Co-operative breeding in vertebrates may emerge due to subordinates delaying dispersal when free breeding habitat is not available (‘habitat saturation’ hypothesis, HS). However, delayed dispersal might also be due to younger individuals postponing dispersal to when they are more competitively able or have more to gain from breeding independently (‘benefits-of-philopatry’ hypothesis, BP) or to when inclusive fitness benefits no longer outweigh the benefits from independent breeding (‘kin selection’ hypothesis, KS). Here, we show in three experiments that both HS and BP determine the extent of co-operative breeding in the cichlid *Neolamprologus pulcher*. Contrary to the KS, individuals significantly avoided settlement with related individuals, and an additional settlement experiment confirmed this result. Our results suggest that kin structure in these cichlids emerges from limits on dispersal, but if such barriers are absent, cichlids prefer to settle with unrelated individuals to maximize the benefits of direct reproductive participation. **Key words:** body size, cichlidae, co-operation, density-dependence, helper, status. [Behav Ecol 22:82–92 (2011)]
individual females). If small females do not reproduce, despite they are dominant and therefore not reproductively suppressed (Heg 2008), they might derive more benefits from philopatry than dispersal due to protection inside their group (e.g., Heg et al. 2004a) and delay dispersal until they are reproductively capable. If small subordinate females are unlikely to reproduce because they are subdominant in groups compared with similar-sized dominant females (see also Heg 2008), reproductive benefits from philopatry are unlikely to apply, and they could derive more reproductive benefits from dispersal and establish a new group as a dominant female.

In the second experiment, we test the hypothesis that both small males and small females delay dispersal because they cannot successfully compete with larger conspecific competitors for free habitat if an empty breeding patch is provided and instead opt for a subordinate position. We test this hypothesis by releasing 5 differently sized males and females into a large tank with 1 empty breeding patch available and expected the largest male and female to gain the dominant breeding positions and the smallest males and females to be accepted as subordinates.

In the third experiment, we test the hypothesis that subordinates delay dispersal because the availability of suitable free habitat is limited (the habitat saturation hypothesis). We induce habitat saturation by sequentially releasing 3 males and 3 females of 3 different size classes inside a large octagonal tank (ringtank) every third day, and they were allowed to settle in any of the 24 breeding patches. This was repeated 12 times, so the population increased from 6 to 72 fish. If the HS applies, all fish should attempt to pair up and breed at initial low densities. With increasing density, only the smaller fish should settle as subordinates, whereas larger fish should be competitively excluded. If the BP applies, smaller fish should attempt to settle as subordinates from the very start, even when free breeding habitat is available.

In the third experiment, we also allowed close kin to settle sequentially (e.g., a large, medium, and small sibling released on days 0, 3, and 6, respectively): If the kin selection hypothesis applies, subordinate cichlids should prefer to settle with close kin (e.g., associate with a related dominant male or female already settled inside the ringtank, see also Jordan et al. 2010). However, sequential settlement under a competitive laboratory setting might make it difficult for cichlids to settle close to kin because their previous association has been disrupted by us. Therefore, we also conducted a fourth experiment, where 4 groups of 6 close kin were released simultaneously and the kin could remain together and settle or disassociate and settle with any of the other fish. Again, if kin selection applies, the siblings are expected to remain together. In experiments 3 and 4, we expected kin to settle with nonkin if they target own reproduction (avoid inbreeding) or avoid competition with close kin for mates.

### MATERIALS AND METHODS

#### Study animals

All experiments were conducted with *N. pulcher* descendants, from parents originally wild caught near the village of Kasakalawe (Zambia) in 1996 and held in separate breeding stock families from that year onwards. The breeding stock has retained substantial genetic variation: The number of alleles and the levels of heterozygosity estimated using microsatellite DNA markers does not differ between our stock (Heg et al. 2006; Heg 2008) and the natural source population (Dierkes et al. 2005). Note that *N. pulcher* is able to recognize conspecifics individually (Hert 1985; Balshine-Earn and Lotem 1998), so relatives released in experiments 3 and 4 should be able to find (experiment 3) or respectively stay together (experiment 4), with their relatives if they wanted to. All individuals in all experiments were sexed by inspection of the gonadal papilla. Individuals were sexed at the end of the experiments when most individuals had reached mature size (>30 mm standard length [SL]), and sexing was more reliable. In experiments 1 and 2, all individuals were correctly sexed, and in experiments 3 and 4, missexing of some individuals resulted in slight alterations in the final number of males and females released (see Table 1).

#### Experiment 1: body size, status, and reproduction

Reproduction of dominant and subordinate females was recorded in 94 groups for an average period of 226 days (range 25–675 days) in tanks of 50–100 l in climate-controlled rooms (study period 23 March 2006 to 2 February 2008). This period encompassed the estimated ages of dominant females in nature (Balshine-Earn et al. 2001; Heg et al. 2005: see area between hatched lines in Figure 2a) and females in the laboratory produce on average 1 clutch every 14 days (e.g., Heg 2008). All group members >20 mm SL were individually marked with fin clips and remarked and measured (body mass in milligrams and body size standard length SL in millimeter) every 30 days. To test whether small dominant females are reproductively capable, the 94 groups included 18 pairs newly formed from a small female and a small male (at the start of the experiment between 28.5 and 42.0 mm SL, females always smaller than the males). Broods were assigned to dominant and subordinate females based on prespawning behavior (cleaning of the pots, courting, and pseudo-spawning with the dominant male, which they do very intensively in the prespawning period, i.e., usually every minute and at least every 5 min) 1–2 days before spawning and actual spawning behavior by daily observations. This method has been proved to be extremely reliable in determining the mother of a brood (Heg et al. 2008b). Tanks were fed daily with Tetramin cichlid flake food. In total, 78 dominant females produced on average 7.0 broods (range 1–22), and their average body size was 59.4 mm SL (range 35.3–80.3 mm). Dominant females who failed to produce broods were replaced with a new dominant female from 400-l tank containing aggregation fish, as this was typical to occur in newly established pairs. In total, 13 subordinate females from 13 different groups produced on average 2.4 broods (range 1–11), and their average body size was 48.9 mm SL (range 32.0–60.4 mm). Note that in 6 of the groups where a subordinate female reproduced, the dominant female did not produce any clutches, although she had produced clutches before the experiment started.

Clutch sizes were determined in the evening and an average egg mass was determined after a sample of eggs per brood were dried for 32 h in a stove at 70 °C. Groups contained between 0 and 30 subordinates that were larger than 25 mm SL. Group sizes changed during the experimental period due to the growth of offspring who were recruited as subordinates and due to expulsion of subordinates, who were removed from the tank (average change in the number of subordinates ± standard deviation [SD]), dominants with broods: 2.8 ± 6.2, range: 0–30, n = 78; subordinates with broods: 0.23 ± 0.44, range: 0–1, n = 13). Therefore, actual number of subordinates present during reproduction were as follows: groups with dominant female broods—average ± SD: 2.0 ± 3.6, range: 0–23, n = 78 and groups with subordinate female broods—average ± SD: 3.4 ± 2.5, range: 1–9, n = 13. Ages of female dominants and subordinates fish at the time of each clutch production were estimated using the Blumberg’s hyperlogistic growth curve equation published elsewhere, i.e., interpolating their size SL at clutch production...
from the monthly body size measurements and using SL to estimate their age (Skubic et al. 2004).

**Experiment 2: competition for breeding positions**

This experiment was conducted from 17 April to 11 June 2006 using 3 different 4004 tanks kept in climate-controlled rooms. The experiment was designed to test how body size affects competition for dominant breeding positions and whether small fish get accepted as subordinates instead. Tanks were fed daily with Tetramin cichlid flake food. Five males and 5 females of different sizes were released simultaneously inside a 4004 tank and allowed to compete for group membership in a single breeding patch containing 2 flowerpot halves for spawning (n = 20 trials, each fish was individually marked, see Table 1 for the body sizes). Fish were all unrelated. Trials ended on average after 7.3 days (±3.8 SD, range 2–16 days) when the first brood was spawned by the pair gaining the dominant breeding position (so called “dominants”), and on this day, group membership was determined for all other fish (accepted as “subordinate” or not accepted and staying in the upper half of the tank as “aggregation”). Fish were removed, and a new trial was initiated until a total sample size of 20 trials was reached; thus in total, 20 trials × 5 size classes × 2 sexes = 200 different individuals were tested.

**Experiment 3: habitat saturation and sequential settlement with kin**

This experiment was conducted from 28 March to 14 September 2007. Cichlids were individually marked with fin clips taken from the dorsal and anal fin, individual body measurements taken (body size SL in 0.1 mm and body mass in 1 mg accuracy) and sexed.

Per trial (n = 3 trials), 72 fish were released in sets (n = 12 sets) of 1 large male, 1 large female, 1 medium male, 1 medium female, 1 small male, and 1 small female every third day into a large octagonal ringtank (7200 L, 60 cm height, 50 cm water column, and 3 cm sand layer of 1 mm grain size). Twelve flowerpot halves (3 large, 5 medium, and 4 small pots) and 2 stone slabs were placed within each of the 24 breeding patches as breeding substrate (see Figure 1 and Supplementary material Figure S1, S2). Cichlid food flakes Tetramin were provided through 4 automated feeders, supplemented daily with Artemia spp. eggs. The water current distributed the food through the whole tank ensuring feeding locations could not be monopolized (see Supplementary material Table S1).

Water temperature (mean ± SD: 27.2 ± 1.5 °C) and illumination cycle (lights on from 08:00 to 21:00 h) were kept constant in a climate-controlled room. The location (patches and pots visited), status (dominant, subordinate, or aggregation), and group membership (pairs and the identity of the dominants assisted by the subordinates) were determined for each individual daily from day 0 (first set released) to day 39 (6 days after the last, 12th set was released). We also recorded the location and counted the number of aggressive behaviors received (from all other individuals already present in the ringtank) during a 5-min observation directly after release for each released individual fish separately. On day 40, all individuals were removed from the ringtank and sexed, and body measurements were taken to determine growth. This whole procedure was repeated 3 times (n = 3 trials). Note that the locations of the breeding patches were slightly different between trial 1, trial 2, and trial 3. In total, 3 trials × 12 sets × 6 fish = 216 different individuals were tested.

**Figure 1**

Setup of experiment 3. Top view of the ringtank during trial 1, showing the 24 breeding patches, each patch contained 12 flowerpot halves and 2 stone slabs used as spawning substrate. See the Supplementary material for the setup of trials 2 and 3.
To test whether individuals settled preferably with related individuals, trios (n = 40 trios) or duos (n = 5 duos) of unequally sized relatives were released in different sets (e.g., large individual on day i, medium individual on day i + 3, and small individual on day i + 6). Note that relatives of unequal sizes were released, which avoids that kin evicted similar-sized kin from their patches (e.g., subordinates matching the size of their dominants are more likely to get evicted: Balshine-Earn et al. 1998), so we reduced the likelihood that kin competition would affect our results and we increased the likelihood that relatives would settle together as well. Sexes varied randomly. These relatives were subordinates from established family groups in 50-l tanks, and because reproduction by subordinates is rare in such small tanks, we assume that all relatives were full siblings (r = 0.5). Only pairwise comparisons of individuals who had successfully settled in any patch on day 39 entered the analyses (i.e., dominants and subordinates). We calculated pairwise the expected and observed numbers of patches shared. Pairwise expected patch sharing was determined using the hypergeometric distribution, based on the actual number of patches occupied by individual $i$ and the actual number of patches occupied by relative $j$ for each potential $i-j$ combination.

**Experiment 4: simultaneous settlement with kin**

This experiment was conducted from 7 November 2007 to 17 March 2008. Cichlids were individually marked with fin clips taken from the dorsal and anal fins, individual body measurements taken (body size SL in 0.1 mm and body mass in 1 mg accuracy), and sexed. Six relatives, for each of the 4 families separately, were released simultaneously into the ringtank ($n = 6$ relatives $× 4$ families = 24 individuals, day 0, Table 1). Relatives were from each sex and size–class combination (Table 1) and were originally subordinates from established family groups in 504 tanks, and because reproduction by subordinates is rare in such small tanks, we assume all relatives were full siblings ($r = 0.5$). Note that body sizes varied between trials (which was determined by the availability of relatives of particular sizes), but within each trial, body sizes were kept comparable between the families released.

The ringtank contained 4 breeding patches, where the 24 individuals could settle, form co-operative groups, and breed (see Supplementary material Figure S3). Cichlid food flakes Tetramin were provided through 4 automated feeders, supplemented daily with *Artemia* spp. eggs. On day 15, we determined for each individual group membership and the patches he/she occupied. Afterward, we added another 4 patches (see Supplementary material), which resulted in 1 some cichlids leaving their groups and forming new groups and 2) previously unsuccessful individuals (aggregation fish) settling. Again, on day 22, we determined for each individual, group membership and the patches he/she occupied. In total, 6 trials were conducted, which gives a total sample size of 144 individuals tested, minus 1 medium male who died shortly after the release into the ringtank. We expected the large fish to form pairs with unrelated large partners to avoid inbreeding. Under the kin selection hypothesis, we expected the medium and small cichlids to remain with their kin. Alternatively, if subordinates target their own participation in reproduction, small and medium cichlids should avoid settlement with kin.

**Ethical statement**

We provided plastic hiding tubes near the surface of the tanks in each experiment. These tubes were used by helpers being evicted from the group (experiment 1, evicted helpers were removed from their group) or fish failing to settle in the breed-
Twenty-nine of 545 dominant female and 2 of 31 subordinate female broods were consumed before egg mass could be determined, but the Log10 transformed before analysis. Coefficient for dominant females, subordinate females have coefficient set to 0.

Table 2
Experiment 1. Female reproduction depending on female body size, status (dominant or subordinate), and number of subordinates >25 mm SL inside their group. Results are shown of 2 general linear mixed model analyses, with individual identifier nested within status as random effect.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>df</th>
<th>Error df</th>
<th>F</th>
<th>P</th>
<th>Coefficient ± SE</th>
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<tr>
<td>Clutch size (n = 576 broods of 91 females)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
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<td>82.7</td>
<td>82.9</td>
<td>&lt;0.0001</td>
<td>0.7517 ± 0.1029</td>
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<td>Body size b</td>
<td>1</td>
<td>73.1</td>
<td>108.8</td>
<td>&lt;0.0001</td>
<td>0.0164 ± 0.0016</td>
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<td>Status c</td>
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<td>125.6</td>
<td>8.0</td>
<td>0.005</td>
<td>0.1969 ± 0.0697</td>
</tr>
<tr>
<td>Number of subordinates</td>
<td>1</td>
<td>506.3</td>
<td>5.3</td>
<td>0.022</td>
<td>-0.0066 ± 0.0029</td>
</tr>
<tr>
<td>Average egg mass (mg, n = 545 broods of 87 females)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td>436.5</td>
<td>1.3</td>
<td>0.26</td>
<td>0.1009 ± 0.0840</td>
</tr>
<tr>
<td>Body size b</td>
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<td>379.0</td>
<td>11.6</td>
<td>0.001</td>
<td>0.0100 ± 0.0030</td>
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<tr>
<td>Body size squared</td>
<td>1</td>
<td>305.7</td>
<td>6.5</td>
<td>0.011</td>
<td>-0.000067 ± 0.000026</td>
</tr>
<tr>
<td>Status c</td>
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<td>0.6</td>
<td>0.46</td>
<td>-0.0142 ± 0.0190</td>
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<tr>
<td>Number of subordinates</td>
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<td>504.6</td>
<td>11.2</td>
<td>0.001</td>
<td>-0.0015 ± 0.0004</td>
</tr>
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</table>

* Log10 transformed before analysis.
* Standard length (SL, millimeters).
* Coefficient for dominant females, subordinate females have coefficient set to 0.
* Twenty-nine of 545 dominant female and 2 of 31 subordinate female broods were consumed before egg mass could be determined, but the likelihood did not depend on female status (likelihood $\chi^2 = 0.07$, df = 1, $P = 0.79$).
females (Figure 2a), but egg mass did not depend on social status (Figure 2b, Table 2). Clutch size and egg mass decreased with the number of subordinates present in the group for both types of females (all interactions between the main effects were nonsignificant, Table 2). The results suggest that small females are reproductively capable and should try to compete for dominant group membership (see experiment 2), settle independently when habitat is available (see experiment 3), and might need to avoid kin when targeting own reproduction (e.g., inbreeding avoidance, see experiments 3 and 4). Note that previous parentage studies have also shown that small males are reproductively capable (i.e., parasitize the reproduction of the dominant males, see references in INTRODUCTION), so similar arguments also apply to the small males as apply to the small females.

Experiment 2: competition for breeding positions

The results of the second experiment show that large females (Figure 2c) and males (Figure 2d) successfully competed for the dominant reproductive position, whereas small cichlids were accepted as subordinates and medium cichlids remained inside the aggregation (Figure 2c–d, Table 3). Dominants accepted on average 1.20 subordinates (±0.70 standard error of the mean [SEM], range 0–2, n = 20 trials; n = 3 × 0 subordinate, n = 10 × 1 subordinate, and n = 7 × 2 subordinates were accepted). Subordinates were of a different sex in the 7 cases where 2 subordinates were accepted. The size difference between the dominants and same-sex subordinates was significantly larger (both for females, Figure 2e, and males, Figure 2b) than the size difference between the dominants and same-sex aggregation fish (ANOVA effect of same-sex status F1,137 = 5.5, P = 0.021; no effects of sex F1,137 = 1.5, P = 0.23, sex × status F1,137 = 0.07, P = 0.79, and trial F1,137 = 0.86, P = 0.64). The results suggest that small and medium cichlids may need to accept a subordinate position when the habitat gets saturated (see experiment 3).

Experiment 3: habitat saturation

The results of the third experiment show that both habitat saturation and competition among differently sized fish determine the extent of group living (Table 4). Cichlids appeared to notice the extent of saturation on release, as 1) the initial number of different breeding patches visited significantly declined through the experiment (GLM, n = 216): day of release (Wald χ² = 12.6, degrees of freedom [df] = 1, P < 0.001, B = −0.024 ± 0.007) and size class (Wald χ² = 12.1, df = 2, P = 0.002) were significant, and the effect of trial was nonsignificant (Wald χ² = 3.0, df = 2, P = 0.22). Small cichlids visited significantly more patches (B = 0) than large cichlids (B = −0.619 ± 0.179, P = 0.001) but did not visit more patches than medium cichlids (B = −0.253 ± 0.160, P = 0.113). Moreover, 2) the number of patches visited are correlated significantly with the frequency of aggression received by the already established fish (Spearman rank correlation, n = 216, rs = 0.78, P < 0.001).

Co-operative breeding emerged directly from the start of each trial (Figure 3a–c; i.e., fish settled as subordinates are the light gray bars on days 3, 4 etc.), where large dominants accepted smaller-sized fish as subordinates (Figure 3i: e.g., all the light gray bars on days 3, 4 etc.), where large dominants accepted smaller-sized fish as subordinates (Figure 3i: e.g., all the light gray bars on days 3, 4 etc.). Due to habitat saturation, the number of fish not accepted as group members steadily increased through each trial, such that at the end of each trial only a few new fish settled inside the patches (Figure 3a–c). Both the number of dominant females (Figure 3d) and males (Figure 3e) gradually increased but leveled off at 15–17 females and 8–9 males at the end of each trial (Poisson Regression, effects of sex Wald χ² = 5.3, df = 1, P = 0.021, days since start trial Wald χ² = 775.3, df = 1, P < 0.0001, sex × days Wald χ² = 11.8, df = 1, P = 0.001, trial Wald χ² = 1.5, df = 2, P = 0.47). The average number of subordinates accepted by each dominant female was 1.92 (±0.26 SEM, range 0–6, n = 48, at day 39) and did not significantly increase during the experiment (Figure 3f, Poisson regression, effects of days since start trial Wald χ² = 0.15, df = 1, P = 0.70, trial Wald χ² = 0.35, df = 2, P = 0.84).
Body size and sex of the individuals cointeracted with habitat saturation to determine the extent of co-operative breeding in these cichlids (Tables 4 and 5): large fish mainly settled as dominants (Figure 3g), medium fish as dominants or subordinates (Figure 3h), and small fish as subordinates (Figure 3i), but all fishes of all size classes were more likely to end up in the aggregation as the experiment progressed (Figure 3g–i). Females were more likely to settle as dominants (because they could mate polygynously), also for a smaller size compared with males, and males of intermediate sizes were more likely to remain inside the aggregation, particularly when the habitat was already saturated (Figure 4, Table 5).

Experiments 3 and 4: sequential and simultaneous settlement with kin

To test the kin selection hypothesis, we also examined whether individuals preferred to settle with relatives in experiments 3 and 4 (relatives were full siblings, see Supplementary material). If cichlids go for kin-selected benefits, small and medium individuals should settle as subordinates with large relatives, that is, with large relatives who successfully settled as dominants. If cichlids go for direct own reproductive participation and avoid inbreeding, they should settle with non-relatives.

In the third experiment, we tested this by releasing trios or duos of relatives sequentially on different days ("sets", e.g., large relative on day 1, medium relative on day 1 + 3, and small relative on day 1 + 6 or vice versa). Then, we compared the pairwise settlement pattern of these fish that either settled as subordinates or dominants, discarding fish with no relatives settled at all. Within duos of settled relatives, subordinates and dominants occupied and defended between 1 and 4 patches of the 24 patches available (1: \( n = 38 \), 2: \( n = 17 \), 3: \( n = 3 \), and 4 patches: \( n = 1 \)). However, only 2 of 29 small and 0 of 22 medium cichlids settled with a large relative, and 5 of 31 small cichlids settled with a medium relative (see Supplementary material Table S2 for the details). Using the hypergeometric distribution, we calculated the pairwise expected number of patches shared between 2 relatives under random settlement (24 patches) and compared these with the pairwise observed patches shared between two relatives (Figure 5a). Small and medium cichlids avoided settlement with related large individuals (Wilcoxon’s test \( z = -5.3, n = 51, P < 0.001 \)), and small cichlids avoided settlement with related medium individuals \( (z = -2.0, n = 31, P = 0.041) \). Only 1 small male and 1 small female subordinate settled with a related dominant female and might acquire kin-selected benefits from their helping behavior, whereas a small dominant female and a large dominant male were inbreeding (see Supplementary material Table S2 for the details). However, one might argue

<table>
<thead>
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<th>Parameter</th>
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<th>( \chi^2 )</th>
<th>( P )</th>
<th>SE subordinate</th>
<th>SE dominant</th>
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<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>8.565 ± 1.488</td>
<td>9.199 ± 2.362</td>
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</tr>
<tr>
<td>Day released</td>
<td>2</td>
<td>33.3</td>
<td>&lt;0.0001</td>
<td>-0.063 ± 0.024</td>
<td>-0.146 ± 0.030</td>
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<tr>
<td>Body size</td>
<td>2</td>
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<td>&lt;0.0001</td>
<td>-0.175 ± 0.033</td>
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<tr>
<td>Sex</td>
<td>2</td>
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<td>&lt;0.0001</td>
<td>0.255 ± 0.480</td>
<td>3.277 ± 0.698</td>
</tr>
<tr>
<td>Trial</td>
<td>4</td>
<td>8.0</td>
<td>0.09</td>
<td>0.923 ± 0.587</td>
<td>-0.316 ± 0.686</td>
</tr>
<tr>
<td>Trial 2</td>
<td>2</td>
<td>-0.514 ± 0.540</td>
<td>-0.360 ± 0.595</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Aggregation is the reference category.
b Standard length SL (millimeters).
c Coefficients for females, males have coefficients set to 0.
d Coefficients for trials 1 (up) and 2 (below); trial 3 has coefficients set to 0.

Figure 3

Experiment 3: habitat saturation and co-operative breeding. The number of individuals of dominant (black), subordinate (gray), and aggregation status (dark gray) during (a) trial 1, (b) trial 2, and (c) trial 3. The average number per trial and day of (d) dominant females and (e) dominant males (\( n = 3 \) each dot). (f) The average number of subordinates per dominant female (\( n = \) between 6 and 48), with the lower and upper dashed lines indicating minimum and maximum. The proportion of (g) large, (h) medium, and (i) small cichlids achieving dominant (black), subordinate (gray), or aggregation status (dark gray) at the end of the experiment (day 39) depending on the day of their release (0–33, \( n = 6 \) each bar).

Table 5

Experiment 3: habitat saturation, body size, sex, and final status achieved. Results are shown of a multinomial regression analysis, with the response variable status achieved (dominant, subordinate, or aggregation) depending on the day of release in the ringtank (0, 3, 6, 9, . . . 33 days), body size, sex, and trial (1, 2, or 3), \( n = 216 \) individuals.
that due to the sequential release of relatives, cichlids had difficulties in finding a vacancy with a relative to settle in.

Therefore, we also conducted a fourth experiment, where 6 relatives of 4 families each (3 size classes × 2 sexes per family) were released simultaneously inside the ringtank (day 0) with only 4 patches to settle in and measured settlement on day 15. Then, we added another 4 patches and again measured the distribution over the patches on day 22 (8 patches). In total, over all trials, 111 of 144 individuals (4 patches available) and 141 of 144 individuals (8 patches available), had settled as a dominant or subordinate (the remainder did not settle and were “aggregation fish” staying near the water surface of the ringtank). Again, we calculated the expected and observed patch overlap for experiment 4 (separately for 4 or 8 patches available). Settled cichlids who had settled relatives inside the ringtank occupied and defended between 1 and 3 patches when 4 patches or 8 patches were available (1: n = 82 or 100, 2: n = 3 or 15, and 3: n = 2 or 1, for 4 or 8 patches separately). Again, pairwise settlement with related individuals was relatively rare: in 62 of 215 cases cichlids settled with relatives (28.8%, 4 patches available) or in 57 of 340 cases (16.8%, with 8 patches available). Settlement was random in respect to the expected patch sharing when 4 patches were available (Figure 5b, Wilcoxon’s test z = −1.1, n = 215, P = 0.25) and cichlids avoided relatives when 8 patches were available (Figure 5c, z = −6.4, n = 340, P < 0.001). We redid these analyses for pairs consisting of a subordinate and a dominant (subordinate i vs. dominant j or dominant i vs. subordinate j). Settlement was random in respect to the expected patch sharing when 4 patches were available (Wilcoxon’s test z = −0.3, n = 113, P = 0.75), and cichlids avoided relatives when 8 patches were available (z = −3.4, n = 187, P = 0.001). Then we redid the analyses for pairs consisting of two dominants. Again, settlement was random in respect to the expected patch sharing when 4 patches were available (Wilcoxon’s test z = −1.6, n = 29, P = 0.11), and cichlids avoided relatives when 8 patches were available (z = −3.6, n = 51, P < 0.001). Finally, we performed a linear stepwise regression through the origin with response variable (pairwise): observed number of patches shared and as independent variables: expected number of patches shared (forcibly entered), focal body size (SL in millimeters), difference in body size with relative (larger relative j − focal relative i, SL in millimeters), focal sex (coded 1 for males or 0 for females), same sex as relative (coded 1 yes,
When 4 patches were available, the observed number of patches shared only significantly increased with the expected number of patches shared (Table 6). When 8 patches were available, the observed number of patches shared significantly increased with the expected number of patches shared and significantly decreased with the focal’s body size (Table 6). Running the same analysis separately for subordinate-dominant pairs revealed only a positive significant effect of the expected number of patches shared on the observed patch sharing (4 patches: $R^2 = 0.353, n = 113, P < 0.001$ and 8 patches: $R^2 = 0.342, n = 187, P < 0.001$). Running the same analysis separately for dominant–dominant pairs revealed only a significant effect of the expected number of patches shared on the observed patch sharing when 4 patches were available (4 patches: $R^2 = 0.530, n = 29, P < 0.001$), but an additional negative significant effect of body size SL when 8 patches were available ($R^2 = 0.513, n = 51, P < 0.001$, effect of body size: $P = 0.01$ with coefficient $-0.004 \pm 0.002$ SE).

Taken together, these results strongly suggest that focal cichlids were mainly avoiding inbreeding or avoiding competition with kin by avoiding settlement with relatives: avoidance of relatives increased with body size when more patches became available (i.e., when breeding became more likely and when avoidance was also more easy to achieve compared with only 4 patches available), particularly for pairs of related dominants of larger size (who are the most likely to participate in reproduction).

**DISCUSSION**

Our experiments show that although small cichlids are productively capable, they are excluded from breeding positions and accept a subordinate helper position instead, where they are unlikely to reproduce themselves (see also Heg et al. 2006; Heg 2008; Heg et al. 2008b). Habitat saturation determined the sizes and sexes of competitors accepted as group members and whether they achieved dominant status. However, habitat saturation appeared not necessary for cooperative breeding to emerge because small individuals also accepted a subordinate position when free habitat was still available, supporting previous results (Heg et al. 2008a). Large cichlids first settled as dominants but were then progressively excluded from groups, and males were less likely to settle than females, leading to polygyny (Limberger 1983). Medium cichlids sometimes settled as dominants, but these

![Figure 5](image-url)

**Figure 5**

Experiments 3 and 4: effects of kinship on settlement in cichlids. Depicted are pairwise for 2 related individuals who both settled successfully: The observed number of patches shared with the related individual versus the expected number of patches shared with the related individual under random settlement. (a) Experiment 3 (24 patches available, $n = 82$ pairwise comparisons); (b) experiment 4 (4 patches available, $n = 215$), and (c) experiment 4 (8 patches available, $n = 340$). Increasing symbol sizes denote increasing sample sizes, and the sample sizes are depicted alongside the symbols.

0 no, status ringtank (coded 1 dominant, 0 subordinate), same status as relative (coded 1 yes, 0 no), and all their two-way interactions (using dummy variables) for 4 patches and 8 patches separately (Table 6). None of the interactions were significant.

<table>
<thead>
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<th>Table 6</th>
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<td><strong>Experiment 4: linear regression through the origin of the observed number of patches shared depending on the expected patches shared under random settlement, parameters of the smaller focal $i$, and differences of these parameters with the larger relative $j$.</strong> Sample sizes are pairwise settlement patterns of a smaller focal $i$ and his/her larger relative $j$</td>
</tr>
<tr>
<td>Parameter</td>
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<tr>
<td>Expected patches shared</td>
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<td>Body size (SL mm)</td>
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<td>Sex (female or male)</td>
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Depicted are the coefficients $B \pm SE$ and their significance $P$ of the two final models shown in bold with $R$ squared (and $P$ values of excluded variables when entered into these two final models).

* Coded yes or no.
were mainly females, and both sexes accepted subordinate positions. Again, due to habitat saturation, later released medium-sized cichlids were excluded from the groups, and this effect was more severe in the males. Small cichlids were accepted as subordinates also after the habitat was well saturated and only ended in the aggregation at the very end of the experiments. Four small females achieved dominance status, whereas none of the small males achieved this. Our results provide additional support to the notion that body size may have important effects on dominance, reproduction, and growth in co-operatively breeding cichlids (e.g., Heg et al. 2004a; 2004b; Bergmüller 2004b; Heg and Hamilton 2008), as is also increasingly found in other taxa (e.g., Clutton-Brock et al. 2001; Gilchrist et al. 2004; Russell et al. 2004; Tibbetts and Dale 2004).

Finally, cichlids rather avoided, than preferred, to settle with relatives, if they had a choice. This indicates that kin selection is not a necessary condition for co-operation to emerge in these cichlids but rather that kin structure in cichlids (Stiver et al. 2004, 2005; Dierkes et al. 2005) emerges due to constraints on dispersal (Heg et al. 2004a, 2004b; Bergmüller et al. 2005; Heg and Hamilton 2008). Kin avoidance is a sensible strategy if cichlids target own direct reproduction, and this would explain why larger sized individuals avoided settlement with kin because larger group members are more likely to participate in reproduction (Heg et al. 2006, 2008b, 2009; Heg 2008; Stiver et al. 2009). However, field data do not show active inbreeding avoidance, at least not in the dominant pair (Stiver et al. 2008), and we also detected 1 case of inbreeding in a dominant pair in experiment 4. Moreover, in a dual-choice situation where subordinates could associate through plexiglass with either a familiar group (probably includes related and unrelated individuals, see Dierkes et al. 2005) or an unfamiliar group, subordinates preferred to associate with the familiar group (Jordan et al. 2010), although actual settlement was not measured in our experiments. Inbred offspring appear completely viable (Heg D., personal observation), suggesting that kin avoidance might actually not curb the inclusive fitness of subordinates and dominants engaging in inbreeding (e.g., Kokko and Ots 2006). Kin avoidance is also a sensible strategy if cichlids thereby avoid competing with kin over valuable resources (e.g., Perrin and Mazalov 2000; Perrin and Lehmann 2001). Important resources in *N. pulcher* are food, shelter, and mates. Kin competition for food and shelters is unlikely to apply in *N. pulcher*, where zooplankton food floats freely through the territories and is also obtained by swimming away from the territories, and hiding shelters can be enlarged to fit all the group members. However, competition for mates may favor avoidance of kin, if mature group members target own reproduction.

Our habitat saturation experiment corroborates previous experimental work indicating that population density may affect sociality in vertebrates (Komdeur 1992; Harris et al. 1995; Lucia et al. 2008). In the only similar experiment to date, conducted in the Seychelles warblers *Acrocephalus sechellensis*, natal territory quality and saturation together determined the dispersal decisions of subordinates and therefore the extent of co-operative breeding (Komdeur 1992; Komdeur and Edelaar 2001; Ridley et al. 2003). We showed that small cichlids prefer co-operative breeding irrespective of saturation (see Figure 3a–c), also under laboratory conditions without predation risk (which is known to affect dispersal in the field: Heg et al. 2004a). However, habitat saturation did lead to progressively less individuals settling, and this effect was stronger for males and stronger for the intermediate (medium) size classes. The competition experiment suggests that this was due to dominant individuals (mostly large fish) only accepting subordinate individuals substantially smaller than themselves (i.e., mostly small fish).

Our results support the viewpoint (Koenig and Dickinson 2004) and theoretical work (Pen and Weissing 2000; Le Galliard et al. 2005) indicating that a life-history approach is needed to integrate all environmental and social factors affecting the individual’s decision at each life stage whether to disperse or stay. We have shown that measurements of the fitness benefits/costs ratio of philopatry versus dispersal during the entire lifetime are necessary to understand settlement and dispersal decisions in co-operatively breeding vertebrates. We have also shown that kin structure appears to be an emergent property of cichlid societies due to limits on dispersal and settlement because in the absence of such limits, cichlids prefer to settle with nonrelatives.

**SUPPLEMENTARY MATERIAL**

Supplementary material can be found at http://www.beheco.oxfordjournals.org/.

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**REFERENCES**


