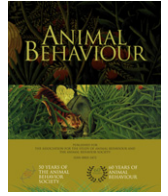


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Partial brood care compensation by female breeders in response to experimental manipulation of alloparental care

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In cooperative breeders, where nonparents participate in brood care, the investment of contributors to offspring care is predicted to be interdependent, reflecting a conflict of fitness interests between care providers. We experimentally manipulated the alloparental care of subordinate helpers in the cooperatively breeding cichlid *Neolamprologus pulcher* to test whether breeders compensate for reduced help, or whether brood care is additive. As predicted by recent theoretical models, breeder females only partially compensated for the reduced help of the subordinate by increasing their brood care effort. After being prevented from contributing to brood care, subordinates increased their brood care effort to a level exceeding that shown before the manipulation. Although this increased effort of helpers reduced the dominant female's workload, the clutch received more care in total post manipulation than during manipulation and before, that is, the brood care response was additive. These experimental results show that load-lightening effects and negotiation over brood care trigger behavioural decisions of dominants and subordinates, as suggested by a recent theoretical model. Together with previous evidence from birds, our results suggest that load lightening is taxonomically widespread in cooperative breeders.

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Alloparental brood care, where individuals provide care for the offspring of other group members, poses a challenge to natural selection theory because it seems to be altruistic, increasing the recipient's fitness at a cost to the acting individual's fitness (Emlen 1984; Bergmuller et al. 2007; West et al. 2007). Nevertheless, apart from many invertebrates such as social insects, it is also common in vertebrate taxa: cooperatively breeding birds deliver food to nestlings (reviewed in Cockburn 1998); cooperatively breeding mammals take turns in babysitting and share the burden of provisioning offspring (Solomon & French 1997; Russell 2004); and helpers in cooperatively breeding fish engage in fanning and cleaning of eggs laid by the dominant female, by removing sand from the breeding shelter and defending the brood against predators (Taborsky 1994).

When several individuals provide care there is great potential for conflict over investment, because an individual's investment should depend on what other group members are doing. Furthermore, life history trade-offs favour holding back current investment

in favour of future potential, if other individuals compensate for such reduction (Johnstone & Hinde 2006). Parents may benefit from help in brood care by reducing their own investment (load lightening, e.g. Brown et al. 1978; Cockburn 1998; Balshine et al. 2001; Russell et al. 2008; Johnstone 2011), which may increase their productivity (Taborsky 1984). Such adjustment has been experimentally demonstrated in several cooperative breeders, mainly birds. For instance, additional provisioning of young by helpers reduces provisioning effort of all other group members in the Arabian babbler, *Turdoides squamiceps* (Wright & Dingemanse 1999), and temporary helper removal in the long-tailed tit, *Aegithalos caudatus*, increases provisioning rate in all other group members (Hatchwell & Russell 1996). Furthermore, per capita chick feeding rate often decreases with increasing number of contributors to care (Komdeur 1994; Cockburn 1998; Wright 1998).

Among vertebrates, the leading paradigm to explain why helpers help is kin selection theory, which explains how the actor's inclusive fitness is increased by caring for related individuals (Hamilton 1964; Griffin & West 2003; Cornwallis et al. 2009). However, in many species, helpers are unrelated to the beneficiaries of their effort and thus kin selection cannot explain why they invest so much in alloparental care (Dunn et al. 1995; Magrath &

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Whittingham 1997; Dierkes et al. 2005; Stiver et al. 2005; Wright et al. 2010). In these systems, direct fitness benefits to helpers such as group augmentation, delayed mating advantages, improved survival because of more resource access and protection, or territory inheritance suggest that alloparental care can be selected for by means other than kin selection (Woolfenden & Fitzpatrick 1978; Reyer 1980, 1984; Taborsky 1984; Magrath & Whittingham 1997; Balshine-Earn et al. 1998; Clutton-Brock et al. 1999; Clutton-Brock 2002, 2009).

In superb fairy-wrens, *Malurus cyaneus*, helpers may use alloparental care as payment to remain tolerated in the territory (Mulder & Langmore 1993), and relatedness seems to be of minor importance for helping decisions in this species (Dunn et al. 1995). An experimental increase in brood-caring individuals relative to the number of nestlings led to a reduction in maternal workload (Russell et al. 2008), and the presence of helpers was associated with increased survival probability of the breeding female (Cockburn et al. 2008). In the cooperatively breeding cichlid fish *Neolamprologus pulcher*, females with helpers were shown to produce larger clutches than those without (Taborsky 1984). Group members in this species were shown to compensate for experimentally reduced participation of individual helpers in defence against intruders (Bergmuller & Taborsky 2005). In general, reactions to reduced help should follow different rules in systems in which helping is mainly a product of kin selection and in those in which helping serves to increase direct benefits.

The African cichlid *N. pulcher* is a model organism of cooperative breeding, allowing experimental manipulation of ecological and social conditions in both field and laboratory (Wong & Balshine 2011). These fish breed in groups composed of a dominant pair and typically 1–25 subordinates of different size classes (Taborsky & Limberger 1981; Balshine et al. 2001; Heg et al. 2005). Subordinates care for the dominants' broods by cleaning and fanning the eggs (Taborsky 1984), by removing sand from the breeding shelter (Bruitjes & Taborsky 2011), and defending the territory against predators, competitors for space and conspecific intruders (Taborsky 1984, 1985; Balshine et al. 2001; Bruitjes & Taborsky 2011). Unrelated helpers are common in this species (Taborsky & Limberger 1981; Dierkes et al. 2005; Stiver et al. 2005), which for these individuals excludes kin selection as an ultimate mechanism selecting for helping and philopatry. This species is one of the few model systems for cooperative breeding in which 'pay to stay' (PTS) has been experimentally investigated, and where trading help for tolerance appears to be an important factor triggering helping behaviour in unrelated subordinate group members (Taborsky 1984, 1985; Balshine-Earn et al. 1998; Balshine et al. 2001; Bergmuller et al. 2005; Bergmuller & Taborsky 2005; Zöttl et al. 2013b).

Hitherto, manipulations of alloparental brood care behaviour of helpers have not been conducted, even though they are crucial to understand load-lightening effects on breeders. Whereas studies of cooperatively breeding birds have mostly investigated alloparental brood care, fish studies often focus on territory defence and maintenance (Taborsky & Limberger 1981; Taborsky 1985; Bergmuller et al. 2005; Bergmuller & Taborsky 2005; Stiver et al. 2005; Heg & Taborsky 2010; Bruitjes & Taborsky 2011; Zöttl et al. 2013a) because it can be easily observed and elicited experimentally. Alloparental brood care of eggs and larvae, however, can only be observed in the laboratory, because the content of breeding shelters is inaccessible to observers in the field. Focusing on alloparental brood care in a different taxonomic group can help to bridge the gap between research on cooperatively breeding birds, mammals and fish.

By conducting a controlled laboratory experiment, we asked how breeders and helpers of *N. pulcher* respond if helpers are

experimentally prevented from performing alloparental brood care. In this species, the female breeder and subordinate helpers are the main contributors to direct brood care, as male breeders rarely participate in the direct care of eggs and larvae (Taborsky 1984; Heg 2008; Heg & Hamilton 2008). Our first aim was to prevent subordinates from participating in alloparental brood care while they were present in the territory and, at the same time, to quantify the breeder female's workload. We predicted that if females benefit from the brood care of helpers by load lightening, the prevention of help should cause an increase in brood care effort by the dominant female, which should be confined to the time period during which helpers do not participate in care. Based on results from biparental care models (McNamara et al. 2003; Johnstone & Hinde 2006) and studies explicitly modelling the situation in cooperatively breeding groups (Hatchwell 1999; Johnstone 2011), we predicted that females should not fully compensate for the lack of help by the subordinate, as offspring mortality in *N. pulcher* is high (Taborsky 1984; Brouwer et al. 2005). Our second aim was to determine the helper's response to experimental prevention of their brood care contribution. We predicted that the helper would subsequently increase its brood care contribution to a level exceeding the investment provided before the manipulation, in order to gain direct benefits by mechanisms such as pay to stay (Gaston 1978), signals of prestige (Zahavi 1995) and group augmentation (Kokko et al. 2000).

METHODS

Experimental Set-up

We used descendants from individuals caught in the wild at Lake Tanganyika near Kasakalawe and Mpulungu, Zambia, in 1996, 2006 and 2009. They were measured, sexed and assigned to groups (cf. Taborsky 1984, 1985) consisting of a pair (male: mean standard length, SL: 6.45 cm, range 5.00–7.70 cm; female: 5.64 cm, range 4.70–6.30 cm; within a pair the female was always smaller than the male, as in nature) and an unrelated, immature helper (2.67 cm, range 2.05–3.30 cm). These groups were kept separately either in undivided ($N = 17$) or halved ($N = 6$) 200-litre tanks. The aquarium bottom was covered with a layer of sand (ca. 3 cm). Each tank contained four to eight clay flowerpot halves serving as breeding substrate, two translucent tubes suspended near the surface to serve as shelters for expelled subordinates, and a suspended filter. The availability of several shelters per fish and suspended refuges (translucent pet bottles) helped to reduce aggression during group formation and during the experimental period. The designated helper was released first and allowed 24–48 h of acclimatization before both pair members were released simultaneously into the tank. Daily observation periods lasting 10 min were started between 1000 and 1700 hours.

Ethical Note

Aggression and expulsion risk in *N. pulcher* increases with decreasing size gap between breeders and helpers (e.g. Zöttl et al. 2013b, c). Choosing a large size gap between helpers and breeder (see above) ensured low levels of aggressiveness. Aggressive behaviours in this species include displays, short chases, ramming and ritualized biting, but subordinate individuals use submissive displays to appease dominant individuals (Bergmuller & Taborsky 2005; for a detailed behavioural description see Hamilton et al. 2005). During our daily checks and frequent observations we never observed persistent chases or biting that inflicted wounds, led to loss of scales or blood being drawn. Aggression experienced did not prevent the fish from feeding and no weight loss was

visually detectable. The experiments were approved by the Veterinäramt Bern (licence 16/09).

General Housing Conditions

The light:dark cycle was set to 13:11 h. Water temperatures were kept constant at 27 ± 1 °C. The fish were fed once ad libitum with commercial flake food (5 days/week) and with frozen food (1 day/week).

Brood Care and Behavioural Observations

Brood care was observed and quantified following a protocol established in previous studies (e.g. Heg & Hamilton 2008). Whenever the dominant female produced a clutch, we recorded for all group members the frequency of egg cleaning, the time spent in the breeding shelter with eggs and the frequency of sand removal from the breeding cavity. *Neolamprologus pulcher* cleans the eggs by micronipping and produces a water flow around the eggs, which is sometimes augmented by fanning, to oxygenate the clutch during development in the brood cavity (Taborsky 1984; Grantner & Taborsky 1998; Heg 2008; Heg & Hamilton 2008). Additionally, an individual's presence in the brood cavity can serve an important protective function against small egg predators (Heg & Taborsky 2010; Bruintjes et al. 2011). Removing sand from the breeding cavity is important, as in nature sand has been shown to be a source of mortality of small young (Taborsky & Limberger 1981). In all observations we recorded these three forms of brood care separately for both the breeder female and the subordinate, and all social interactions between the helper and both breeders.

Male care was omitted because breeder males in this species rarely provide any direct care as measured in this experiment (Taborsky 1984). The mean time males spent in the brood cavity (i.e. all observations across treatments) was only 1.98 s, compared to 40.98 s for the female and 101.80 s for the helper, and similar patterns emerged for egg cleaning (male: 0.38 events/observation; female: 15.80 events/observation; helper: 29.26 events/observation) and removing sand from the shelter (male: 0 events/observation; female: 0.77 events/observation; helper: 5.01 events/observation). Hence, direct brood care by males was always below 1.5% of the brood care a clutch received in total.

Experimental Treatments

When the breeder female produced a clutch, we assigned each group to start either with the treatment or with the control condition. The respective alternative condition was applied on the next day, reflecting a within-subject design with 23 breeding groups.

Four of these 23 groups produced two clutches during the experimental period, so we repeated the experiment in these four cases and averaged the two repeated measurements to avoid pseudoreplication.

In both control and treatment periods, we first conducted a 10 min baseline observation to determine brood care and social interactions as described above (Fig. 1). In the treatment condition, the helper was subsequently confined to a limited area within the territory by placing a clear Plexiglas cylinder over it when it was inside one of the shelters. Thereby it was prevented from participating in brood care while still being present in the territory and visible to the breeders. In the control condition, we conducted the same manipulation with the clear Plexiglas tube but leaving the helper outside of the tube (Fig. 1). The cylinder remained in the tank for 3 h. In the last 10 min of these 3 h, we conducted a second observation following the same protocol as outlined above. We refer to this observation, during which in the treatment but not in the control the helper was prevented from performing alloparental brood care, as the 'manipulation' (Fig. 1). To control for the experience of confinement that helpers received in the treatment condition, we confined the helper in the Plexiglas cylinder for 2 min also in the control condition, right after the last observation in the 'manipulation' phase of the experiment. Subsequently, we removed the cylinder in both treatment and control conditions and waited for 20 min before recording the last 10 min focal observation (referred to as 'post manipulation' phase; Fig. 1).

Statistical Analyses

To analyse whether female breeders compensate for the lack of alloparental brood care we investigated three aspects of brood care behaviour. We modelled their brood care behaviour in the treatment and control conditions before, during and after the manipulation by fitting GLMMs using the software R version 2.14.0 (R Development Core Team 2011) and the packages lme4 (Bates et al. 2011) and nlme (Pinheiro et al. 2012). We added time as a fixed factor with three levels (baseline, manipulation, post manipulation), and set the manipulation phase as reference for comparison with the other two phases, baseline and post manipulation. We also added the pair identity as a random factor.

To model the response of helpers to being prevented from participating in brood care we used the baseline and post manipulation observations of both the treatment and control periods and fitted GLMMs including the time (baseline, post manipulation) and the type of manipulation (treatment, control) as fixed factors. In addition, we included their interaction in all models and added the pair identity as a random factor. For analyses where the exact *P* value (GLMM with Gaussian error structure, R package lme4, Bates

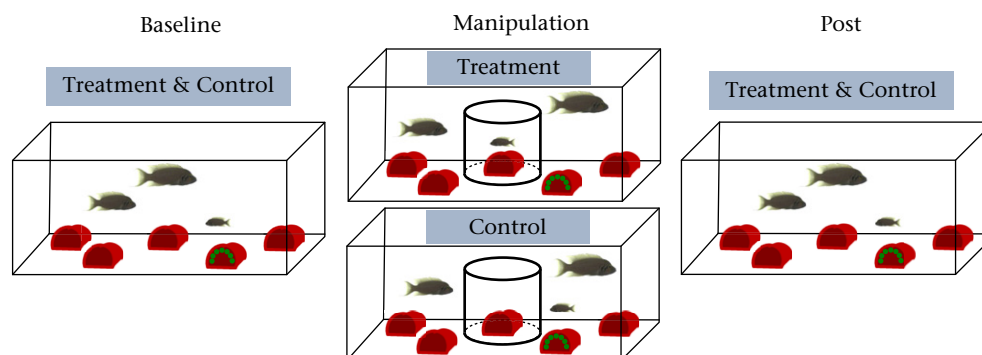


Figure 1. Schematic illustration of the experimental design. In the treatment condition during the manipulation phase, the helper was confined to a limited area within the territory using a clear cylinder which prevented alloparental brood care. In the control treatment the helper was able to conduct alloparental brood care unhindered.

et al. 2011) is not returned, we used likelihood ratio tests to calculate the exact *P* value for the crucial interaction terms and inspection of *t* values for the estimation of significance for main factors (Crawley 2007).

All data were log or square-root transformed before we fitted models with Gaussian error structure. Model validation was conducted subsequently by inspecting the residuals, predicted and fitted value plots. If the models did not comply with the requirements (e.g. if the residuals differed significantly from a normal distribution), we used Poisson errors to model count data (applies to frequency of removing sand from the cavity).

RESULTS

Brood Care of Female Breeder

Brood care behaviour of the female breeder depended on treatment. In the treatment period, the lack of brood care help from the subordinate caused the female breeder to (1) increase her time spent in the breeding shelter (Table 1, Fig. 2a), (2) remove sand more often from the breeding shelter containing eggs (Table 1, Fig. 2c) and (3) increase the frequency of egg cleaning (Table 1, Fig. 2e). As predicted, the time spent in the breeding shelter, the frequency of digging out the cavity and the frequency of egg cleaning all decreased significantly after the subordinate was released and provided help again (Table 1, Fig. 2a, c, e). In contrast to the treatment phase, no change in brood care behaviour of the dominant female was detected in control trials (Table 1, Fig. 2a, c, e).

Response of the Subordinate

Subordinates changed their behaviour in response to the experimental manipulation. They increased their investment in alloparental brood care after they were prevented from helping for 3 h. They spent more time in the breeding shelter (Table 2, Fig. 2b) and removed sand more often from the breeding shelter (Table 2,

Fig. 2d) than during the baseline condition, but they did not show more egg cleaning (Table 2, Fig. 2f).

Total Brood Care

The total time that brood-caring group members spent in the breeding cavity, the total frequency of sand removals and the total egg-cleaning frequencies were all reduced by the experimental prevention of helpers from contributing to brood care (Table 3, Fig. 3a, b, c), although the breeding female increased her workload significantly (Table 1, Fig. 2a, c, e). This reveals that the female breeder did not fully compensate for the lack of help in the manipulation phase of the treatment condition. In the control condition no significant changes were detected (Table 3). Once the helper was released in the treatment condition, it increased its contribution to alloparental care (Table 2), resulting in an increase in the total amount of time brood-caring individuals spent in the breeding shelter (Fig. 3a), as indicated by the significant time*^{*}treatment interaction (Table 2). The total frequency of sand removals from the breeding cavity increased with the duration of the experiment in the treatment condition and tended to increase also during the control condition (Table 3, Fig. 3b). The significant time*^{*}treatment interaction suggests that this increase was significantly more pronounced in the treatment than in the control condition (Table 2, Fig. 3b), suggesting that more sand was removed from the breeding cavity after the subordinate had been prevented from conducting brood care for 3 h. The total egg-cleaning frequency was not different between the control and treatment conditions of the experiment (Table 2, Fig. 3c).

Social Interactions between Group Members

After the subordinate had been prevented from helping for 3 h, the subordinate did not show higher submission rates towards male and female breeders than in the baseline condition (Table 4) and neither of the breeders attacked the subordinate more often (Table 4).

DISCUSSION

As predicted by the load-lightening hypothesis, the female breeders partially compensated for the lack of help by increasing their workload. Johnstone (2011) explicitly modelled effects of increased help on parental workload in cooperative groups, and demonstrated that load lightening should occur independently of whether helpers are related or unrelated to beneficiaries. A likely explanation for the incomplete compensation is that the marginal costs of care exceed the marginal benefits for the breeder females and hence they use help provided to reduce their own workload (Hatchwell 1999; Russell et al. 2008; Johnstone 2011). Experimentally preventing help caused the female breeder to increase her workload, as suggested by Johnstone's (2011) model. This pattern is also consistent with studies on alloparental investment in birds (Hatchwell 1999) and with traditional models of biparental care (Johnstone & Hinde 2006). However, empirical tests of biparental care in noncooperative species have yielded mixed evidence, with some studies supporting the predictions and others rejecting them (Johnstone & Hinde 2006). Our study demonstrates that incomplete compensation for reduced effort from other care contributors also happens in cooperative breeders in which helpers provide alloparental brood care in addition to the parental care of breeders, suggesting that similar mechanisms as those responsible for stable investment strategies in biparental care might also trigger investment strategies of helpers and breeders in cooperatively breeding groups. The alloparental brood care compensation found in this

Table 1
Comparison of brood care behaviours of the female breeder

	Estimate±SE	<i>t</i>	<i>P</i>
Time in breeding shelter during treatment			
Manipulation (Intercept)	4.1±0.19	21.4	<0.001
Baseline	-0.84±0.13	-6.57	<0.001
Post manipulation	-0.75±0.13	-5.92	<0.001
Time in breeding shelter during control			
Manipulation (Intercept)	4.81±0.67	7.18	<0.001
Baseline	0.34±0.43	0.79	0.43
Post manipulation	0.19±0.43	0.43	0.66
Frequency of sand removal during treatment			
Manipulation (Intercept)	-2.38±0.83	-2.89	0.004
Baseline	-1.69±0.37	-4.61	<0.001
Post manipulation	-1.59±0.35	-4.51	<0.001
Frequency of sand removal during control			
Manipulation (Intercept)	-6.94±2.26	-3.07	0.002
Baseline	0.29±0.79	0.36	0.72
Post manipulation	0.69±0.73	0.95	0.34
Frequency of egg cleaning during treatment			
Manipulation (Intercept)	5.20±0.56	9.29	<0.001
Baseline	-1.81±0.39	-4.61	<0.001
Post manipulation	-1.87±0.39	-4.76	<0.001
Frequency of egg cleaning during control			
Manipulation (Intercept)	1.68±0.27	6.13	<0.001
Baseline	-0.28±0.25	-1.16	0.25
Post manipulation	0.12±0.25	0.47	0.64

The baseline and the post manipulation period are contrasted against the manipulation period (reference category) in the treatment and control conditions of the experiment. *N* = 23 groups for all analyses.

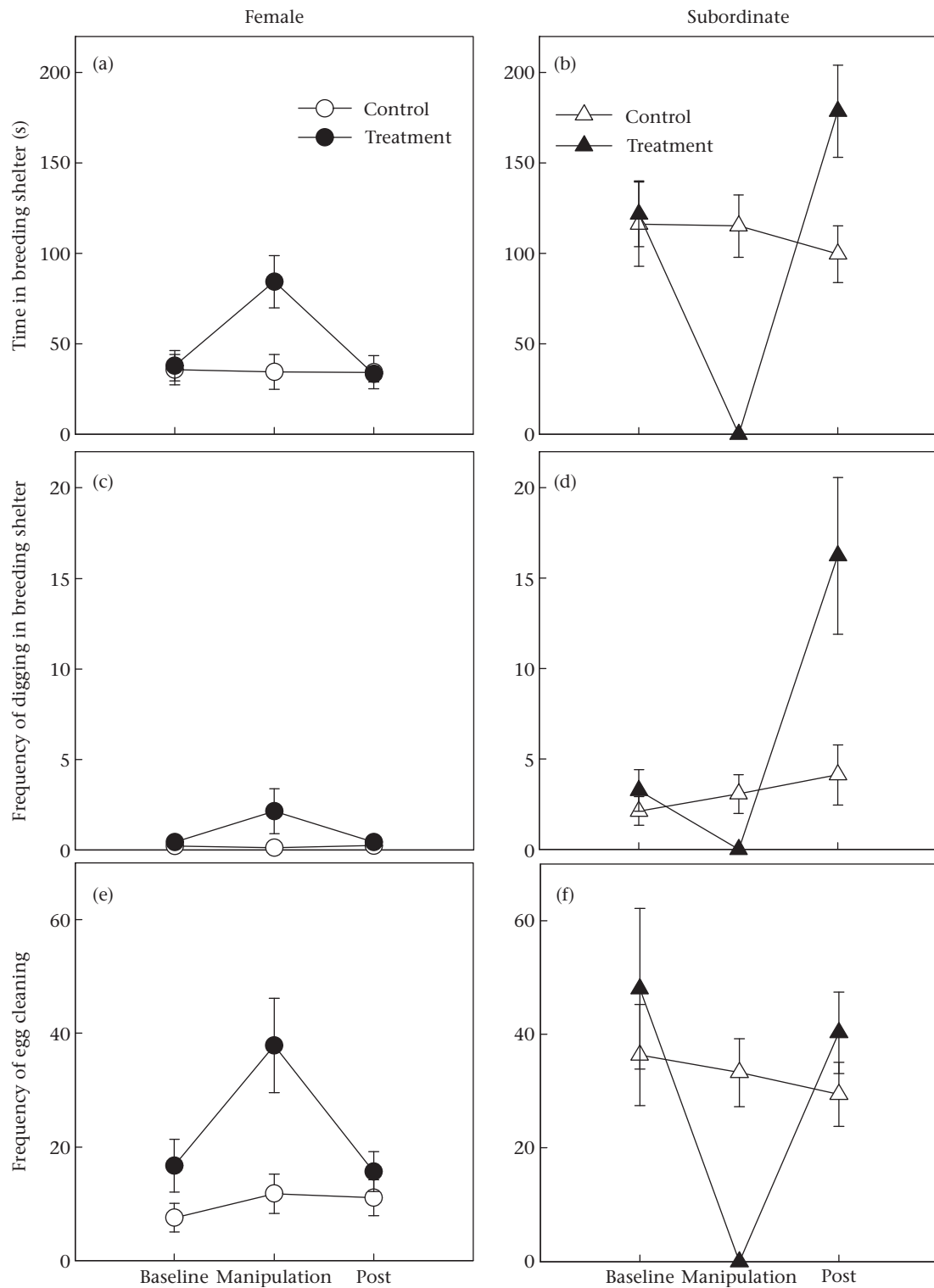


Figure 2. (a) and (b) Time in breeding shelter, (c) and (d) frequency of digging and (e) and (f) frequency of egg cleaning of (a), (c) and (e) female breeders and (b), (d) and (f) subordinates in the treatment and control conditions of the experiment, during baseline, manipulation and post manipulation (post) periods. Depicted are mean \pm SE of untransformed raw data. $N = 23$ groups for all analyses. See Tables 1 and 2 for statistical results.

study is also consistent with investment compensation in cooperative territory defence in *N. pulcher*, in which other group members increased territory defence when a helper was experimentally prevented from participating (Bergmüller & Taborsky 2005). However, it was unclear from previous studies to what extent group

members compensate for the experimental manipulation of a helper's contribution to territory defence, that is, if their investment represents full or partial compensation (Bergmüller & Taborsky 2005). Our new results on alloparental brood care show, however, that compensation by breeders is merely partial.

Table 2
Alloparental brood care and total brood care in response to the experimental manipulation

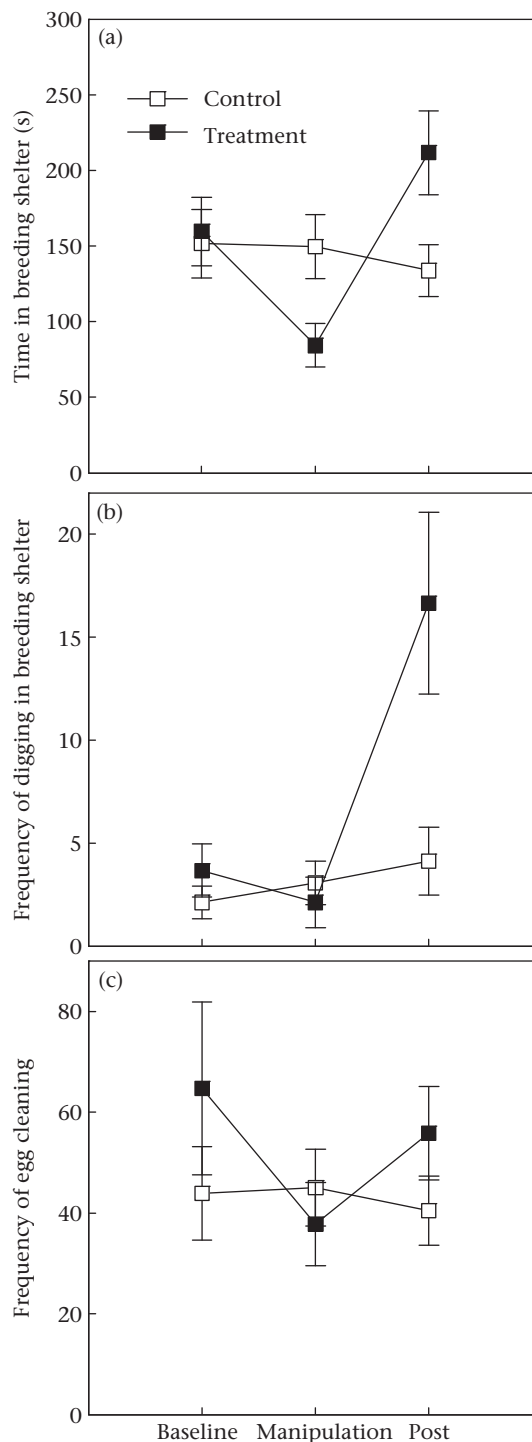
	Estimate±SE	t	P
Time in breeding shelter by the subordinate			
Control (Intercept)	4.5±0.21	21.4	
Time	-0.03±0.08	-0.40	NS
Treatment	-0.17±0.25	-0.69	NS
Time*Treatment	0.22±0.11	1.97	0.047
Frequency of sand removal from the breeding shelter by the subordinate			
Control (Intercept)	0.48±0.31	1.52	0.13
Time	0.20±0.12	1.64	0.11
Treatment	-0.21±0.38	-0.56	0.57
Time*Treatment	0.08±0.10	2.20	0.03
Frequency of egg cleaning by the subordinate			
Control (Intercept)	3.67±0.19	19.78	
Time	-0.05±0.07	-0.72	NS
Treatment	-0.01±0.22	-0.06	NS
Time*Treatment	0.08±0.10	0.78	0.43
Total time in breeding shelter (female breeder and subordinate)			
Control (Intercept)	4.86±0.19	25.36	<0.001
Time	-0.05±0.07	-0.76	0.45
Treatment	-0.21±0.20	-1.01	0.31
Time*Treatment	0.2±0.09	2.19	0.03
Total frequency of sand removal (female breeder and subordinate)			
Control (Intercept)	0.54±0.31	1.76	0.08
Time	0.19±0.12	1.59	0.12
Treatment	-0.19±0.38	-0.49	0.63
Time*Treatment	0.36±0.17	2.14	0.036
Total frequency of egg cleaning (Female breeder and subordinate)			
Control (Intercept)	3.45±0.23	14.74	<0.001
Time	0.01±0.09	0.06	0.95
Treatment	0.09±0.26	0.35	0.72
Time*Treatment	0.06±0.11	0.52	0.60

The table shows the amount of alloparental brood care provided by the subordinate in the baseline and post manipulation periods (time) and the total amount of brood care (care of female breeder + alloparental care of subordinate) that a clutch received in the treatment and control conditions (treatment). The control condition was set as the reference category and estimates denote the difference to the treatment condition. $N = 23$ groups for all analyses.

Table 3
Total amount of brood care in the three experimental periods

	Estimate±SE	t	P
Total time in breeding shelter during treatment			
Manipulation (Intercept)	8.41±0.91	9.26	<0.001
Baseline	3.46±0.80	4.34	<0.001
Post manipulation	5.35±0.80	6.71	<0.001
Total time in breeding shelter during control			
Manipulation (Intercept)	4.76±0.15	32.788	<0.001
Baseline	0.05±0.11	0.05	0.62
Post manipulation	-0.05±0.11	-0.44	0.67
Total frequency of sand removal during treatment			
Manipulation (Intercept)	-0.30±0.39	-0.76	0.44
Baseline	0.54±0.18	3.00	0.003
Post manipulation	2.06±0.15	13.54	<0.001
Total frequency of sand removal during control			
Manipulation (Intercept)	0.38±0.31	1.21	0.22
Baseline	-0.33±0.18	-1.85	0.06
Post manipulation	0.30±0.15	1.96	0.05
Total frequency of egg cleaning during treatment			
Manipulation (Intercept)	2.93±0.246	12.17	<0.001
Baseline	0.68±0.19	3.53	<0.001
Post manipulation	0.81±0.19	4.22	<0.001
Total frequency of egg cleaning during control			
Manipulation (Intercept)	3.44±0.18	19.11	<0.001
Baseline	0.02±0.15	0.12	0.90
Post manipulation	0.03±0.15	0.18	0.86

The table shows the total amount of brood care (care of female breeder + alloparental care of subordinate) that a clutch received during baseline, manipulation and post manipulation periods in the control and treatment conditions. $N = 23$ groups for all analyses.

**Figure 3.** (a) Total time in breeding shelter (sum of breeder female and helper), (b) total frequency of digging and (c) total frequency of egg cleaning in the treatment and control conditions of the experiment, during baseline, manipulation and post manipulation (post) periods. Depicted are mean \pm SE of untransformed raw data. $N = 23$ groups for all analyses. See Table 3 for statistical results.

Brood care is costly in *N. pulcher*, as fanning more than doubles energy expenditure compared to basal metabolic rates (Grantner & Taborsky 1998), and in addition it causes opportunity costs owing to its burden on time budgets (Taborsky & Grantner 1998). The participation of helpers in these costly tasks increases the fitness of breeders, as females with helpers can lay larger clutches (Taborsky 1984). In our experiments, helpers were still present and visible

Table 4
Social interactions between breeders and subordinates

	Estimate±SE	t	P
Subordinate submission towards the male breeder			
Control (Intercept)	0.98±0.22	4.47	<0.001
Time	-0.01±0.09	-0.07	0.95
Treatment	-0.21±0.29	-0.71	0.48
Time*Treatment	0.11±0.13	0.82	0.41
Subordinate submission towards the female breeder			
Control (Intercept)	1.95±0.19	10.44	<0.001
Time	-0.06±0.07	-0.89	0.37
Treatment	-0.10±0.22	-0.45	0.66
Time*Treatment	0.11±0.10	1.18	0.24
Male breeder aggression towards the subordinate			
Control (Intercept)	0.039±0.29	1.36	0.18
Time	-0.05±0.12	-0.48	0.63
Treatment	-0.09±0.37	-0.24	0.81
Time*Treatment	0.14±0.17	0.87	0.39
Female breeder aggression towards the subordinate			
Control (Intercept)	1.38±0.20	6.80	<0.001
Time	-0.01±0.08	-0.05	0.96
Treatment	0.04±0.26	0.15	0.88
Time*Treatment	-0.02±0.12	-0.16	0.87

The tables shows the results of GLMMs on submission and aggression frequency in the treatment and control conditions during the baseline and post manipulation periods. The control condition was set as the reference category and estimates denote the difference to the treatment condition. $N = 23$ groups for all analyses.

to the female breeder during the time they were prevented from participating in brood care, which is important because theoretical models predict that individuals should respond differently to absence of a caring individual than to neglect (McNamara et al. 2003; Johnstone & Hinde 2006; Johnstone 2011). Hence, if breeding females anticipate that lazy subordinates might compensate for their behaviour later on (Bergmuller & Taborsky 2005), it might be beneficial for female breeders to compensate only partly when subordinates reduce their effort, thereby creating a demand for the subsequent increase in brood care by the idle subordinates.

In superb fairy-wrens female breeders also incompletely compensate for missing alloparental care, suggesting that they value the reduction in costs of care more than any benefits they gain from extra provisioning of their current offspring (Russell et al. 2008; Johnstone 2011). However, in Russell et al.'s (2008) study, the manipulation was conducted over a whole breeding cycle, which hence did not allow breeders to expect or enforce compensatory reactions from helpers. Our study shows that incomplete compensation of breeders can happen within a short time period, and also when the subordinate is present in the territory and therefore subsequent compensatory increase in alloparental brood care of helpers might be expected. Studies of cooperatively breeding birds have shown that increased provisioning may reduce care by other individuals in the group (Wright & Dingemanse 1999) or vice versa, when alloparental care is reduced by removing helpers completely from the territory (Hatchwell & Russell 1996). Together with these results our findings suggest that brood care investment by group members commonly depends on the behaviour of other caregivers in the group.

Consistent with models of PTS (Gaston 1978; Kokko et al. 2002; Hamilton & Taborsky 2005) and with mechanisms such as group augmentation (Kokko et al. 2000) and signals of prestige (Zahavi 1995), subordinate helpers increased the time spent in the brood cavity and the investment in removing sand after having been prevented from performing brood care. This induced a load-lightening effect in the female breeder, which decreased all measured parameters of brood care. Contrary to predictions of PTS, subordinates did not receive more aggression from breeders even though they did not show more submission towards them, suggesting that increased alloparental brood care is sufficient to

compensate for previous neglect. This finding is consistent with results from a previous study on *N. pulcher*, which suggested that another helping behaviour, engagement in cooperative territory defence, may serve as appeasement similar to submissive behaviour (Bergmuller & Taborsky 2005). However, the apparent lack of a causal relationship between aggression against subordinates and helping rates in our study should be interpreted with caution, because punishment could have occurred just after subordinates were released from the clear tube, when behavioural recordings had not yet started. In addition, the intensity of aggressive acts may have increased, which could not be quantified by our behavioural protocol. To demonstrate conclusively a causal relationship between aggression and help provided by a subordinate, it would be necessary to manipulate the possibility of punishing, that is, social interaction, in addition to manipulating the subordinate's cooperative effort. As pointed out by recent reviews (Cant 2011; Raihani et al. 2012), such experiments are currently missing in cooperatively breeders owing to the intricate demands of such interventions.

Subordinates increased their time inside the breeding shelter, during which they often fanned the eggs to enhance the oxygen supply in addition to guarding them. Furthermore, they put more effort into removing sand from the breeding shelter. In contrast, they did not increase egg cleaning after being prevented from doing so. It is possible that subordinates are coerced to compensate for previous neglect (Zöttl et al. 2013b) by providing particularly costly behaviours such as removing sand from shelters (which increases BMR six-fold; Grantner & Taborsky 1998).

In conclusion, our study demonstrates that cooperatively breeding fish respond to manipulations of alloparental care as predicted by theoretical models (Johnstone 2011) on a behavioural timescale. They flexibly adjust their investment within a short time period in relation to the behaviour of other group members. Our experimental results suggest that load lightening by helpers is an important factor determining parental brood care investment in cooperatively breeding cichlids. These results provide new insights into mechanisms regulating how cooperatively breeders respond to other group members' investment in brood care, in a system in which helpers are coerced to provide benefits to dominant breeders, and relatedness is of minor importance. The response of helpers is consistent with hypotheses explaining direct fitness benefits to helpers by pay to stay, signals of prestige and group augmentation. In this species, individuals apparently benefit from group augmentation (Heg et al. 2005), and recent evidence suggests that dominants coerce subordinates to provide alloparental brood care, that is, that they pay to stay (Zöttl et al. 2013b). The study closes a major gap between work on cooperatively breeding birds and fish, as to our knowledge it is the first study investigating load lightening with respect to alloparental brood care experimentally in cooperatively breeding fish.

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