

Testis size depends on social status and the presence of male helpers in the cooperatively breeding cichlid *Julidochromis ornatus*

Satoshi Awata,^a Dik Heg,^b Hiroyuki Munehara,^c and Masanori Kohda^a

^aLaboratory of Animal Sociology, Department of Biology and Geosciences, Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi, Osaka 558-8585, Japan, ^bDepartment of Behavioural Ecology, Zoological Institute, University of Bern, Wohlenstrasse 50a, CH-3032 Hinterkappelen, Switzerland, and ^cUsujiri Fisheries Station, Field Science Center for Northern Biosphere, Hokkaido University, 152 Usujiri, Hakodate, Hokkaido 041-1613, Japan

Cooperatively breeding animals, in which helpers may participate in reproduction with dominant breeders, are ideal species for examining intraspecific variation in testis size because they often exhibit both monogamous breeding (low risk of sperm competition) and polyandrous breeding (high risk) within a population. However, little is known about testis investment as a result of sperm competition in these animals. The substrate-brooding cichlid fish *Julidochromis ornatus* has a cooperatively breeding system, in which some males mate monogamously and other males reproduce as dominant breeders or helpers within cooperatively breeding groups, in which male helpers frequently sire young. We examined the relationship between testis investment and male social status in relation to the risk of sperm competition. As predicted from sperm competition models, in groups with male helpers, both the male breeders and the male helpers invested more in testes mass, compared to breeding males without male helpers. We also found a positive relationship between the testes mass of male breeders and their male helpers, suggesting that males increase their investment in reproductive capability under the risk of sperm competition. Sperm competition models also predict that larger testes are associated with increased siring success. Our paternity analysis supported this prediction; we found a positive relationship between testis investment by male helpers and the number of offspring they sired. **Key words:** cooperative breeding, Lake Tanganyika, paternity, social status, sperm competition risk, testis investment. [*Behav Ecol* 17:372–379 (2006)]

Sperm competition occurs when sperm from two or more males compete for egg fertilization (Parker, 1970, 1998). This phenomenon is common in a variety of animals and has considerable force in evolutionary adaptations in males, for example, morphological, physiological, and behavioral adaptations (Birkhead and Møller, 1992, 1998; Smith, 1984). One of the most widespread adaptations is varying testis investment according to the level of sperm competition. Theoretical models predict that across species, testis investment should increase with the risk or intensity of sperm competition (Parker, 1982, 1998; Parker et al., 1996). This prediction has been strongly supported by comparative studies across taxa in a wide range of vertebrates (fishes: Stockley et al., 1997; frogs: Jennions and Passmore, 1993; birds: Møller, 1991; bats: Hosken, 1997; primates: Harcourt et al., 1981) and invertebrates (butterflies: Gage, 1994). The same prediction for testis investment and sperm competition risk also applies within a species, especially in fishes with alternative male reproductive tactics (reviewed by Petersen and Warner, 1998; Taborsky, 1994, 1998). However, only one study has shown that male reproductive success increases with testis investment (Preston et al., 2003).

Cooperative breeding is a system in which more than two adults provide care to young at a single nest. In many cases,

group members consist of a pair of breeders and closely related helpers (Brown, 1987; Emlen, 1991). In some birds, however, dominant breeders and unrelated helpers take care of the brood jointly, and such males frequently share their paternity with one female (e.g., dunnoek *Prunella modularis*: Burke et al., 1989; Davies, 1992; pukeko *Porphyrio porphyrio*: Jamieson, 1997; Jamieson et al., 1994; Galapagos hawk *Buteo galapagoensis*: Faaborg et al., 1995). Moreover, these species usually exhibit both pair breeding and cooperative breeding within a population. In this case, sperm competition is expected to occur between competitive but cooperating males and to be absent in monogamous pairs and cooperatively breeding groups that include only female helpers. Davies (1992) found the support for this hypothesis; the number of copulations by polyandrous males was about 2.5 times greater than by monogamous males. To our knowledge, however, no study has addressed the relationship between testis investment, sperm competition, and reproductive success in relation to male social status within cooperatively breeding animals. On the one hand, dominant breeding males may suppress male helpers participating in reproduction (leading to “high reproductive skew” societies, see for review Johnstone, 2000). Suppression may be mediated directly by, for example, mate guarding of receptive females by dominant males or indirectly by, for example, repeated aggressive interactions with subordinates leading to, for example, elevated cortisol levels, lowered sex steroids, and in the end reduced reproductive capacity of subordinates (Clarke et al., 2001; Creel, 2001). On the other hand, dominant males may not be able to suppress subordinate male reproduction or may concede reproduction to subordinates to ensure their continued group membership, in case subordinates are helpful

Address correspondence to S. Awata, who is now at the Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, 3422 Sesoko, Motobu, Okinawa 905-0227, Japan. E-mail: h066112@sci.u-ryukyu.ac.jp.

Received 25 July 2005; revised 2 November 2005; accepted 22 December 2005.

(leading to “low reproductive skew” societies: Johnstone, 2000).

Julidochromis ornatus is a substrate-brooding cichlid fish endemic to Lake Tanganyika. It has a cooperative breeding system and exhibits a complex social system; monogamous, cooperative, and harem breeding can be found within the same population (Awata et al., 2005). Most helpers of both sexes are unrelated to the dominant breeders, and male breeders frequently share paternity with male helpers (i.e., low reproductive skew: Awata et al., 2005). Interestingly, the number of offspring sired by the male breeders and male helpers varies considerably among groups. One possibility accounting for such variation in offspring number is the testis investment by the two competing males.

In this study, we examined the relationships between testis investment, social status, and reproductive success in *J. ornatus*. Specifically, we tested the following hypotheses. First, if male helpers are reproductively suppressed by the dominant breeding males, we expected male helpers to have a lower relative testes mass compared to the male breeders. Alternatively, if male helpers are not reproductively suppressed or only help male breeders with which they can successfully compete for fertilizations (e.g., low-quality male breeders with relatively small testes), male helpers have similar or higher relative testes mass compared to the male breeders. Second, if male breeders compete for fertilizations with the male helpers, these male breeders should invest relatively more in testes mass compared to breeder males without helpers or breeder males with only female helpers. Third, if we assume that relative testes mass shows a positive relationship with sperm production (e.g., Leach and Montgomerie, 2000; Marconato and Shapiro, 1996; Schärer and Robertson, 1999) and that both males compete for fertilizations simultaneously (as suggested by the paternity data in Awata et al., 2005) and assess each other's reproductive capability by, for example, spawning behavior, we predict that male breeders and male helpers should progressively invest more in their gonads. This should lead to a positive correlation between the relative testes investment of the two competing males, and the male breeder or the male helper with the highest relative testes investment should sire the highest proportion of offspring in the current brood. Finally, we assessed whether the proportion of offspring sired by the different males may have been confounded by, for example, the effects of male and/or female body size or gonad weight on the total reproductive output.

METHODS

Study species

J. ornatus is a small benthic cichlid fish that inhabits shallow rocky shores in the southern part of Lake Tanganyika and limited areas of the northern part (Kohda et al., 1996; Konings, 1998). Both sexes are similar in color, shape, and body size (up to 10 cm in total length, TL; Awata and Kohda, 2004). This fish has various breeding systems; pair breeding, cooperative breeding, and harem breeding coexist within a single population, and the same breeding members repeatedly spawn at the nest (Awata et al., 2005). Breeding takes place inside narrow clefts in or between rocks. Regardless of their social status, all breeding members of both sexes take care of their young until the fry become independent.

In cooperatively breeding groups, the number of helpers is usually one, and male helpers are more numerous than female helpers (Awata et al., 2005). Molecular genetic relatedness analyses have revealed that most helpers (>85%) are unrelated to the dominant breeders, and the remaining helpers are related to either one of the breeders (Awata et al.,

2005). Mature helpers of both sexes frequently participate in reproduction with the dominant breeders. Occasionally, the largest males within a population have large territories and access to two or three breeding nests, where female breeders and male helpers care for the young (male harem; Awata et al., 2005). This is also the case for the largest females, which gain access to two or three males in separate nests for breeding (female harem).

Study sites, field observations, and fish sampling

The field study was conducted from September to December 1998 and August to November 1999 at Kasenga Point (8° 42.9' S, 31° 08.1' E), Wonzye Point (8° 43.5' S, 31° 07.8' E), and Nkumbula Island (8° 45.5' S, 31° 05.7' E) near Mpulungu, Zambia, on the southern shore of Lake Tanganyika. The three study sites were on rocky shores at water depths of 3–12 m, where *J. ornatus* was abundant. Newly hatched young were found throughout the study periods at all study sites. Scuba was used for all underwater observations and fish sampling.

At Kasenga Point, in both years, many groups of *J. ornatus* (e.g., monogamous pairs or cooperatively breeding groups) reproduced, and their young were visible in and around the nest crevices. Breeders and helpers of the groups were readily identifiable by their individual body color patterns. During observations, their swimming routes, number of nest visits, and time spent at nests (within 15 cm of the nest entrance) were recorded. Most individuals were observed for 15 min each on 3 or 4 different days. Each harem owner that had a large home range was observed for 20–45 min on 5–8 different days. After the observations, almost all the adults and young were captured using gillnets or hand nets with the help of an anesthetic (30% clove oil diluted in ethanol). Of 66 groups captured, 40 were monogamous pairs without helpers, 13 were pairs with a male helper, three were pairs with a female helper, three were pairs with several helpers (one male and one to two female helpers), four were female harems (two, two, two, and three nests controlled by each harem owner), and three were male harems (two, three, and three nests).

At Wonzye Point and Nkumbula Island, when breeders and their young were found, the breeding systems were assessed through 5- to 10-min observations. After the assessment, all breeders, helpers, and young were caught. In total, we collected seven monogamous pairs without helpers, six pairs with a male helper, one pair with a female helper, and two pairs with several helpers (one female and two to four male helpers) at Wonzye Point, and we collected four monogamous pairs without helpers and six pairs with a male helper at Nkumbula Island.

On the day of sampling, fish were sacrificed by overanesthesia with clove oil diluted in ethanol. TL (0.1 mm) was measured using calipers (Mitutoyo Corp., Kanagawa, Japan), and wet body weight (to 0.001 g) and wet gonad weight (to 0.001 g) were determined using an electronic balance (PM460, Mettler-Toledo GmbH, Greifensee, Switzerland). Some gonads lighter than 0.002 g were fixed with 10% formaldehyde solution and were weighed to the nearest 0.0001 g using another electronic balance (AG245, Mettler-Toledo GmbH, Greifensee, Switzerland) in Japan.

Parentage assignment

After the morphological measurements, all the fish were preserved in 99% ethanol for genetic analysis of parentage. DNA was extracted from breeders, helpers, and young collected at the three study sites, and the kin relationships between breeders and helpers and the parentage of the young were determined using up to four primer sets. The results of

the parentage assessment of the Kasenga population were reported by Awata et al. (2005). We obtained similar results for the Wonzye and Nkumbula populations, that is, most helpers (>80%) were unrelated to the dominant breeders and frequently shared paternity or maternity with the dominants. In 23 cooperatively breeding groups (three study sites combined), a male helper could have participated in reproduction, and the paternity results of these groups were used to assess whether males showing a high gonadal investment sired more young.

Definition of male types and data selection

Male types were categorized into four groups: (1) male breeders without helpers, (2) male breeders with male helpers, (3) male breeders with a female helper only, and (4) male helpers. These four male types were also found within female harems as follows. In female harems, the female harem owner gained access to two or three breeding nests, each with a male breeder (Awata et al., 2005). Of a total of nine nests controlled by the harem females, two contained only one male breeder (=a male breeder without helpers), two had a male breeder with a female helper, and five had a male breeder with male helpers.

In male harems, the male harem owner cruised widely and gained access to two or three breeding nests, each with a female breeder (Awata et al., 2005). Of a total of eight nests controlled by the harem males, five contained one female breeder and one male helper, two contained one female breeder and one male and one female helper, and one contained only a female breeder. In this situation, male helpers are assumed to have a higher risk of sperm competition as harem owners increase their time spent at the nest and their number of nest visits. Therefore, we analyzed male harem data separately from other breeding systems. The data from the three harem groups were pooled because of the small sample size.

Data analysis

We used general linear models (GLMs) to examine the difference in testis investment in males of different social status following Tomkins and Simmons (2002). First, we constructed a GLM with log gonad weight (LGW) as the dependent variable and the following as independent variables: male types (fixed categorical effect: breeders without helpers, breeders with male helpers, or helpers), location (fixed categorical effect: Kasenga Point, Wonzye Point, or Nkumbula Island), and log soma weight as a covariate, including all interactions.

Second, because LGW did not depend on the location (see Results), we constructed a full GLM similar to the first model, excluding location from the main effects. The full GLM allowed us to test whether male types differed in their LGWs and whether LGW depended on log soma weight, independent of other factors. The full model is important for the interpretation of the results because (1) males differed in body sizes (see Results), so any changes in LGW depending on social status may come about by body size differences alone (i.e., due to allometric scaling), and (2) males of different types may not only show differences in LGWs (i.e., different intercepts) but also different allometric scaling of the LGW on log soma weight (i.e., different slopes, see Tomkins and Simmons, 2002), making the interpretation of the results more complex.

Third, because only male types and log soma weight showed a significant effect on LGW and none of the interactions were significant (see Results), we also constructed a final GLM incorporating only these two factors. We assessed which male types differed significantly from each other by multiple com-

parison analyses within the final GLM. To illustrate differences between the male types and relate the relative LGW to reproductive success, the residuals of LGW from the expected LGW were calculated from the final GLM (see Results), where

$$\begin{aligned} \text{Expected LGW} = & \text{weighted average intercept} \\ & + \text{coefficient} \times \log \text{ soma weight,} \end{aligned}$$

with weighted average intercept = constant + average of the coefficients for the four male types weighted by their sample sizes.

Thus, the residual LGW is defined as the difference from the expected LGW of an average male in the population of a given body size.

Likewise, we related LGW to log soma weight for females using GLM and used the residuals of LGW from this relationship to assess relative gonadal investment of the females and related these with the body measurements of males and females to reproductive success (see below).

To test whether males with a relatively high gonadal investment (i.e., high residual LGW) sired more offspring, we calculated the difference in residual LGWs for the two males in a group (i.e., residual LGW for the male helper – residual LGW for the male breeder) and related this difference to the proportion of offspring sired by the male helper using a GLM with a weighted logit link (also known as weighted logistic regression, Crawley, 2002) and the number of offspring sired using GLMs with a log link (also known as Poisson regressions, Crawley, 2002).

Finally, we assessed whether the previous result might have been confounded by differences in total reproductive output depending on, for example, male breeder, male helper, or female breeder soma weight or gonad weight, using a GLM with a log link (Crawley, 2002).

Statistical analyses were performed using the statistical software program SPSS 12.0 and R 1.0.8 (Crawley, 2002). Because behavioral data and data of brood size were not normally distributed, we used nonparametric statistics for the analyses. When we used GLMs and partial correlation coefficient analyses, all values were log transformed to produce a normal distribution. All probabilities reported are two tailed.

RESULTS

Male body size and social status

Body sizes overlapped widely among the males of different social status: male breeders with male helpers (mean TL \pm SD = 70.3 \pm 10.0 mm, range = 49.4–86.6 mm, n = 35), male breeders with a female helper (63.3 \pm 5.6 mm, range = 56.5–71.5 mm, n = 6), male breeders without helpers (58.3 \pm 10.5 mm, range = 38.4–82.6 mm, n = 53), and male helpers (51.1 \pm 8.3 mm, range = 37.9–71.3 mm, n = 40). Body sizes differed significantly among the four male types (ANOVA, $F_{3,130} = 25.62$, $p < .0001$) due to male helpers being significantly smaller than all other males (Tukey's honestly significant difference, Tukey HSD, all $p < .05$). Male breeders with male helpers were larger than those without helpers ($p < .0001$), and no significant differences were detected between male breeders with a female helper and monogamous males without helpers ($p = .63$) or male breeders with male helpers ($p = .34$).

Testis investment and social status

The first GLM revealed that sampling locality had no significant effect on LGW (location: $F_{2,122} = 0.82$, $p = .44$; location \times status: $F_{4,110} = 0.58$, $p = .68$; location \times log soma weight: $F_{2,110} = 0.98$, $p = .38$; location \times status \times log soma weight: $F_{4,110} = 0.68$,

Table 1

Results from the GLMs of LGWs on four male types, with the covariate log soma weight

Independent variable	Full GLM				Final GLM			
	df	F	p	Coefficient \pm SE	df	F	p	Coefficient \pm SE
Constant	1	322.29	<.0001	-4.97 ± 0.16	1	1521.44	<.0001	-4.99 ± 0.14
Male type	3	17.86	<.0001		3	32.29	<.0001	
Breeder without helpers				-1.73 ± 0.24				-1.70 ± 0.19^a
Breeder with male helpers				-0.79 ± 0.43				-0.63 ± 0.24^b
Breeder with a female helper				-0.42 ± 1.19				-1.47 ± 0.38^a
Helper				0				0 ^c
Log soma weight	1	7.69	.006	1.06 ± 0.27	1	65.03	<.0001	1.12 ± 0.14
Male type \times log soma weight	3	0.36	.78					
Breeder without helpers				0.08 ± 0.34				
Breeder with male helpers				0.16 ± 0.40				
Breeder with a female helper				-1.04 ± 1.19				
Helper				0				

Superscripted letters indicate significantly different male types in LGW (all $p < .01$, except male breeder with male helpers versus male breeder with a female helper with $p = .028$) from six pairwise comparisons using the sequential Bonferroni test (Rice, 1989).

$p = .61$). Therefore, we combined data from the three study sites for further analyses.

The second GLM analysis showed a significant effect of log soma weight and male type on LGW, whereas the interactions were not significant (Table 1). This shows that LGW follows a simple relationship with these two factors (Figure 1), and therefore the third and final GLM was constructed to compare the different male types and to calculate residual LGW for each male for subsequent analyses (see Methods, Table 1,

Figure 1). As predicted, multiple comparisons within this final GLM showed that the LGWs of both male breeders with male helpers and the helpers themselves were significantly higher than the LGWs of male breeders without helpers and the male breeders with a female helper only (Table 1). Moreover, the LGWs of male helpers were significantly higher than those of male breeders with male helpers. We found no significant difference in LGWs between the male breeders without helpers and those with a female helper (Table 1).

Testis investment and sperm competition

As predicted, males invested more in reproductive capacity under the risk of sperm competition as the residual LGWs of the male breeder and the male helper within his group were positively correlated (Figure 2). The residual LGW of the female breeder was also correlated with the residual LGW of her male helper (Pearson's correlation, $r = .61$, $n = 22$, $p = .002$). Partial correlation coefficient analyses showed that when the residual LGW of the female breeder was held constant, the residual LGW of the male breeder was still positively correlated with the residual LGW of the male helper (Table 2); same results were also obtained within populations (Kasenga: $df = 9$, partial $r = .84$, $p = .001$; Wonzye: $df = 2$, partial $r = .89$, $p = .11$; Nkumbula: $df = 2$, partial $r = .99$, $p = .012$). When the residual LGW of the male breeder was held constant, a significant correlation remained between the residual LGWs of the female breeder and the male helper. In monogamous pairs without helpers, the residual LGW of the male breeder was not related to the residual LGW of the female breeder (Pearson's correlation, $r = .03$, $n = 53$, $p = .82$).

Male harem owners visited each nest 0.08–3.33 times per 10 min and stayed at the nest for a total of 0.06–3.41 min per 10 min. The residual LGWs of male helpers tended to increase with the frequency of nest visits by harem males (Kendall's rank correlation, $\tau = 0.52$, $n = 7$, $p = .099$) but, more importantly, showed a positive correlation with the time spent at the nest by the harem male ($\tau = 0.81$, $n = 7$, $p = .011$).

Testis investment, paternity, and brood size

Microsatellite analyses of paternity showed that in total, 18 individual male breeders and 17 individual male helpers gained paternity in the 23 groups with a male helper

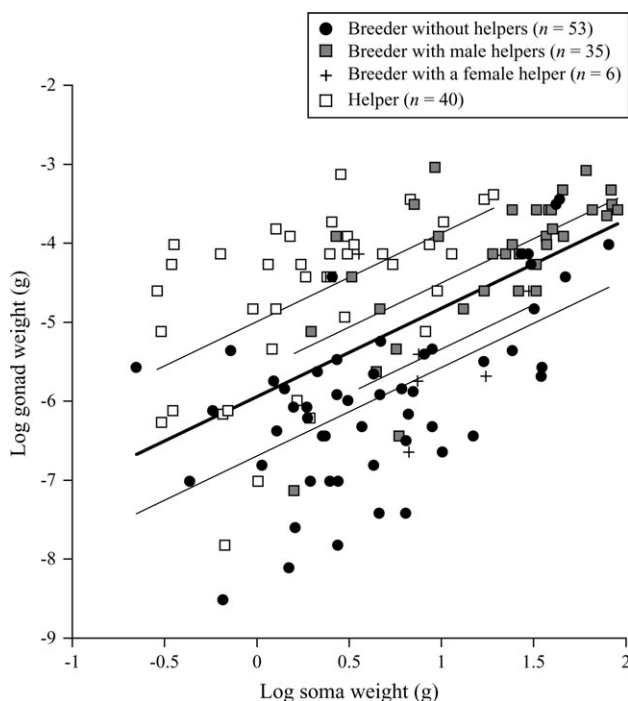


Figure 1

LGWs differed significantly between the four different types of males and scaled allometrically with their log soma weight. Note that the slopes did not differ significantly, and therefore the bold regression lines depicted for the four types of males are from the final GLM given in Table 1. Also depicted is the average relationship between log soma weight and LGW (bold thick line: from the final GLM in Table 1), used to calculate for each male his residual LGW.

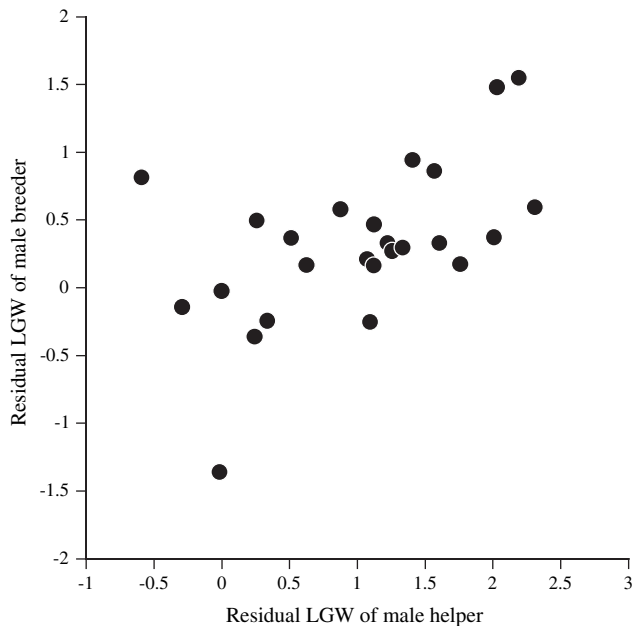


Figure 2

The residual LGWs of male breeders were positively related to the residual LGWs of their male helpers (Pearson's correlation, $r = .54$, $n = 25$, $p = .005$).

(including two groups where no offspring were produced at all). The median number of young per nest fathered by male breeders and male helpers was 2.0 (range = 0–21, $n = 23$) and 1.0 (range = 0–10, $n = 23$), respectively. The number of young fathered by male breeders and male helpers did not differ (Wilcoxon signed-rank test, $T = 79.00$, $n = 21$, $p = .35$, excluding the two groups with no offspring), and the proportion of offspring sired by the male helpers was close to 40% (mean \pm SD = $41.1 \pm 36.9\%$, median = 37.5%, range = 0–100%, $n = 21$).

Contrary to the expectation, the proportion of offspring sired by the male helper did not depend on his relative investment in testes (Figure 3a, GLM with weighted logit link, effect of the difference in residual LGW of the male helper minus the male breeder: $z = -0.36$, $n = 21$, $p = .72$). This seemed due to the two males increasing their investment in testes concordantly (see Figure 2), leading to a positive relationship, albeit not significantly for the male breeders, between residual LGW and the number of young sired by the two males (Figure 3b,c, GLMs with log link, effect of residual LGW on the number of offspring sired for the male helpers: $z = 2.79$, $n = 21$, $p = .005$; for the male breeders: $z = 0.15$, $n = 21$, $p = .88$, deleting the outlier with 21 offspring sired did not change this result, $z = 1.13$, $n = 20$, $p = .26$).

Table 2

Pairwise partial correlations between the residual LGWs of the three major group members, after the correlation with the other group member was controlled for

Relationship	df	Partial r	p
Male breeder LGW versus female breeder LGW	19	-.26	.25
Male breeder LGW versus male helper LGW	19	.69	.0006
Female breeder LGW versus male helper LGW	19	.61	.003

r , correlation coefficient.

The above results may have been confounded by differences in productivity between the groups depending on the body measurements of the breeders and the helpers. As in the males, the females showed a significant positive correlation between LGW and log soma weight (Pearson's correlation, $r = .49$, $n = 134$, $p < .0001$), and we used a GLM to calculate the residual LGW for each female. There were no significant differences in brood sizes among the three study sites (Kruskal-Wallis test, $H = 2.08$, $n_{\text{Kasenga}} = 13$, $n_{\text{Wonzye}} = 5$, $n_{\text{Nkumbula}} = 5$, $p = .35$), so we combined these data. We found a significant positive effect of the male helper size and the female breeder size on the total number of offspring produced by the group, whereas the effect of female breeder relative gonad size was also positive but just not significant (Table 3, $n = 22$, excluding one group where no body and gonad measurements of the female breeder were available). Moreover, and unexpectedly, the number of offspring produced significantly decreased with the body size of the male breeder (Table 3). However, we note that the body measurements of all group members were highly correlated.

DISCUSSION

Our results show that testis investment varies in relation to the level of sperm competition in *J. ornatus* as follows. First, male breeders with male helpers invested more in testes than all other male breeders. This suggests that male breeders increased their investment in testes only when their potential share in paternity is under risk by male helpers participating in reproduction. Second, male helpers invested more in testes than male breeders. Apparently, male helpers are not reproductively suppressed in this species. On the contrary, it may explain the high level of male helper paternity in this study population (Awata et al., 2005). Third, within a breeding group, testes investment of male breeder and male helper correlated positively. Again, this might be due to both group member males competing for paternity, and to gain the largest share, both males might progressively invest more in sperm production leading to a positive correlation in testes mass. However, there are also several alternative explanations for such a positive correlation in testes mass between both males (see below).

To our knowledge, this is the first evidence within a cooperatively breeding animal that males increase their testis investment under the risk of sperm competition, a pattern otherwise found in between-species comparisons (see review Birkhead and Møller, 1998). In *J. ornatus*, monogamous males without helpers and male breeders with a female helper had relatively small testes. In contrast to many other Lamprologini cichlids (Konings, 1998; Kuwamura, 1986; Nagoshi and Yanagisawa, 1997), the clutch size in *J. ornatus* is rather small (no more than 40 mature eggs in the ovary of monogamous females; Awata, 2005), suggesting that only a limited quantity of sperm is needed for successful fertilization. If we assume that sperm production is costly, as found in other species (e.g., Dewsbury, 1982; Nakatsuru and Kramer, 1982; Shapiro et al., 1994), this might explain why these male breeders have notably smaller testes than both male breeders with male helpers and the male helpers. We assume that the larger testes allow more and larger ejaculates of sperm in order to win the intra-sexual competition for egg fertilization, as found in other species (e.g., Leach and Montgomerie, 2000; Marconato and Shapiro, 1996; Schärer and Robertson, 1999).

Body size, social status, and testes investment

In many fishes, males show alternative mating tactics in relation to their body sizes (Gross, 1996; Taborsky, 1994). In

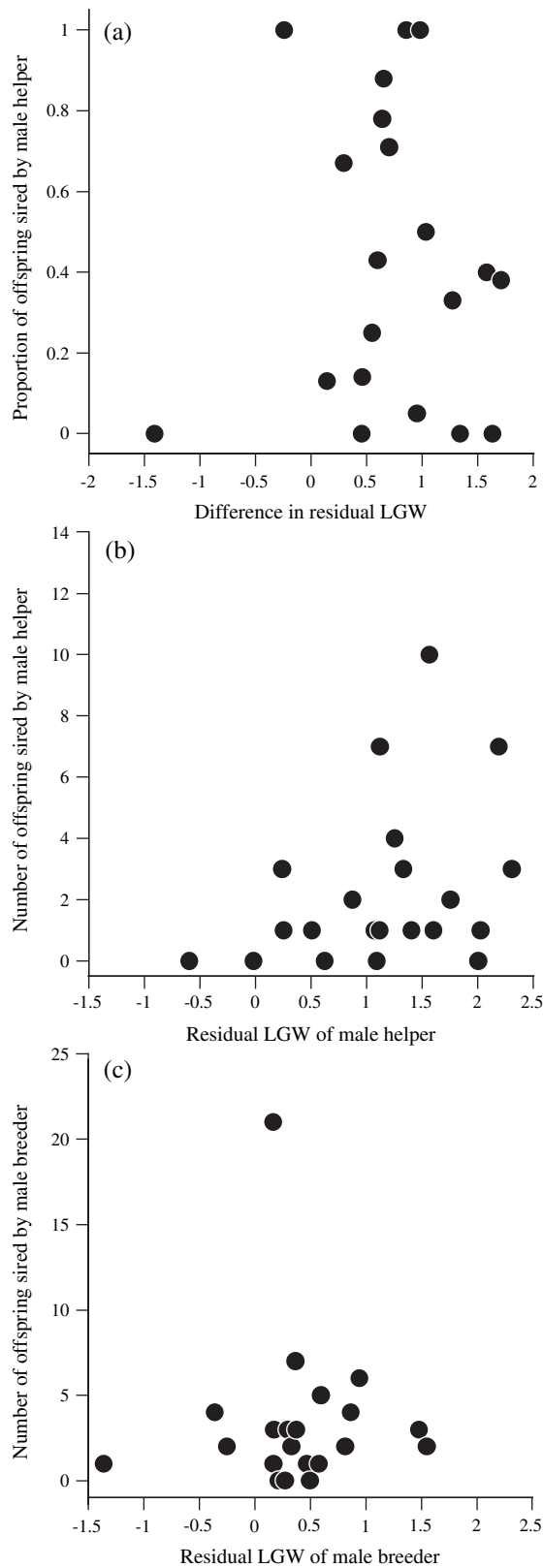


Figure 3
Effects of residual LGWs on the reproductive success of the male group members. (a) The proportion of offspring sired by the male helper did not depend on his relative investment in testes (residual LGW of male helper – residual LGW of his male breeder). The number of young sired significantly depended on the residual LGW for (b) male helpers but not (c) male breeders. See text for statistics.

Table 3
Effects of male and female body measurements on group productivity

Independent variable	df	z	p	Coefficient ± SE
Constant	1	4.10	<.001	2.20 ± 0.54
Log soma weight				
Male breeder	1	-2.84	<.005	-1.32 ± 0.47
Female breeder	1	4.17	<.001	0.69 ± 0.16
Male helper	1	2.59	<.01	0.93 ± 0.36
LGW ^a				
Female breeder ^b	1	1.76	.078	0.47 ± 0.27

Depicted are results from a GLM with log link on the total number of offspring produced in the group.

^a The following nonsignificant variables were excluded from the model: LGW of male helper and LGW of male breeder.

^b Note that LGW of male helper and LGW of female breeder were highly correlated (see Table 2) and thus interchangeable.

many cases, sexual selection favors different ontogenies and allocation patterns to soma investment and testes investment depending on these mating tactics. For instance, bourgeois males (Taborsky, 1997), which defend a mating territory and attract females, have large colorful bodies with relatively small testes, whereas parasitic males are small and dull colored and have large testes (Gross, 1996; Petersen and Warner, 1998; Taborsky, 1994, 1998). Therefore, in many fishes, there is a strong relationship between mating tactic, testis investment, and morphology, such as body size and color. In our study population, however, the body sizes of male *J. ornatus* of different social status overlapped widely. In particular, all four male types were found in the body size range from 50 to 70 mm TL, and testes mass showed a similar allometric scaling with soma mass in all males (i.e., same slopes with different intercepts). Moreover, we found no difference in coloration among male types (Awata S, personal observations). These results indicate that testis investment by male *J. ornatus* is not related to morphology but only to the risk of sperm competition. Indeed, there is a high possibility that males strategically allocate energy to testis investment with increasing levels of sperm competition, as an effect of the social environment on each male. It would be worthwhile to experimentally alter the social position of individual males (e.g., male helpers become male breeders) or group composition (e.g., removing male helpers) and study the effects on sperm production of both male breeders and (former) male helpers.

Testes investment and within-group male-male competition

We found that in cooperatively breeding groups with male helpers, both male helpers and male breeders invested more in their gonads than monogamous males did, and no difference was found in the siring success of male breeders and male helpers. These results indicate that male breeders in *J. ornatus*, which are socially dominant over male helpers, cannot or do not fully control egg fertilization by these helpers, and selection would favor gonadal investment by male breeders rather than mate guarding (Alonzo and Warner, 2000). Indeed, we found no evidence for mate guarding occurring in *J. ornatus* as a way to ensure paternity (Awata S, personal observations), as has been found in, for instance, dunnocks (Davies, 1992). This type of society in which strong reproductive suppression by dominants is apparently absent and reproductive skew is low between breeders and unrelated

helpers is similar to some cooperatively breeding birds (e.g., dunnock: Davies, 1992; pukeko: Jamieson et al., 1994) and mammals (African lion *Panthera leo*: Packer et al., 2001; banded mongoose *Mungos mungo*: Cant, 2000; De Luca and Ginsberg, 2001; Gilchrist et al., 2004; spotted hyena *Crocuta crocuta*: Engh et al., 2002).

The positive correlation in relative testes mass of the male breeders and their male helpers suggests that reproductive competition is mediated through sperm competition in our study species. We can think of three nonmutually exclusive explanations for such a positive correlation and the resulting effects on sperm production and paternity. First, if group member males are able to assess whether the female is ready to spawn within the next couple of days (e.g., by increased female nest-building behavior like carrying sand from the breeding shelter, swelling of female's abdomen, or increased chemical cues excreted by females), all males within the group might respond by a short-term increase in sperm production. A significant positive correlation was detected between the relative gonad mass of female breeders and male helpers but not detected between male and female breeders in both pairs with and without male helpers. These results suggest that increasing relative testes mass is not affected by the breeding cycle of the female breeders.

Second, group member males might increase their investment in gonads irrespective of the breeding cycle of their female in anticipation of sperm competition occurring if she spawns. The partial correlation between the relative gonad investment of male breeders and male helpers was significant and positive, supporting the second hypothesis. Third, high-quality male breeders might attract or acquire high-quality male helpers, with quality in both males correlating positively with testes mass.

Our results show that males are sensitive to the risk of sperm competition and invest in gonads accordingly. This conclusion is also supported by our results from the male harem situation. Male harem owners increased both the number and duration of their visits to the nests where their male helpers had relatively large testes. In other studies of fishes, birds, insects, and spiders, there is evidence that males can assess sperm competition risks at a given mating and increase their ejaculate size or ejaculation rate in response to the risk of sperm competition (Candolin and Reynolds, 2002; Elgar et al., 2003; Evans et al., 2003; Wedell et al., 2002).

Testes investment and paternity

When males cannot monopolize access to females, high investment in testis may be the only remaining direct means to ensure siring success (Parker, 1982, i.e., increasing ejaculate frequency and size). Indeed, the number of young sired by male helpers in our study species increased with their relative testes mass, while no such tendency was found in male breeders. We did not find an effect of the difference in relative testes mass of both males and the proportion of offspring sired, which might be due to relative testes mass of the male breeders showing a high correlation with the relative testes mass of their male helpers. Nevertheless, the results reveal that male helpers may gain direct fitness benefits by increasing their investment in gonads, and in turn, this may determine the helper's willingness to cooperate and show direct or indirect brood care behavior, as found in other species (e.g., Burke et al., 1989; Davies, 1992). Empirical evidence that larger testes are associated with increasing siring success under sperm competition has been found in the Soay sheep, *Ovis aries* (Preston et al., 2003), and our finding may be the first documented case in fishes.

CONCLUSION

Our results show that testis investment varies according to the risk of sperm competition as predicted. Furthermore, male helpers may gain direct fitness benefits by increasing their investment in testes. Experiments are needed to corroborate these results, to establish whether, when, how fast, and how much males change their investment in testes mass and sperm production depending on their social status (e.g., by inducing male helpers to become male breeders) and the risk of sperm competition (e.g., by altering the group composition by, e.g., removing male helpers), and to establish whether this affects paternity in the predicted direction.

We thank Michio Hori, Haruki Ochi, and the members of the Japanese Research Team of Lake Tanganyika, who arranged our research at the lake, and the staff of the Lake Tanganyika Research Unit, who assisted us in the field. We are also grateful to Tetsu Sato, Masaoki Takagi, Daisuke Gomagano, Mark A. Elgar, and three anonymous reviewers for their valuable comments on an earlier draft of the manuscript. This study was supported by a Grant-in-Aid for Overseas Scientific Research (No. 10041178) from the Japanese Ministry of Education, Science, Sports, and Culture to M.K. and was partly supported by an Osaka City University Grant for Graduate Course Students and by the 21st Century Centers of Excellence program of the University of the Ryukyus to S.A. This study was also supported by the Swiss National Science Foundation (No. 3100A0-108473) to D.H. The research presented here was conducted under permission of fish research in Lake Tanganyika from the Zambian Ministry of Agriculture, Food, and Fisheries and complies with the current laws of Zambia.

REFERENCES

- Alonzo SH, Warner RR, 2000. Allocation to mate guarding or increased sperm production in a Mediterranean wrasse. *Am Nat* 156:266–275.
- Awata S, 2005. Social system, reproduction of helpers and sperm competition in the cooperatively breeding fishes (*Julidochromis* spp.) in Lake Tanganyika (PhD dissertation). Osaka: Osaka City University.
- Awata S, Kohda M, 2004. Parental roles and the amount of care in a bi-parental substrate brooding cichlid: the effect of size differences within pairs. *Behaviour* 141:1135–1149.
- Awata S, Munehara H, Kohda M, 2005. Social system and reproduction of helpers in a cooperatively breeding cichlid fish (*Julidochromis ornatus*) in Lake Tanganyika: field observations and parentage analyses. *Behav Ecol Sociobiol* 58:506–516.
- Birkhead TR, Møller AP, 1992. Sperm competition in birds: evolutionary causes and consequences. London: Academic Press.
- Birkhead TR, Møller AP, 1998. Sperm competition and sexual selection. London: Academic Press.
- Brown JL, 1987. Helping and communal breeding in birds: ecology and evolution. Princeton, New Jersey: Princeton University Press.
- Burke T, Davies NB, Bruford MW, Hatchwell BJ, 1989. Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. *Nature* 338:249–251.
- Candolin U, Reynolds JD, 2002. Adjustments of ejaculation rates in response to risk of sperm competition in a fish, the bitterling (*Rhodeus sericeus*). *Proc R Soc Lond B* 269:1549–1553.
- Cant MA, 2000. Social control of reproduction in banded mongooses. *Anim Behav* 59:147–158.
- Clarke FM, Mithé GH, Bennett NC, 2001. Reproductive suppression in female Damaraland mole-rats *Cryptomys damarensis*: dominant control or self-restraint? *Proc R Soc Lond B* 268:899–909.
- Crawley MJ, 2002. Statistical computing. An introduction to data analysis using s-plus. Chichester: John Wiley and Sons Ltd.
- Creel S, 2001. Social dominance and stress hormones. *Trends Ecol Evol* 16:491–497.
- Davies NB, 1992. Dunnock behaviour and social evolution. Oxford: Oxford University Press.
- De Luca DW, Ginsberg JR, 2001. Dominance, reproduction and survival in banded mongooses: towards an egalitarian social system? *Anim Behav* 61:17–30.

- Dewsbury DA, 1982. Ejaculate cost and male choice. *Am Nat* 119: 601–610.
- Elgar MA, Champion de Crespigny FE, Ramamurthy S, 2003. Male copulation behaviour and the risk of sperm competition. *Anim Behav* 66:211–216.
- Emlen ST, 1991. Evolution of cooperative breeding in birds and mammals. In: *Behavioural ecology: an evolutionary approach*, 3rd ed (Krebs JR, Davies NB, eds). Oxford: Blackwell Scientific Publications; 301–337.
- Engh AL, Funk SM, van Horn RC, Scribner KT, Bruford MW, Libants S, Szykman M, Smale L, Holekamp KE, 2002. Reproductive skew among males in a female-dominated mammalian society. *Behav Ecol* 13:193–200.
- Evans JP, Pierotti M, Pilastro A, 2003. Male mating behavior and ejaculate expenditure under sperm competition risk in the eastern mosquitofish. *Behav Ecol* 14:268–273.
- Faaborg J, Parker PG, DeLay L, de Vries TJ, Bednarz JC, Maria Paz S, Naranjo J, Waite TA, 1995. Confirmation of cooperative polyandry in the Galapagos hawk (*Buteo galapagoensis*). *Behav Ecol Sociobiol* 36:83–90.
- Gage MJG, 1994. Associations between body size, mating pattern, testis size and sperm lengths across butterflies. *Proc R Soc Lond B* 258:247–254.
- Gilchrist JS, Otali E, Mwanguhya F, 2004. Why breed communally? Factors affecting fecundity in a communal breeding mammal: the banded mongoose (*Mungos mungo*). *Behav Ecol Sociobiol* 57: 119–131.
- Gross MR, 1996. Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol Evol* 11:92–98.
- Harcourt AH, Harvey PH, Larson SG, Short RV, 1981. Testis weight, body weight and breeding system in primates. *Nature* 293:55–57.
- Hosken DJ, 1997. Sperm competition in bats. *Proc R Soc Lond B* 264:385–392.
- Jamieson IG, 1997. Testing reproductive skew models in a communally breeding bird, the pukeko, *Porphyrio porphyrio*. *Proc R Soc Lond B* 264:335–340.
- Jamieson IG, Quinn JS, Rose PA, White BN, 1994. Shared paternity among non-relatives is a result of an egalitarian mating system in a communally breeding bird, the pukeko. *Proc R Soc Lond B* 257:271–277.
- Jennions MD, Passmore NI, 1993. Sperm competition in frogs: testis size and a 'sterile male' experiment on *Chiromantis xerampelina* (Rhacophoridae). *Biol J Linn Soc* 50:211–220.
- Johnstone RA, 2000. Models of reproductive skew: a review and synthesis. *Ethology* 106:5–26.
- Kohda M, Yanagisawa Y, Sato T, Nakaya K, Niimura Y, Matsumoto K, Ochi H, 1996. Geographical colour variation in cichlid fishes at the southern end of Lake Tanganyika. *Environ Biol Fish* 45:237–248.
- Konings A, 1998. Tanganyika cichlids in their natural habitat. El Paso: Cichlid Press.
- Kuwamura T, 1986. Parental care and mating systems of cichlid fishes in Lake Tanganyika: a preliminary field survey. *J Ethol* 4:129–146.
- Leach B, Montgomerie R, 2000. Sperm characteristics associated with different male reproductive tactics in bluegills (*Lepomis macrochirus*). *Behav Ecol Sociobiol* 49:31–37.
- Marconato A, Shapiro DY, 1996. Sperm allocation, sperm production and fertilization rates in the bucktooth parrotfish. *Anim Behav* 52:971–980.
- Møller AP, 1991. Sperm competition, sperm depletion, paternal care, and relative testis size in birds. *Am Nat* 137:882–906.
- Nagoshi M, Yanagisawa Y, 1997. Parental care patterns and growth and survival of dependent offspring in cichlids. In: *Fish communities in Lake Tanganyika* (Kawanabe H, Hori M, Nagoshi M, eds). Kyoto: Kyoto University Press; 175–192.
- Nakatsuru K, Kramer DL, 1982. Is sperm cheap? Limited male fertility and female choice in the lemon tetra (Pisces, Characidae). *Science* 216:753–755.
- Packer C, Pusey AE, Eberly LE, 2001. Egalitarianism in female African lions. *Science* 293:690–693.
- Parker GA, 1970. Sperm competition and its evolutionary consequences in the insects. *Biol Rev* 45:525–567.
- Parker GA, 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. *J Theor Biol* 96:281–294.
- Parker GA, 1998. Sperm competition and the evolution of ejaculates: towards a theory base. In: *Sperm competition and sexual selection* (Birkhead TR, Møller AP, eds). London: Academic Press; 3–54.
- Parker GA, Ball MA, Stockley P, Gage MJG, 1996. Sperm competition games: individual assessment of sperm competition intensity by group spawners. *Proc R Soc Lond B* 263:1291–1297.
- Petersen CW, Warner RR, 1998. Sperm competition in fishes. In: *Sperm competition and sexual selection* (Birkhead TR, Møller AP, eds). London: Academic Press; 435–463.
- Preston BT, Stevenson IR, Pemberton JM, Coltman DW, Wilson K, 2003. Overt and covert competition in a promiscuous mammal: the importance of weaponry and testes size to male reproductive success. *Proc R Soc Lond B* 270:633–640.
- Rice WR, 1989. Analyzing tables of statistical tests. *Evolution* 43: 223–225.
- Schärer L, Robertson DR, 1999. Sperm and milt characteristics and male v. female gametic investment in the Caribbean reef fish, *Thalassoma bifasciatum*. *J Fish Biol* 55:329–343.
- Shapiro DY, Marconato A, Yoshikawa T, 1994. Sperm economy in a coral reef fish, *Thalassoma bifasciatum*. *Ecology* 75:1334–1344.
- Smith RL, 1984. Sperm competition and the evolution of animal mating systems. London: Academic Press.
- Stockley P, Gage MJG, Parker GA, Møller AP, 1997. Sperm competition in fishes: the evolution of testis size and ejaculate characteristics. *Am Nat* 149:933–954.
- Taborsky M, 1994. Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. *Adv Study Behav* 23:1–100.
- Taborsky M, 1997. Bourgeois and parasitic tactics: do we need collective, functional terms for alternative reproductive behaviours? *Behav Ecol Sociobiol* 41:361–362.
- Taborsky M, 1998. Sperm competition in fish: 'bourgeois' males and parasitic spawning. *Trends Ecol Evol* 13:222–227.
- Tomkins JL, Simmons LW, 2002. Measuring relative investment: a case study of testes investment in species with alternative male reproductive tactics. *Anim Behav* 63:1009–1016.
- Wedell N, Gage MJG, Parker GA, 2002. Sperm competition, male prudence and sperm-limited females. *Trends Ecol Evol* 17:313–320.