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ORIGINAL PAPER

Group composition, relatedness, and dispersal in the cooperatively breeding cichlid *Neolamprologus obscurus*

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Abstract Cooperative breeding has been studied intensively in many species of birds and mammals but remain less well studied in fish. We report a remarkable new example of a cooperatively breeding cichlid from Lake Tanganyika, *Neolamprologus obscurus*. Using field observations and microsatellite DNA analyses, we studied group structure, helping behavior, relatedness, and dispersal of this species. We present four major observations. First, large territorial breeding males mated with one to eight breeding females, each of which was territorial and unrelated to another. Second, one to ten smaller fish ("subordinates") of both sexes were allowed to stay inside the breeding females' territories. Subordinates were often highly related to both the respective breeding male and female and performed territory defense and shelter maintenance, which is regarded as helping behaviors. Third, one to three subordinate males, similar in size to breeding females, were allowed to stay inside a breeding male's territory but were not tolerated in the breeding females' territories. Pairwise relatedness suggests these individuals are usually sons of the respective breeding male. Fourth, pairwise relatedness estimates suggest that juveniles delay dispersal and assist their mothers in raising offspring. As female subordinates grow up, they leave the father's territory and disperse into other groups. In contrast, male subordinates leave their mother's territory but remain within the territory of their father. The described social system makes *N. obscurus* a promising new model species to study the evolution of cooperative breeding.

Keywords Cooperative breeding · Social system · Related helper · Delayed dispersal · Fish

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Introduction

A large number of bird and mammal species have been reported to engage in cooperative breeding (Lukas and Clutton-Brock 2012; Feeney et al. 2013). Cooperative breeding is defined as a breeding system in which individuals other than parents remain in the breeder's territory and assist in raising young (Koenig and Dickinson 2004; Cockburn 2006; Lukas and Clutton-Brock 2013). In contrast to birds and mammals, only a few fish species are known to breed cooperatively, despite a wide variety of mating and parental care systems (e.g., Taborsky 1994, 2001; Kohler 1998; Wisenden 1999; Awata et al. 2005; Heg et al. 2005; Heg and Bachar 2006). Those that have been described as cooperative breeders are almost all cichlids of the tribe Lamprologini endemic to Lake Tanganyika (Heg and Bachar 2006). Helpers of cooperative breeding cichlids participate in territory defense (attacking predators and territory competitors), territory maintenance (digging and removing debris from shelters), and fry care (cleaning and fanning eggs and defending young)

(Taborsky and Limberger 1981; Kohler 1998; Awata et al. 2005; Heg et al. 2005). Cichlids represent an excellent model system to study the evolution and behavioral ecology of cooperative breeding using both field data and laboratory experiments (Wong and Balshine 2010). However, detailed data on cooperative breeding cichlids have been gathered from only a few species (see Heg and Bachar 2006 for a complete list), notably *Neolamprologus pulcher/brichardi* (*N. pulcher* and *N. brichardi* are synonymous species according to molecular data, see Duftner et al. 2007). Other Lamprologines, such as *Neolamprologus multifasciatus* (Rossiter 1993; Sato and Gashagaza 1997; Kohler 1998; Schradin and Lamprecht 2000, 2002), *Neolamprologus savoryi* (Heg et al. 2005), *Julidochromis ornatus* (Awata and Kohda 2004; Awata et al. 2005; Heg and Bachar 2006) *Julidochromis marlieri* (Yamagishi and Kohda 1996), and *Chalinochromis brichardi* (MK et al., unpublished data) have been less well studied.

These species show a broad array of mating systems, ranging from monogamy and polygyny (e.g., *N. pulcher*: Desjardins et al. 2008; *N. multifasciatus*: Kohler 1998; *N. savoryi*: Heg et al. 2005) to classical polyandry (e.g., *J. marlieri*: Yamagishi and Kohda 1996; *J. ornatus*: Awata et al. 2005) and cooperative polyandry (*J. ornatus*: Awata et al. 2005, 2006; *C. brichardi*: MK et al., unpublished data). While the subordinates of cooperatively polyandrous species are mostly unrelated to the dominants of both breeders (Awata et al. 2005, 2006 showed more than 80 percent of the helpers were unrelated to the dominant breeders in *J. ornatus*), monogamous and polygynous species show age dependent relatedness between dominants and subordinates, and relatedness declines with age of the subordinates (*N. pulcher*: Dierkes et al. 2005; *N. savoryi*: DH et al., unpublished data).

The dispersal patterns of cooperative breeders are relatively well documented in birds and mammals—e.g., in Florida scrub jays (Woolfenden and Fitzpatrick 1990), Seychelles warblers (Eikenaar et al. 2007, 2008), and dwarf mongoose (Creel 1994). Usually, the dispersal patterns of birds are female biased, while they are male biased in mammals (Greenwood 1980, 1983; Greenwood and Harvey 1982; Clarke et al. 1997; Clutton-Brock and Lukas 2012). Sex-dependent differences in delayed natal dispersal directly affect group composition, the opportunity to avoid inbreeding and competition with relatives for resources and/or mates (Pusey and Wolf 1996; West et al. 2002). It also affects the probability of assisting kin, or non-kin, in raising their offspring (e.g., Griffin and West 2003; Koenig and Dickinson 2004). Whereas dispersal in birds and mammals can be studied directly using individually marked group members, information in fish is less detailed and more difficult to obtain in nature due to the challenges of marking fish (most cooperatively breeding species are <10 cm in total length, and subordinates are even smaller). Nevertheless, in *N. pulcher* short-term observations (e.g., Bergmüller et al. 2005), individual genotyping across years (e.g., Stiver et al. 2004), and evidence from pairwise relatedness analyses (e.g., Dierkes et al. 2005; Stiver et al. 2005, 2007) together suggest that males disperse farther and more often than females, and that subordinates are more related to breeding females than to breeding males. This further indicates higher turnover rates among males due to breeder dispersal or death. However, to our knowledge, natural dispersal has not yet been studied in any other species of cooperatively breeding cichlid.

The purpose of this study is to advance our knowledge of cooperative breeding cichlids by adding information on group structure, helping behavior, within group relatedness and dispersal patterns of the previously unstudied Lake Tanganyika cichlid *N. obscurus*. Based on a literature search and personal communications, Heg and Bachar (2006) concluded that at least 19 Lamprologini species are cooperative breeders, while the status of *N. obscurus* was inconclusive. As the cooperative breeding system of *N. obscurus* has not yet been described, we start with a comprehensive description of group composition, body sizes, territoriality, behavior, and reproductive maturity using gonad sizes. Next, we use within- and between-group pairwise relatedness analyses, based on microsatellite DNA, to estimate opportunities for kin selection and sex-dependent dispersal.

Methods

Study species

Neolamprologus obscurus is a small cichlid (8 cm total length in maximum) endemic to Lake Tanganyika, where it lives under stones in sediment-rich intermediate substrates near shorelines, typically at depths of 5–35 m (Poll 1978; Konings 1998; HT personal observation). *N. obscurus* occupy territories in which they dig out shelters under stones, which they use for breeding, foraging, and protection from predators and conspecifics. The species' diet consists mainly of benthic animals, such as insect larvae and shrimp, but may also include zooplankton (HT, unpublished data).

Field observations

The study was conducted at the southern tip of Lake Tanganyika, at Nkumbula Island near Mpulungu (8° 45.2′ S, 31° 05.2′ W), Zambia. Data were collected by SCUBA diving from September to November 2010. The study site was located at a depth of 6.5–8 m along a steep sandy slope with many partially exposed stones (typical diameter, 10–30 cm). It measured approximately 20×7 m. This area was subdivided into a 0.5×0.5-m grids using a rope to more easily map the home range of each individual. We used the resulting detailed

topographic map of all stones to trace the swimming tracks of every individual observed during the study (i.e., breeding males, independent males, breeding females, single males, and helpers; see "Definition of social rank"). Fish were individually identified by their size and distinct natural markings, which consisted of a series of unique gray lines on the head and body (Appendix 1).

Definition of social rank

We recorded two-dimensional swimming tracks of the fish as a continuous line on the topographic map and used the maximum extent of each individual's swimming tracks to determine its home range.

We observed several large males in the study area. These males had the largest home ranges and sometimes showed aggressive behaviors against each other at the boundaries of their respective territories. Their home ranges overlapped with sexually mature females and other individuals that typically showed submissive and/or social behaviors (see "Behavioral observation" for the definition of submissive behavior and social behavior). These males had mature testes (see results of the gonad analysis), and we therefore defined them as breeding males (Fig. 1a–d).

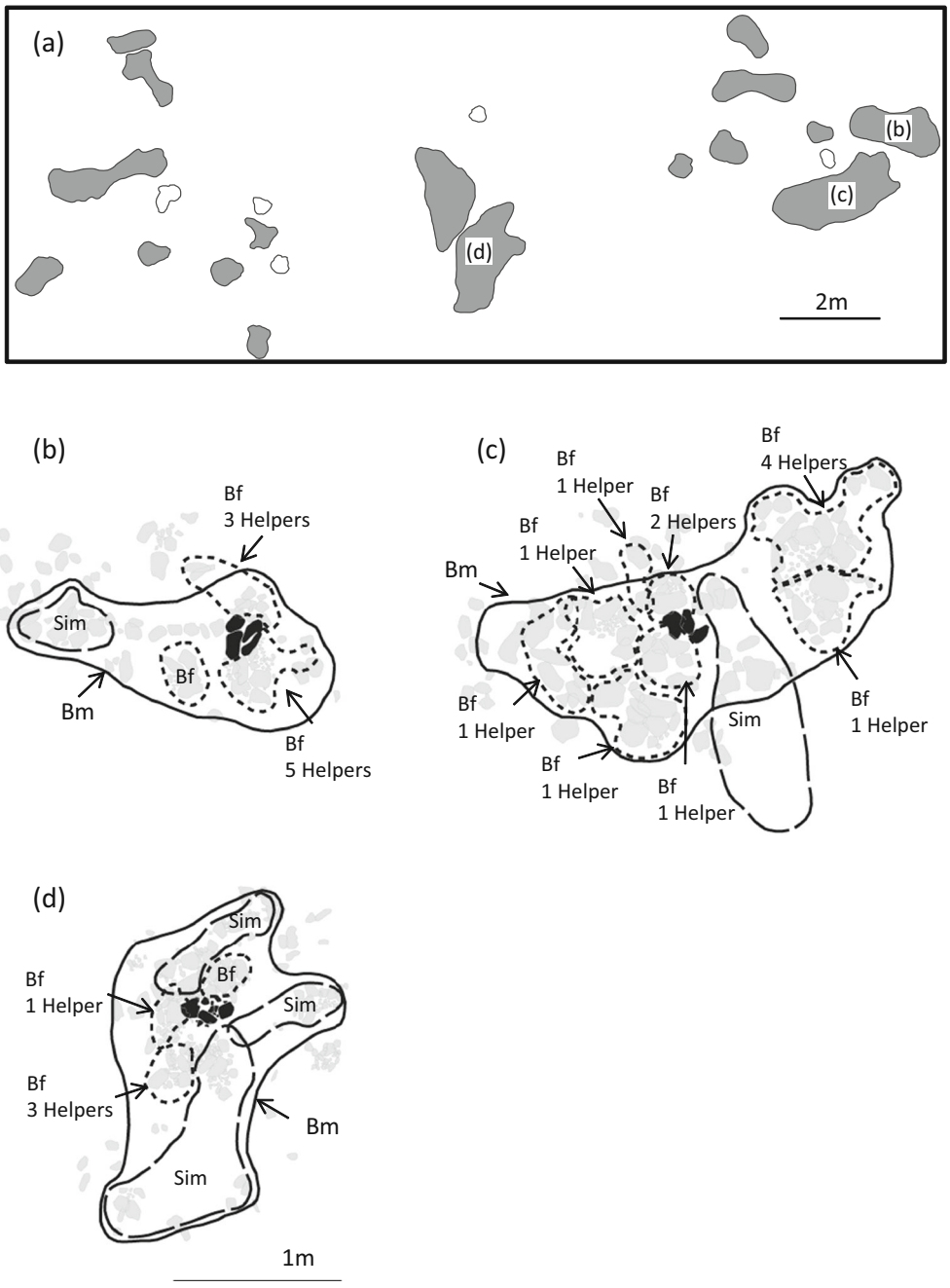

Fig. 1 **a** Home range distribution of *N. obscurus* in the study area (20×7 m): home ranges of breeding males (*shaded*) and independent males (*open*). The *bar* represents 2 m in (**a**). **b**–**d** Schematic representations of home ranges of breeding males (*Bm*; *solid line*), breeding females (*Bf*; *dotted line*), and single males (*Sim*; *broken line*) of three different groups (the number of helpers is listed per breeding female). **b** A typical group; **c** the group with the most breeding females; **d** the group with the most single males. Shelters frequently used by the breeding male are in *black*, whereas all other shelters and stones are shown in *gray*. The *bar* represents 1 m in (**b**)–(**d**)

We also found males whose territory did not overlap with any breeding females but remained inside the home range of a breeding male. These males showed aggressive behavior toward their neighboring breeding females and defended their own shelters, but showed submissive and social behaviors (especially by swimming side by side; HT, unpublished data) toward, and were tolerated by, the cohabitant breeding male. We defined these as single males (Fig. 1b, c). Finally, we found that some males fought against neighboring breeding males, but their territories did not overlap with any other individuals and they remained alone. We defined these as independent males (Fig. 1a).

We found several females inside the home ranges of breeding males. These females' home ranges rarely overlapped, and they showed aggressive behaviors against each other. Almost all had mature ovaries (see results of the gonad analysis), and we therefore defined them as breeding females (Fig. 1b–d).

We also found smaller individuals within the home ranges of breeding females. These small individuals were tolerated by, and typically used the same shelters as, the breeding female but were attacked if they stayed near neighboring females' home ranges (Fig. 1b–d). They showed aggressive behavior toward both con- and heterospecific individuals, including other breeding females, but showed submissive behavior toward their own breeding female, and removed sand from her shelter. Similar behaviors have been described as costly helping behaviors in other cichlid species (Taborsky 1984, 1985; Grantner and Taborsky 1998; Heg et al. 2005; Heg and Bachar 2006). Thus, we consider them likely to be costly in *N. obscurus* as well and defined these individuals as helpers.

Finally, we observed small fish inside the shelter of some breeding females. These individuals were small (below 19 mm), rarely overlapped in size with helpers (Table 1), and never emerged from the shelter during observations. They neither removed sand nor were aggressive toward intruders, and we therefore defined them as juveniles.

We defined all individuals that lived within a breeding male's home range as group members (i.e., the breeding male and all associated breeding females, single males, helpers, and juveniles) and individuals that overlapped only with breeding females as subgroup members (i.e., the breeding female, helpers, and juveniles).

Behavioral observation

We randomly selected 14 groups and four independent males for focal behavioral observations in the study area (N=14 breeding males, 38 breeding females, 11 single males, 54 helpers, and 4 independent males). Each individual was observed three times within 1 day. Each observation lasted 10 min, summing up to a total of 30 min per individual. Observations were made during three time periods: between 9:00 and 10:30, 10:30 and 12:00, and 14:00 and 15:30. Juveniles were not observed because they did not emerge during the observation period. We recorded pecking frequency as a proxy of feeding behavior both in the water column and on the substrate, the frequency of sand digging from the shelter and sand removal from the home range, the frequency of aggressive behaviors (including overt aggression such as bites, chases, fast approaches, mouthfights, and restrained aggression such as opercula spreading, also called puffed throat, S-shaped body posture, and fin spreading), the frequency of submissive behaviors (tail-quivering), and the frequency of social behaviors (soft body contact, also called bumping) toward con- and heterospecific individuals. These behaviors are similar to behaviors in *N. pulcher*/*brichardi* (described in Taborsky 1984, 1985) and *N. savoryi* (Heg et al. 2005). We further found that some individuals swam side by side with other individuals that approached the focal individual's home range. This behavior has also been reported in *Neolamprologus meeli* as "swim together" behavior (Sunobe and Munehara 2003), and we included it as social behavior because it was only observed among group members (HT, unpublished data).

Table 1 Mean body size (SL, mm)±SD and range of each social rank in *N. obscurus*

Social rank	Number	Mean body size (SL, mm)±SD	Range	Statistical difference
Breeding male	17	42.56±3.91	35.40–47.95	a
Independent male	5	35.06±5.66	30.20–44.25	b
Single male	47	31.81±4.65	22.50–37.20	b
Breeding female	13	30.24±3.36	20.95–36.00	b
Helper	57	22.98±3.26	17.45–32.40	
Helper male	26	24.36±3.79	18.35–32.40	c
Helper female	26	22.17±2.13	17.95–25.70	c
Juvenile	40	12.80±3.86	6.45–18.80	d

Letters in statistical difference denote statistical difference by Tukey HSD tests

Fish sampling

After behavioral observations, all *N. obscurus* inside the study area were captured using gillnets and hand-nets with the help of 30 % clove oil diluted in ethanol and brought to the laboratory. Here, we measured standard length (SL; to the nearest 0.05 mm) and wet body weight (BW; to the nearest 0.001 g). Sex of fish >18 mm was determined by inspection of the genital papilla; sexing is unreliable in individuals <18 mm. After measurement, the fish were anesthetized and euthanized using an overdose of the anesthetic FA100 (10 % solution of eugenol; Tanabe Seiyaku Inc.). The right pelvic fin was preserved in 99.9 % ethanol, and the remaining body was fixed in 10 % formalin solution. After fixation, all *N. obscurus* bodies were dissected for gonad measurement (GW; mg).

Microsatellite analysis

Genomic DNA was extracted from all of the ethanol-preserved fin tissue samples using the AquaPure Genomic DNA Purification Kit (Bio-Rad). We used seven microsatellite loci for genotyping: 758/773 (Schliewen et al. 2001), Chb1 (Munehara et al. 2001), Pzeb1 and Pzeb3 (van Oppen et al. 1997), TmoM11 (Zardoya et al. 1996), and UME002 and UME003 (Parker and Kornfield 1996). Each forward primer was labeled with a fluorescent dye (FAM, HEX, NED, and VIC). DNA was amplified using the Qiagen Type-it Multiplex PCR Kit, arranging loci with non-overlapping size ranges in each dye, to thus allow co-amplification of all microsatellite loci in a single polymerase chain reaction (PCR). PCR was conducted in a 5-μl volume containing 1 μl genomic DNA and 2× Qiagen Type-it Multiplex PCR Master Mix and microsatellite primer pairs with varying concentrations from 0.03 to 0.09 μM, according to the intensity of the respective amplification products. Amplification was performed using a GeneAmp® PCR System 9700 (Applied Biosystems), with the following program: one cycle at 94 °C for 5 min; 35 cycles at 94 °C for 30 s, 52 °C for 40 s, and 72 °C for 70 s; and one cycle at 72 °C for 20 min. PCR products were analyzed using an ABI 3130xl Genetic Analyzer (Applied Biosystems) and automatically analyzed using GeneMapper® (Applied Biosystems). Characteristics of the seven microsatellite loci are listed in Appendix 2. One breeding male was not included in the analyses because low DNA quality yielded an unreliable microsatellite result.

Statistical analysis

We used separate linear models (LM), generalized models (GLM), or generalized linear mixed models (GLMM) for the analyses. Residuals for all models were checked for overdispersion and heterogeneity (Bolker et al. 2009). All statistical analyses were performed in R version 2.14.0 (R Development Core Team 2011). GLMMs were performed by using the lme4 package (Bates et al. 2011).

Group composition and group structure

To investigate the difference of body size between each social rank, we used linear models with Gaussian error structure, followed by Tukey HSD post hoc tests. Using separate GLMs, we tested the effect of breeding male body size on breeding male home range size, largest female body size, group size, number of breeding females, or helpers, or juveniles within his group; respectively. In the model of home range size of the breeding male, we used a GLM with gamma error structure and log link function, and in the model of largest female body size, we used a GLM with Gaussian error structure and identity link function. In the rest of the models, we used GLMs with poisson error structure and log link function. We also tested the effects of breeding female body size on breeding female home range size, her subgroup size, number of helpers, or juveniles within her subgroup; respectively, using separate GLMMs. In each GLMM, the identity of the subgroup number was incorporated as a random factor. In the model of breeding female home range size, we used a GLMM with gamma error structure and log link function. In the rest of the model, we used GLMMs with Poisson error structure and log link function. In these LM, GLMs and GLMMs analyses, we performed likelihood ratio test to examine the significance of the explanatory variable.

Behavior

To investigate the difference in digging, aggressive, and feeding behaviors between social ranks, we used separate GLMs for each behavior, respectively. In each of the models, we used GLMs with Poisson error structure and log link function. We set breeding female as reference category to compare each behavior between social ranks in each of the model, because breeding female of other cichlids usually contribute most to territory defense and maintenance and to caring for the offspring (DH, personal observation).

Gonadal analysis

To assess differences in gonadal mass among the four different social ranks of male *N. obscurus* (breeding male, independent male, single male, and male helper) and two different social ranks of females (breeding female and female helper), we compared gonadal mass among social ranks for each sex by using GLMs with gamma error structure and log link function, followed by Tukey HSD post hoc test. We performed likelihood ratio test to examine the significance of the explanatory variable in GLMs. Next, to examine differences in gonadal investment among social ranks for each sex, we

followed Tomkins and Simmons (2002) and used LMs in which log-transformed gonadal mass was compared among social rank, with log-transformed soma mass (log (body mass −gonadal mass)) as covariate, including the interaction between social rank and log soma mass.

Kinship structure

Dyadic estimates of KINSHIP genetic relatedness (Goodnight and Queller 1999) were calculated using KINGROUP v.2.0 software (Konovalov et al. 2004) using background allele frequencies from Konovalov and Heg (2008). We used pairwise relatedness to establish whether this fish lives in kin structured groups by comparing among non-group members, group members, and subgroup members of each pairwise social ranks. Relatedness was analyzed with Mann–Whitney U tests with Bonferroni corrected p values to avoid type I errors.

Results

Group composition and group structure

In total, we found 17 breeding males, 47 breeding females, 13 single males, and 5 independent males in the study area (Fig. 1a). The 17 breeding males had one to eight breeding females each (median, quartiles=3, 1, 3, N=17) and zero to three single males (median, quartiles=0, 0, 1, N=17) in their home ranges and occasionally visited the group members' respective shelters. Conversely, the independent males were solitary. Body sizes differed between social ranks (GLM: χ^2_6=344.56, P<0.001) and breeding males were larger than independent males (Tukey HSD test: z=−4.06, P<0.001; Table 1). Male body size showed a significant relationship with the size of his territory and the number of group members and sizes of his group members (Tables 2 and 3 (breeding male body size (SL))). Breeding females and single males were smaller than breeding males (Tukey HSD test: z=−11.99, P<0.001 and z=−8.04, P<0.001, respectively; Table 1) but were of similar size to each other (Tukey HSD test: z=1.38, P=0.80; Table 1). Female body size also showed significant relationship with the size of her territory and the number and sizes of her subgroup members (Tables 2 and 3 (breeding female body size (SL))).

Breeding females tolerated up to 10 helpers (median, quartiles per subgroup=1, 0, 2, N=47) and up to four juveniles in their home ranges (median, quartiles per subgroup=0, 0, 1, N=47). Larger breeding males and larger breeding females had more helpers and juveniles (Table 3 (breeding male body size (SL) and breeding female body size (SL))). Of 57 helpers, 26 were males and 26 were females. For the remaining five, assessing their sex was impossible due to small gonads. Body sizes of male and female helpers did not significantly differ (Tukey HSD test: z=−2.17, P=0.29; Table 1).

Behavior

All breeding males, independent males, breeding females, and single males had their own shelters within their home range (Fig. 1b–d). Helpers and juveniles typically used the same shelter as the breeding female of their respective subgroup. Fish often entered their shelter and spent time inside their shelters during the behavioral observation period (Table 4). Furthermore, breeding males moved freely within their home ranges and frequently entered breeding females' shelters (median and quartiles/30 min=3, 2, 3.5, N=14) and the shelter of single males (0, 0, 1, N=14). Breeding females and single males attempted to prevent breeding males from entering their home ranges by side-by-side swimming or from entering their shelters by using intense bumping to push them away from the entrance of their shelters (median and quartiles of behavior toward the breeding male/30 min: breeding female, 1, 0, 1, N=38; single male, 1, 0.5, 1, N=11). Breeding males and females showed no significant difference in digging/removing sand but males showed increased aggression toward intruders (Tables 4 and 5). On the other hand, single males

Table 2 Description of home ranges (cm^2), (sub)group sizes, largest female size (mm), and group composition of breeding males and females

Variable	Number	Mean±SD	Range
Breeding male home range size	14	6649.0±6859.4	942.1–21,462.1
Breeding female home range size	38	711.1±590.6	170.0–3032.7
Group size	17	7.94±5.83	2–19
Largest female size within the group	17	31.55±2.97	24.00–36.00
Number of breeding females per group	17	2.77±2.05	1–8
Number of helpers per group	17	3.35±3.84	0–12
Number of juveniles per group	17	2.35±2.74	0–10
Subgroup size	47	2.31±1.79	1–11
Number of helpers per subgroup	47	1.21±1.76	0–10
Number of juveniles per subgroup	47	0.85±1.23	0–4

Table 3 Effects of breeding male body size (SL) and breeding female body size (SL) on home range, (sub)group size, and numbers of the different group members from separate GLMs

Variable	Number	β±SE	χ^2	P
Effect of breeding male body size (SL, mm)				
Breeding male home range size	14	9.79±1.78	23.61	<0.001
Largest female size within the group	17	0.54±0.14	12.18	<0.001
Group size	17	0.14±0.03	31.54	<0.001
Number of breeding females within the group	17	0.12±0.05	7.95	0.004
Number of helpers within the group	17	0.22±0.05	26.55	<0.001
Number of juveniles within the group	17	0.24±0.06	20.73	<0.001
Effect of breeding female body size (SL, mm)				
Breeding female home range size	38	3.04±0.64	17.64	<0.001
Subgroup size	47	0.16±0.04	21.11	<0.001
Number of helpers within the subgroup	47	0.37±0.07	37.49	<0.001
Number of juveniles within the subgroup	47	0.24±0.06	16.95	<0.001

Depicted are the regression coefficients with standard error with results of likelihood ratio test

showed less digging/removing sand from their shelters compared with breeding females and showed more aggression toward intruders (Tables 4 and 5). Helpers dug and removed sand from the breeding female's shelters of the same subgroup and showed aggressive behavior toward con- and heterospecifics but did so significantly less frequently than breeding females (Tables 4 and 5). Helpers also showed submissive behavior mostly toward breeders of their own group (median and quartiles/30 min=0.5, 0, 1.75, N=54), except for five cases in which it was directed toward breeders of another group or subgroup. Feeding rates of fish in each social rank differed significantly (Tables 4 and 5).

Table 4 Description of time spending inside the shelter and mean frequency of digging, aggressive, and feeding behaviors in each social rank

Behavior	Social rank	Number	Mean±SD/30 min
Time spend inside the shelter (min)	Breeding male	14	6.13±5.39
	Breeding female	38	13.59±8.42
	Independent male	4	16.69±7.43
	Single male	11	10.34±9.66
	Helper	54	19.02±9.47
Digging	Breeding male	14	19.00±17.89
	Breeding female	38	17.24±17.65
	Independent male	4	24.25±20.81
	Single male	11	13.00±13.83
	Helper	54	4.67±8.22
Aggressive	Breeding male	14	11.21±4.44
	Breeding female	38	5.45±4.64
	Independent male	4	4.75±4.27
	Single male	11	8.27±7.73
	Helper	54	0.89±1.97
Feeding	Breeding male	14	84.14±148.42
	Breeding female	38	77.66±81.21
	Independent male	4	31.75±36.28
	Single male	11	65.91±48.93
	Helper	54	48.72±63.70

Reproductive potential of each social rank

Gonads of independent males, single males, and helpers of both sexes appeared very thin and underdeveloped, and accordingly, we found significant difference in gonad masses between social ranks in each sex (GLM: χ^2_3=185.83, P<0.001 in males, χ^2_1=99.49, P<0.001 in females). Gonad masses of independent males, single males, and male and female helpers weighed significantly less than those of both breeding males and females (Tukey HSD test: z=−7.36, P<0.001, z=−10.36, P<0.001, z=−11.19, P<0.001, respectively, in males; z=−11.13, P<0.001 in females; Table 6). The analysis of testis investment in males showed a significant interaction of social rank and log soma mass, while the same was true of ovary investment in females (LM: t=−7.14, P<0.001 in males, t=−2.86, P=0.006 in females; Fig. 2; Table 7).

Relatedness

We calculated pairwise mean relatedness among breeding males, breeding females, single males, and helpers/juveniles and compared among same subgroup members, same group but not the same subgroup members, and different group members of each social rank (Fig. 3). The mean relatedness of breeding males vs. helpers/juveniles from the same group was significantly higher than that of individuals from different

Table 5 Effect of social rank on digging, aggressive, and feeding behaviors

Response variable	Explanatory variable	β±SE	z	P
Digging	Intercept	2.85±0.04	72.86	<0.001
	Social rank			
	Breeding male	0.10±0.07	1.34	0.18
	Breeding female	0 (reference)	–	–
	Independent male	0.34±0.11	3.14	0.002
	Single male	−0.28±0.09	−3.06	0.002
	Helper	−1.31±0.07	−17.63	<0.001
Aggressive	Intercept	1.70±0.07	24.39	<0.001
	Social rank			
	Breeding male	0.72±0.11	6.82	<0.001
	Breeding female	0 (reference)	-	-
	Independent male	−0.14±0.24	−0.57	0.57
	Single male	0.42±0.13	3.32	<0.001
	Helper	−1.81±0.16	−11.32	<0.001
Feeding	Intercept	4.35±0.02	236.43	<0.001
	Social rank			
	Breeding male	0.08±0.03	2.33	0.02
	Breeding female	0 (reference)	–	–
	Independent male	−0.89±0.09	−9.87	<0.001
	Single male	−0.16±0.04	−3.96	<0.001
	Helper	−0.47±0.03	−17.39	<0.001

groups (mean relatedness±SE, within group: 0.33±0.03, N=90; between group: 0.03±0.01, N=1430; Mann–Whitney U test, z=9.40, P<0.001; Fig. 3a); we found the same trend for breeding females vs. helpers/juveniles from the same subgroup, and between subgroups within the same group (mean relatedness±SE, within subgroup, 0.42±0.02, N=93; between subgroup from the same group, 0.15±0.01, N=301; Mann–Whitney U test, z=8.63, P<0.001; Fig. 3b). These results suggest that helpers and juveniles are related to breeding males of the same group and to females of the same subgroup.

Table 6 Mean gonad mass in each social rank of male and female of *N. obscurus*

Social rank	Number	Gonad mass (mg)	Statistical difference
Males			
Breeding males	17	4.8±3.7	a
Independent males	5	0.2±0.4	b
Single males	13	0.2±0.4	b
Male helper	26	0.4±0.6	b
Females			
Breeding females	47	3.2±2.2	a
Female helper	26	0.3±0.5	b

Letters in statistical difference denote statistical difference by Tukey HSD tests

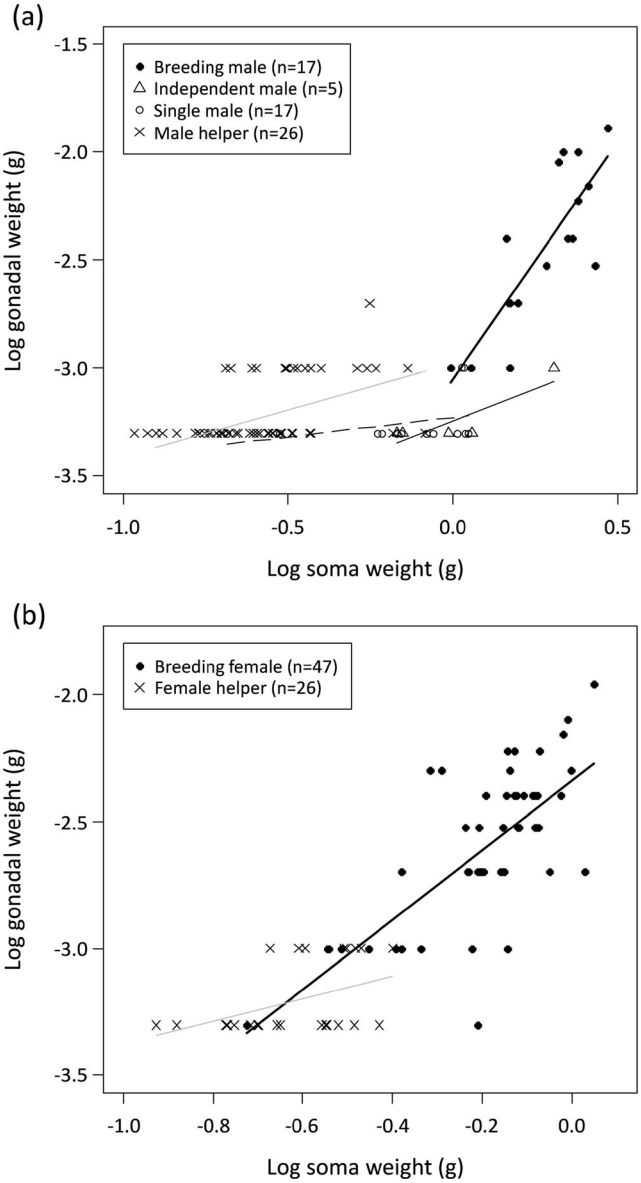

Fig. 2 Relationships between log soma mass and log gonadal mass in each **a** male and **b** female social rank. The *thick solid*, *thin solid*, *dashed*, and *gray solid lines* represent breeding males and females, independent males, single males, and male and female helpers, respectively

Furthermore, mean relatedness among helpers/juveniles also declined from subgroup to non-subgroup, to non-group members (mean relatedness±SE, within subgroup, 0.39±0.02, N=181; between subgroups from the same group, 0.20±0.01, N=346; and between groups, 0.04±0.00, N=3938; Mann–Whitney U test, within subgroup vs. between subgroup, z=8.63, P<0.001; between subgroup vs. between group, z=10.98, P<0.001; Fig. 3b), suggesting that helpers and juveniles are full siblings in the same subgroup and are half siblings in the same group.

The relatedness of breeding males to single males was highly variable within the same group (range of relatedness=−0.30 to 0.67), and the mean relatedness among

Table 7 Effects of log soma mass and social rank on log gonad mass in males and females

Variable	β±SE	t	P
Males			
Intercept	−3.05±0.09	−34.88	<0.001
Social rank			
Breeding male	0 (reference)	–	–
Single male	−0.18±0.10	−1.74	0.09
Independent male	−0.19±0.11	−1.73	0.09
Helper male	0.07±0.12	0.64	0.53
Log soma mass	2.20±0.29	7.64	<0.001
Social rank×log soma mass			
Breeding male×log soma mass	0 (reference)	-	-
Single male×log soma mass	−2.02±0.35	−5.76	<0.001
Independent male×log soma mass	−1.59±0.50	−3.18	<0.001
Helper male×log soma mass	−1.77±0.32	−5.51	0.002
Females			
Intercept	−2.34±0.06	−41.42	<0.001
Social rank			
Breeding female	0 (reference)	–	–
Helper female	−0.60±0.22	−2.67	<0.001
Log soma mass	1.37±0.22	6.25	<0.001
Social rank×log soma mass			
Breeding female×log soma mass	0 (reference)	–	–
Helper female×log soma mass	−0.93±0.40	−2.32	0.02

breeding males and single males was much higher than that between different groups (mean relatedness±SE, within group, 0.32±0.12, N=8; between group, 0.07±0.02, N=136; Mann–Whitney U test, z=2.17, P=0.03; Fig. 3a). This result indicates that some single males were offspring, or full or half siblings of the breeding male. The within-group relatedness of single males to helpers/juveniles was also much higher than that between groups (mean relatedness±SE, within group, 0.21±0.04, N=49; between group, 0.02±0.01, N=806; Mann–Whitney U test, z=4.97, P<0.001; Fig. 3c), suggesting that single males are half siblings of helpers and juveniles of the same group.

Finally, the mean relatedness of breeding males vs. breeding females within the same group was similar to the mean relatedness between groups (mean relatedness±SE, within group, 0.05±0.05, N=41; between group, 0.03±0.01, N=647; Mann–Whitney U test, z=0.07, P=0.95; Fig. 3a), suggesting that breeding females are not related to breeding males of the same group. Furthermore, mean relatedness among breeding females within the same group was not significantly different compared with the mean relatedness between breeding females with other groups (mean relatedness±SE, within group: 0.13±0.03, N=59; between group, 0.06±0.01, N=844; Mann–Whitney U test, z=1.93, P=0.10; Fig. 3b), indicating that breeding females of the same group are unrelated.

Discussion

We provided the first comprehensive description of the cooperative breeding system of *N. obscurus*, a Lake Tanganyika cichlid previously unknown to show such behavior. We also obtained novel results on group structure, reproductive potential, and relatedness.

Cooperative breeding

Helpers of *N. obscurus* were allowed to remain inside breeders' home ranges, and assisted the breeding pair with territory maintenance (digging/removing sand from shelters) and defense (aggressive behavior toward intruders). Furthermore, helpers showed submissive behaviors mostly toward the breeding male and female of their own group and subgroup, which has also been reported in other cooperatively breeding cichlids, e.g., *N. pulcher*/*brichardi* (Taborsky 1984, 1985; Wong and Balshine 2010) and *N. savoryi* (Heg et al. 2005). However, we did not observe direct brood care behavior, such as cleaning and fanning of eggs or caring for fry, maybe because eggs were laid inside the shelter and juvenile spent all the time inside the shelter. Still, as most helpers remained in or near the shelters in which juveniles spend most of their time and chased intruders away from the breeding female's home range, they are likely to provide survival benefits to juveniles. Further observations and experimental verifications will help to better understand the effects of helping behavior on survival (e.g., Brouwer et al. 2005).

Most of the *N. obscurus* helpers were closely related to the breeding pair of the same group or subgroup. In many cases, helpers were genetically related to both breeders, which would facilitate kin selected benefits in this species (Dierkes et al. 2005). However, high variability in pairwise helper vs. breeding pair relatedness of the same group and/or subgroup (as in other cichlid species, e.g., *N. pulcher*: Dierkes et al. 2005; *N. multifasciatus*: Kohler 1998) suggests that some of the helpers were not related to the breeding pair of their own group and/or subgroup. Thus, opportunities for kin selected benefits could exist for many but not all helpers in *N. obscurus*.

Group structure, mating systems, and reproductive potential

The mating system of *N. obscurus* is harem polygyny, wherein larger males retain more females and offspring. This pattern of mating appears to be common in cooperatively breeding cichlids: *N. multifasciatus* and *N. savoryi* also show a haremic mating system (Kohler 1998; Heg et al. 2005), and the majority of females were also part of a polygynous group in a detailed study of *N. pulcher*/*brichardi* (Desjardins et al. 2008).

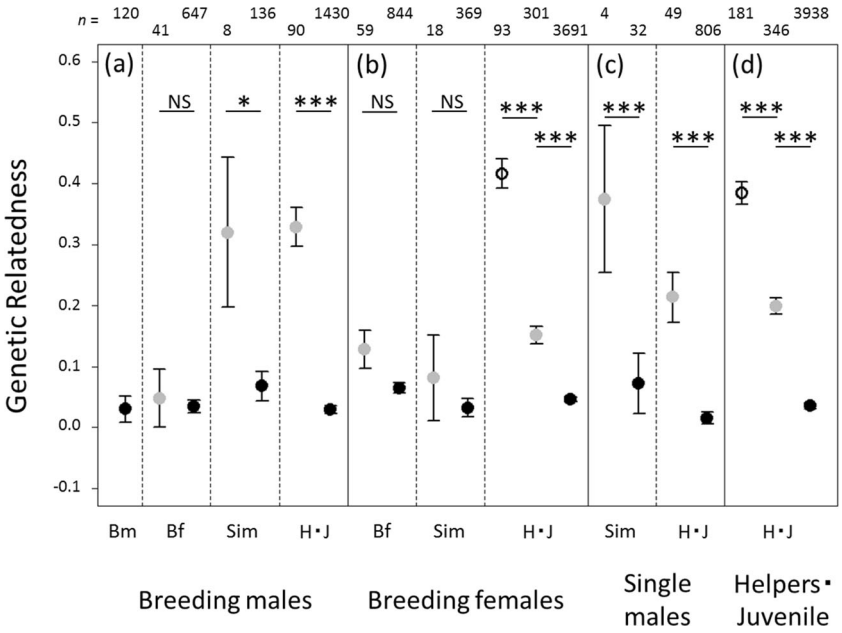

Fig. 3 Mean relatedness (±SE) of **a** breeding males (*Bm*), **b** breeding females (*Bf*), **c** single males (*Sim*), and **d** helpers/juveniles to members of the same subgroup (*white circle*), different subgroups within the same group (*gray circle*), and between non-group members (*black circle*). A group is defined by the home range of the breeding male and a subgroup is defined by the home range of the breeding female (see results and examples in Fig. 1b–d). *NS* not significant; $^{**}P<0.01$; $^{***}P<0.001$ from Mann–Whitney *U* tests after Bonferroni correction

The relationship between soma mass and gonads of both sexes indicates that males and females follow a clear ontogenetic trajectory. They increase their soma (but not their gonads, which remain regressed) up to ca. 0.0 log soma mass in male and ca. −0.5 log soma mass in female (Fig. 2). At that time point, the probability of males and females to acquire a territory or breeding position starts to rapidly increase and at the same time their gonads are also developed. Interestingly, there is no indication of a flattering-off of this effect in breeding males and females, which may be due to older and larger breeding males having more females to fertilize, and older and larger breeding females laying relatively more eggs compared with younger and smaller breeding females.

In *N. obscurus*, breeding females and single males were significantly smaller than breeding males. The ovaries of breeding females were significantly larger than those of female helpers, and testis size of single males were similar to those of male helpers but smaller than those of breeding males. The growth rates of breeding females and single males did not differ in the field (HT, unpublished data), indicating that females can start breeding earlier and at a smaller size than males. In contrast, single males cannot easily compete for territorial vacancies or mates because in doing so, they must compete with other males (see, e.g., Heg et al. 2011). The testis mass of single and helper males suggest reproductive suppression by the breeding male, which was also found in *N. pulcher*, or investment in growth at the expense of gonads (Fitzpatrick et al. 2005, 2008). If the benefit of staying inside the natal group exceeds the costs of dispersal for single males (e.g., due to predation risk, competitive costs, and energetic costs for digging a new shelter), delayed dispersal from the natal group will be the better choice (Heg et al. 2004).

Single males

Notably, single males which were highly related to breeding males but did not help them were observed in approximately 45 % of breeding male home ranges. Pairwise relatedness suggests that single males were often offspring, or full or half siblings of the breeding male of the same group. Such males were only rarely reported in *N. savoryi* and *N. pulcher* ("independents" in Heg et al. 2005; Wong and Balshine 2010). Single males removed sand from their shelters and showed aggressive behavior toward intruders of their home ranges, while their shelters were often entered by the breeding male. Why single males were tolerated by breeding males remains unknown, but we propose two non-mutually exclusive explanations. First, the diet of *N. obscurus* consists largely of benthic animals, including shrimp, mostly found between and under the shelter rocks. Thus for *N. obscurus*, the shelter is potentially also an important feeding resource, and breeding males might exploit the shelters of single males accordingly. Single males maintain their shelters not to be buried by the sand, and thus breeding males were able to access to the feeding resource in his territory. Second, single males provide benefits to their breeding male due to shared territory defense; i.e., the likelihood of an intruder leaving the breeding male's home range is increased by the presence of single males. In both cases, the likelihood of toleration might be enhanced because single males do not impose significant costs on the fitness of breeding males (as they are prevented from participating in reproduction by breeders, or they are strategically suppressed to invest their gonads; Fitzpatrick et al. 2005). As most single males are highly related to the breeding male of their own group, parental nepotism should also allow single males to stay (e.g., Ekman et al. 1999).

From the perspective of single males, there also might be several possible reasons to stay. First, living in a group might be beneficial for single males, e.g., due to group augmentation (Kokko et al. 2001). Second, single males may be in the process of "budding-off" their own territory, while waiting to grow sufficiently to recruit their own females (e.g., Komdeur and Edelaar 2001). Indeed, some of the single males extended their home ranges and overlapped with small females outside of the breeding male's home range, which support this idea (HT, unpublished data). Third, a chance of territory inheritance exists for single males if the breeding male disperses or dies (Balshine-Earn et al. 1998). All of the points are not mutually exclusive, and future work will resolve the factors affecting single males to stay inside the breeding male's home range.

Dispersal patterns inferred from pairwise relatedness estimates

We used pairwise relatedness estimates to infer likely patterns of dispersal in *N. obscurus*, as direct dispersal observations could not be obtained. We expected that individual *N. obscurus* typically disperse to obtain an immediate breeding position or join a new group as a helper (Stiver et al. 2004; Wong and Balshine 2010). Many helpers were highly related to the breeding female and breeding males, suggesting that juveniles are mostly the retained offspring of these breeding pairs, which became helpers in their natal group. Furthermore, the relatedness of single and breeding males of the same group were high, while that of breeding females and males were low. As single males and breeding females were of similar size, dispersal patterns are most likely sex-dependent in *N. obscurus*. While female helpers will disperse from their natal group and become breeding females in other groups, male helpers will become single males in their natal group or remain independent.

The low relatedness among breeding females both within and among groups adds more support to the notion that breeding females may immigrate into breeding male's territory from other groups. The distance between the nearest groups in our study area was 0.38 m±0.35 SD (N=17 groups; Fig. 1a). Thus, movement between neighboring groups should be relatively easy for *N. obscurus*. Alternatively, females may not disperse, and a high turnover occurs among breeding males (i.e., death or emigration). This explanation is less likely, however, because if the breeding male is replaced, the relatedness of not only breeding females and males but also of single and breeding males should drop (except if a male full sibling of the current breeding male takes the breeder position). The discrepancy between these two pairwise estimates corroborates our prediction that females will show natal dispersal, whereas males are more likely to stay, at least until they are sufficiently grown. In a previous study of *N. pulcher* dispersal, males dispersed farther and more often than females (Stiver et al. 2004, 2007). In birds, males may benefit most from philopatry, as a male's territory quality can influence both mate attraction and the survival of young (Pusey 1987). We conclude that female *N. obscurus* might disperse from the natal group earlier than males, and that this difference will reflect intersexual differences in the timing of reproductive onset associated with harem mating systems. Additional work is needed to determine precisely how dispersal varies with individual sex and size to fully explore the relationship between rank change and dispersal, and to shed more light on the factors driving dispersal patterns in cooperative breeding cichlids.

Conclusion

In summary, we introduced a remarkable new example of cooperative breeding system in the Lake Tanganyika cichlid *N. obscurus* and provided evidence for sex biased dispersal in this species. The fact that a recent phylogeny places *N. obscurus* in a different lineage than all other cooperatively breeding cichlids (Sturmbauer et al. 2010) underlines the potential of this species helping us to understand the evolution of cooperative systems in fish.

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Ethical standards The research presented here was conducted with permission from the Zambian Ministry of Agriculture, Food and Fisheries and complies with current Zambian law. We treated fish in compliance with the guidelines of the Animal Care and Use Committee of Osaka City University, the Japan Ethological Society, and the Ichthyological Society of Japan.

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