Original Article

Task-dependent workload adjustment of female breeders in a cooperatively breeding fish

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Parental investment affects the future survival and reproductive success of breeders. Therefore, breeders should optimize the amount of care they invest into the current offspring. In cooperative breeding systems, the amount of breeders' parental care is influenced by the behavior of brood-care helpers. Such workload adjustment is expected to depend on the task that needs to be fulfilled. While investment rules of breeders in respect to single tasks are well investigated in many bird and mammal species, little is known about behavioral adjustment of breeders when dealing with multiple tasks. Here, we examined the workload adjustment in multiple tasks of female breeders in the cooperatively breeding fish *Neolamprologus obscurus*. By combining behavioral observations with helper removal experiments in a wild population, we show that female territory defense and offspring care significantly decreased with increasing helper number. Furthermore, the workload invested in these tasks significantly increased after the removal of a helper, suggesting load-lightening effects in territory defense and offspring care. On the other hand, female territory maintenance behavior (i.e., excavating sand from the breeding shelter) did not correlate with helper number. While sand excavation significantly increased after the helper removal experiment, the size of the excavated stone area decreased after the helper removal in the recent study, suggesting that sand excavation may have additive effects for the breeders. These results demonstrate and underline the importance of task-dependent workload adjustment in cooperative breeders.

Key words: **additive effects, brood care, cichlid, group living, load-lightening effects, *Neolamprologus obscurus***

INTRODUCTION

In animals that provide parental care to their offspring, parents should adjust and optimize their care effort according to their future reproductive success (Stearns 1992). In cooperatively breeding animals, individuals other than breeders assist in raising young (Brown 1987; Solomon and French 1997; Koenig and Dickinson 2016). In such cooperatively breeding systems, behavioral decisions of breeders about how much they invest in parental care depend on the amount of help they receive from helpers. Numerous empirical and theoretical studies have shown that breeders adjust their workload in 2 ways (Hatchwell 1999; Heinsohn 2004): first, the behavior of helpers may contribute to reduce the effort of breeder's parental care, known as *load-lightening effects* (Brown 1978; Crick

1992; Hatchwell and Russell 1996). Due to the helper's compensatory care, breeders may reduce their own investment and in turn increase their future reproduction (Brown et al. 1978; Wright and Dingemanse 1999; Russell et al. 2008; Sharp et al. 2013; Zöttl et al. 2013a; Brouwer et al. 2014). Second, the amount of breeders' workload may remain constant irrespective of the behavior of helpers. Here, helpers increase the current breeding success of breeders due to *additive effects* (Emlen and Wrege 1991; Liebl et al. 2016). Recent theoretical studies predict that such differences in parental responsiveness to helping behavior can be explained by the shape of the cost function of the respective parental care task (Johnstone 2011). In addition, increasing evidence from both theoretical and empirical studies suggest that multiple factors, such as relatedness to breeders, sex of helpers, number of offspring, or female cost of reproduction, are associated with the workload adjustment of breeders (MacColl and Hatchwell 2003; Russell et al. 2007; Savage et al. 2013; Brouwer et al. 2014; Savage et al. 2015). This argument

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might be especially true in species where helping individuals show task specialization (Bruintjes and Taborsky 2011). Although most studies have focused on single parental care tasks (Hatchwell and Russell 1996; Wright and Dingemanse 1999; Gilchrist and Russell 2007; Russell et al. 2008; Sharp et al. 2013, but see Clutton-Brock et al. 2004; Zöttl et al. 2013a), behavioral adjustments of breeders are not necessarily the same when it comes to different tasks. Here, it is possible that breeders benefit from load-lightening effects in some tasks, and from additive effects in others (Johnstone 2011).

In cooperatively breeding fishes, territory defense against predators, territory maintenance, and egg care (providing oxygen and cleaning the eggs) are the most important tasks for individuals engaging in parental care (see Taborsky 2016 for a review). Behavioral decisions of breeders depending on helpers' workload are well investigated in the cooperatively breeding African cichlid *Neolamprologus pulcher*. Here, a field study showed that breeders' total workload is negatively correlated with the number of helpers in their territory (Balshine et al. 2001). In addition, laboratory experiments showed that when helpers were prevented from providing help, breeders increased their territory defense (Bergmüller and Taborsky 2005) and alloparental brood care (Zöttl et al. 2013a), suggesting load-lightening effects in this species. Helping behavior is usually prolonged in fishes, that is, helpers do not only help during the parental phase (Bergmüller and Taborsky 2005; Heg et al. 2005; Heg and Bachar 2006; Tanaka et al. 2015). Thus, breeders may benefit from helping behavior also when helpers are not directly engaging in parental care. However, to our knowledge, the behavioral decisions of breeders during the non-parental care period are rarely investigated. Furthermore, while several fishes are known to breed cooperatively (Taborsky and Limberger 1981; Yamagishi and Kohda 1996; Kohler 1998; Awata et al. 2005; Heg et al. 2005; Heg and Bachar 2006; Tanaka et al. 2015), little is known about breeders' behavioral adjustments in most of the species. Such lack of knowledge is contrasting the rich evidence in birds (e.g., Crick 1992; Hatchwell 1999; Liebl et al. 2016) and mammals (e.g., Clutton-Brock et al. 2004; Gilchrist and Russell 2007), making it difficult to compare the evolution of cooperative breeding among these different taxonomic groups.

To understand breeders' behavioral decisions in cooperatively breeding fishes more generally, we investigated the behavioral adjustment of breeders' workload and the effect of helpers in the understudied cooperatively breeding cichlid fish *Neolamprologus obscurus*. *N. obscurus* is a small benthic fish, which inhabits rocky-sandy habitats in Lake Tanganyika (Konings 1998). In this species, breeder females form a breeding unit with up to 10 helpers (hereafter termed "group"). Breeder males are either monogamous or monopolize several of such female groups (Tanaka et al. 2015). Group members occupy excavated cavities, which they dig under stones. These cavities serve as shelters from predation for all group members, including fry (Tanaka et al. 2016) and provide the spawning substrate for the female (Tanaka et al. 2015). Moreover, the cavities serve as trap for benthic invertebrates, which are the main food resource of *N. obscurus* (Tanaka et al., *in revision*). Therefore, defending and maintaining the excavated cavities is of high importance for breeders and helpers. Helpers join the female in territory defense and maintenance (Tanaka et al. 2015). Such helping behaviors are thought to be costly in terms of time and energy investment. For example, digging behavior raised the metabolic rate of *N. pulcher* by 6.1 times compared to the resting metabolic rate (Taborsky and Grantner 1998). A recent study showed that the investment of helpers in territory defense and maintenance is lower

compared to the breeder females in *N. obscurus*, leaving the extent of beneficial effects of helpers unclear (Tanaka et al. 2015). Helpers might provide task-dependent load-lightening or additive effects, which may be beneficial in terms of females' current and future reproductive success.

To test how helpers affect behavioral adjustments in different parental care tasks of breeder females, we first conducted behavioral observations of breeding groups under natural conditions. If females adjust their effort according to the number of helpers, we expect their workload to decline with increasing number of helpers, while we expect no such relationship when females engage in parental care irrespective of helper numbers. Next, we conducted a removal experiment of helpers to examine the behavioral response of breeder females. To elucidate whether helper effects are task dependent, we measured 3 different parental care tasks as well as foraging behavior. If the contributions of helpers lead to load-lightening effects, we expect an increased workload of breeder female after removing a helper from their group. On the other hand, if the benefits of helping behavior are additive, we expected the amount of breeder females' workload to be the same throughout the experiment. Finally, in order to investigate whether the breeders modify their behavioral decision also outside the reproductive period we tested whether the effect of helpers changed with the presence of fry.

METHODS

Study species and study site

The study was 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). The underwater landscape at this site consists of a steep sandy slope with partially exposed stones. *N. obscurus* inhabits this site in depths between 5 and 13 m. Data were collected by SCUBA diving. Data collection always started after a 5-min habituation time, during which the observer remained motionless in front of the respective territory. Fish with a body size larger than 17 mm standard length (hereafter SL, measured from the tip of the snout to the posterior end of the last vertebra) can frequently be found outside the excavated cavities, where they engage in territory defense and maintenance, while fish below 17 mm SL rarely leave the shelter (Tanaka et al. 2016). We therefore defined fish above 17 mm SL as helpers and fish below 17 mm SL as juveniles, following Tanaka et al. (2016).

Behavioral observations under natural conditions

All observations were conducted between August 20th and December 17th 2013 at 2 separate sites of the study population: a shallow (6–8 m depth) and deep one (11–13 m depth, see Tanaka et al. 2016 for details). The 2 study sites were at minimum 21.5 m apart. At each site, we installed a 4 × 8 m grid, subdivided into 1 × 1 m cells using rope in order to acquire the position of each groups. First, all groups inside the 2 study sites were marked by placing numbered stones close to the respective territory. Second, all fish larger than 17 mm were individually identified using their body color patterns, which were noted down on water resistant paper. Third, we assessed the social rank of all individuals by observing each group for 10 min (see Tanaka et al. 2015 for the definition of each social rank), and recorded the respective group compositions. Afterwards, we randomly chose 20 focal groups from each of the 2 study sites. We recorded the behavior of these 40 breeder females and their helpers for 20 min. We recorded aggressive behaviors

shown to con- and heterospecific territory intruders (sum of overt and restrained aggression, see Tanaka et al. 2015 for details about each type of aggression), all digging near or inside the excavated cavities as measure of territory maintenance, visits to the excavated cavities as a proxy of brood care (cf. Balshine et al. 2001) and pecking on the substrate as a proxy of feeding on benthic invertebrates. Breeder females usually attempt to keep the breeder males away from their territory, presumably due to competition for benthic food resources (Tanaka et al. 2015). In addition, breeder males usually monopolize several breeder females inside their territories (median = 3 breeder females per breeder male, $n = 16$ in each study site), and rarely contribute to territory maintenance, defense, or brood care of their females. Therefore, we did not focus on the behavior of breeder males in this study.

Within 2 weeks after the behavioral observations, we caught all fish, including juveniles, of the focal groups using hand nets and 30% clove oil diluted in ethanol as anesthetic. All the fish were brought back to the field station in Mpulungu city. We anesthetized all fish using the anesthetic FA100 (10% solution of eugenol; Tanabe Seiyaku Inc.) and measured their SL to the nearest 0.05 mm in the laboratory using a caliper. Samples were then used in further studies (Tanaka H, unpublished data). Juveniles only rarely leave the excavated cavities (Tanaka et al. 2015), and checking for the existence of juveniles without opening and disturbing the excavated cavities is difficult. For this reason, we conducted the sampling within 2 weeks after the behavioral observations were finished, and regarded all the groups containing juveniles as being under parental care.

Helper removal experiment

We conducted the helper removal experiment close to the deep study site from November 15th to December 17th 2013 and from July 28th to December 23th 2015. We randomly chose 36 groups, which consisted of a breeder female and of more than one helper. All focal groups were marked by placing numbered stones near to the excavated cavities. Next, we observed each focal group for 10 min to record the group composition and assess the social rank by the behavioral interactions among group members. Afterwards, we observed the behavior of the breeder female for 30 min (hereafter termed "initial observation"). We recorded the amount of aggressive behaviors shown to con- and heterospecific territory intruders, digging behavior in the excavated cavities, visits to the excavated cavities, and pecking on the substrate inside the territory. Within 10 min after the initial observation, we removed one helper from the group, using hand nets and hooks with dried shrimp as bait. Helper removal was conducted when all the group members except the target helper were in the excavated cavities, in order to keep the potential disturbance as small as possible. Out of 36 groups, we randomly chose 18 in which we released the captured helper 5 min after the catching as a control treatment. After 5 min of either helper removal or release, we started the second behavioral observation of the respective breeder female for 30 min to investigate their immediate response to the helper removal (termed "first post observation" hereafter). To investigate whether the behavioral responses of breeder females change over time, a third 30-min behavioral observation was conducted between 3 and 4 h after the end of the initial observation (termed "second post observation" hereafter). Afterwards, we caught all focal group members and measured all fish including juveniles as described

before. Caught fish were sacrificed and used for subsequent analyses (Tanaka H, unpublished data).

Statistical analysis

Data were analyzed using R version 3.1.1 (R Core Team 2014). We fitted generalized linear mixed models (GLMMs) using the lme4 package (Bates et al. 2011). In all models, either a Poisson error distribution with log link, or a gamma error distribution with log link was assumed. All tests were 2-tailed. We predicted that the different behaviors show different responses to the treatment as a central hypothesis to our research questions. Therefore, we did not correct test statistics for multiple testing in order to avoid type II errors. Still, results would not qualitatively change with a Bonferroni-adjusted α level of 0.0125.

To test the effect of the helper number on the observed female behavior, we fitted separate GLMMs on the 4 behavioral measures of the breeder females. We included number of helpers, body size of the breeder female, and presence of juveniles as explanatory variables in all of the 4 models. In the analysis of the frequency of visits to the excavated cavities, we only analyzed groups where juveniles were present ($n = 15$). A previous study revealed that the shallow and deep site differ in their ecological settings (Tanaka et al. 2016). To account for this, we included study site ("shallow" or "deep") as a random factor. We further included the ID of the breeder male as a random factor to correct for analyzing several females belonging to the same male.

To analyze the helper removal experiment, we fitted 4 GLMMs on the different behaviors of the breeder females. We included observation period ("initial observation", "first post observation", or "second post observation", with "initial observation" as reference), treatment ("control" or "experiment", with "control" as reference), body size of breeder female, body size of removed helper, and existence of juveniles as explanatory variables. Furthermore, to compare the difference of female behavior before and after the helper removal between the experiment and control treatment, we included the interaction between observation period and treatment. As the factors, "observation period" and "treatment" had 3 and 2 levels, respectively, the output of the interaction term will show 2 effects, one comparing the difference between control and experiment in the initial observations and first post observation period, and a second comparing these differences in the initial and second post observation period. Note that the term "treatment" of these models return the results of behavioral differences between control and experimental treatment in the initial observation period.

Next, to analyze the direction of behavioral changes before and after the helper removal, we fitted GLMMs on the experimental treatment alone. Again, the 4 behavioral measures of the breeder female served as response variable. We included observation period as explanatory variable in these models. We did not include the body size of the breeder female, the body size of removed helper, and the presence of juveniles in these models, since we did not find any significant effects in the initial model (see Results). In all GLMMs, we incorporated group ID as a random factor to account for measuring each group 3 times. We set "initial observation" (for the variable "observation period") and "control" (for the variable "treatment") as a reference category when the model included observation period or treatment. Again, we only used the groups where we found juveniles for the analysis of the frequency of visits into the excavated stone area ($n = 17$).

RESULTS

Behavioral observations

Breeder females' amount of aggression towards con- and hetero-specific intruders and visits to the excavated cavities significantly decreased with increasing number of helpers, while the frequency of feeding on the substrate increased with increasing number of helpers (Figure 1a, c and d; Table 1). The body size of the breeder female was positively correlated to the frequency of aggression and feeding on the substrate, and negatively correlated to the frequency of visits to the excavated cavities (Table 1). Sand removal by the breeder female was not significantly correlated to the number of helpers (Figure 1b; Table 1). The presence of juveniles did not affect the amount of aggression, visits to the excavated cavities, or substrate feeding, while breeder female having juveniles showed less sand removal (Table 1).

Helper removal experiment

Females in the experimental and control treatments differed in their behavioral reaction to the treatment as indicated by a significant interaction term (Figure 2a–d; Table 2). Body size of the breeder female and body size of the removed helper as well as the presence

of juveniles had no significant effect on all of the tested female behaviors (Table 2). The frequency of each behavior during the initial observation period did not differ between control and experimental treatment (term "Treatment" of the respective models in Table 2). We found that aggression towards intruders significantly decreased, while female sand removal, visits to the excavated cavities and feeding on substrate remained unchanged after the helper removal in the control treatment (term "Observation period" of the respective models in Table 2). To examine the influence of helper removal on the experimental treatment groups, we analyzed the data sets of experimental groups separately from that of control groups. In the experimental treatment, aggression towards intruders and visits to the excavated cavities both significantly increased after the helper removal (Table 3). On the other hand, the amount of female sand removal significantly increased only in the second post observation, and the frequency of feeding on substrate by female significantly decreased only during first post observation, respectively (Table 3).

DISCUSSION

While workload adjustment of breeders in single tasks has been described in a wide range of cooperatively breeding systems,
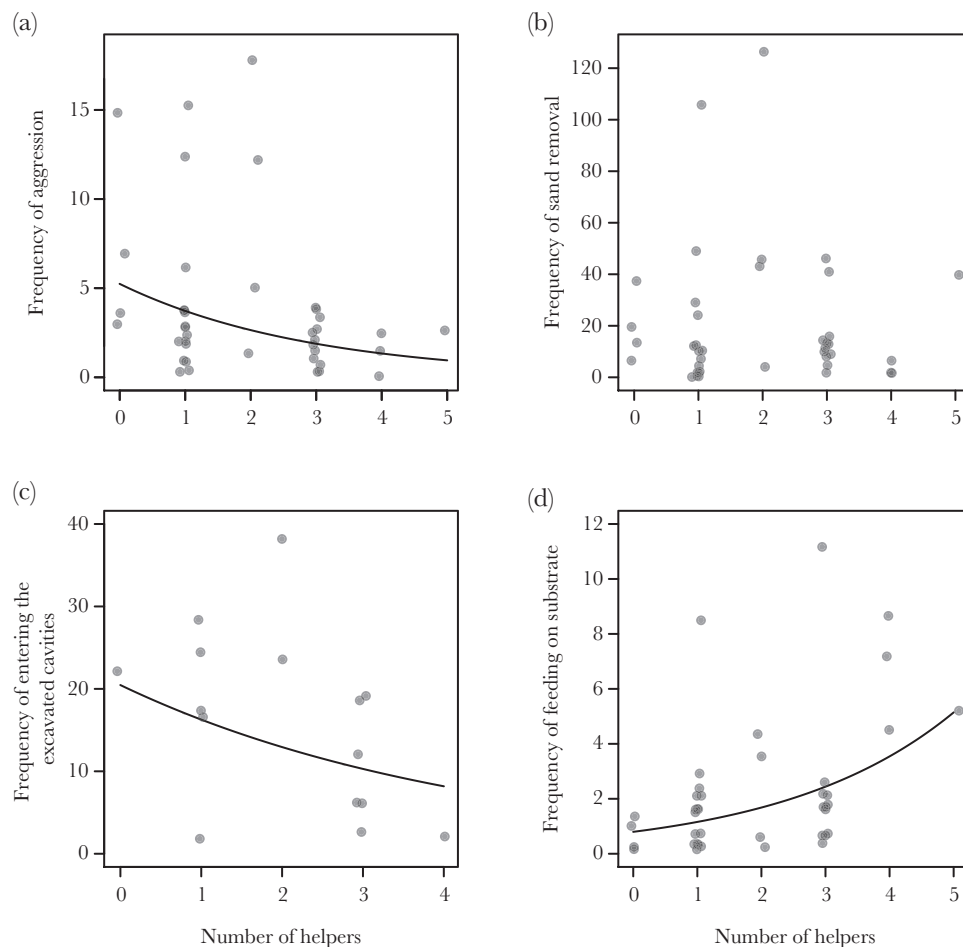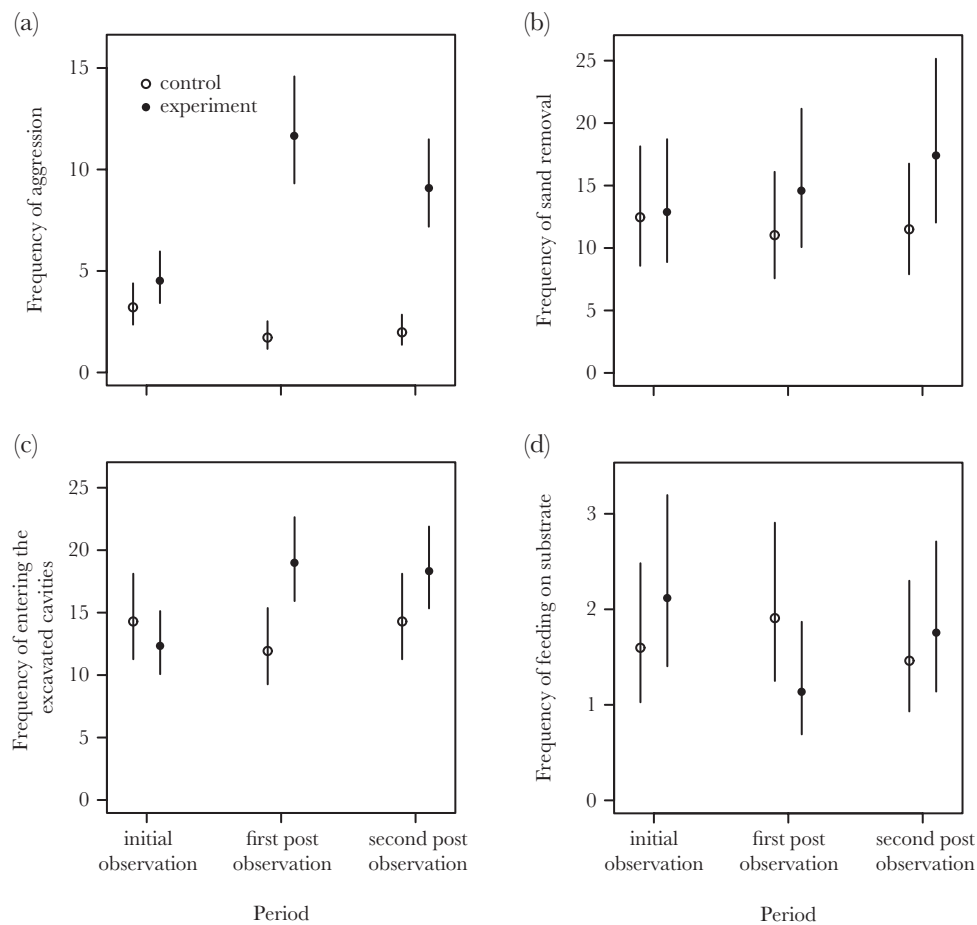

Figure 1

The relationship between the number of helpers and the (a) frequency of aggression toward intruders, (b) frequency of sand removal, (c) frequency of visit to the excavated cavities, and (d) frequency of feeding on the substrate of breeder females. Provided plots are residuals from the predicted GLMMs, corrected for breeder female body size and existence of juveniles. Plots are jittered on the x axis to avoid overlap of points, with its' color half transparent. The regression lines indicate the predicted relationship from the GLMMs (see Table 1 for statistical results).

Results of GLMMs investigating the factors affecting the behavior of breeder females during the observational study

Models and explanatory variables	β ± SE	z-value	P-value
Frequency of aggression ($N = 40$)			
Intercept	−2.77 ± 1.33	−2.09	0.04
Number of helpers	−0.34 ± 0.10	−3.38	0.0007
Body size	0.12 ± 0.04	3.26	0.001
Existence of juveniles	0.04 ± 0.32	0.12	0.90
Frequency of sand removal ($N = 40$)			
Intercept	2.69 ± 1.18	2.16	0.02
Number of helpers	0.03 ± 0.07	0.49	0.62
Body size	−0.003 ± 0.03	−0.08	0.94
Existence of juveniles	−0.54 ± 0.22	−2.45	0.01
Frequency of visit into excavated cavities ($N = 15$)			
Intercept	5.43 ± 1.58	3.43	0.0006
Number of helpers	−0.23 ± 0.09	−2.53	0.01
Body size	−0.07 ± 0.05	−1.45	0.15
Frequency of feeding on substrate ($N = 40$)			
Intercept	3.08 ± 1.54	1.99	0.05
Number of helpers	0.37 ± 0.13	2.95	0.003
Body size	−0.09 ± 0.05	−1.96	0.05
Existence of juveniles	−0.17 ± 0.43	−0.41	0.68

Figure 2

Behavioral changes of breeder females before and after the helper removal experiment. Plots show predicted values of the (a) frequency of aggression toward intruders, (b) frequency of sand removal, (c) frequency of visit to excavated cavities, and (d) frequency of feeding on substrate of control (white) and experimental (black) treatment obtained from respective GLMMs (see Table 2 for statistical results). Vertical bars indicate the 95 percent confidential intervals.

Table 2

Results of the GLMMs from the helper removal experiment

Models and explanatory variables	$\beta \pm$ SE	z-value	P-value
Frequency of aggression ($N = 36$)			
Intercept	1.64 ± 1.47	1.12	0.26
Observation period (initial observation as reference)			
First post observation	−0.63 ± 0.21	−2.95	0.003
Second post observation	−0.49 ± 0.20	−2.40	0.02
Treatment (control as reference)			
Experiment	0.34 ± 0.22	1.55	0.12
Observation period × treatment (initial observation × control as reference)			
First post observation × experiment	1.58 ± 0.25	6.36	<0.0001
Second post observation × experiment	1.19 ± 0.24	4.89	<0.0001
Body size of breeder female	−0.01 ± 0.04	−0.16	0.87
Body size of removed helper	−0.01 ± 0.02	−0.40	0.69
Existence of juvenile	0.17 ± 0.15	1.08	0.28
Frequency of sand removal ($N = 36$)			
Intercept	1.18 ± 2.42	0.49	0.62
Observation period (initial observation as reference)			
First post observation	−0.12 ± 0.09	−1.38	0.17
Second post observation	−0.08 ± 0.09	−0.93	0.35
Treatment (control as reference)			
Experiment	0.03 ± 0.28	0.11	0.91
Observation period × treatment (initial observation × control as reference)			
First post observation × experiment	0.25 ± 0.12	2.07	0.04
Second post observation × experiment	0.38 ± 0.12	3.28	0.001
Body size of breeder female	0.07 ± 0.07	1.03	0.30
Body size of removed helper	−0.05 ± 0.04	−1.30	0.20
Existence of juvenile	0.35 ± 0.25	1.41	0.16
Frequency of visit into excavated cavities ($N=17$)			
Intercept	4.75 ± 1.41	3.38	0.0007
Observation period (initial observation as reference)			
First post observation	−0.18 ± 0.15	−1.20	0.23
Second post observation	0.00 ± 0.14	0.00	0.99
Treatment (control as reference)			
Experiment	−0.15 ± 0.16	−0.90	0.37
Observation period × treatment (initial observation × control as reference)			
First post observation × experiment	0.61 ± 0.19	3.26	<0.0001
Second post observation × experiment	0.40 ± 0.18	2.16	0.03
Body size of breeder female	−0.06 ± 0.03	−1.84	0.07
Body size of removed helper	0.01 ± 0.02	0.70	0.48
Frequency of feeding on substrate ($N = 36$)			
Intercept	0.47 ± 2.30	0.20	0.84
Observation period (initial observation as reference)			
First post observation	0.18 ± 0.22	0.80	0.43
Second post observation	−0.09 ± 0.24	−0.37	0.71
Treatment (control as reference)			
Experiment	0.28 ± 0.31	0.90	0.37
Observation period × treatment (initial observation × control as reference)			
First post observation × experiment	−0.80 ± 0.34	−2.34	0.02
Second post observation × experiment	−0.10 ± 0.33	−0.30	0.76
Body size of breeder female	0.03 ± 0.06	0.51	0.61
Body size of removed helper	−0.05 ± 0.04	−1.12	0.26
Existence of juvenile	0.08 ± 0.24	0.33	0.74

simultaneous behavioral adjustment to multiple tasks have rarely been investigated (but see Clutton-Brock et al. 2004; Zöttl et al. 2013a). In this study, we examined whether and how helping behavior affects the breeder's workload in the cooperatively breeding cichlid fish *N. obscurus* into multiple tasks. We show that under natural conditions, the investment of breeder females in aggressive behavior towards intruders and into care for offspring is negatively correlated with the number of helpers. These observational findings were supported by the helper removal experiment, where the frequency of these 2 behaviors significantly increased after the helper removal. These results suggest that helpers are lightening the load of the breeder female in territory defense and offspring care. On the other hand, sand removal by the breeder female did not correlate with the number of helpers. Although sand removal significantly increased 3–4 h after helper removal, a further study showed that the size of the excavated stone area decreased 1 week after the helper removal (Tanaka et al. *in revision*), suggesting that sand removal of helpers is a rather additive effect in *N. obscurus*.

Our results provide the first evidence of task-dependent workload adjustment of breeders in cooperatively breeding animals. Such task-dependent workload adjustment of breeders in *N. obscurus* can be explained by the balance of cost and benefit ratios, predicted by Hatchwell (1999) and Johnstone (2011). The additive effect of helpers on sand removal in *N. obscurus* may be explained by the multiple

Models and explanatory variables	$\beta \pm SE$	z-value	P-value
Frequency of aggression in experiment ($N = 18$)			
Intercept	1.50 ± 0.14	11.04	<0.0001
Observation period (initial observation as reference)			
First post observation	0.95 ± 0.13	7.51	<0.0001
Second post observation	0.70 ± 0.13	5.33	<0.0001
Frequency of sand removal in experiment ($N = 18$)			
Intercept	2.48 ± 0.23	10.66	<0.0001
Observation period (initial observation as reference)			
First post observation	0.12 ± 0.08	1.56	0.12
Second post observation	0.30 ± 0.08	3.92	<0.0001
Frequency of visit into excavated cavities in experiment ($N = 10$)			
Intercept	2.55 ± 0.11	23.57	<0.0001
Observation period (initial observation as reference)			
First post observation	0.43 ± 0.11	3.83	0.0001
Second post observation	0.40 ± 0.1	3.49	0.0004
Frequency of feeding on substrate in experiment ($N = 18$)			
Intercept	0.74 ± 0.19	3.95	<0.0001
Observation period (initial observation as reference)			
First post observation	-0.62 ± 0.26	-2.38	0.02
Second post observation	-0.19 ± 0.23	-0.82	0.42

functions of the excavated cavities. In *N. obscurus*, excavated cavities serve as food trap for capturing benthic invertebrate prey in addition to the shelter for all group members including offspring. Prey capture linearly increases according to the size of the excavated stone area, suggesting that the extension of excavated cavities by removing sand will directly increase the food abundance in the territory (Tanaka et al., *in revision*). As food is limited under natural conditions, there should be no upper limit after which additional digging of excavated cavities will not be beneficial anymore. In contrast, the cost to benefits ratio of other helping tasks, that is, territory defense and offspring care, will decline with increasing frequency of the behavior at a given threshold. For instance, the benefits of aggression towards territory intruders depend on the frequency of intruder visits to the territory, and excessive aggressive behavior to a territory intruder may not significantly increase the benefits anymore. Likewise, the benefits of providing oxygen to the eggs by circulating water with the pectoral fins will reach a plateau at a given amount of such fanning behavior. Therefore, if helpers stably engage in territory defense and offspring care, breeder females will be safe to reduce their workload in the presence of helpers. In *N. obscurus*, most of the helpers are closely related to the breeder female and helpers will gain indirect fitness benefits through helping (Tanaka et al. 2015). This will increase their propensity to consistently help the breeder female (cf. Kokko et al. 2002), and will be a favorable condition for females to reduce their workload in the presence of helpers.

Our observational results show that females did not adjust their behavior depending on the presence or absence of juveniles. These findings are supported by the outcome of the helper removal experiment. Instead, females modify their behavioral decisions and rely on helpers' behavior also when they are not engaging in immediate parental care. Thus, helpers are beneficial to breeder females irrespective of their breeding status. Indeed, both observational and experimental data showed that female feeding behavior increased with increasing number of helpers and decreased after helper removal. Thus, having helpers appears to allow females

to invest more time into feeding and in turn improve their future reproductive success. This workload adjustment of breeders may be different from cooperative breeders found in other animal taxa, especially in birds where food provisioning to offspring is the main task of helpers (but see Crick 1992 for the evidence of load-lightening effects on food storage, nest building and territory defense). Helpers of other cooperatively breeding fishes usually show territory defense and maintenance without offspring (Bergmüller and Taborsky 2005; Heg et al. 2005; Heg and Bachar 2006), indicating the possibility of breeders' workload adjustment outside the reproductive period in other fish species as well.

Unexpectedly, we found a significant decrease of female sand removal behavior in groups engaged in parental care. There might be 2 non-mutually exclusive explanations for this finding. First, the presence of young might attract predators specialized in preying on eggs and small fish (Hori 1997), which increase the need of defense against such predators. Digging behavior is costly compared to other tasks, both in terms of time and energy spent (cf. Taborsky and Grantner 1998). Thus, females have to carefully allocate their time and energy budget between offspring defense and territory maintenance, which might eventually lead to a reduction of digging behavior. Second, extensive territory maintenance might result in increased sand invasion to the excavated cavities, which will be harmful to the immobile eggs. While both scenarios are plausible to explain our results, we cannot test them with the current data. Another unexpected result was the decreased aggression of breeder females in the control treatment of the helper removal experiment. One possibility might be that helpers increased their workload in response to the temporal helper removal treatment, and females reduced their workload accordingly. While the helper removal period was short, helpers may increase their workload to avoid punishment from dominants after preventing them from helping. Indeed, such pay-to-stay system has been demonstrated in *N. pulcher* (Balshine et al. 1998; Bergmüller and Taborsky 2005; Zöttl et al. 2013b; Fischer et al. 2014). Alternatively, the number of predators and food/shelter competitors might have fluctuated

during the day. Indeed, census data of a previous study (Tanaka et al. 2016) suggest a lower occurrence of shelter competitors in the afternoon (Tanaka, unpublished data). We are not able to discern these 2 explanations with our present data, leaving these questions open for future studies.

Another interesting point is the possibility of helpers' behavioral adjustment to variation in group size. For instance, all group members including helpers reduced their provisioning rates in response to an increased number of male helpers in red-winged fairy-wrens (Brouwer et al. 2014). Similarly, helpers in *N. obscurus* might reduce per capita workload with increasing group size. On the other hand, in systems where helpers are "coerced" to help by other group members, helpers will consistently help without any change in their workload amount, even if group size increases. In such scenarios only socially dominant individuals might reduce their workload (e.g., Zöttl et al. 2013b; Fischer et al. 2014).

Load-lightening effects in territory defense and offspring care are also found in *N. pulcher* (Balshine et al. 2001; Bergmüller and Taborsky 2005; Zöttl et al. 2013a). In addition, in the cooperatively breeding cichlid *Julidochromis ornatus*, dominant breeders spent significantly less time inside their nest when helpers were present (Awata et al. 2005) and increase shelter visits and defense behavior after helper removal (Bruintjes et al. 2013). Similarly, breeders with helpers leave the nest and engage in feeding behavior more readily in the cooperatively breeding *Chalinochromis brichardi* (Kohda et al., unpublished data), suggesting potential load-lightening effects by helpers in these species as well. Taken together, load-lightening effects might be widespread among cooperatively breeding fishes. In contrast, additive effects in sand digging shown in this study, which will increase the excavated cavities and food abundance (Tanaka et al. *in revision*), are rarely described thus far.

In summary, we provide first evidence of task-dependent workload adjustment of breeders in cooperatively breeding animals. Considering evidence from other species, load-lightening effects might be widespread in cooperatively breeding fishes. These results contribute to a better understanding of the importance of broodcare helpers and the evolution of cooperative breeding in animals.

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Data accessibility: Analysis reported in this article can be reproduced using the data provided by Tanaka et al. (2017).

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