean number of digging acts per 10 min) in relation to (a) aggressiveness (mean number of within-group aggressive acts per 10 min), (b) activity level (mean number of zone changes per 10 min) and (c) risk responsiveness (mean latency (s) to approach a novel object). Circles: related helpers; triangles: unrelated helpers. See text for statistics.



**Figure 4.** Territory defence by helpers (mean number of aggressive acts towards a conspecific intruder per 10 min). (a) aggressiveness, (b) activity level and (c) risk responsiveness. Circles and black line: related helpers; triangles and grey line: unrelated helpers. See text for statistics.

we had already demonstrated that relatedness did not affect the amount of territory defence carried out (Fig. 2b), and that individual levels of risk responsiveness were not affected by whether the individual was related or unrelated to the breeders.

## DISCUSSION

Helping effort in this study was variable between, but repeatable within, individuals. Further, some individuals were always helpful, but examination of the data (Fig. 1) suggests that individuals that carried out most territory defence were not always also contributing most to maintaining the territory. Although we have previously demonstrated that *N. pulcher* can recognize kin independently of familiarity (Le Vin et al. 2010), relatedness to the breeding pair had no clear effect on the amount or form of helping performed. We found no evidence that related helpers ( $r = 0.5$ ) carried out more risky territory defence, which carries the risk of injury (Balshine-Earn et al. 2001), or that unrelated helpers ( $r = 0$ ) did more, low-risk, territory maintenance. Helper aggressiveness, activity levels and risk responsiveness were variable between, but repeatable within, individuals, so constitute behavioural types, but were uncorrelated with each other. One issue was that individuals would not behave normally on their own, so their behavioural types had to be assayed within a group or pair, which might have modulated their responses and thus the relationships (or lack of) between behavioural types (e.g. Nelson et al. 2008). In contrast to relatedness, we found significant relationships between these behavioural types and helping effort, with more aggressive, risk-prone or more active individuals carrying out more territory defence than more submissive, risk-averse or inactive individuals. That more aggressive offspring were more helpful suggests that social rank, a function of an individual's aggression to group members, independent of size and age, might predict helping performance. Risk-prone helpers performed more territory maintenance than risk-averse helpers. So, when controlling for group size, structure, familiarity between group members and helper size, consistent individual differences in behaviour explained more variation in helping effort than relatedness.

Some individuals were generally more helpful than others rather than specialising in certain forms of helping, as has been shown in other cooperatively breeding species (e.g. Traniello & Rosengaus 1997; Arnold 2000; Arnold et al. 2005). Task specialization of helpers independent of body size has been demonstrated recently in *N. pulcher*, however (Bruintjes & Taborsky 2011). That two key categories of helping behaviour were correlated within individuals in our study is intriguing, possibly suggesting the existence of a 'helping personality' that may also exist in other social species. However, in our study this correlation was of borderline significance ( $P = 0.05$ ) and may have been driven by a few very helpful individuals, so this remains to be further explored. As with other behaviours that have been measured in the personality literature (e.g. Sih et al. 2004; Arnold et al. 2007; Arnold & Taborsky 2010), helping might have a heritable component and be influenced by a 'social niche' experienced earlier in life (Bergmüller & Taborsky 2010), thus representing a fairly fixed behavioural pattern. Thus, as has been previously suggested, behavioural type might be both a cause and consequence of interindividual variation in life history trajectory (Wolf et al. 2007; Schürch & Heg 2010a). More long-term data collected under standard conditions are needed to investigate the long-term stability and consequences of helping.

Aggression, activity and risk responsiveness were repeatable over time within individuals and therefore constituted 'behavioural types' (or personality traits) but were uncorrelated with each other so did not form a behavioural syndrome, as found in other species

(Huntingford 1976; Riechert & Hedrick 1993; Coleman & Wilson 1998; Wilson & Godin 2009). Schürch et al. (2010a) found a behavioural syndrome correlating aggression, risk responsiveness and exploration in both males and females after sexual maturity in *N. pulcher*. However, no such behavioural syndrome appeared to exist in our highly standardized groups, with helpers initially unfamiliar to breeders and to each other, and matched for size, age and mass (see also Witsenburg et al. 2010). Within individuals, aggression, activity and risk responsiveness were independently related to the propensity of an individual to defend, but not maintain, the territory. These relationships might arise for a number of reasons. First, *N. pulcher* that performed more aggressive acts towards their own group members were more likely to attack an intruder. Aggressive individuals might appear detrimental to a group, particularly in a species showing complex social interactions (Arnold & Taborsky 2010), by potentially escalating competition for shelter, reducing growth and increasing the risk of injury to conspecifics (Huntingford et al. 2006; Riebli et al. 2010). However, there may be a trade-off, as these aggressive individuals, who might also have high social ranking, should be tolerated because they could also be keen to protect the group from intruders (Heg & Taborsky 2010). Second, risk-prone individuals may be more inclined to approach a 'novel' conspecific, and hence have more opportunities to defend, than a risk-averse individual. Finally, in terms of activity and territory defence, individuals that swim about their territory more may also be more likely to come across an intruder, so defend more, than a less active individual (Schürch & Heg 2010a). Therefore, having individuals within a group that are aggressive, more willing to take risks or be active, in addition to carrying out defence, may be essential to maintain group stability (Schürch & Heg 2010b). Variation in behavioural types among group members has been posited to result in reduced social conflict because each group member will assume a social role commensurate with its behavioural type (Bergmüller & Taborsky 2007, 2010). Cooperatively breeding species present a good model system for testing this hypothesis because of the complex social interactions and multiple social niches available to group members throughout their lives.

Not all forms of helping will have the same causes and consequences, or indeed costs and benefits, for either individuals or the group. The propensity to dig sand out of the breeding shelter appeared to be a more flexible trait than territory defence: with no relationships between territory maintenance and activity levels or aggressiveness and risk responsiveness only marginally correlated. Further, exploration and territory maintenance were correlated only in females in the study of Schürch & Heg (2010a). Variation in body condition and thus physical ability to help did not predict helping effort, although other unmeasured factors linked with condition or experience might have been important. Interestingly, we found that the offspring of wild-caught and captive-bred parents did not differ significantly in their helping or behavioural types. The sex of the helpers, which regrettably we did not know in our study, might have influenced their contributions to defending or digging, although the evidence from previous studies is inconclusive (Stiver et al. 2005; Bruintjes & Taborsky 2008). The divergent patterns of relationships between different forms of helping and three behavioural traits traditionally measured in animal personality studies, plus the disparity between *N. pulcher* studies (Bergmüller & Taborsky 2007; Schürch & Heg 2010a), illustrates that optimal helping effort may be adaptive depending on the (social) environment.

Our finding that relatedness of helpers did not generally predict the amount or form of helping performed appears to contradict previous studies on *N. pulcher* in the wild (Stiver et al. 2005). They found that helpers related to the breeding female, and helpers unrelated to the breeding male showed most territory defence, but found no such correlation between relatedness and overall helping

effort. However, their helpers were on average only related to the breeding female at the level of first cousins ( $r = 0.125$  compared with  $r = 0.5$  in our study) and unrelated to the breeding male ( $r = 0.5$  in our study), and only about 16% were first order relatives of the breeders within groups. In an analogous laboratory study, Stiver et al. (2005) observed that unrelated helpers ( $r = 0$ ) carried out more combined territory defence and maintenance than related helpers (offspring of both breeders, i.e.  $r = 0.5$ ), contrary to our results. The inconsistencies between our results may be due to differences in the experimental set-up and/or behaviours measured. In the laboratory, Stiver et al. (2005) used groups that had been established for at least two years and contained varying numbers of either only related or only unrelated helpers, rather than a mixture of both, as in natural groups and in our study. By contrast, we standardized group size, familiarity and relatedness. We have previously shown that *N. pulcher* can recognize kin that they have never met before via phenotype matching based on olfactory cues. Moreover, we controlled for age of helpers: older and larger helpers, which in the wild would usually be unrelated to the breeders (Dierkes et al. 2005), have been shown to defend more than smaller individuals (Taborsky & Limberger 1981; Bruintjes & Taborsky 2011). We are aware that by establishing artificial groups and controlling for multiple factors, we might have caused some social disruption, altering the behaviour of group members compared with the wild. Hopefully, this would have been standardized across groups, unlike naturally occurring social variation. Consistent with this we found no clear differences in helper or total group behaviour between groups in which helpers were quick or slow to be accepted (see Appendix). Both Stiver et al.'s (2005) field and laboratory studies measured natural levels of helping, rather than experimentally manipulating helping requirements, for example by filling in the breeding shelters or introducing a conspecific intruder as we did. Finally, the measure of help reported in Stiver et al. 2005 included the frequency of breeding shelter visits, a measure of direct brood (egg) care. Thus, the discrepancy between the results of these two studies might have been caused by the different combinations of helping behaviours measured.

In our study, it appeared that kin-selected benefits alone cannot explain helping effort in *N. pulcher*. Consequently, the direct benefits accrued by unrelated helpers must be equal to the combined (direct and indirect) benefits the related helpers received (Kokko et al. 2002). Overall, aggressive, risk-prone or active individuals showed more defence against intruders than submissive, risk-averse and inactive helpers. Therefore, it is important to consider multiple factors, including relatedness, body condition and behavioural type, when investigating complex behaviours such as helping. Consistent individual differences in behaviour appear to predict participation and performance in a range of contexts, including complex social interactions.

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## APPENDIX

In this appendix we assess: (1) the effect of population origin on helper behaviour; (2) whether the stock fish were inbred such that our relatedness estimates might have been inflated; and (3) whether individuals in groups that were slow to accept the helpers behaved differently from more harmonious groups.

### Comparing Fish from Different Populations

Adults were transported from the University of Bern, Switzerland to the University of Glasgow in February 2007. The fish were a mixture of wild-caught ( $N = 10$ ) and captive-bred ( $N = 10$ ) adults. The wild-caught individuals originated from Nkumbula Island, near Mpulungu, Zambia in 2006. The captive-bred individuals were the offspring of wild fish caught at Kasakalawe, near Mpulungu, Zambia, in 1996. As captive-bred and wild-caught fish can behave differently, we compared the behaviour of the offspring of these adults. The focal offspring from the two populations did not differ significantly in any of the behaviours analysed (Table A1). Independent samples *t* tests were used to statistically analyse the behavioural data.

**Table A1**

Comparison of the behaviours of offspring of wild-caught (wild) and captive-born (captive) adult fish used in the study

Behavioural trait	Wild mean±SE (N)	Captive mean±SE (N)	<i>t</i>	<i>df</i>	<i>P</i>
Territory maintenance	9.79±2.27 (18)	7.31±2.83 (16)	-0.76	32	0.45
Territory defence	9.06±2.21 (18)	6.77±1.68 (16)	-0.81	32	0.42
Mean aggressiveness	1.55±0.40 (18)	0.98±0.24 (16)	-1.18	32	0.24
Mean activity levels	37.25±5.88 (12)	34.50±3.61 (16)	0.34	26	0.74
Mean risk responsiveness	255.2±60.6 (12)	301.9±45.8 (16)	0.63	26	0.54

Two helping behaviours were analysed: territory maintenance (mean number of digging acts per 10 min) and territory defence (mean number of agonistic to intruder per 10 min). Three behavioural types were measured: mean aggressiveness to other group members in the general observation periods; mean activity levels (number of zone changes per 10 min) and mean risk responsiveness (time to approach the novel object). The results of an independent samples *t* test are shown.

### Assessing Relatedness of Original Breeders

To check that our populations were not inbred, which could have confounded our results, we analysed relatedness between males and females within a breeding pair, based on multilocus microsatellite markers. Fish that shared more alleles than expected by chance were said to be related.

### Methods

DNA samples collected from the stock population were used to ensure that they were not inbred. Furthermore, DNA samples were collected from the offspring to assess whether maximum likelihood relatedness scores between individuals had any influence on kin recognition or breeding. DNA was extracted either from a fin clip or by swabbing (Le Vin et al. 2011). Fin clips were taken from the caudal fin of each fish (approximately 5 mm) and swabbing was carried out by running a Barloworld Scientific sterile rayon-tipped swab six times down the length of the body of the fish (A. Bell, personal communication). Both fin clips and swabs were stored in 100% alcohol at 4 °C before DNA extraction. Individuals from which both types of samples were collected were used to confirm that swabbing is a reliable noninvasive sampling method to provide high quality DNA in this species (Le Vin et al. 2011). Fin clips were collected at the end of breeding or experiments, in case altering the phenotype of an individual altered their behaviour. The specific method of DNA collection used in each part of the experiment is clarified within the relevant methodology sections below.

DNA from fin clips was extracted using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, U.S.A.). DNA from swabs was extracted using an extraction protocol for swabs in the QIAamp DNA Micro Handbook (Qiagen), using QIAshredder spin columns (Qiagen) and the DNeasy Blood and Tissue Kit (Qiagen). Individuals were then genotyped at up to eight microsatellite loci (see Table A2). The forward primer of each pair was labelled with the ABI fluorescent dyes NED (yellow), HEX (green) and 6-FAM (blue). Products were

amplified by multiplex PCR, using the default reagent concentrations recommended by the kit instruction manual (Qiagen Inc., Crawley, U.K.). Two multiplex PCRs, one at 53 °C and the other at 60 °C, were carried out. Thermocycling was performed on a DNA Engine Dyad (MJ Research, Waltham, MA, U.S.A.) using the following programme: initial denaturation for 15 min at 95 °C, followed by 34 cycles of denaturation at 94 °C for 30 s, annealing at either 53 °C for 60 s or 60 °C for 90 s and extension at 72 °C for 90 s, followed by a final 10 min extension at 72 °C. Multiplexed products (1:160 dilutions) were genotyped using an ABI 3730 sequencer (by The Sequencing Service, University of Dundee, Dundee, U.K.). Genotypes were read, corrected by eye and analysed using GeneMapper version 4.0 (Applied Biosystems, Foster City, CA, U.S.A.); ROX GS400HD was used as the size standard ladder. Relatedness between individuals was then calculated using the program ML-Relate (Kalinowski et al. 2006), which calculates maximum likelihood estimates of relatedness based on allele sharing.

### Results

All stock fish used as breeders were successfully genotyped at a minimum of seven loci and the average number typed per individual was 7.98. One individual at one locus (UME003PT) did not amplify. Overall, relatedness between the breeders was low, with only five of the 56 pairs having a relatedness score above that of cousins ( $r = 0.125$ ) and 41 of the pairs having a relatedness score of 0. Although there was little evidence of overall homozygote excess, at TmoM13PT for the captive-bred fish and TmoM11PT for the wild-caught fish, the observed heterozygosity was lower than that expected. Furthermore, locus TmoM27PT had very low heterozygosity across both populations. Relatedness scores between breeding pairs from the two populations were not found to differ significantly (Mann–Whitney *U* test;  $U = 243$ ,  $N = 56$ ,  $P = 0.11$ ). However, the fry survival of the wild-caught population was found to be significantly greater than the fry survival of the captive-bred population ( $U = 22$ ,  $N = 29$ ,  $P < 0.001$ ). Overall, relatedness between individuals in the adult breeding stock was very low, so the F1s to be used in the experiments should have been genetically diverse. Relatedness scores within pairs did not affect the propensity to breed or not to breed ( $U = 354$ ,  $N = 56$ ,  $P = 0.44$ ). Some individuals never bred and so may have been infertile. However, even when they were removed from the analysis, relatedness did not affect propensity to breed ( $U = 111$ ,  $N = 37$ ,  $P = 0.88$ ).

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### Comparison of Groups

#### Methods

In total, 24 groups were established, but in 17 of these breeders did not initially accept helpers, instead forcing them to leave the breeding shelter and/or to hide in the refuge tubes. Using a technique used by previous studies, we tried to encourage acceptance of helpers by restraining the breeders for up to 24 h in mesh cages within the experimental tank. Breeders were then released from the cage and the group was reassessed to see if the helpers had been accepted. Helpers were perceived to be accepted into the group when they had free access to swim around the tank, including in and around the breeding shelters ( $N = 10$ ). Thus, in seven of the 24 original groups, helpers were accepted straight away ('quick to accept helpers'); in 10, helpers were accepted after the breeders had been restrained ('slow to accept helpers'); the remaining groups never accepted the helpers, so we could not

assess helping behaviour in such groups. We then compared the behaviour of the helpers within the first two types of groups (slow or quick to accept helpers) using parametric or nonparametric tests as appropriate. We also compared the helping effort and numbers of aggressive interactions of whole groups. See the main text for descriptions of how each behaviour was measured.

### Results

*Behaviour of individual helpers.* Helper acceptance (quick or slow) did not explain variation in helper territory defence, territory maintenance, aggressiveness (mean across nine observation periods), activity levels or risk responsiveness (Table A2).

*Aggression and helping of groups.* The mean number of aggressive interactions within groups over the nine observation periods did not differ between groups that were slow or quick to accept helpers (independent samples  $t$  test:  $t_{1,32} = 1.13$ ,  $P = 0.27$ ). The mean

amount of territory defence (independent samples  $t$  test with unequal variances:  $t_{1,18.26} = 1.23$ ,  $P = 0.24$ ) and territory maintenance (independent samples  $t$  test:  $t_{1,32} = -0.15$ ,  $P = 0.88$ ) carried out by whole groups did not differ between groups that were slow or quick to accept helpers.

**Table A2**

Comparison of the behaviour of helpers in groups that were slow and quick to accept helpers

Behavioural trait	Mann–Whitney $U$ test	$N$	$P$
Territory maintenance	117.00	14, 20	0.44
Territory defence	88.50	14, 20	0.071
Mean aggressiveness	125.50	14, 20	0.54
Mean activity levels	69.50	12, 16	0.22
Mean risk responsiveness	125.00	12, 16	0.19

Results from nonparametric Mann–Whitney  $U$  tests.  $N$  = number of helpers in quick groups, number of helpers in slow groups.