

# Subordinate removal affects parental investment, but not offspring survival in a cooperative cichlid

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## Summary

1. Subordinates in cooperative breeding systems may provide help to dominant pairs, who can benefit by either an increased total investment in their current brood or a reduced personal contribution to this investment. In the social cichlid *Julidochromis ornatus*, one large male subordinate generally spends 90% of his time in close proximity to the breeding shelter, whereas the dominants only spend 50% of their time close to the shelter.

2. We experimentally removed the large subordinate for 30 days (approximating one breeding cycle) to study the investment strategies of dominants and the effects on offspring survival, while accounting for subordinate immigration. Experimental groups were compared with control groups, from which subordinates were also caught but not removed. On day one following removal, we tested whether dominants overcompensated, fully compensated or undercompensated for absence of the subordinate on several parental behaviours. Moreover, we tested whether the pairs' potential compensatory behaviour remained high seven days following large subordinate removal.

3. One day following removal, dominants increased their time spent in the territory and their frequency of breeding shelter visits and defence, compared with the pre-removal phase and control groups. The dominant pair overcompensated for the loss of subordinate help in their breeding shelter visits, fully compensated in defence and undercompensated their time spent in the territory. Seven days after large subordinate removal, behavioural differences between treatments had disappeared. However, when distinguishing between groups with or without a new immigrant subordinate, dominant pairs only diminished investment in the presence of an immigrant, suggesting a compensatory role of the large subordinate. Finally, survival of juvenile group members was not affected by the treatment.

4. Our experiments indicate that the presence of a large subordinate does not increase the dominant pairs' current reproductive success, but instead allows them to reduce their personal contribution to investment in the current brood. In addition, we illustrate that dominants may show strikingly different compensatory responses depending on the type of behaviour and emphasize the importance of immigrant subordinates to relieve dominants from costly compensatory responses in cooperative breeding systems.

**Key-words:** cichlidae, compensation, cooperative breeding, helping behaviour, investment, Lake Tanganyika, load-lightening, offspring survival

## Introduction

Parents of iteroparous species need to trade-off their investment in obtaining current versus future reproductive benefits, because increases in care for current brood might

reduce future reproductive output (Trivers 1972). An evolutionary sexual conflict is expected over the optimal levels of investment provided by each parent (Trivers 1972; for a review see Houston, Szekely & McNamara 2005). This conflict has received much theoretical and empirical attention (Chase 1980; Houston & Davies 1985; Wright & Cuthill 1990a; McNamara, Gasson & Houston 1999;

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McNamara *et al.* 2003; Houston, Szekely & McNamara 2005). Theoretical expectations concerning the optimal level of investment for a parent depend on the model type that is used (e.g. dynamic or sequential) and critically depend on the assumptions (e.g. whether parents invest a fixed level of effort or adjust their effort in direct response to that of their partners). Nevertheless, most theoretical models predict that when one parent decreases its investment in the brood, the other parent should only partially compensate for this (Chase 1980; McNamara, Gasson & Houston 1999; McNamara *et al.* 2003; but see Johnstone & Hinde 2006). However, by manipulating the level of care provided by one parent, empiricists have found a range of outcomes with regard to the partners' behavioural strategies, including no response (e.g. Slagsvold & Lifjeld 1990), undercompensation (e.g. Wright & Cuthill 1989) and full compensation (e.g. Wright & Cuthill 1990a,b; see Harrison *et al.* 2009 for a review about compensatory responses). Full compensation entails an increase in effort of the focal parent to compensate completely for the reduced effort of the manipulated partner. Accordingly, in undercompensation, the combined effort of both parents decreases after the manipulation.

In cooperatively breeding species, subordinates (often called helpers) assist the dominant pair in various costly tasks (Stacey & Koenig 1990; Solomon & French 1997). Compared with biparental species, conflict over investment towards young is thus more complex and variable, as at least three individuals determine the total level of investment into the offspring (Hatchwell & Russell 1996). Accordingly, predicting the level of individual investment is more difficult, and theoretical models that tackle the behavioural negotiations over care are rare (but see Härdling, Kokko & Arnold 2003; Carranza *et al.* 2008; Johnstone 2011). Empirical and correlational studies in cooperative breeders have reported a variety of different patterns of investment by subordinates and dominant pairs, including the following: (i) additive investment by subordinates and dominants (e.g. Reyer 1990; Emlen & Wrege 1991; Browning *et al.* 2012); (ii) investment by subordinates may cause dominant pairs to invest less (i.e. 'load-lightening': Brown *et al.* 1978; Crick 1992; Dunn & Cockburn 1996; Hatchwell & Russell 1996; Wright & Dingemanse 1999; Legge 2000; MacColl & Hatchwell 2003; Russell *et al.* 2008); and (iii) investment by subordinates may cause dominant pairs to invest more (Valencia *et al.* 2006). However, only few studies used experimental techniques, and most of these have focussed on the short-term effects of subordinate removal on parental investment (i.e. subordinates were temporarily removed for 3–8 h, Hatchwell & Russell 1996; brood sizes were temporarily manipulated to alter subordinate: offspring ratios for 4 h, Clutton-Brock *et al.* 2001; brood sizes were temporarily manipulated for less than a day, Russell *et al.* 2008). Continuing experimental effects have been less intensively studied. This is unfortunate, because group members might be able to compensate for the absence of

subordinates and respond to increased need on a short-term basis (e.g. by increasing their feeding levels for a few hours), but might not be able to sustain this on a long-term basis. We aimed to study the effects of subordinate removal on investment by dominants, in particular whether dominants are able to compensate several parental behaviours for large subordinate absence over a sustained period, how this translates into reproductive success and if immigration of a large subordinate influences investment strategies of dominant pairs.

In the cooperative breeding fish *Julidochromis ornatus*, groups consist of a dominant pair and up to six subordinates of different sizes and sex (Heg & Bachar 2006). Nevertheless, dominants are often only assisted by a single large male subordinate, who spends almost all his time in close proximity to the breeding shelter, whereas the dominant pair is frequently absent (Awata, Munehara & Kohda 2005; Heg & Bachar 2006). These large, sexually mature subordinates help the pair by performing various cooperative behaviours such as territory defence, territory maintenance and brood care (Awata, Munehara & Kohda 2005; Heg & Bachar 2006; Kohda *et al.* 2009). Although large subordinates are typically male, this is not always the case and sometimes there is more than one. Group living appears to be beneficial for large subordinates, as newly formed groups are joined by a large subordinate within days (Heg & Bachar 2006) and large male subordinates can gain high direct reproductive benefits by sharing in reproduction (Awata, Munehara & Kohda 2005). However, conflicts between the dominant pair and the large subordinate are bound to arise because of differences in the optimal level of investment in juvenile group members. Subordinate presence on the territory is enforced by the dominant pair; if the large subordinate ventures out too far from the territory, it is aggressively chased back by both dominants (Awata & Kohda 2004; Heg & Bachar 2006). Furthermore, evidence suggests that large subordinates may have a substantial influence on the group's functioning and reproductive output (Awata, Munehara & Kohda 2005; Awata *et al.* 2006), but experiments testing effects of subordinate presence on the investment strategies of dominant pairs are lacking.

To investigate the impact of the main subordinate on the investment strategies of the dominant pair and their subsequent reproductive output, we performed experimental removals of the large subordinate in the field lasting 30 days. We then studied behavioural compensation of the pair on day 1 and day 7 as a response to the removal of their large subordinate on day 0. We hypothesized that (i) the large subordinate lightens the load of the dominant pair, as in groups with a large subordinate the dominant pair is often absent (Heg & Bachar 2006). (ii) We expected the pair to fully compensate for the absence of the large subordinate to sustain the survival rate of their offspring (i.e. increasing territory presence, defence effort and breeding shelter visits). Alternatively, if the pair provides a fixed amount of care which is independent of that provided by the large subordinate, fry and small subordinates will

receive less help in absence of the large subordinate and their survival rate will drop. (iii) We expected an increase in immigration in the removal treatment compared with the control treatment on day 30 post-removal. However, as subordinate immigration occurred exceptionally fast in several groups (see Results), we added immigration analyses for day 1 and day 7 post-removal *a posteriori*, and added the following hypothesis: (iv) dominants in groups with an immigrant subordinate have a lower investment than those groups without a new immigrant.

## Material and methods

### STUDY SITE AND SPECIES

The research was performed by SCUBA diving at the southern tip of Lake Tanganyika, Kasakalawe Bay, near Mpulungu, Zambia, from September to November 2005. This experiment was approved by the Zambian Ministry of Agriculture, Food and Fisheries, and it complies with present laws of Zambia.

*Julidochromis ornatus* is a small cooperative breeding cichlid with large dominant individuals reaching 77 mm standard length [SL] (Heg & Bachar 2006). Up to six subordinates larger than 20 mm SL can be present in a group (Heg & Bachar 2006), and maturity is reached at 32 mm SL (corresponding to 38 mm total length as reported in Awata, Munehara & Kohda 2005). Dominant females are often bigger than dominant males (Awata & Kohda 2004), and this pattern was consistent in all groups used in this experiment. *J. ornatus* is a substrate-breeding cichlid (Kuwamura 1997) that typically uses large stones with a cavity underneath as its breeding shelter (Heg & Bachar 2006). The fish defend the territory against con- and heterospecific individuals, perform territory maintenance (digging and carrying sand) and brood care (fanning and cleaning eggs; Heg & Bachar 2006). Genetically, most large subordinates are unrelated to the dominant pair, and subordinates of both sexes can contribute to reproduction in their group (male subordinates have been shown to sire 44% of the brood and female subordinates 56%; Awata, Munehara & Kohda 2005).

### BEHAVIOURAL OBSERVATIONS

All focal observations were performed for 15 min between 8:40 and 16:40 h, and no treatment differences were detected between the time of day of the observations (GLM;  $t_{29} = -0.44$ ,  $P = 0.662$ ). We recorded the frequency of breeding shelter visits and frequency of defence (including bites, chases, mouth fights, frontal approaches, spreading of the opercula, head-down displays and S-shaped bends directed at con- and heterospecific fishes; see Taborsky 1984, for a detailed description of these behaviours in closely related Lamprologin species). Additionally, we recorded the amount of time spent in the territory (in seconds; territory is defined as the area <50 cm from the breeding shelter), and once every minute, we estimated the focal fish's distance to the shelter (in cm) to obtain spacing behaviour (the maximum distance recordable was 4 m). As time spent in the territory was negatively correlated with distance to the shelter (Spearman rank correlation:  $r_s = -0.918$ ,  $n = 245$ ,  $P < 0.001$ ), below we only show time spent in the territory.

### EXPERIMENTAL REMOVALS

Groups consisted of a dominant pair and  $5.5 \pm 3.3$  subordinates >15 mm SL (mean  $\pm$  SD; range 1–13; mean size subordinates  $29.0 \pm 11.6$  mm SL). In the removal treatment, one large subordinate (>40 mm SL) per group was removed from its territory for

30 days ( $n = 14$ ; one female and 13 male); in two groups, there were two large subordinates, and in these cases, we removed the largest one. In the control groups ( $n = 17$ ), large subordinates were caught, sexed (all 17 large subordinates were males), measured, fin-clipped and released immediately after handling. As with the removal groups, there were two control groups where two large subordinates were present, and again, we removed the largest one. The average body size difference between the large subordinate and the 2nd largest subordinate was 18.8 mm (18.3 mm in the control and 19.5 mm in the removal treatment). No difference was detected between body size differences of the large subordinate and the 2nd largest subordinate between treatments (Independent *t*-test:  $t_{26} = 0.74$ ,  $P = 0.736$ ). Moreover, no differences between treatments in the number and size of large subordinates were detected (see Table 1 for details). Group size, number of subordinates >30 mm, dominant female and dominant male sizes did not differ between groups in the control and removal treatments (Table 1). During the post-removal observation (coded 'day 0' throughout), we sequentially observed the three largest group members (dominant female, dominant male and large subordinate) in randomized order. Immediately afterwards, all group members were caught with help of tent nets and the anaesthetic eugenol (Kreiberg 2000) and were measured, sexed and marked by carefully excising a single fin ray of the dorsal and/or anal fin. All group members were then returned to their territories, apart from the large subordinate in the removal treatment. These individuals were stored in a large underwater cage ( $2 \times 2 \times 2$  m) and returned to their territories 30 days after start of the experiment. One and seven days after catching, the second and third observations were performed (coded 'day 1' or 'day 7' throughout). In the removal groups, only the dominant pair was observed, whereas in the control groups, we observed the dominant pair and the large subordinate. All observations were performed in randomized order.

Immigration of large subordinates was monitored on day 1, 7 and 30 by direct observations of all mature group members. Note that the behaviour of these immigrants was not summed for the total group effort for the compensatory effects analysis. We determined the effect of the large subordinate on survival of juvenile group members by counting all group members measuring

**Table 1.** Pre-experimental body sizes of dominant females, dominant males and large subordinates; number of subordinates >40 and >30 mm SL per group and total group size per treatment

	Control treatment	Removal treatment	<i>t</i> -value	<i>P</i>
Dominant female body sizes (SL)	65.6 $\pm$ 3.9	66.4 $\pm$ 4.9	$t_{29} = -0.49$	0.630
Dominant male body sizes (SL)	62.2 $\pm$ 3.5	62.5 $\pm$ 3.4	$t_{28} = -0.29$	0.776
Largest subordinate body sizes (SL)	49.6 $\pm$ 4.2	50.4 $\pm$ 3.6	$t_{29} = -0.55$	0.586
No. subordinates (>40 mm SL) /group	1.1 $\pm$ 0.3	1.2 $\pm$ 0.4	$t_{29} = -0.29$	0.776
No. subordinates (>30 mm SL) /group	2.1 $\pm$ 1.3	1.6 $\pm$ 0.7	$t_{29} = 1.23$	0.227
Total group size (incl. dominant pair)	13.4 $\pm$ 7.6	11.1 $\pm$ 5.6	$t_{29} = 0.93$	0.361

Shown are means  $\pm$  SD for standard length [SL] in mm and independent samples *t*-tests were used to test for treatment differences.

3–32 mm SL on day 0 (pre-removal) and again on day 30 in all groups for both treatments. Survival was calculated as the percentage survival of juvenile individuals.

## STATISTICS

The majority of the data were analysed using R (version 2.12.0; R Development Core Team 2010); survival was analysed with PASW statistics (version 18.0.0, IBM, Chicago, IL, USA). All data were checked for normality with Kolmogorov–Smirnov tests.

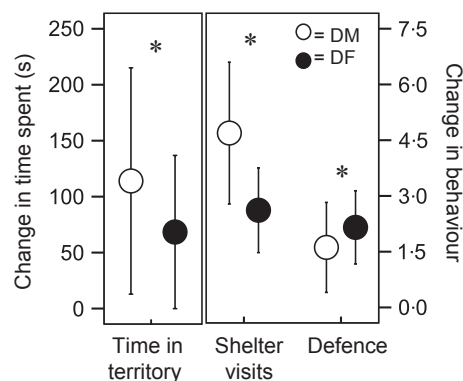
The frequencies of breeding shelter visits and defence were Poisson-distributed and analysed with generalized linear mixed models (GLMM) using the Laplacian approximation fit, whereas time in the territory showed a normal distribution and was analysed with general linear mixed models (GLMM) using restricted maximum likelihood methods. We first tested whether breeding shelter visits, defence and time in the territory differed between the control and removal treatments on day 0 (pre-removal phase) using GLMMs; no differences between the treatments were detected (see Appendix in Additional Supporting Information [ASI] Table S1). The same behaviours were then analysed comparing day 0 (pre-removal) and day 1 between treatments using GLMMs, including all two- and three-way interactions. The models included treatment, status (dominant female or dominant male), phase (day 0 or day 1) and all interaction effects (two- and three-way) as fixed effects (model 1). Additionally, individual identifiers nested within territory identifier were included as random effects on the intercept, to account for the nested structure and repeated measures design of the study. Subsequently, we built the same GLMM models excluding the three-way interaction effect treatment\*status\*phase (model 2). Model 2 was tested against model 1 (Crawley 2007), and in all instances, the three-way interaction was not significant. Therefore, we present the results of model 1 throughout. All two-way interactions remained in the models to account for potential differential effects on the dominant female or dominant male depending on the treatments or phases of the experiment. The same modelling procedures were repeated for analysing potential behavioural differences between day 0 and day 7 comparing both treatments, where again the three-way interactions proved nonsignificant in any model and were excluded.

Compensatory behavioural responses in the removal treatment were analysed using general or generalized linear models GLM, comparing day 1 and 7 (the post-removal phases) versus day 0 (the pre-removal phase, phase), including territory identifiers as random effects on the intercept, to account for the repeated measures design. Note that compensatory behaviour was analysed by summing the behaviours of the dominant pair and the large subordinate on day 0 and only the dominant pair on day 1 and 7 for the removal groups (for time in the territory, frequency of breeding shelter visits and frequency of defence). To test for the impact of new immigrants on compensatory responses by dominant pair, we used exactly the same GLMs and added immigration as an additional factor. Survival data were tested with a weighted binomial regression GLM with a logit-link.

## Results

### DAY 1 POST-REMOVAL

In the removal treatment, dominant males and females increased their time spent in the territory, frequency of breeding shelter visits and frequency of defence one day after large subordinate removal compared with the control treatment and the pre-removal phase (Fig. 1; Table 2 GLMMs). In absence of the large subordinate, dominant



**Fig. 1.** Change of behaviours over time (day 1 after removal minus day 0 pre-removal) for dominant males and females (DM; DF) in the removal treatment groups. The left side of the graph shows the change in time spent in the territory (in seconds), whereas the middle part of the graph shows the change in number of breeding shelter visits, and the right side shows the change in frequency of defence per 15 min. Shown are means  $\pm$  SE of the difference;  $n = 13$  (lost one observation); \* denotes  $P < 0.05$ .

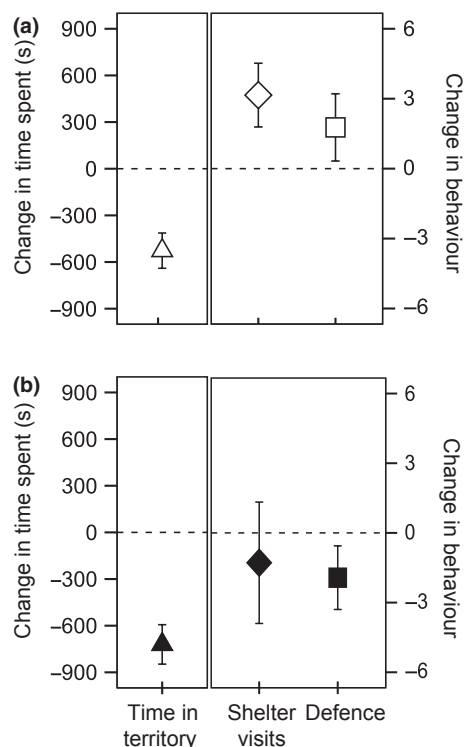
**Table 2.** Results of GLMMs for time spent inside the territory, frequency of breeding shelter visits and frequency of defence on day 0 (the pre-removal observation) and day 1 after large subordinate removal for the control and removal treatments; only data of the dominant pairs were used

Behaviour	Fixed and interaction Effects	$t$ -value/ $z$ -value	$P$
Time inside territory	Intercept	$t_{55} = 4.57$	<0.001*
	Status	$t_{29} = -0.34$	0.739
	Treatment	$t_{29} = 1.02$	0.317
	Phase	$t_{55} = -1.52$	0.133
	Treatment*phase	$t_{55} = 2.30$	0.036*
	Treatment*status	$t_{29} = -1.69$	0.102
	Status*phase	$t_{54} = 0.96$	0.341
Breeding shelter visits	Intercept	$z = 1.22$	0.223
	Status	$z = -0.88$	0.380
	Treatment	$z = 0.14$	0.890
	Phase	$z = -1.8$	0.066
	Treatment*phase	$z = 5.32$	<0.001*
	Treatment*status	$z = -0.97$	0.334
	Status*phase	$z = 1.77$	0.076
Defence	(intercept)	$z = -0.42$	0.675
	Status	$z = -0.87$	0.385
	Treatment	$z = -0.03$	0.976
	Phase	$z = -1.74$	0.083
	Treatment*phase	$z = 4.04$	<0.001*
	Treatment*status	$z = -0.07$	0.947
	Status*phase	$z = 0.74$	0.460

Shown are the GLMMs with corresponding fixed and interaction effects;  $t$ -values ( $t$ ) for normally distributed data with corresponding degrees of freedom;  $z$ -values ( $z$ ) for Poisson-distributed data with log-link; and  $P$ -values ( $P$ ).

\* $P < 0.05$ .

pairs overcompensated for breeding shelter visits (GLM; day 0 vs. day 1:  $z = 2.308$ ,  $P = 0.021$ ; Fig. 2a) and tended to overcompensate for defence, as defence tended to be higher on day 1 post-removal than on day 0 (GLM;



**Fig. 2.** Compensatory responses of the dominants per 15 min in the removal experiment on (a) day 1 (day 1 after subordinate removal minus pre-removal observation data) and (b) on day 7 (day 7 after subordinate removal minus pre-removal observation data). Note that the pre-removal data include behaviours of the three largest individuals, whereas on day 1 and 7, only the behaviours of the dominant pair are used. The left side of both graphs show the change in time spent in the territory (triangles), whereas the right sides show the change for the number of breeding shelter visits (diamonds) and the change in the frequency of defence (squares). Shown are means  $\pm$  SE; in (a)  $n = 13$  (lost one observation), whereas in (b)  $n = 14$ .

$z = 1.81$ ,  $P = 0.071$ ; Fig. 2a). Moreover, in the removal treatment, less time was spent in the territory on day 1 after removal (data of the dominant pair only) compared with day 0 (the pre-removal phase; data of the dominant pair and the large subordinate summed; GLM;  $t_{25} = -4.64$ ,  $P < 0.001$ ; Fig. 2a), suggesting an under-compensatory response of the dominants to the absence of the large subordinate. No new large subordinate immigrants had joined any group on day 1 in both treatments.

#### DAY 7 POST-REMOVAL

On day 7, dominant males and females in the removal treatment did not show any difference in time spent in the territory or in defence compared with the control treatment and the pre-removal phase (Table 3 GLMMs), but they made more breeding shelter visits than compared with the pre-removal phase. No such effects were detected in the control treatment. Independent of the treatment, dominant females tended to increase the frequency of breeding shelter visits more than dominant males 7 days after

**Table 3.** Results of GLMMs of the dominant pairs for time spent inside the territory, frequency of breeding shelter visits and frequency of defence on day 0 (the pre-removal observation) and day 7 after large subordinate removal for the control and removal treatments

Behaviour	Fixed and interaction effects	$t$ -value/ $z$ -value	$P$
Time inside territory	Intercept	$t_{59} = 4.96$	$<0.001^*$
	Status	$t_{29} = -0.75$	0.460
	Treatment	$t_{29} = 0.59$	0.558
	Phase	$t_{59} = -0.80$	0.429
	Treatment*phase	$t_{59} = 0.09$	0.932
	Treatment*status	$t_{29} = -1.01$	0.321
	Status*phase	$t_{59} = 1.48$	0.144
Breeding shelter visits	(intercept)	$z = 1.94$	0.053
	Status	$z = -1.75$	0.080
	Treatment	$z = -0.34$	0.738
	Phase	$z = -1.63$	0.104
	Treatment*phase	$z = 2.94$	$0.003^*$
Defence	Treatment*status	$z = -0.49$	0.622
	Status*phase	$z = 3.62$	$<0.001^*$
	(intercept)	$z = -0.18$	0.860
	Status	$z = -0.39$	0.696
	Treatment	$z = 0.35$	0.729
	Phase	$z = -1.47$	0.142
	Treatment*phase	$z = 1.10$	0.273
Treatment*status	$z = -0.70$	0.485	
Status*phase	$z = 0.55$	0.581	

Shown are the GLMMs with corresponding fixed and interaction effects;  $t$ -values ( $t$ ) for normal-distributed data with corresponding degrees of freedom;  $z$ -values ( $z$ ) for Poisson-distributed data log-link; and  $P$ -values ( $P$ ).

\* $P < 0.05$ .

removal (Table 3). The dominant pair fully compensated for the absence of the large subordinate in their breeding shelter visits in the removal treatment (GLM; day 0 vs. day 7:  $z = -0.54$ ,  $P = 0.590$ ; Fig. 2b) on day 7. Moreover, less time was spent in the territory and less defence was shown seven days after removal (data of the dominant pair only) compared with the pre-removal phase in the removal treatment (data of the dominant pair and the large subordinate summed; GLM; time in the territory,  $t_{25} = -6.29$ ,  $P < 0.001$ ; defence,  $z = -2.47$ ,  $P = 0.013$ ; Fig. 2b). Note that the data presented above have not been controlled for immigrant subordinates.

#### IMMIGRATION DAY 7 POST-REMOVAL

On day 7, five of 14 groups in the removal treatment (36%) had been joined by a new male subordinate ( $>30$  mm) compared with two of 17 groups (12%) in the control treatment (Fisher's exact test;  $P = 0.200$ ).

To test for the impact of new immigrants on the behaviour of the dominants, we divided groups in the removal treatment into those with a new large subordinate immigrant seven-day post-removal and those without such an immigrant. Control groups were not included nor analysed because (1) only two control groups had an immigrant

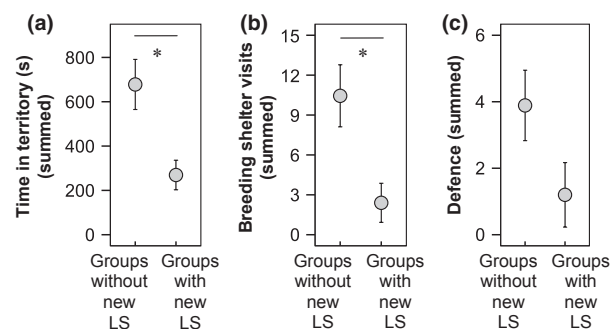
(increase from one to two large subordinates), which was different from the removal groups (i.e. five groups increased from zero to one large subordinate); (2) the magnitude or absence of the additive effect of the second large subordinate on the pair's summed behaviour could not be validly tested, due to the small sample size ( $n = 2$ ). In the removal treatment, however, dominants spent less time in the territory (GLM;  $t_{13} = -2.53$ ,  $P = 0.027$ ; Fig. 3) and performed fewer breeding shelter visits (GLM;  $t_{13} = -2.23$ ,  $P = 0.046$ ; Fig. 3) in groups with an immigrant ( $n = 5$ ) compared with groups without one ( $n = 9$ ), whereas no difference was detected for the frequency of defence (GLM;  $t_{13} = -1.53$ ,  $P = 0.153$ ; Fig. 3, ASI Table S2; see ASI Fig. S1 for the comparison day 7 vs. day 0). Note that for all these analyses, the behaviour of the dominant male and female was summed, and the behaviour of the new immigrant was disregarded.

#### IMMIGRATION ONE-MONTH POST-REMOVAL

One month after the removal of the large subordinate, ten of 14 groups from the removal treatment (71%) had been joined by a new subordinate immigrant >30 mm SL, whereas in the control treatment, four of 17 groups (24%) had been joined by a new subordinate immigrant >30 mm SL (Fisher's exact test;  $P = 0.012$ ). This shows that immigration was affected by the experimental removal and was higher in the removal treatment than the control treatment for at least a cumulative period of 30 days.

#### SURVIVAL OF JUVENILES

In total, 43 of 90 juveniles survived in the removal treatment and 37 of 93 survived in the control treatment after 30 days (odds ratio, 95% confidence interval: 1.38, 0.74–2.60). This difference was not significant (average % offspring survival per group  $\pm$  SE: removal:  $58.5 \pm 9.7$  and control:  $44.2 \pm 9.3$ ; weighted binomial GLM;  $\chi^2 = 0.42$ ,  $P = 0.519$ ; effect size  $\pm$  SE:  $-0.32 \pm 0.51$ ). However,



**Fig. 3.** The dominant pairs' summed (a) time spent in the territory, (b) number of breeding shelter visits and (c) number of defence rates observed per 15 min in the removal treatment on day 7 (7 days after large subordinate [LS] removal) for groups with a new large subordinate immigrant ( $n = 5$ ) and for those without ( $n = 9$ ). Shown are means  $\pm$  SE. \* denotes  $P < 0.05$ .

immigration into the experimental groups (see previous section) might have diluted the validity of this analysis. To account for this effect, we tested whether the survival of juvenile individuals differed between removal groups with a large subordinate immigrant and those without. Survivorship of juvenile individuals did not differ between these two types of groups (weighted binomial GLM;  $\chi^2 = 0.47$ ,  $P = 0.492$ ). Furthermore, no treatment differences were seen in reproductive output during the one-month experimental period: of 31 groups, two broods in the removal treatment and one in the control treatment produced new offspring (Fisher's exact test;  $P = 0.576$ ).

#### Discussion

Large subordinate removal caused the dominant pair to increase the time they spent in the territory as well as their frequencies of breeding shelter visits and defence at day 1, whereas no such effects were apparent in the control groups. These results show that dominants strategically increase their investment in the current brood to compensate for the loss of care that an absent large subordinate would have otherwise provided. Thus, large subordinates appear to 'lighten the load' for dominant pairs by reducing the need for them to perform costly cooperative behaviours and maintain a presence in the territory. Seven days after subordinate removal, however, almost all behaviours of the dominant pair had returned to their pre-removal level and – apart from breeding shelter visits – no differences in behaviour between the removal and control groups were detected. Our data suggest that the dominants could afford to lower their compensatory effort in many of the removal groups, because they were able to recruit immigrant large subordinates to help. Not surprisingly, survival of juveniles was not impacted by the treatment, as absence of the large subordinate was compensated for by the dominant pair and in part by a new subordinate immigrant.

One day after large subordinate removal, dominants overcompensated for the loss of investment in terms of the number of breeding shelter visits, fully compensated the defence effort and undercompensated the time spent in the territory. This illustrates that dominants may show strikingly different compensatory responses depending on the type of behaviour: a key finding of our study that warrants further experimental investigation in cooperatively breeding animals. For instance, to maintain the survival chances of juvenile group members, dominants needed to defend the juvenile individuals, which may explain the full compensatory response for defence behaviour. Additionally, time in the territory was undercompensated, as already one mature individual can guard and defend the juveniles and the territory against most intruders. The overcompensation in the number of breeding shelter visits may suggest an immediate emerging conflict between the pair members over the main care-giving position now that the large subordinate is absent, where the smaller-sized pair member is usually 'forced' (through subdominance) to provide the

majority of care (Awata & Kohda 2004). Clearly understanding the fitness benefits of the various behaviours (e.g. guarding, defence, breeding shelter visits, egg care) will need direct experimental manipulation of each behaviour separately, taking into account whether behaviours are likely to show additive effects on fitness. In practice, long-term manipulation of single behaviours (of single individuals) might prove to be difficult (at least in the field), and probably, only proxies of fitness can be measured (instead of e.g. offspring survival).

Load-lightening by subordinates has been shown in various cooperatively breeding birds and mammals through correlative studies (e.g. Brown *et al.* 1978; Rothe *et al.* 1993; Lacey & Sherman 1997; Clutton-Brock *et al.* 2000; MacColl & Hatchwell 2003), but few experimental tests have been performed. Wright & Dingemanse (1999) showed that subordinate Arabian babblers (*Turdoides squamiceps*) increased chick provisioning when receiving supplemental food, which decreased provisioning levels of the dominant pair. Hatchwell & Russell (1996) showed that subordinate removal in long-tailed tits (*Aegithalos caedatus*) caused the dominant pair to compensate fully for the reduced provisioning rate and concluded that subordinates 'lighten the load' of the pair. Nevertheless, all results from removal experiments have to be interpreted with some caution because not only the amount of help might be altered, but group size is also reduced (Jennions & Macdonald 1994; Cockburn 1998; Russell *et al.* 2008). To avoid reduction in group size, some studies have tested load-lightening experimentally by altering helper to offspring ratios (Clutton-Brock *et al.* 2001; Russell *et al.* 2008; Browning *et al.* 2012). Our study demonstrates that dominants assisted by a large subordinate spend less time in the territory, perform fewer breeding shelter visits and defend less. This is likely to be advantageous for dominants, as apart from saving energy, they could benefit from the following: (i) an increased number of breeding attempts, (ii) increased personal survival because of the reduced current reproductive costs (Hatchwell 1999) and (iii) the extra resources of the pair could potentially be used for additional spawning possibilities, as polygamy is widespread in *J. ornatus* (Awata, Munehara & Kohda 2005). Even though male subordinates often gain a share in reproduction, the absolute quantity of young sired by the dominant male they are assisting is equal to that of same-sized dominant males with no large subordinate in their group (Awata, Munehara & Kohda 2005). This suggests that the costs of sharing part of reproduction with a large subordinate may be outweighed by the benefits of decreasing their own investment in the territory (Awata & Kohda 2004).

In birds and mammals, young are often directly provided for and an extra helping subordinate can make a large contribution to the amount of food chicks receive (see Lazarus & Inglis 1986; 'unshared investment'), whereas in cooperatively breeding fish, fry have to feed for themselves and can only indirectly benefit from the presence of the dominant pair and/or subordinates, through protection from preda-

tors ('shared investment'). Although additional subordinates may contribute to guarding or defence (shared investment), this is unlikely to have large additive effects on offspring fitness. Therefore, contrary to the situation in most birds and mammals, group size in *J. ornatus* might be less important for overall survival of fry (Awata, Munehara & Kohda 2005; Awata *et al.* 2010). Nevertheless, if the pair and large subordinate both reduce their investment in protection duties leading to absence of all three individuals from the territory, offspring may need to hide more and feed less (Heg & Taborsky 2010) or otherwise suffer an increased predation risk (Heg *et al.* 2004).

Based on previous work (see Heg & Bachar 2006), we expected to find some immigration of large subordinates occurring in our study groups. However, contrary to expectation, we detected fast and high levels of immigration (on day 7, 36% of the removal treatment groups had a large immigrant compared with 12% of the control groups). One month after removal, the difference in immigration was significant, reaching 71% in the removal groups against 24% in the control groups. In cooperatively breeding fishes, only one previous study performed experimental subordinate removal and tested for offspring survival effects one week later; yet, this study in *Neolamprologus pulcher* did not report any subsequent immigration of new subordinates (Brouwer, Heg & Taborsky 2005). Subordinate immigration in cooperatively breeding birds generally follows a different route; groups are joined by subordinates that failed their own reproductive attempt (e.g. in long-tailed tits, Russell & Hatchwell 2001), or by unrelated subordinates that failed to find a suitable mate (e.g. in pied kingfishers, *Ceryle rudis*, Reyer 1984). In pied kingfishers, subordinate immigration appears dependent on parental energy expenditure, as immigrants are only tolerated in the case of large broods (Reyer & Westerterp 1985). In our study species, however, new immigrants seem to be tolerated by dominants in all situations, potentially due to the savings in costly parental behaviour and releasing the dominant pair to pursue polygamous mating. Our findings of rapid immigration are consistent with the fact that large subordinates in *J. ornatus* may gain substantially from joining a group through shared reproduction, despite providing the largest share of investment (Awata, Munehara & Kohda 2005). In turn, immigration also affected the compensatory response of the pair. Taking immigration into account, the dominant pair of removal groups continued high investment without new immigrants, but immediately reduced their investment when a new large subordinate had joined their group.

No effect of large subordinate removal was found on survival of juvenile group members one month later when comparing the removal and control groups. This was not unexpected, as absence of the large subordinate was compensated for by both dominants and partly by newly immigrated subordinates as well. Additionally, survivorship within the removal treatment did not differ between groups with and without a new large subordinate immigrant. This

suggests that the new large subordinate has a similar impact on the survival of juvenile group members as the dominant pair, and therefore, the large subordinate seems effectively to lighten the load of dominant individuals. In various bird species where dominants (under) compensated for the absence of subordinates, equal offspring survival between control and removal groups has been reported (Zahavi 1990; Leonard & Horn 1996; Magrath 2001; but see Brown *et al.* 1982; Mumme 1992; Komdeur 1994). However, in a different cooperatively breeding cichlid (*N. pulcher*), subordinate removal decreased offspring survival (Brouwer, Heg & Taborsky 2005), perhaps due to a lack of compensation by the remaining group members. One notable difference between these two species is that in *N. pulcher*, all subordinates perform defence against potential predators (Bruitjes, Hekman & Taborsky 2010; Bruitjes & Taborsky 2011; Bruitjes *et al.* 2011), whereas in *J. ornatus*, only the mature large subordinate and the dominant pair are involved in defence (Awata *et al.* 2010).

## Conclusions

We conclude that the effects of a large male subordinate on dominant investment were consistent across all three types of analyses: comparing control with removal groups in the post-removal phase, comparing observations of removal groups between pre- and post-removal phases and comparing removal groups with or without a new immigrant on day 7. Our study shows that large subordinate presence is valuable for dominants in a cooperative breeding system. After losing their large subordinate, the dominants compensated by increasing the time they spent in the territory and their frequencies of breeding shelter visits and defence, suggesting that they normally save energy when a large subordinate group member is present. In cases where they were able to recruit a new large subordinate, dominants changed their investment to previous lower levels. Large subordinates might improve the future reproductive success of dominants, or alternatively they may enhance the dominants' current reproductive success by freeing them up from costly activities and allowing them more time to engage in multiple mating.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** The dominant pairs' summed difference in behaviour per 15 min between day 7 and day 0 in the removal treatment (day 7 after removal minus pre-removal data) for groups with a new large subordinate recruit (LS;  $n = 5$ ) and for those without ( $n = 9$ ).

**Table S1.** Results of GLMMs for time spent in the territory, frequency of breeding shelter visits and defence rates of the pre-removal observation.

**Table S2.** Summed behaviours of dominants pairs from the control and removal treatment with and without a new large subordinate (LS) recruit at day 7.

**Table S3.** Results of GLMs for time spent in the territory, frequency of breeding shelter visits and defence rates in removal treatment groups with and without new large subordinate recruits.