



Predation risk promotes delayed dispersal in the cooperatively breeding cichlid *Neolamprologus obscurus*



Hirokazu Tanaka ^{a,*}, Joachim G. Frommen ^b, Tetsumi Takahashi ^{c,d}, Masanori Kohda ^a

^a Laboratory of Animal Sociology, Department of Biology and Geosciences, Graduate School of Science, Osaka City University, Osaka, Japan

^b Department of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Hinterkappelen, Switzerland

^c Institute of Natural and Environmental Sciences, University of Hyogo, Hyogo, Japan

^d Division of Nature and Environmental Management, Museum of Nature and Human Activities, Hyogo, Japan

ARTICLE INFO

Article history:

Received 8 October 2015

Initial acceptance 18 November 2015

Final acceptance 6 April 2016

MS. number: 15-00860R

Keywords:

brood care helpers
ecological constraint
fish
migration
Neolamprologus pulcher
predation pressure
relatedness

Delayed dispersal of offspring from the natal territory is an important process in the evolution of cooperative breeding. Ecological constraints such as habitat saturation can promote delayed dispersal. Thus far, the role of predation risk in the evolution of cooperative breeding systems has received less attention, although it is understood as an important ecological factor shaping dispersal patterns and social structure in animals. To bridge this gap, we analysed group structure and dispersal patterns of helpers within a population of the cooperatively breeding cichlid *Neolamprologus obscurus*. We conducted behavioural observations for a period of 3 months at two study sites within this population, which differed in predation risk and availability of unoccupied space. Furthermore, by combining field observations with molecular analyses, we assessed group structure to unravel the dispersal patterns of helpers. At the high predation risk location, we found that helpers (1) had a lower dispersal propensity, (2) dispersed shorter distances and (3) were generally larger. Furthermore, molecular analyses and body size measurements suggest that helpers may stay longer in the natal territory under high predation risk than under low predation risk. Groups contained more helpers in the high predation risk site, which indicates that high predation pressure leads to delayed dispersal of helpers. Unoccupied potential dispersal areas were larger in the high predation risk site, suggesting that habitat saturation did not account for the dispersal patterns. Our results suggest that predation pressure is the main factor limiting the dispersal of helpers in the cooperatively breeding cichlid fish *N. obscurus*, and might be overlooked in other studies focusing on the evolution of cooperative breeding systems.

© 2016 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

In cooperative breeding systems, helpers forgo their own reproductive opportunities and assist dominants of their social group in raising young (Brown, 1987; Koenig & Dickinson, 2004; Solomon & French, 1997; Taborsky, 1994). Helpers and breeders are often highly related as a result of delayed dispersal by offspring from the natal territory (Brown, 1987; Ekman, Hatchwell, Dickinson, & Griesser, 2004). The delayed dispersal creates groups of kin (Drobniak, Wagner, Mourc, & Griesser, 2015) and provides opportunities for indirect fitness benefits via kin-directed helping behaviour to take place (Covas & Griesser, 2007; Ekman, Baglione, Eggers, & Griesser, 2001; Ekman, Hatchwell, Dickinson, & Griesser, 2004). These indirect fitness benefits are usually

regarded as being a fundamental factor promoting the evolution of complex social systems, such as cooperative breeding or eusociality (Bourke, 2014; West, Griffin, & Gardner, 2007). Thus, in order to understand the evolution of cooperative breeding systems, it is crucial to understand the factors triggering dispersal decisions of offspring.

Dispersal decisions of helpers usually depend on the costs and benefits of staying in the natal territory (Hatchwell & Komdeur, 2000). The ecological constraint hypothesis (Emlen, 1982) predicts that ecological opportunities, such as the availability of suitable territories (Emlen, 1984; Selander, 1964; Woolfenden & Fitzpatrick, 1984) or mating partners (Maynard Smith & Ridpath, 1972; Reyer, 1984; Rowley, 1965), influence the dispersal of offspring. However, recent theoretical (Kokko & Lundberg, 2001) and empirical studies (e.g. Baglione et al., 2005; Ekman et al., 2001; Gardner, Magrath, & Kokko, 2003; Kokko & Ekman, 2002; Lucia et al., 2008; Russell, 2004) have questioned the general validity of

* Correspondence: H. Tanaka, Laboratory of Animal Sociology, Department of Biology and Geosciences, Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi, Osaka 558-8585, Japan.

E-mail address: hirok.tanak@gmail.com (H. Tanaka).

the effect of habitat saturation. Interestingly, even though predation risk is well known for being one of the most important factors triggering group living (Alexander, 1974; Krause & Ruxton, 2002), it has received little attention in studies explaining delayed dispersal (but see Groenewoud et al., 2016; Heg, Bachar, Brouwer, & Taborsky, 2004; Rasa, 1987; Russell, 2004). When predation risk is high, dispersal is assumed to be risky, especially when individuals have to sample unfamiliar environments in order to find a suitable dispersal option (Bocedi, Heinonen, & Travis, 2012; Clobert, Galliard, Cote, Meylan, & Massot, 2009). Thus, high predation risk may force helpers to stay in the natal territory. Additionally, natal groups can serve as refuges, or 'safe havens' for helpers (Bergmüller, Heg, Peer, & Taborsky, 2005; Kokko & Ekman, 2002), and helpers staying in a group may gain benefits from early detection of predators (Hollén & Radford, 2009) or a reduction of per capita anti-predator effort (Jungwirth, Josi, Walker, & Taborsky, 2015). Therefore, high predation risk is predicted to directly reduce the dispersal probability of helpers, and comparing dispersal patterns between sites differing in predation pressure is indispensable. Still, studies investigating dispersal patterns under different levels of predation risk in cooperative breeders are scarce (but see Groenewoud et al., 2016; Heg et al., 2004).

In fish, cooperative breeding has been reported in several cichlid species endemic to Lake Tanganyika (Awata, Munehara, & Kohda, 2005; Heg & Bachar, 2006; Heg, Bachar, & Taborsky, 2005; Kohler, 1998; Taborsky, 1994; Tanaka et al., 2015). Many of them live under variable environmental conditions, in which they are confronted with different levels of predation pressure (Groenewoud et al., 2016; Kohler, 1998). This makes cooperatively breeding cichlids a highly suitable study system to investigate the influence of predation risk on dispersal under natural conditions. Thus far, the effects of predation risk on dispersal patterns of helpers are only reported from *Neolamprologus pulcher*. Here, field studies suggest that high predation pressure reduces the survival rate and the likelihood of dispersal of helpers, which supports the ecological constraint hypothesis (Groenewoud et al., 2016; Heg et al., 2004; Heg & Taborsky, 2010). Additionally, the availability of suitable habitats constrains dispersal of helpers in laboratory experiments (Bergmüller, Heg, & Taborsky, 2005; Heg, Rothenberger, & Schürch, 2010). In contrast, field observations suggest that habitat saturation cannot fully explain delayed dispersal of helpers (Heg, Heg-Bachar, Brouwer, & Taborsky, 2008). The cause of delayed dispersal in other cooperatively breeding fish has been elusive, leaving open the question of what general mechanisms trigger dispersal of helpers in fish.

In the present study, we investigated how predation risk and habitat saturation affect the dispersal pattern of helpers in a wild population of the cooperatively breeding fish *Neolamprologus obscurus*. Preliminary investigations revealed high variation in several ecological factors in this population (H. Tanaka, personal observation). Groups in deeper water face more predators and a less saturated habitat than groups in shallow water. This scenario offers an ideal opportunity to study the factors affecting dispersal patterns of helpers. If dispersal of helpers is mostly limited by predation pressure, we expect (1) a lower frequency of dispersal events, (2) shorter dispersal distances and (3) a higher relatedness among group members in groups occurring in deeper regions of our study site. In contrast, if dispersal is mainly driven by the availability of unoccupied territories, we expect more dispersal events and a lower within-group relatedness in groups occurring in deep water. In the present study, we first systematically assessed predation risk and unoccupied potential territories at two different depths of the study population. Then, we compared group composition and dispersal patterns of helpers by direct observation of dispersal events for a period of 3 months. Finally, we used genetic

samples for microsatellite analyses to further assess patterns of relatedness at the two sites.

METHODS

Study Species

Neolamprologus obscurus is a small benthic cichlid fish endemic to Lake Tanganyika. It lives in narrow crevices under stones on sediment-rich substrate and mainly feeds on benthic animals, such as insect larvae and shrimps (Hori, n.d.). The breeding system is characterized by a mixture of monogamous and polygynous breeding groups (Tanaka et al., 2015). Dominant males accept one to eight reproductive dominant females in their territory. Each dominant female defends her own shelter and territory where up to 10 helpers can be present. Helpers are allowed to enter the shelter of the respective dominant female and engage in shelter maintenance (e.g. removing sand and debris) and defence against conspecific and heterospecific intruders (Tanaka et al., 2015). According to pilot observations, fish in the study population start to participate in shelter maintenance and defence when they reach 17 mm standard length (SL; H. Tanaka, personal observation). Therefore, we defined fish above 17 mm SL as helpers, and fish smaller than 17 mm SL as juveniles. Furthermore, up to three potentially reproductive males (called 'single male', see Tanaka et al., 2015 for a detailed description) may reside inside the territory of a dominant male. Home ranges of single males do not overlap with those of any other fish except for the dominant male. Most single males are closely related to the dominant male, and frequently show submissive behaviour to them (Tanaka et al., 2015). Finally, solitary individuals are occasionally found outside the territory of the dominant male, which do not form any group but defend their own shelter.

Study Site and General Methods

Field observations were conducted at the southern tip of Lake Tanganyika, at Nkumbula Island near the city of Mpulungu, Zambia (8°45.2'S, 31°05.2'W). Data were collected by SCUBA diving from 20 August to 17 December 2013. The underwater landscape of this site comprises a steep sandy slope with partially exposed stones, and *N. obscurus* can be found at this site through depths of 5–13 m. Within this range we chose two separate study sites: a shallow (6–8 m depth) and a deep (11–13 m depth) one. We installed a 4 × 8 m grid subdivided by 1 × 1 m cells using strings in each study site to allow habitat measurements and to easily assess the relative positions of territories within each site. The two study sites were at minimum 21.5 m apart. The topographies of the ground in each study site were recorded using a Canon G15 digital camera, and subsequently mapped onto a personal computer. These topographical maps were used to calculate dispersal distances, and to measure the size of vacant territories and shelter areas.

Assessment of Predation Risk

To assess predation risk, we measured (1) the entrance size of the shelters used by resident *N. obscurus*, (2) the number and the body size of predators, shelter competitors and food competitors, and (3) the occurrence of predator attacks in addition to the number of fish showing signs of bites at the two study sites (see [Supplementary Material 1, 2](#)).

Neolamprologus obscurus digs out sand from existing rock crevices, and thus entrance size is limited by digging activity and crevice size itself. The size of the shelter entrance may directly affect the ease of intrusion by predators, and will directly link to

predation risk for small fish (Hixon & Beets, 1993). To compare the size of the shelter entrances, we randomly selected 50 shelter entrances in both study sites. We measured the maximum diameter of a hypothetical circular shelter entrance by fitting a rolled sheet of water-resistant paper into the shelter entrance to extrapolate the actual entrance size.

To measure numbers and body sizes of predators, we conducted nine censuses for each study site between 0900 and 1500 hours during the dispersal recording period (see below). Censuses were carried out at least 7 days apart. During each census, all cells of the respective grid were observed for 1 min each (i.e. 32 min per grid). We identified all fish species, counted individuals and visually estimated their body size (total length (TL) to the nearest 1 cm). A total of 25 fish species were recorded and categorized as predators, shelter competitors, food competitors or others following Hori (1997) and Abe (1997) (see [Supplementary Material 3](#)).

During the study period, we witnessed some predation events of *N. obscurus* ([Supplementary Material 1](#)). These predation events were recorded by ad libitum observation during a total of 67.0 h behavioural observations at the shallow and 85.0 h at the deep study site. We assessed the TL of all of the fish that were attacked to the nearest 5 mm, using previous recordings (see [Fish Density](#) for details). In addition, we found heavily scarred and/or injured *N. obscurus*, which had most likely been attacked by predators ([Supplementary Material 2](#)). We counted these individuals in both study sites, and measured their body size after sampling (see [Fish Sampling](#) for details).

Habitat Measurement

Both study sites contained several patches of stones partly submerged in the sand, which constitute potential shelters for *N. obscurus*. To evaluate the availability of these unoccupied potential shelters, we photographed 15 randomly selected cells of each grid in order to calculate the area of stones that was unoccupied by any fish (called 'unoccupied stone area' hereafter). To assess whether these unoccupied stone areas were large enough to be used as shelters for potential *N. obscurus* dispersers, we compared them with the stone area of shelters that were occupied by a dominant female and her helpers (called 'shelter area' hereafter). To calculate the shelter area, we randomly selected shelters of 20 *N. obscurus* groups from both study sites. The respective sizes were measured from the digital pictures using Image J ([Rasband, 2014](#)).

Fish Density

We observed and counted all *N. obscurus* within the two study sites. First, we identified each individual by its natural stripe pattern on the body and visually estimated the TL to the nearest 5 mm. Then, we assessed group memberships of all fish and counted all individuals except for juveniles (i.e. dominants, single males, helpers and solitary individuals) within the two study sites.

Recording of Dispersal Events

After the assessment of group membership in the two study sites, we randomly selected 16 dominant males at each site, thus considering a total of 32 males. We observed each of these males three to six times for 20 min and mapped their movements onto the topographical map. All female groups inside the 32 territories of dominant males served as focal groups. Thus we observed 48 female groups at the shallow and 56 at the deep study site. All focal groups were marked by placing numbered stones near the shelters of each group. Dispersal patterns of all helpers ($N_{\text{shallow}} = 56$,

$N_{\text{deep}} = 131$) were monitored for a 3-month period from 8 September to 7 December. We recorded the positions of these 187 focal helpers once a week. Furthermore, we recorded whether individuals were found in a shelter other than their home shelter. These individuals were checked every day to verify the dispersal event. Observed dispersers all joined an already established group, and no fish dispersed into a vacant area. Most of the dispersers were attacked by members of the new group they joined, and moved between their initial home group and the new group several times. We defined a successful dispersal when these individuals stopped moving between the new and their home group and stayed with the new group for at least a week. Furthermore, we noted all unidentified immigrants from outside the study sites. These fish usually visited several new groups. We followed these fish until we found their original group, and we added these groups onto the topographical map. These individuals were then used as additional focal individuals and were observed until a successful dispersal event occurred. At the end of the 3-month dispersal recording period, we counted the dispersers/immigrants, and measured dispersal distances (cm) by using the topographical map.

Fish Sampling

At the end of the 3-month dispersal recording period, we captured all focal individuals (i.e. the helpers of the 104 groups and additional immigrants into the two grids) as well as all other *N. obscurus* inside the territories of the 32 dominant males (i.e. dominant males, dominant females, single males and juveniles) by using gillnets, hand-nets and 30% clove oil diluted in ethanol. In total, 384 fish were caught. We measured SL and TL to the nearest 0.05 mm in the laboratory. We failed to catch five dominant females ($N_{\text{shallow}} = 3$, $N_{\text{deep}} = 2$), 14 helpers ($N_{\text{shallow}} = 4$, $N_{\text{deep}} = 10$) and two juveniles ($N_{\text{shallow}} = 1$, $N_{\text{deep}} = 1$). Furthermore, four fish were lost due to predation events (two dominant females and two helpers at the deep study site). For these fish, the TL was estimated during the observations (see [Fish Density](#)), and SL was calculated based on the correlation between TL and SL derived from data of fish collected for this study. After the size measurements, fish were anaesthetized and euthanized using an overdose of the anaesthetic FA100 (10% solution of eugenol; Tanabe Seiyaku Inc.). We dissected the fish, and their sex was determined on the basis of gonadal development. The right pelvic fin or the caudal fin was preserved in 99.9% ethanol for DNA extraction. We caught an additional 40 matured fish from outside the two quadrats for background allele frequency calculation in the subsequent microsatellite analysis (see below). These individuals were immediately released back to their shelters after taking a small tissue sample from their caudal fin while scuba diving.

Microsatellite Analysis and Relatedness Calculations

To estimate within-group relatedness, we extracted genomic DNA from the ethanol-preserved fin tissue samples by using the Wizard Genomic DNA Purification Kit (Promega, Tokyo, Japan). We used 11 microsatellite loci for genotyping (characteristics of the microsatellite loci are listed in [Appendix 1](#)). Each of the 11 microsatellite forward primers was labelled with a fluorescent dye (FAM, HEX, NED, and VIC). DNA was amplified using the Qiagen Type-it Multiplex PCR Kit (Qiagen, Tokyo, Japan). Loci with nonoverlapping size ranges were arranged in each dye, to allow co-amplification of all microsatellite loci in a two-polymerase chain reaction (PCR). PCR was conducted in a 5 μ l tube containing 1 μ l of genomic DNA and 2 \times Qiagen Type-it Multiplex PCR Master Mix and microsatellite primer pairs with varying concentrations from 0.03 to 0.10 μ M, according to the intensity of the respective amplification products. Amplification was

performed using GeneAmp PCR System 9700 (Applied Biosystems, Yokohama, Japan), with the following programme: 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 analysed using an ABI 3130 Genetic Analyzer (Applied Biosystems) and automatically analysed using GeneMapper 4.0 (Applied Biosystems). Hardy–Weinberg equilibrium tests were conducted using the Cervus software (Marshall, Slate, Kruuk, & Pemberton, 1998). Due to a low quality of purified DNA, microsatellite amplification failed for three dominant females ($N_{\text{shallow}} = 3$), five helpers ($N_{\text{shallow}} = 4$, $N_{\text{deep}} = 1$), one single male ($N_{\text{shallow}} = 1$), two juveniles ($N_{\text{shallow}} = 1$, $N_{\text{deep}} = 1$) and one matured individual from outside the two grids.

Dyadic estimates of relatedness (Goodnight & Queller, 1999) among helpers/juveniles and the dominant female of the same group were estimated for each group, using KINGROUP ver.2.0 software (Konovalov, Manning, & Henshaw, 2004). We used 11 microsatellite loci for the entire extracted DNA to calculate background allele frequencies following Konovalov and Heg (2008a). Using this method, we were able to estimate the outbred population allele frequency even when sample genotypes contained individuals from multiple generations and when the actual pedigree was assessed simultaneously using the same genetic markers (Konovalov & Heg, 2008b).

Statistical Analysis

Data analysis was conducted using R version 3.1.1 (R Core Team, 2014). In order to compare data obtained at the two study sites, we fitted linear models (LMs), generalized linear models (GLMs), linear mixed models (LMMs) or generalized linear mixed models (GLMMs). LMMs and GLMMs were performed by using the lme4 package (Bates, Maechler, & Bolker, 2011). Model validation was conducted by inspecting the residuals of the applied models, and either a Poisson error structure or a gamma error structure was applied for the analysis of non-normally distributed data. In all LMs, GLMs, LMMs and GLMMs that contained 'study site' as an explanatory variable, the shallow study site was set as the reference category. To examine the significance of the explanatory variable, we performed a likelihood ratio test. All of the tests were two tailed.

Predation risk

To compare differences in shelter entrance size between the two study sites, we fitted a GLM with gamma error distribution and log link. Shelter entrance size served as the response variable and study site as the explanatory variable. For the analysis of number and size of predators, shelter competitors and food competitors, we used GLMMs with Poisson error structure and log link (analysis of fish number), and gamma error structure and log link (analysis of fish body size). We fitted the three fish categories as separate response variables, study site was set as the explanatory variable and the date of the census was included as a random factor.

Territory availability

To compare the size difference of unoccupied stone areas between the two study sites, we fitted a GLM with gamma error structure and log link. In the model, size of unoccupied stone areas was set as a response variable and study site was set as an explanatory variable. To evaluate whether these potential territories were large enough to serve as shelters for potential dispersers, we compared their size with the size of occupied territories by fitting a GLM with a gamma error structure and a log link. We set size as the response variable and territory type (shelter area or unoccupied stone area) and study site as explanatory variables. Shelter area was set as the reference category.

Body Size and Group Composition

To compare the difference in fish body size between the two study sites, we fitted an LM with Gaussian error structure and an identity link. In the model, we set body size as a response variable, and social rank (dominant male, dominant female, single male and helper) and study site as explanatory variables. To analyse group composition, we fitted a GLM with Poisson error structure and log link to test for differences in group size between the two study sites. We set number of helpers as the response variable, and study site and body size of dominant female as explanatory variables, because helper numbers are usually related to the body size of the dominant female (Tanaka et al., 2015).

Dispersal of helpers

The proportion of dispersed helpers was compared between the two study sites by using a Fisher's exact probability test. A GLM with gamma error structure and log link was fitted to test for differences in dispersal distance between the two study sites. We set dispersal distance as the response variable, and study site as the explanatory variable. To compare body size differences of dispersed individuals between the two study sites, we used an LM with Gaussian error structure and identity link. Body size of dispersers served as the response variable and study site as the explanatory variable.

Relatedness

To investigate whether the within-group relatedness between helpers/juveniles and their respective dominant female differed between the two study sites, we first fitted an LMM with Gaussian error structure and identity link. We set relatedness as the response variable and study sites as the explanatory variable. To test whether the relationship between body size and relatedness between helpers/juveniles and their dominant female differed between the two study sites, we fitted an LMM with Gaussian error structure and identity link. In this analysis, we set relatedness as the response variable and the interaction term of study sites and helpers/juveniles' body size as explanatory variables. As we found a significant interaction term (see Results), we fitted two further LMMs with Gaussian error structure and identity link for the respective study site separately. In all of the LMMs, we used the dyadic relatedness between a single helper/juvenile and its respective dominant female as the response variable and group ID was incorporated as a random factor.

Ethical Note

The study complies with the current laws of the Republic of Zambia and was carried out in agreement with the Department of Fisheries: Ministry of Agriculture and Cooperatives. Data collection followed the ASAB/ABS (2012) guidelines for the treatment of animals in behavioural research and teaching.

RESULTS

Ecological Difference

The sizes of the shelter entrances were larger at the deep study site than at the shallow site (shallow: mean entrance size \pm SD (mm) = 18.12 \pm 6.19, $N = 50$; deep: 35.92 \pm 12.53, $N = 50$; GLM: $\beta = 0.68 \pm 0.07$, $\chi^2_1 = 96.38$, $P < 0.001$). Predators were more abundant at the deep study site, while there was no significant difference in the abundance of food competitors or shelter competitors (Table 1). Additionally, all fish of all three classes were larger at the deep study site (Table 2). We observed four predation events by spiny eels, *Caecomastacembellus moorii*, at the deep study site (shallow: 0 events/67.0 h; deep: 4 events/85.0 h; mean SL of

Table 1

Median number and quartiles of each fish category observed during the censuses, and results of the GLM comparing fish communities between the two study sites

Fish category	Study site	N	Median, quartiles	$\beta \pm SE$	χ^2	P
Predator	Shallow	9	1, 1, 7	Reference	–	–
	Deep	9	17, 8, 28	1.76±0.07	101.62	<0.0001
Shelter competitor	Shallow	9	17, 13, 23	Reference	–	–
	Deep	9	18, 14, 22	0.05±0.11	0.20	0.65
Food competitor	Shallow	9	19, 15, 20	Reference	–	–
	Deep	9	19, 14, 19	0.03±0.11	0.08	0.78

Table 2

Results of the GLM comparing mean body size (TL mm) of each fish category between the two study sites, shelter competitors and food competitors

Fish category	Study site	N	Mean TL±SD mm	$\beta \pm SE$	χ^2	P
Predator	Shallow	27	50.0±7.8	Reference	–	–
	Deep	157	68.1±28.8	0.51±0.08	37.47	<0.0001
Shelter competitor	Shallow	165	49.9±10.9	Reference	–	–
	Deep	160	65.0±12.3	0.26±0.02	104.36	<0.0001
Food competitor	Shallow	154	44.9±12.2	Reference	–	–
	Deep	162	65.9±24.4	0.37±0.04	92.04	<0.0001

predated fish ± SD (mm) = 30.88 ± 6.02; [Supplementary Material 1](#)), as well as five injured fish (shallow: 0/123 individuals; deep: 5/216 individuals; mean SL of injured fish ± SD (mm) = 29.02 ± 3.71, N = 5; [Supplementary Material 2](#)).

The areas of unoccupied stones were significantly larger at the deep study site (shallow: mean area cm²/m² = 871.3 ± 372.3, N = 15; deep: 1599.3 ± 671.8, N = 15; GLM: $\beta = 0.61 \pm 0.15$, $\chi^2_1 = 15.18$, P < 0.001). Furthermore, the areas of unoccupied stones were significantly larger than the shelter area in the respective study sites, suggesting that fish can potentially use these unoccupied stones in both of the study sites (shallow: mean shelter area cm²/m² = 216.2 ± 68.4, N = 20; deep: 364.6 ± 130.1, N = 20; [Table 3](#)).

Fish Density

The overall number of *N. obscurus* at the deep study site was comparable to that of the shallow study site (shallow: 284 individuals/32 m²; deep: 299 individuals/32 m²). In contrast, the number of helpers at the deep study site was about 1.7 times higher than at the shallow study site (shallow: 105 individuals/32 m²; deep: 179 individuals/32 m²).

Body Size and Group Composition

Neolamprologus obscurus were significantly larger at the deep study site ([Fig. 1](#), [Table 4](#)). The number of helpers increased with the body size of dominant females in both study sites, and more helpers per group were found at the deep study site ([Fig. 2](#), GLM: dominant female's body size: $\beta = 0.17 \pm 0.03$, $\chi^2_1 = 35.03$, P < 0.001; study site: $\beta = -0.82 \pm 0.33$, $\chi^2_1 = 6.59$, P = 0.01).

Table 3

Results of GLMs comparing the size of the shelter area at the two study sites

Variable	$\beta \pm SE$	df	χ^2	P
Intercept	4.80±0.15			
Study site		1	38.64	<0.0001
Shallow	Reference			
Deep	0.56±0.09			
Type of stone area		1	249.5	<0.0001
Shelter area	Reference			
Unoccupied stone area	1.44±0.09			

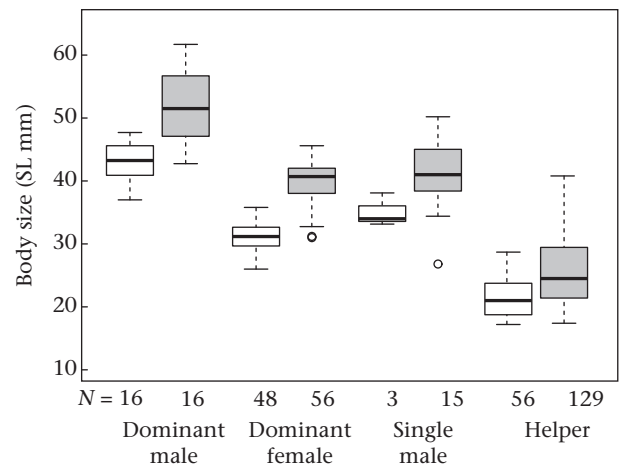


Figure 1. Body size of *N. obscurus* of each social rank at both study sites. Box plots show medians (thick horizontal lines), quartiles (boxes), 10% and 90% percentiles (dashed vertical lines with caps) and outliers (circles). White boxes indicate the shallow and grey the deep study site. Sample sizes are shown below each box.

Dispersal Events and Distance

We observed 13 helper dispersal events during the observation period. Dispersal propensity tended to be higher at the shallow study site (shallow: 7/56 helpers; deep: 6/129 helpers; Fisher's exact test: P = 0.07). The dispersal distance was significantly shorter at the deep than at the shallow study site (shallow: mean

Table 4

Results of the LM analysing the influence of study site and social rank on body size of individual *N. obscurus* at the two study sites

Variable	$\beta \pm SE$	df	χ^2	P
Intercept	38.84±0.50			
Study site		1	124.19	<0.0001
Shallow	Reference			
Deep	6.23±0.51			
Social rank		3	504.35	<0.0001
Dominant male	11.83±0.90			
Dominant female	Reference			
Single male	2.30±1.15			
Helper	-12.56±0.55			

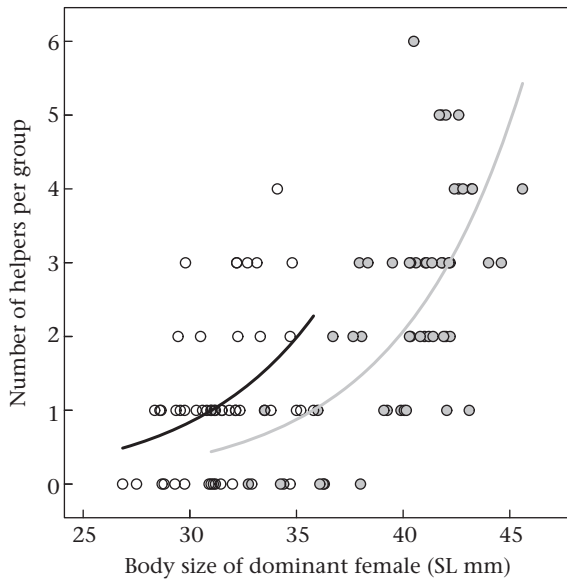


Figure 2. The relationship between the body size of dominant females and the number of helpers per group at the two study sites. White circles and the black regression line indicate the shallow study site ($N = 48$), grey circles and the grey line the deep one ($N = 56$).

dispersal distance \pm SD (cm) = 144.57 \pm 68.36; deep: 55.00 \pm 36.44; GLM: $\beta = -0.97$, $\chi^2_1 = 8.84$, $P = 0.003$). Dispersing individuals were significantly larger at the deep than at the shallow study site (shallow: mean SL \pm SD (mm) = 23.31 \pm 3.91; deep: 30.43 \pm 3.27; LM: $\beta = 7.12$, $\chi^2_1 = 9.82$, $P = 0.002$).

Relationship Between Relatedness and Helper Size

The within-group relatedness between helpers/juveniles and dominant females was higher at the deep study site (shallow: mean relatedness \pm SE = 0.22 \pm 0.03, $N = 68$; deep: 0.39 \pm 0.02, $N = 146$; LMM: $\beta = 0.16$, $\chi^2_1 = 12.55$, $P = 0.0004$). Moreover, the relationship between the body size of helpers/juveniles and the relatedness between helpers/juveniles and the respective dominant female differed between study sites (Fig. 3; LMM: effects of study site:

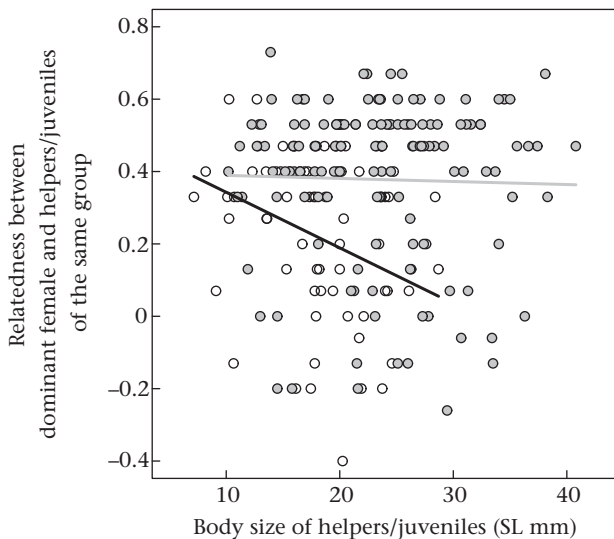


Figure 3. The relationship between the body size of helpers/juveniles and their relatedness to the respective dominant female. White circles and the black regression line indicate the shallow study site ($N = 68$), grey circles and the grey line the deep one ($N = 146$).

$\beta = -0.098 \pm 0.114$; effects of helper/juvenile body size: $\beta = -0.015 \pm 0.005$; effects of interaction term: $\beta = 0.015 \pm 0.005$, $\chi^2_1 = 7.17$, $P = 0.007$). While relatedness decreased significantly with increasing body size of helpers/juveniles at the shallow site (LMM: $\beta = -0.015 \pm 0.005$, $\chi^2_1 = 6.41$, $P = 0.01$), there was no such relationship at the deep site (LMM: $\beta = -0.001 \pm 0.002$, $\chi^2_1 = 0.15$, $P = 0.70$).

DISCUSSION

Predation risk is an important cause of sociality in many animal species (Caro, 2005; Krause & Ruxton, 2002). Interestingly, in studies investigating dispersal decisions of cooperative breeders, it has been largely overlooked (but see Heg et al., 2004; Rasa, 1987; Russell, 2004). We aimed at filling this gap by investigating the link between predation risk, group structure and dispersal decisions in a wild population of the cooperatively breeding fish *N. obscurus*. We found that the entrance size of shelters used by *N. obscurus* was larger in deeper water, which may offer better hunting conditions for predators. Indeed, there were more predators at the deep study site and predation events, as well as injured individuals, were only documented there. Additionally, unoccupied potential territories were more abundant at the deep than at the shallow study site. These findings suggest that the study population was substructured into a deep, risky site with ample space to establish new territories and a shallow, less risky site.

These ecological differences were reflected in dispersal patterns of helpers. Dispersal events tended to occur less often at the deep study site, despite the 1.7 times higher number of potential dispersers. In addition, dispersal distances were shorter and the dispersed helpers were larger at the deep study site. The micro-satellite analyses showed that the within-group relatedness between the dominant female and the respective helpers/juveniles was higher at the deep study site. Moreover, intragroup relatedness showed a significant decline with increasing body size of helpers/juveniles at the shallow study site, while there was no such relationship at the deep study site. These results suggest that dispersal is more restricted at the deep study site, and indicate that predation risk is a more important factor than habitat saturation for the dispersal of helpers in *N. obscurus*.

Predation risk can affect the dispersal of helpers in two phases: (1) during their search for a potential new group or a vacant territory and (2) until they have been accepted in the new group or have created a new shelter. In the process of actual dispersal, a disperser visits several groups, probably to seek out the optimal new group or a vacant territory for successful dispersal. During this time, they normally receive aggression from members of the new group (cf. Bergmüller, Heg, Peer, & Taborsky, 2005; Bergmüller, Heg, & Taborsky, 2005; Jungwirth, Walker, & Taborsky, 2015) and move between the new group and their home group before the successful settlement in the new group takes place. Predation risk may directly impair these behaviours. The relative short dispersal distance and the larger body size of dispersed helpers in the high-predation study site support this idea. When helpers disperse to a vacant territory, they have to establish a new shelter. In *N. obscurus*, individuals usually excavate sand and create a shelter up to 15 cm deep (H Tanaka, personal observation). Predators, especially spiny eels, often try to enter the shelter, seeking potential prey. Therefore, dispersers with incomplete, shallow shelters will face an increased predation risk. In addition, the costs of subsequent solitary defence of the territory will be high. The energetic cost of digging and creating a new shelter (cf. Grantner & Taborsky, 1998) may also affect dispersal decisions. In *N. obscurus*, frequency of sand digging for maintaining the shelter is six to 10 times higher than in other cooperatively breeding fish (Heg et al., 2005; Taborsky, 1985; Tanaka et al., 2015). Therefore, under high predation risk,

delaying dispersal and forming a group with relatives will benefit both offspring and parents (Dickinson, Ferree, Stern, Swift, & Zuckerberg, 2014; Ekman, Sklepkovych, & Tegelström, 1994; Griesser, 2003), even when the habitat is not fully saturated.

The larger body size of fish living at the deep study site can be explained in several ways. First, high predation risk selects for increased growth rates. Body size enlargement triggered by predation pressure has been shown in several other fish species (e.g. Brönmark & Miner, 1992; Frommen et al., 2011; Stratmann & Taborsky, 2014). Second, helpers may reach larger sizes because they stay longer in their natal territory, where they receive protection from dominants and other helpers. This assumption is supported by the finding that groups contained more helpers at the deep study site. Third, a small shelter entrance will set an upper limit for the body size at the shallow study site. Most fishes show indeterminate growth during their lifetime and the body size is often determined by the shelter or substrate that they use (e.g. Takahashi, Watanabe, Munehara, Rüber, & Hori, 2009). These hypotheses are not mutually exclusive, and may jointly explain the difference in body size between the two study sites in *N. obscurus*.

The remarkable differences in population structure of *N. obscurus* are expected to have broader implications for the evolution of cooperative breeding in this species. For example, differences in dispersal patterns and subsequent within-group genetic structure will eventually affect potential indirect fitness gains through cooperation. In addition, the more viscous population structure at the deep study site will increase the probability of inbreeding, which might have detrimental effects on inbreeding depression (see Koenig & Haydock, 2004 for a review on inbreeding depression in other cooperatively breeding systems). Finally, the differences in body size might imply differences in life history traits between the two study sites, such as the onset of reproductive timing or life expectancy.

Although factors affecting delayed dispersal in cooperative breeders have been intensively discussed (see Hatchwell & Komdeur, 2000), the impact of predation risk has received little attention. Studies on the cooperatively breeding fish *N. pulcher* have shown effects of predation risk on dispersal patterns that are comparable to that described in the present study (Groenewoud et al., 2016; Heg et al., 2004, 2008). Taking this into account, predation risk appears to be a major cause of delayed and limited dispersal in cooperatively breeding fish and might be an overlooked force in the evolution of cooperative breeding in general.

Acknowledgments

We thank Masaya Morita, Michio Hori and the staff of the Lake Tanganyika Research Unit (LRTU), Department of Fisheries, Republic of Zambia, especially Harris Phili, Danny Syninyza and Henry Simpembwa for their field support. We are grateful to Nobuyuki Kutsukake and Michael Griesser for discussions on dispersal, family formation and cooperative breeding in mammals and birds. We thank Haruki Ochi for discussion of predation and habitat preference in cichlid fish in Lake Tanganyika, and we are grateful to Theresa Burt de Perera, Stefan Fischer, Frank Groenewoud, Arne Jungwirth and an anonymous referee for helpful comments on the manuscript. This work was financially supported by JSPS KAKENHI Grant Numbers 25304017, 23570033 and 4501 to M.K.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.anbehav.2016.04.019>.

References

- Abe, N. (1997). Ecology of non-cichlids in the littoral zone of Lake Tanganyika. In H. Kawanabe, M. Hori, & M. Nagoshi (Eds.), *Fish communities in Lake Tanganyika* (pp. 241–256). Kyoto, Japan: Kyoto University Press.
- Albertson, R., Streebman, J., & Koehler, T. (2003). Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 5252–5257.
- Alexander, R. D. (1974). The evolution of social behavior. *Annual Review of Ecology and Systematics*, 5, 325–383.
- ASAB/ABS. (2012). Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour*, 99, 1–IX.
- Awata, S., Munehara, H., & Kohda, M. (2005). Social system and reproduction of helpers in a cooperatively breeding cichlid fish (*Julidochromis ornatus*) in Lake Tanganyika: field observations and parentage analyses. *Behavioral Ecology and Sociobiology*, 58, 506–516.
- Baglione, V., Marcos, J. M., Canestrari, D., Griesser, M., Andreotti, G., Bardini, C., et al. (2005). Does year-round territoriality rather than habitat saturation explain delayed natal dispersal and cooperative breeding in the carrion crow? *Journal of Animal Ecology*, 74, 842–851.
- Bates, D., Maechler, M., & Bolker, B. (2011). *lme4: Linear mixed-effects models using Eigen and R package version 0.999375-39*. <http://CRAN.R-project.org/package=lme4>.
- Bergmüller, R., Heg, D., Peer, K., & Taborsky, M. (2005). Extended safe havens and between-group dispersal of helpers in a cooperatively breeding cichlid. *Behaviour*, 142, 1643–1667.
- Bergmüller, R., Heg, D., & Taborsky, M. (2005). Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. *Proceedings of the Royal Society B: Biological Sciences*, 272, 325–331.
- Bocedi, G., Heinonen, J., & Travis, J. M. J. (2012). Uncertainty and the role of information acquisition in the evolution of context-dependent emigration. *American Naturalist*, 179, 606–620.
- Bourke, A. G. F. (2014). Hamilton's rule and the causes of social evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369, 20130362.
- Brandtmann, G., Scandura, M., & Trillmich, F. (1999). Female-female conflict in the harem of a snail cichlid (*Lamprologus ocellatus*): behavioral interactions and fitness consequences. *Behaviour*, 136, 1123–1144.
- Brönmark, C., & Miner, J. G. (1992). Predator-induced phenotypic change in body morphology in crucian carp. *Science*, 258, 1348–1350.
- Brown, J. L. (1987). *Helping and communal breeding in birds*. Princeton, NJ: Princeton University Press.
- Caro, T. (2005). *Antipredator defenses in birds and mammals*. Chicago, IL: University of Chicago Press.
- Clobert, J., Gaillard, L., Cote, J., Meylan, S., & Massot, M. (2009). Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology Letters*, 12, 197–209.
- Covas, R., & Griesser, M. (2007). Life history and the evolution of family living in birds. *Proceedings of the Royal Society B: Biological Sciences*, 274, 1349–1357.
- Dickinson, J. L., Ferree, E. D., Stern, C. A., Swift, R., & Zuckerberg, B. (2014). Delayed dispersal in western bluebirds: teasing apart the importance of resources and parents. *Behavioral Ecology*, 25, 843–851.
- Drobnjak, S., Wagner, G., Mouroucq, E., & Griesser, M. (2015). Family living: an overlooked but pivotal social system to understand the evolution of cooperative breeding. *Behavioral Ecology*, 26, 805–811.
- Ekman, J., Baglione, V., Eggers, S., & Griesser, M. (2001). Delayed dispersal: living under the reign of nepotistic parents. *Auk*, 118, 1–10.
- Ekman, J., Hatchwell, B. J., Dickinson, J. L., & Griesser, M. (2004). Delayed dispersal. In W. D. Koenig, & J. L. Dickinson (Eds.), *Ecology and evolution of cooperative breeding in birds* (pp. 35–47). Cambridge, U.K.: Cambridge University Press.
- Ekman, J., Sklepkovych, B., & Tegelström, H. (1994). Offspring retention in the Siberian Jay (*Perisoreus infaustus*): the prolonged brood care hypothesis. *Behavioral Ecology*, 5, 245–253.
- Emlen, S. T. (1982). The evolution of helping. I. An ecological constraint model. *American Naturalist*, 119, 29–39.
- Emlen, S. T. (1984). Cooperative breeding in birds and mammals. In J. R. Krebs, & N. B. Davis (Eds.), *Behavioural ecology* (pp. 305–337). Oxford, U.K.: Blackwell.
- Frommen, J. G., Herder, F., Engqvist, L., Mehli, M., Bakker, T. C. M., Schwarzer, J., et al. (2011). Costly plastic morphological responses to predator specific odour cues in three-spined sticklebacks (*Gasterosteus aculeatus*). *Evolutionary Ecology*, 25, 641–656.
- Gardner, J., Magrath, R., & Kokko, H. (2003). Stepping stones of life: natal dispersal in the group-living but noncooperative speckled warbler. *Animal Behaviour*, 66, 521–530.
- Goodnight, K. F., & Queller, D. C. (1999). Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology*, 8, 1231–1234.
- Grantner, A., & Taborsky, M. (1998). The metabolic rates associated with resting, and with the performance of agonistic, submissive and digging behaviours in the cichlid fish *Neolamprologus pulcher* (Pisces: Cichlidae). *Journal of Comparative Physiology B*, 168, 427–433.
- Griesser, M. (2003). Nepotistic vigilance behaviour in Siberian jay parents. *Behavioral Ecology*, 14, 246–250.
- Groenewoud, F., Frommen, J. G., Josi, D., Tanaka, H., Jungwirth, A., & Taborsky, M. (2016). Predation risk drives social complexity in cooperative breeders. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 4104–4109.

- Hatchwell, B. J., & Komdeur, J. (2000). Ecological constraints, life history traits and the evolution of cooperative breeding. *Animal Behaviour*, 59, 1079–1086.
- Heg, D., & Bachar, Z. (2006). Cooperative breeding in the Lake Tanganyika cichlid *Julidochromis ornatus*. *Environmental Biology of Fishes*, 76, 265–281.
- Heg, D., Bachar, Z., Brouwer, L., & Taborsky, M. (2004). Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proceedings of the Royal Society B: Biological Sciences*, 271, 2367–2374.
- Heg, D., Bachar, Z., & Taborsky, M. (2005). Cooperative breeding and group structure in the Lake Tanganyika cichlids *Neolamprologus savoryi*. *Ethology*, 111, 1017–1043.
- Heg, D., Heg-Bachar, Z., Brouwer, L., & Taborsky, M. (2008). Experimentally induced helper dispersal in colonially breeding cooperative cichlids. *Environmental Biology of Fishes*, 83, 191–206.
- Heg, D., Rothenberger, S., & Schürch, R. (2010). Habitat saturation, benefits of philopatry, relatedness, and the extent of co-operative breeding in a cichlid. *Behavioral Ecology*, 22, 82–92.
- Heg, D., & Taborsky, M. (2010). Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS One*, e10784.
- Hixon, M. A., & Beets, J. P. (1993). Predation, prey refuges, and the structure of coral-reef fish assemblages. *Ecological Monographs*, 63, 77–101.
- Hollén, L., & Radford, A. (2009). The development of alarm call behaviour in mammals and birds. *Animal Behaviour*, 78, 791–800.
- Hori, M. (1997). Structure of littoral fish communities organized by their feeding activities. In H. Kawanabe, M. Hori, & M. Nagoshi (Eds.), *Fish communities in Lake Tanganyika* (pp. 275–298). Kyoto, Japan: Kyoto University Press.
- Hori, M. (n.d.). [Data book of stomach contents analysis Vol. II (version 2.0): Zambia 1988–1989]. Unpublished data.
- Jungwirth, A., Josi, D., Walker, J., & Taborsky, M. (2015). Benefits of coloniality: communal defence saves anti-predator effort in cooperative breeders. *Functional Ecology*, 29, 1218–1224.
- Jungwirth, A., Walker, J., & Taborsky, M. (2015). Prospecting precedes dispersal and increases survival chances in cooperatively breeding cichlids. *Animal Behaviour*, 106, 107–114.
- Koenig, W. D., & Dickinson, J. L. (2004). *Ecology and evolution of cooperative breeding in birds*. Cambridge, U.K.: Cambridge University Press.
- Koenig, W. D., & Haydock, J. (2004). Incest and incest avoidance. In W. D. Koenig, & J. L. Dickinson (Eds.), *Ecology and evolution of cooperative breeding in birds* (pp. 142–156). Cambridge, U.K.: Cambridge University Press.
- Kohler, U. (1998). *Zur Struktur und Evolution des Sozialsystems von Neolamprologus multifasciatus (Cichlidae, Pisces), dem kleinsten Schneckenbuntbarsch des Tanganyikasees*. Aachen, Germany: Shaker Verlag.
- Kokko, H., & Ekman, J. (2002). Delayed dispersal as a route to breeding: territorial inheritance, safe havens, and ecological constraints. *American Naturalist*, 160, 468–484.
- Kokko, H., & Lundberg, P. (2001). Dispersal, migration, and offspring retention in saturated habitats. *American Naturalist*, 157, 188–202.
- Konovalov, D. A., & Heg, D. (2008a). Estimation of population allele frequencies from small samples containing multiple generations. In *Proceedings of 6th Asia-Pacific Bioinformatics Conference* (pp. 321–331).
- Konovalov, D. A., & Heg, D. (2008b). A maximum-likelihood relatedness estimator allowing for negative relatedness values. *Molecular Ecology Resources*, 8, 256–263.
- Konovalov, D. A., Manning, C., & Henshaw, M. T. (2004). KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. *Molecular Ecology Notes*, 4, 779–782.
- Krause, J., & Ruxton, G. D. (2002). *Living in groups*. New York, NY: Oxford University Press.
- Lucia, K. E., Keane, B., Hayes, L. D., Lim, Y. K., Schaefer, R. L., & Solomon, N. G. (2008). Philopatry in prairie voles: an evaluation of the habitat saturation hypothesis. *Behavioral Ecology*, 19, 774–783.
- Marshall, T. C., Slate, J., Kruuk, L., & Pemberton, J. M. (1998). Statistical confidence for likelihood based paternity inference in natural populations. *Molecular Ecology*, 7, 639–655.
- Maynard Smith, J., & Ridpath, M. G. (1972). Wife sharing in the Tasmanian native hen, *Tribonyx mortierii*: a case of kin selection? *American Naturalist*, 106, 447–452.
- Munehara, H., Awata, S., Katoh, R., Kohda, M., & Sunobe, T. (2001). Primer sequences and cross-species amplification for parentage discrimination of Tanganyikan cichlid fishes. *Bulletin of Fisheries Sciences, Hokkaido University*, 52, 131–133.
- van Oppen, M. J. H., Rico, C., Deutsch, J. C., Turner, G. F., & Hewitt, G. M. (1997). Isolation and characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Molecular Ecology*, 6, 387–388.
- Parker, A., & Kornfield, I. (1996). Polygyny in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environmental Biology of Fishes*, 47, 345–352.
- R Core Team. (2014). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rasa, O. A. E. (1987). The dwarf mongoose: a study of behavior and social structure in relation to ecology in a small, social carnivore. *Advances in the Study of Behavior*, 17, 121–163.
- Rasband, W. S. (2014). *ImageJ* (pp. 1997–2014). Bethesda, Maryland, USA: U. S. National Institutes of Health. <http://imagej.nih.gov/ij/>.
- Reyer, H. U. (1965). Investment and relatedness: a cost/benefit analysis of breeding and helping in the pied kingfisher (*Ceryle rudis*). *Animal Behaviour*, 32, 1163–1178.
- Rowley, I. (1965). The life history of the superb fairy wren, *Malurus cyaneus*. *Emu*, 64, 251–297.
- Russell, A. F. (2004). Mammals: comparisons and contrasts. In W. D. Koenig, & J. L. Dickinson (Eds.), *Ecology and evolution of cooperative breeding in birds* (pp. 210–227). Cambridge, U.K.: Cambridge University Press.
- Schliwien, U., Rassmann, K., Markmann, M., Markert, J., Kocker, T., & Tautz, D. (2001). Genetic and ecological divergence of a monophyletic cichlid species pair under fully sympatric conditions in the Lake Ejagham, Cameroon. *Molecular Ecology*, 10, 1471–1488.
- Selander, R. K. (1964). In *Speciation in wrens of the genus Campylorhynchus* (Vol. 74, pp. 1–224). University of California Publications in Zoology.
- Solomon, N., & French, J. (1997). *Cooperative breeding in mammals*. Cambridge, U.K.: Cambridge University Press.
- Stratmann, A., & Taborsky, B. (2014). Antipredator defences of young are independently determined by genetic inheritance, maternal effects and own early experience in mouthbrooding cichlids. *Functional Ecology*, 28, 944–953.
- Taborsky, M. (1985). Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. *Behaviour*, 95, 45–75.
- Taborsky, M. (1994). Sneakers, satellites and helpers: parasitic and cooperative behaviour in fish reproduction. *Advances in the Study of Behavior*, 23, 1–100.
- Takahashi, T., Watanabe, K., Munehara, H., Rüber, L., & Hori, M. (2009). Evidence for divergent natural selection of a Lake Tanganyika cichlid inferred from repeated radiations in body size. *Molecular Ecology*, 18, 3110–3119.
- Tanaka, H., Heg, D., Takeshima, H., Takeyama, T., Awata, S., Nishida, M., et al. (2015). Group composition, relatedness and dispersal in the cooperatively breeding cichlid *Neolamprologus obscurus*. *Behavioral Ecology and Sociobiology*, 69, 169–181.
- West, S. A., Griffin, A. S., & Gardner, A. (2007). Evolutionary explanations for cooperation. *Current Biology*, 17, 661–672.
- Woolfenden, G. E., & Fitzpatrick, J. W. (1984). *The Florida Scrub Jay: Demography of a cooperative-breeding bird*. Princeton, NJ: Princeton University Press.
- Zardoya, R., Vollmer, D. M., Craddock, C., Streelman, J. T., Karl, S., & Meyer, A. (1996). Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (Pisces: Perciformis). *Proceedings of the Royal Society B: Biological Sciences*, 263, 1589–1598.

APPENDIX

Table A1
Summary of 11 microsatellites used in the study

Locus	N	N _A	H _O	H _E	HWE	Null	Source
758/773	416	16	0.779	0.767	0.12	–0.0141	Schliwien et al. (2001)
chb1	416	22	0.906	0.927	0.01	0.0112	Munehara, Awata, Katoh, Kohda, and Sunobe (2001)
GM264	416	9	0.714	0.7	0.35	–0.0067	Albertson, Streelman, and Kocher (2003)
NP101	416	21	0.873	0.874	0.57	–0.0009	Brandtmann, Scandura, and Trillmich (1999)
TmoM7	416	2	0.488	0.499	0.70	0.0106	Zardoya et al. (1996)
TmoM11	416	9	0.368	0.42	0.04	0.0609	Zardoya et al. (1996)
Pzeb1	416	11	0.615	0.597	0.32	–0.0244	van Oppen, Rico, Deutsch, Turner, and Hewitt (1997)
Pzeb3	416	8	0.762	0.726	0.12	–0.0273	van Oppen et al. (1997)
ULI2	416	17	0.853	0.853	0.71	–0.0007	Schliwien et al. (2001)
UME002	416	6	0.262	0.267	0.81	0.0089	Parker and Kornfield (1996)
UME003	416	19	0.892	0.895	0.63	0.0015	Parker and Kornfield (1996)

The table shows the number of adults successfully typed (N), the number of different alleles (N_A), observed (H_O) and expected heterozygosities (H_E), P values of Hardy–Weinberg equilibrium tests (HWE, all loci were nonsignificant after Bonferroni correction) and estimated null allele frequencies (Null).