Classical polyandry in the reversed sex-role substrate breeding cichlid *Julidochromis marlieri*

（逆転した性役割の基質産卵魚 *Julidochromis marlieri* における古典的一妻多夫）

平成**30**年度

Cirhuza Mushagalusa Deo

(キルフザ　ムシャガルサ　デオ**)**

Acknowledgements

After this long, very long journey, I would like to express my thanks to all my friends and colleagues that contributed, directly or indirectly, daily or occasionally, to improve my life and this work. Without their support, the end of this project would not have been possible.

Firstly, I would like to express my sincere gratitude to my advisor Professor Dr. Masanori Kohda for the continuous support of my PhD study and related research, for his patience, motivation, and immense knowledge in fish behaviour. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my PhD study. I want to thank Professor Dr. Satoshi Awata for his prompt help in DNA microsatellite analysis and continuous scientific guidance during my stay at Osaka City University. Satoshi-sensei, thank you very much for your patience and words of encouragement during my very long journey. I am very grateful to Dr. Kazutaka Ota for the valuable discussion and statistical support by showing me how to precisely handle and understand behavioural data correctly.

I am grateful to the staff of Lake Tanganyika Research Unit and “Maneno group” members for assistance and access to the laboratory and research facilities in the field. Particularly, my respective sincere thanks to Professors H. Kawanabe, M. Hori, T. Takahashi and Dr. H. Tanaka, who provided me an opportunity to join the group and to let me feel that I was part of the team.

I would like to thank my colleagues of the Laboratory of Animal Sociology (Osaka City University) and my fellow doctoral students for the patience, cooperation and of course friendship. In particular, I am sincerely very grateful to my friend Dr. Shun Satoh for

enlightening me the first glance of scuba diving and underwater research at the sea and at Lake Tanganyika.

In addition, I would like to express my gratitude to the staff of the Centre de Recherche en Hydrobiologie (CRH-Uvira), particularly Professor Dr. Pascal Masilya for assistance in research facilities and study permits. I am grateful to Professor Dr. M. Nshombo for accepting co-advise of my PhD project and for assisting me to overcome several complicated situations in the field. I want to express my grateful to Mr. Kisaro Muzaire and Mr. Amisi Aochi for their helpful and daily assistance during several research trips and for the last minute favors at Lake Tanganyika. Last but not the least; I would like to thank my family: my wife, my sons and my daughter for their patience and kind encouragements. I am also grateful to my parents, my brothers and sisters for supporting me spiritually throughout writing this thesis and my life in general.

Funding was provided by the Japanese Society for the Promotion of Science (JSPS) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan and by Osaka City University.

Chapter 1

General introduction

Polyandry, i.e. mating with multiple males within one reproductive event, expands the scope of classical sexual selection theory. Sexual selection theory predicts divergent mating tactics for males and females. This arises from competition over mates, typically through male-male competition and female choice (Darwin 1871; Trivers 1972; Bateman 1948). It is much more common for males to have multiple female partners during the nesting season (Klemme et al. 2006a). Increasingly, in some species, however, females are also being found to have multiple male partners, even to the point of raising broods in which siblings have different fathers (Davies et al. 1992; Andersson 2005; Andersson and Ahlund 2012; Goymann et al. 2016). Thus, polyandry can generate competition among females for access to multiple males, and impose sexual selection on female traits that influence their mating success (Emlen and Oring 1977; Andersson 1994, 2004; Reynolds 1996; Klug et al. 2010; Kvarnemo and Simmons 2013).

Among animals, polyandry takes two basic forms (Oring 1986; Betts and Jenni 1991) where biologists commonly distinguish between classical- and cooperative polyandry (Davies 1991; Andersson 1994; Arnold and Duvall 1994; Klug et al. 2013). Cooperative polyandry occurs when two or more males form stable social units with a single female in the same reproductive effort and provide care of young at a single nest (Jenni 1974; Arnold and Owens 1998, 1999; Clutton-Brock et al. 2002; Awata et al. 2006a; Heinsohn et al. 2007; Koenig et al. 2016). Cooperative breeding is a rare behaviour, but known to occur in many birds belonging to the avian order Charadriiformes (Jenni 1974; Edwards and Naeem 1993; Arnold and Owens 1999; Andersson 2005). Polyandry coupled with cooperative breeding is also found to occurs among a variety of mammals (Hrdy 2009; Schradin et al. 2009) such as canids (Wagner et al. 2007) and callitrichids (tamarins and marmosets) (Goldizen 1990; Schaffner and French 2004). In contrast to birds and mammals, only few fish species are known to breed cooperatively, despite a wide variety of mating and parental care systems (Heg and Bachar 2006). Most cases of cooperative breeding reported in fish have been described in the lamprologine cichlid species (~25 species) endemic to Lake Tanganyika (reviewed in Taborsky and Limberger 1981; Taborsky 1994; Yamagishi and Kohda 1996; Kohler 1998; Awata et al. 2005; Heg et al. 2005; Heg and Bachar 2006; Tanaka et al. 2015; Taborsky 2016; Tanaka et al. 2016; Taborsky and Wong 2017; Tanaka et al. 2018a, 2018b). In these animals, group members include a pair of breeders and closely related helpers (Emlen 1991, Awata et al. 2006a, 2006b; Wong and Balshine 2011; Li et al. 2015; Taborsky 2016; Tanaka et al. 2016; Tanaka et al. 2018b). This makes lamprologine cichlids a unique model to investigate alternative pathways to the evolution and maintenance of polyandry.

Classical polyandry, the main focus of this thesis, is a mating system in which a single female engages in separate reproductive efforts and mates, either sequentially or simultaneously with two or more males during a single breeding cycle. Each male has his own nest, incubates the eggs and provides care for his young, typically with no or little help from the female (reviewed in Jenni 1974; Oring and Lank 1986; Okuda 1999; Butchart 2000; Jones et al. 2000; Jones and Avise 2001; Owens 2002; Berglund and Rosenqvist 2003; Ligon and Burt 2004; Emlen and Wrege 2004; Goymann et al. 2004; Andersson 1994, 2002, 2005; Koenig et al. 2016). Although classical polyandry is of great interest in relation to parental investment, sex role and sexual selection theory, this mating system occurs in animals where the sex roles are reversed, that is, females compete more intensely than males over mates (Oring 1986; Ligon 1999; Eens and Pinxten 2000; Goymann et al. 2004; Berglund et al. 1989, 2005; Andersson 2005). The evolution of classical polyandry, however, is debated and not well understood. Andersson (2005) suggested classical polyandry to generally take place in three main steps. In step one, mainly or only male care has evolved in many different animal taxa of invertebrates, amphibia, fishes and birds as well (Reynolds et al. 2002; Andersson 2004, 2005). A next step occurs when females become able to lay more eggs than a male can care for, mainly due, for example, to evolutionary changes of habitat and foraging. In step 3, some females (more larger than males) compete to produce clutches for several males and take advantage of higher fecundity by laying a clutch to a second male while the first mate is still caring for clutch (Jenni 1974; Berglund et al. 1989; Jones et al. 2001a, 2001b; Jones and Avise 2001; Berglund and Rosenqvist 2003; Andersson 2004, 2005; Kvarnemo and Simmons 2013).

Furthermore, classical polyandry is only known in animals in which male incubate or carry the eggs (e.g. birds and fish). These animals include principally the tinamous (Tinamiformes), shorebirds (Charadriiformes), buttonquail (Gruiformes), and coucals (Centropodinae), all of which have small clutch sizes and precocial young, except in the coucals with larger clutches and altricial young (for reviews see Jenni and Collier 1972; Hays 1972; Oring and Knudson 1972; Jenni 1974; Vernon 1971, 1975; Emlen and Oring 1977; Erckmann 1983; Lenington 1984; Oring 1982, 1986; Colwell and Oring 1988; Ligon 1999). In fishes, classical polyandry associated with sex-role reversed is reported exclusively in species where the males gestate the offspring in specialized pouches and make a substantial investment in development of embryos (Jones and Avise 2001; Jones et al. 2001b; Partridge et al. 2013). All are belonging to the family Syngnathidae with species of pipefishes and seahorses (Dawson 1985; Lourie et al. 1999). During copulation the female transfers unfertilized eggs to the male's ventral surface, with elaborated brood pouches where fertilization takes place and transfers nutrients from male to offspring (Herald 1959; Haresign and Shumway 1981; Dawson 1985; Berglund et al. 1986a, 1986b, 1989; Rosenqvist 1990; Jones and Avise 2001; Partridge et al. 2013).

Unlike birds, pipefish and seahorse species, rather no further species with classical polyandry associated with sex-role reversed is known, particularly in other fish species which may build nests, fan eggs, or carry developing young in their mouths (Breder and Rosen 1966; Ridley 1978; Jones and Avise 2001; Partridge et al. 2013; Andersson 2005; Koening et al. 2016). Regardless, there is little or no field data in many polyandrous taxa, either cooperative- (Sefc 2001; Tanaka et al. 2018a, 2018b) or classical (Andersson 2005; Kohda personal communication) polyandry, particularly in tropical fishes. Seemingly, cichlid fish of Lake Tanganyika provide an unending source of information to understand this breeding system (Tanaka et al. 2016). Like some higher mammals and birds, many cichlid species are monogamous, biparental pair that cares for eggs and young until independence. Typically, the male is larger, more colourful and dominant than his mate, and both, often, participate in protecting the eggs and fry from predators. Though cooperative breeding (i.e., breeding pairs assisted by helpers) is common in lamprologine species at Lake Tanganyika (see above), polyandry breeding, however, was signaled only in the genus *Julidochromis* with up to 6 species described (Taborsky and Limberger 1981; Taborsky 1994; Yamagishi and Kohda 1996; Balshine-Earn et al. 1998; Sunobe 2000; Sefc 2001; Awata and Kohda 2004; Awata et al. 2005; Awata et al. 2006a, 2006b; Heg and Bachar 2006). *Julidochromis* species exhibit intraspecific variation in mating systems, ranging from monogamy to cooperative polyandry with sex-role reversal (Taborsky and Limberger 1981; Taborsky 1994; Sunobe 2000; Sefc 2001; Awata and Kohda 2004; Awata et al. 2005; Awata et al. 2006a, 2006b; Ito et al. 2017, 2018), that provide excellent opportunities for comparative analysis in polyandry. Increasingly, preliminary studies by Yamagishi and Kohda (1996) suggested that large female of *Julidochromis marlieri* Poll (1956) may mate to two males simultaneously at separate nesting sites, in addition to being involved in monogamy and cooperative breeding. Since Yamagishi and Kohda's paper, there is virtually not as much information from the field on the breeding behaviour of *J. marlieri*. Thus, I chose this fish as the target animals, and studied quantitatively in more detail and longer periods in the field.

The overall aim of this thesis is to describe the mating system of *J. marlieri* and find quantitative evidence for classical polyandry associated with sex-role reversal in this species. A closer look at this and other *Julidochromis* species might therefore shed new light on the evolution of reversed sex-roles, reversed sexual size dimorphism, polyandry, and sexual selection. In chapter 2, I study the social breeding system, group composition, size distribution and sex role patterns, and examine the extent of classical polyandry by field observations and molecular analyses in *J. marlieri*. The evolution of such sex role reversal in *J. marlieri*, sometimes combined with polyandry, remains poorly understood at Lake Tanganyika (Andersson 2005). In chapter 3, I show the spawning pattern, group structure and how large classical polyandrous females of *J. marlieri* will lay and split egg clutches into different separate nests in order to increase their reproductive success and gain paternity level in the field. The reproductive pattern and reason for why female may reproduce repeatedly and raise broods for several males is elucidated here as the male reproductive effort. In chapter 4, I examine the social status, parental effort, average paternity (proportion of young sired) and testes size investment of males involved in polyandry and compared with monogamous males in relation to sperm competition arising in *J. marlieri*. The investment in testes size is presumably advantageous when male–male competition occurs via sperm competition.

Chapter 2

Classical polyandry in a nest brooding cichlid fish: Evidence from field observations and molecular data

Abstract

Classical polyandry, often associated with reversed sex-roles, has been reported mainly from bird species, but empirical studies with quantitative data are scarce. Evolution of this mating system is not well understood, and for the understanding, the quantitative research of this mating system outside of avian species such as fish will provide a good opportunity. Here I document this interesting mating system from the cichlid *Julidochromis marlieri* in Lake Tanganyika, that care for brood at nest and exhibiting size dimorphism. From field observations and DNA analysis, I found followings: 1) Females were territorial each other, and larger than males in all mating units, 2) Their territories enclosed several nests at which small males were attending, 3) Females that had several nests spawned eggs at these nests, and were sired by the males at each nest, 4) These polyandrous females were larger than monogamous females, 5) Males attended nests longer and foraging less than females, and 6) Polyandrous females that had multiple nests spawned more number of clutches and produced more of independent young than females that had one nest. Together all, my results suggest, larger females spawned egg clutches into two or more nests in their territories, and their mating system is classical polyandry associated with reversed sex-role, whereby these females would obtain advantage in breeding. The present paper provides the first evidence of classical polyandry in fish that brood in the nest, and I compare the mating system with those documented from birds, and consider the scenario of social evolution among the related fish species.

Keywords: Classical polyandry, reversed sex-role, cichlid fish, Lake Tanganyika, paternal care, size advantage

Introduction

In classical polyandry in birds, females compete more strongly than males over mates, and a female is mated with more than one male, each of whom raises his offspring with little or no help from her (Oring 1986; Andersson 2004, 2005). Although it being rare, this breeding system is of great interest because the traditional sex-roles are reversed, and females compete over males who takes care of the offspring (Andersson 2005). As exceptions test a rule in general, classical polyandry offers unique possibilities for testing sexual selection and breeding systems theory, which was developed mainly based on knowledge of animals with traditional sex-roles (e.g., Emlen and Oring 1977; Clutton-Brock 1991, 2009; Andersson 1994, 2005; Owens et al. 1994; Berglund and Rosenqvist 2003; Wiebe and Kempenaers 2008).

Classical polyandry has been discovered in several of bird families particular in such as shorebirds Charadrii. In spite of its great interest, quantitative studies with the field research are limited, and the evolution of classical polyandry is not well understood (reviewed for example, by Jenni 1974; Williams 1975; Emlen and Oring 1977; Oring 1986; Clutton-Brock 1991, 2002; Ligon 1999; Eens and Pinxten 2000; Bennett and Owens 2002; Owens 2002; Berglund and Rosenqvist 2003). It is suggested that future development of a general model for the evolution of polyandry seems unlikely, and insufficient data exist to conclude whether a single explanation for the evolution of classical polyandry is possible (Erckman 1983; Betts and Jenni 1991). Polyandry and uni-parental care remains a puzzle (Clutton-Brock 1991, 2009) and classical polyandry is probably the most interesting, and certainly is the least well understood among the recognized mating systems in vertebrates (Ligon 1999; Andersson 2004, 2005). In terms of causal aspects of ecology and life-history, we still do not know why some lineage have adopted, or maintained, this unusual breeding system in birds (Bennet and Owens 2002; Andersson 2005).

Until now, classical polyandry other than birds has been documented from fish of several pipefishes Syngnathidae (e.g. Oring 1986; Berglund et al. 1989) and one case of cardinal fish of Apogonidae (Okuda 1999). However, these fish provide care on or in their body (pouch or mouth), and there have been no examples as in the cases of birds in which eggs are deposited in nests and cared at nest sites in fish. If such polyandrous fish that breed in nests is discovered, it provides a good opportunity for examination by direct comparison of the hypotheses from bird classical polyandry. The mating system of the cichlid fish *Julidochromis marlieri* will be a candidate for the study of classical polyandry, as suggested by the preliminary studies (Yamagishi and Kohda 1996; Sunobe 2000; Andersson 2005). Thus, I chose this fish as the target animals, and studied quantitatively in more detail and longer periods.

Females of this fish are larger than males, and largest females have territories that enclose two nests at which small males are attending (Yamagishi and Kohda 1996; Sunobe 2000). Females are more aggressive than males of the same size (Barlow and Lee 2005). Fish of this genus spawn eggs at nest such as rock crevice or narrow space beneath rock, and they perform bi-parental care of the eggs and young (Awata and Kohda 2004; Awata et al. 2005; Kohda et al. 2009). Interestingly, in fish of this genus, the role of caretakers is not sexually determined, but by relative size of the sexual pair, that is, smaller members take care of young independent of sex as in *J. ornatus* and *J. transcriptus* (Awata and Kohda 2004; Kohda et al. 2009). Thus, it will be plausible that the largest females have two or more nests and spawn clutches at the respective nest where the small males take care of the offspring more than the females (Yamagishi and Kohda 1996; Sunobe 2000). If so, such mating system will be classical polyandry, probably associated with the sex-role reversal.

To consider the evolution of social systems, comparative studies on the different sociality among closely related species are effective (Emlen and Oring 1977; Oring 1986; Andersson 2005). The genus *Julidochromis* consist of 6 species and the closely related genus *Chlinochromis* having 2 species (Strumbauer et al. 2010; Dey et al. 2017; Tanaka et al. 2018a, 2018b). Mating systems were studied in several of them, and so I can conduct comparative study on the social evolution of classical polyandry. I studied the mating system and reproductive strategy of *J. marlieri* in the same population of the Yamagishi and Kohda's study. Here I show the results of my field observations for five months and molecular data to examine the paternity and maternity of their offspring. I compare the mating system of *J. marlieri* with those documented from birds and discuss the scenario of social evolution of classical polyandry of this fish with considering social systems and phylogenetic relationships of the related species.

Materials and methods

Field observations

I conducted field studies at Pemba Point (Lat. 3^{o} 61' S, Long. 29^{o}15' E), 25 km south from Uvira City, Democratic Republic of the Congo. Yamagishi and Kohda (1996) suggested the classical polyandry of *Julidochromis marlieri* based on their preliminary field observations at this point. I made a study quadrat of 20 m x 22 m with 2 m x 2 m mesh grid by a thin thread on a rocky bottom, which included the study area of Yamagishi and Kohda (1996). In this quadrat of 3-8 m water depth, there lived a plenty of *J. marlieri*. I conducted field observations in the quadrat from the beginning of April to the end of August 2018, three days a week always including every Wednesday. I observed behaviour of fish inside and sometimes around the quadrat. Underwater note was recorded for each fish identified by the individually different colour patterns (see Yamagishi and Kohda 1996; Awata 2005; Awata et al. 2005). Sex of a fish was estimated based on their body size and finally identified by the examination of gonads of fish captured after the observation period. From the beginning of April, I recorded fish colour patterns inside the quadrat, and identified almost all individuals and their nests site by the end of April. Nests of this fish were crevices on rocks, interstice between rocks, and narrow space beneath a rock, and the nest sites were located easily throughout the study area. I recorded the size and the number of young inside the nests on every Wednesday during the study period, and I checked the apparition of new young also in other observation days. I counted the number of young categorized into three classes: small (7-15 mm in TL just after hatching), middle (15-25 mm) and large (25-35 mm) in or around the nest entrance. Eggs inside nests were not visible, except in one case.

Small young just after york-sac absorption appeared in or around the nest entrance and I could count the number. Therefore, when I found small young (7–10 mm) at a nest, I regarded them as young of a new clutch, and the number of these small young was used to determine the relative size of the clutch of a female. I checked the clutch deposition and the growth and number of young at each nest at least once a week. When I observed young that were at eight weeks and grew into large young around 3 cm TL, I regarded them as successfully grown young. Young larger than 3 cm TL were often observed to leave nests (Mushagalusa, personal observation), and young of 3 cm is regarded as the best indicator of the number of independent young.

Behaviours of identified fish were observed for 10 min 11 times or more per individual with intervals of a week or more. I recorded fish swimming trace, foraging site and number, time for staying at nest (within 30 cm from nest entrance), numbers of entering nests and attacking conspecific and hetero-specific fish on the territory map. From the records of swimming trace, I determined their territories (m^2), of which borders corresponded to the point of female-female aggression. I observed each fish 1 m or more apart from the subject fish and did not affect the behaviour of the fish.

Definitions of mating system and males

Almost all resident fish except for young were individually identified, and were followed during the 3.0 to 4.5 months during the study period. I defined the mating systems observed in the study area as follows (Yamagishi and Kohda 1996):

Monogamy: A female and a male attended the nest without aggressions and defended young of one or more clutches against their predators.

Cooperative polyandry: A large female and more than one male attended a nest. I call the largest male alpha, the second largest beta and the third largest (if present) gamma. They were often not exclusive one another. One or more clutches were observed to be deposited at the nests, and parental care by these participants was observed.

Classical polyandry: A large territorial female occupied and visited two or more nests at which an alpha male and sometimes beta and gamma males attended. These fish were often not aggressive each other, and young at these nests were guarded by the attending males and the females. This system encompasses both monogamy if in a nest a large female with an alpha male attended, and cooperative polyandry if, in addition to alpha male, beta and gamma attended in a nest.

The number of observation days varied among the mating units; observation duration of classical polyandry (mean = 16.3 weeks ± 2.9 SD, n = 14) was shorter than cooperative polyandry (19.1 ± 1.6 SD, n = 16) and monogamy (18.5 ± 2.0, n = 12) (Mann

Whitney U-test, Bonferroni significance, between classical polyandry and cooperative polyandry, *p* = 0.003, between classical polyandry and monogamy, *p* = 0.034).

Fish sampling and measurements

Fish were collected in a week from 20 August 2018. I used standing nets of 4 m long and 1.5 m height (7 mm x 7 mm mesh) and hand nets. When capturing young or small adults inside nests, I used solution of clove oil diluted with ethanol. Captured fish were killed with over doze of clove oil on the land near the study site. After sacrificed, whole bodies of young and cut pectoral fins of adults were put in 99% ethanol for subsequent DNA analysis. Bodies of adults were preserved in 10 % formalin solution.

In the laboratory at the CRH Uvira Station (DR Congo), I measured the body size (TL, to 0.01 mm) of the adult fish and young. After measurements, fish were dissected and sexed by morphological observation of gonads. If ripe eggs were found in the ovaries, I also counted the number of eggs.

Parentage analyses

Genomic DNA was isolated from fin-clip samples of 356 fish, using the Gentra Puregene Kit (Applied Biosystems, Foster City, CA, USA) (see Table S4). Extracted DNA were preserved in -30 ˚C until PCR reactions. These samples were genotyped at 8 microsatellite loci: Adew8 (Ota et al. 2014), Chb1 (Munehara et al. 2001), Pzeb1, Pzeb3 and Pzeb4 (van Oppen et al. 1997), TmoM25 (Zardoya et al. 1996) and UME002 and UME003 (Parker and Kornfield 1996). Microsatellites were amplified using two sets of multiplex PCR reactions: Adew8, Chb1, Pzeb1, Pzeb4 and UME003 (Set I) and Pzeb4, TmoM7 and UME002 (Set II). PCR reactions were carried out in 7 μL of final reaction mixture, containing 1 x Type-it Microsatellite PCR kit (Qiagen, Hilden, Germany), approximately 50 ng of template DNA, the fluorescently labelled forward primer (6-FAM, ATTO565, HEX, NED, Yakima Yellow) and the non-labelled reverse primer. The final concentration of the primers was optimized for each marker. The cycling protocol consisted of an initial denaturation step of five min at 95°C, followed by 35 cycles of denaturation at 95°C for 30 sec; annealing at 60°C for 90 sec; and extension at 72°C for 30 sec; and a final extension at 60 ˚C for 30 min. PCR products were electrophoresed on a ABI3730XL Genetic Analyzer with the GeneScan 500 Liz Size Standard (Applied Biosystems, Foster City, CA, USA) at Macrogen Japan Corp. (Kyoto, Japan). The data were analyzed using Peak scanner software 1.0 (Applied Biosystems, Foster City, CA, USA).

Analyses of marker polymorphism, null allele frequencies, and deviations from Hardy–Weinberg equilibrium (HWE) per locus were performed using CERVUS version 3.0.7 (Marshall et al. 1998; Wang 2004). A summary of the marker polymorphism statistics is shown in Table S1. The mean expected heterozygosity was 0.70, and the mean polymorphic information content (PIC) was 0.66, with an average of 12 alleles per locus (n = 130 fish including one young having individual specific alleles; Table S2). The combined non-exclusion probability for all loci was estimated as 0.00058 for the second parent. No evidence of significant deviations from HWE was observed at the eight loci. Fragment sizes at eight microsatellite loci of all 356 fish are shown in Table S3.

Ethical notes

When the fish being sampled, they were put in overdose clove oil solution and fish die without pain. The research was conducted under permission of fish research in Lake Tanganyika from the Congo Ministry of Research and Technology, and Ministry of Agriculture, Food and Fisheries and complies with the current law in DR Congo.

Statistical analyses

Statistical analyses were performed using R version 3.3.2 (R Core Team 2016) and SPSS (ver. 16.0; Chicago, IL, USA) for Windows statistical software package. I used linear models (LMs), generalized linear models (GLMs) or generalized linear mixed models (GLMMs) for the analyses in the lme4 package. All statistical tests were two-tailed, and alpha was set at 0.05. All reported *P*-values were two tailed, and results were considered statistically significant at *P*-values of < 0.05.

Results

Social units

A total of 12 monogamous, 16 cooperative polyandrous, and 14 classical polyandrous breeding-units were observed inside or around the study area (Fig. 1). The territories of the females were considerably larger than males, but rarely overlapped one another. Classically polyandrous females had two (n = 10) or three nests (n = 4). Females other than the territory owners rarely visited these nests. One to three males, alpha-, beta and gamma males, attended one nest, and had small home ranges around the nest, widely overlapping with each other and with the female territory (Fig. 1). Of the 14 classically polyandrous females, clutches were observed at two and three nests in 7 and 3 territories, respectively.

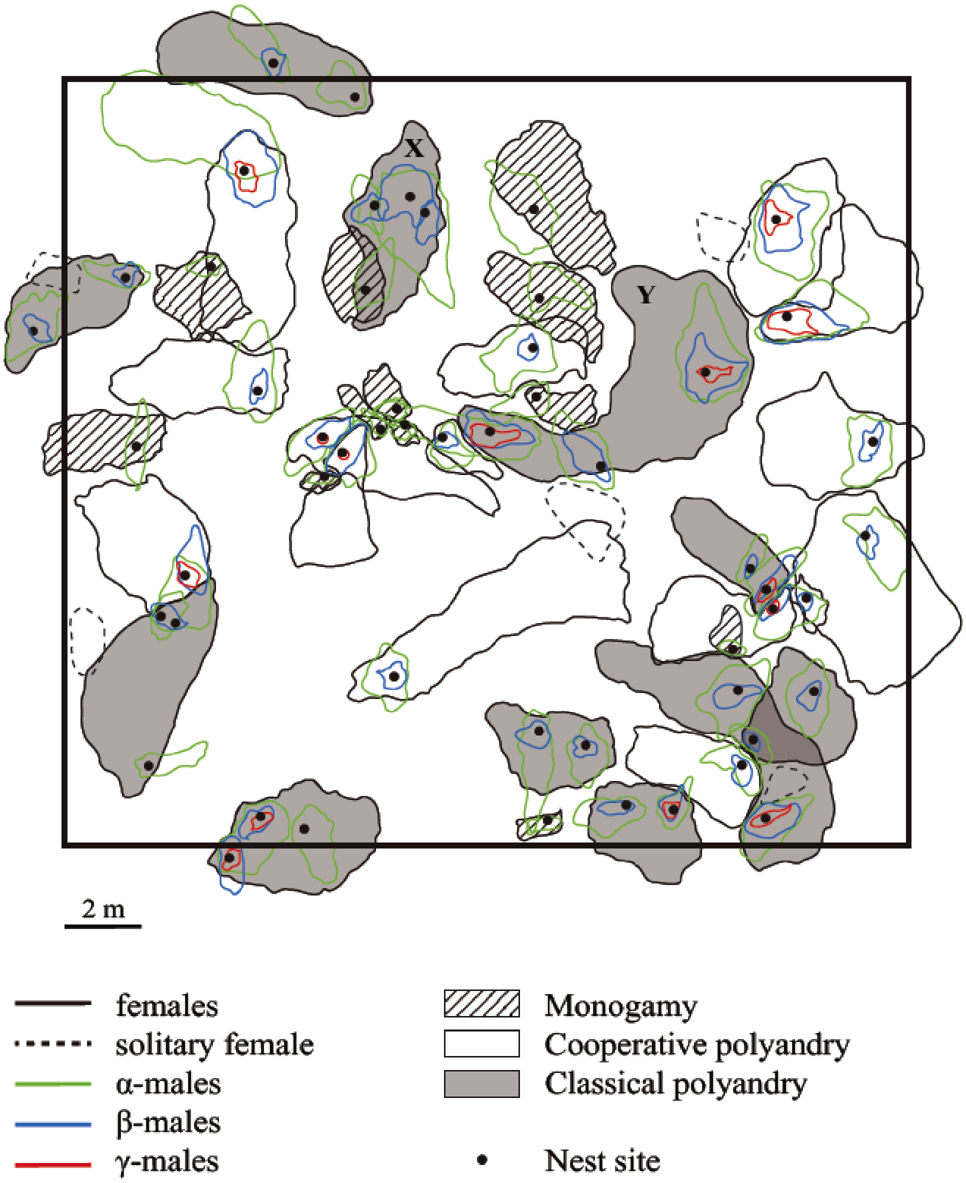


Figure 1. Territory map of females (solid bold line) in classical polyandry (shadowed area), cooperative polyandry (open area), monogamy (oblique lines area) and solitary non-reproductive females (bold chained line) in and near the study area at middle June 2018. Male territories overlap within each female territory, and alpha (solid thin green line), beta male (thin blue line), gamma (thin red line) males are indicated. Dots are location of nest sites. The female of classical polyandry X disappeared at the early July but the vacant being taken by a new female immediately, of which territory is not shown. The classical-polyandrous female with Y extended her territory to cover the most left nest site from the middle June.

Fish size distributions

Sizes differed among mating systems within each female group according to the statuses of members (GLMM, $x^2 = 214.45$, $p < 0.001$). Female sizes were not different between classical polyandry (total length TL ± SD in mm = 92.33 ± 7.93, n = 14) and cooperative polyandry (89.51 ± 11.39, n = 16), but monogamous females (70.98 ± 15.82, n = 12) were smaller than the former two females (Tukey post hoc test, F = 11.20, $p < 0.0001$) (Fig. 2).

Table 1. Body size ratio (mean ± SD) according to the social status in classical polyandry, cooperative polyandry and monogamy mating systems in fish of *J. marlieri*. Data in parentheses represent the sample size.

Breeding system	Female / αmale	αmale / βmale	βmale / γmale
Classical polyandry	1.29 ± 0.17 (28)	1.42 ± 0.18 (24)	1.40 ± 0.29 (7)
Cooperative polyandry	1.22 ± 0.18 (16)	1.37 ± 0.15 (16)	1.38 ± 0.21 (7)
Monogamy	1.32 ± 0.20 (11)	–	–
Total average	1.28 ± 0.18 (55)	1.40 ± 0.17 (40)	1.39 ± 0.24 (14)

Within classical polyandry and cooperative polyandry, sizes of alpha-males (72.35 ± 9.34, n = 28 and 74.34 ± 8.29, n = 15), beta-males (53.36 ± 9.05, n = 24 and 54.64 ± 8.30, n = 16) and gamma-males (45.98 ± 11.36, n = 7 and 43.22 ± 4.21, n = 7) were different each other (all $p < 0.001$), but almost similar in each social status. Alpha-males of monogamy (54.95 ± 15.57, n= 12) were smaller than alpha males of cooperative - and classical polyandry (F = 12.81, $p < 0.0001$), but were not different from beta males (Fig. 2). As such, body sizes of members attending at a nest were apparently different whereupon the size ratio between the fish of neighboring social status was around 1.28 or more in the three mating systems of *J. marlieri* (Table 1). Sizes of single females were 75.5 ± 6.3 mm (n = 5), in a range of 67.3 – 82.5 mm TL (see Fig. 1).

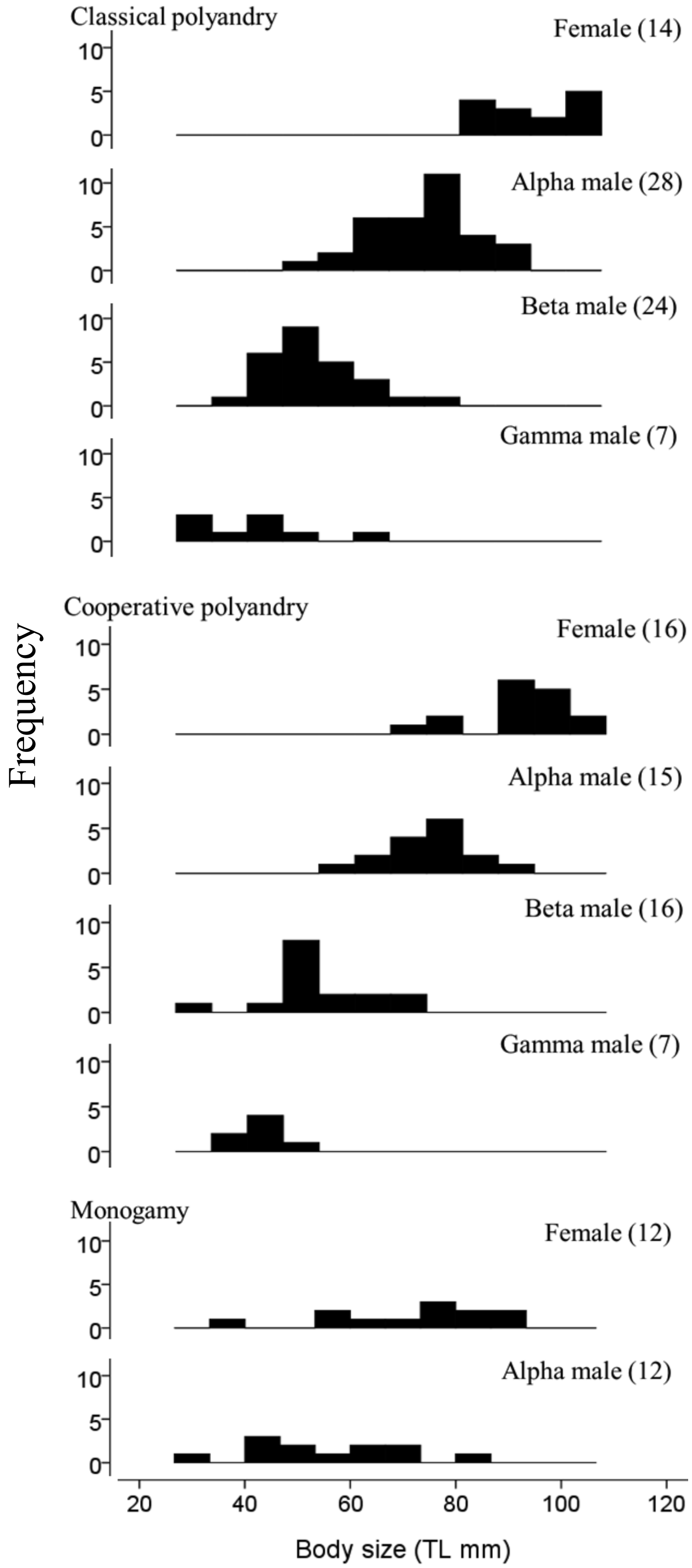


Figure 2. Histogram of body size (total length mm) of monogamous, cooperative polyandrous and classical polyandrous group members of *Julidochromis marlieri* in the study area. Numbers in parentheses are sample sizes. See text for means and statistics.

Territory size of individuals

Territory size was the largest in females and followed by alpha-males, beta-males and gamma-males in classical - and cooperative polyandry (GLMM, $x^2 = 192.61$, $p < 0.001$) (Fig. 3). Similarly, territory size of monogamous females (2.23 ± 2.01 m^2, n = 12) was larger than their mates (0.71 ± 0.72 m^2, n = 12) (Tukey test, $p = 0.028$). The territory sizes of females were not different between classical- (7.92 ± 4.15, n = 14) and cooperative (6.43 ± 3.62 m^2, n = 16) polyandry ($p > 0.05$). Territory size of monogamous females and males were smaller than those of polygamous mating (females: $p < 0.0001$ and males: $p < 0.0001$; Fig. 3).

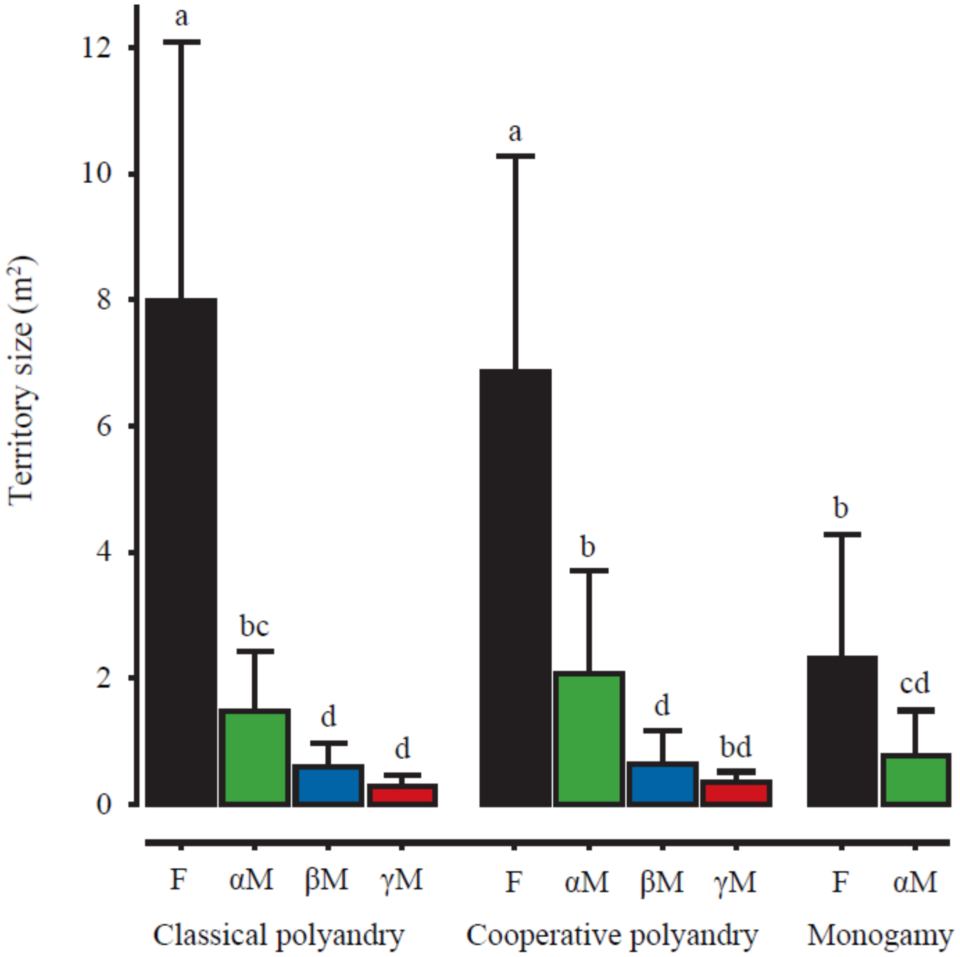


Figure 3. Territory sizes (mean ± SD m^2) of monogamous, cooperative polyandrous and classical polyandrous group members of *J. marlieri* in the study area. Different letters denote statistic differences.

Stability of memberships

Except two cases, all the members of breeding units were observed during the observation period, indicating the mating system of this population is stable. One of classical polyandrous female (X in Fig. 1, ca. 105 mm TL) that had three nests disappeared at the late June. Immediately after the disappearance the vacant territory and the nests were taken over by a new female (89.3 mm). The previous alpha males similar size to the coming females left the nest and the beta male attained an alpha-male rank at two nests. The previous alpha males moved to an unoccupied area near the previous site, and fed frequently for three weeks and disappeared thereafter. The female Y visited two nests, and extended her territory to cover the other nest (the left nest in her territory) at the middle June, that had not been occupied by any females.

Territory defense against conspecifics

Both males and females defended their territories against conspecifics of the same sex. A total of 157 attacks against the neighbours were observed in females, and a total of 22 attacks were between alpha males (including monogamous males). The frequency of female-female attacks (0.40 times ± 0.45 SD/ 10 min, n = 13) was significantly higher than that of male-male attacks (0.04 ± 0.09/ 10 min, n = 28) in classical polyandry (Tukey post hoc test, $p < 0.001$). This was also the case for cooperative polyandry (0.33 ± 0.35 vs. 0.06 ± 0.12, n = 16, $p < 0.001$) and for monogamy (0.47 ± 0.33 vs. 0.05 ± 0.18, n = 11, $p < 0.001$). Attacks between beta males were not observed.

Time of nest attending and foraging frequency

Time staying at nest, frequency of nest entrance and foraging frequency are shown in Fig. 4. Monogamous females stayed at nests longer, entered nest more frequently and foraged less frequently than females of cooperative and classical polyandries (Tukey test, all $p < 0.01$).

The staying time, nest entrance frequencies at the nest and foraging frequencies were not different between the latter two (all $p > 0.05$; Fig. 4a,b,c).

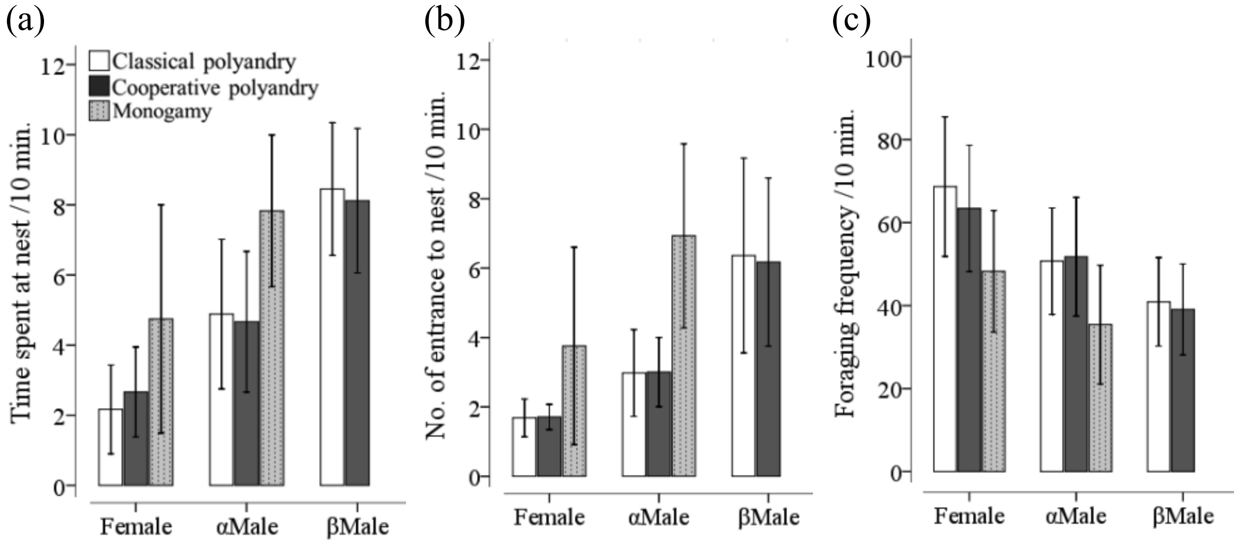


Figure 4. The (mean ±SD) time staying at nests, frequency of entering nests and foraging frequency of female, alpha- and beta males among classical polyandry, cooperative polyandry and monogamy mating systems of *J. marlieri*.

Number of eggs in ovaries

Total number of mature eggs in ovaries were significantly correlated with female body size (Pearson correlation test, r = 0.60, $p < 0.0001$, n = 35). Since body size of classical- and cooperative polyandrous females was larger than that of monogamous females (see Fig. 2), the former two females had large number of eggs than the latter (Tukey test, $F_{1,33} = 4.59$, $p = 0.040$, Fig. 5). The maximum number of eggs was 150 in the large females of classical polyandry and cooperative polyandry.

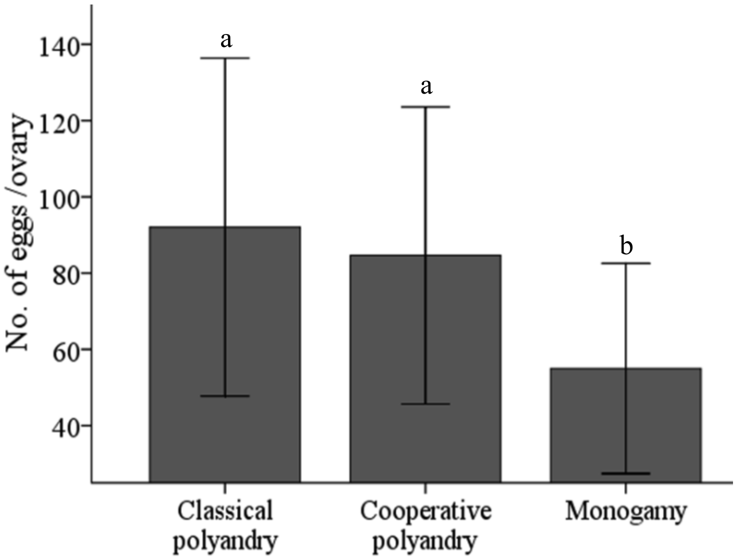


Figure 5. Mean number (± SD) of ripe eggs in ovaries of females captured in the study area of the present study in function of female body size. Fish are grouped into three mating systems.

Clutch numbers, numbers of small young and independent young, and survival rates of young

Fourteen females of classical polyandry deposited a total of 58 clutches in their nests (mean ± SD = 4.07 ± 2.28 clutches per female), 16 females of cooperative polyandry deposited 39 clutches (2.44 ± 1.54) and 12 monogamous females did 19 clutches (1.58 ± 1.32) (Fig. 6a). The numbers of clutch per female were significantly different among the mating systems (GLMM, $\chi^2 = 16.14$, $p = 0.0003$). Multiple comparisons revealed that classical polyandrous females more frequently spawned than cooperative polyandrous females and monogamous females, but clutch numbers of cooperative polyandrous females did not differ those of monogamous females.

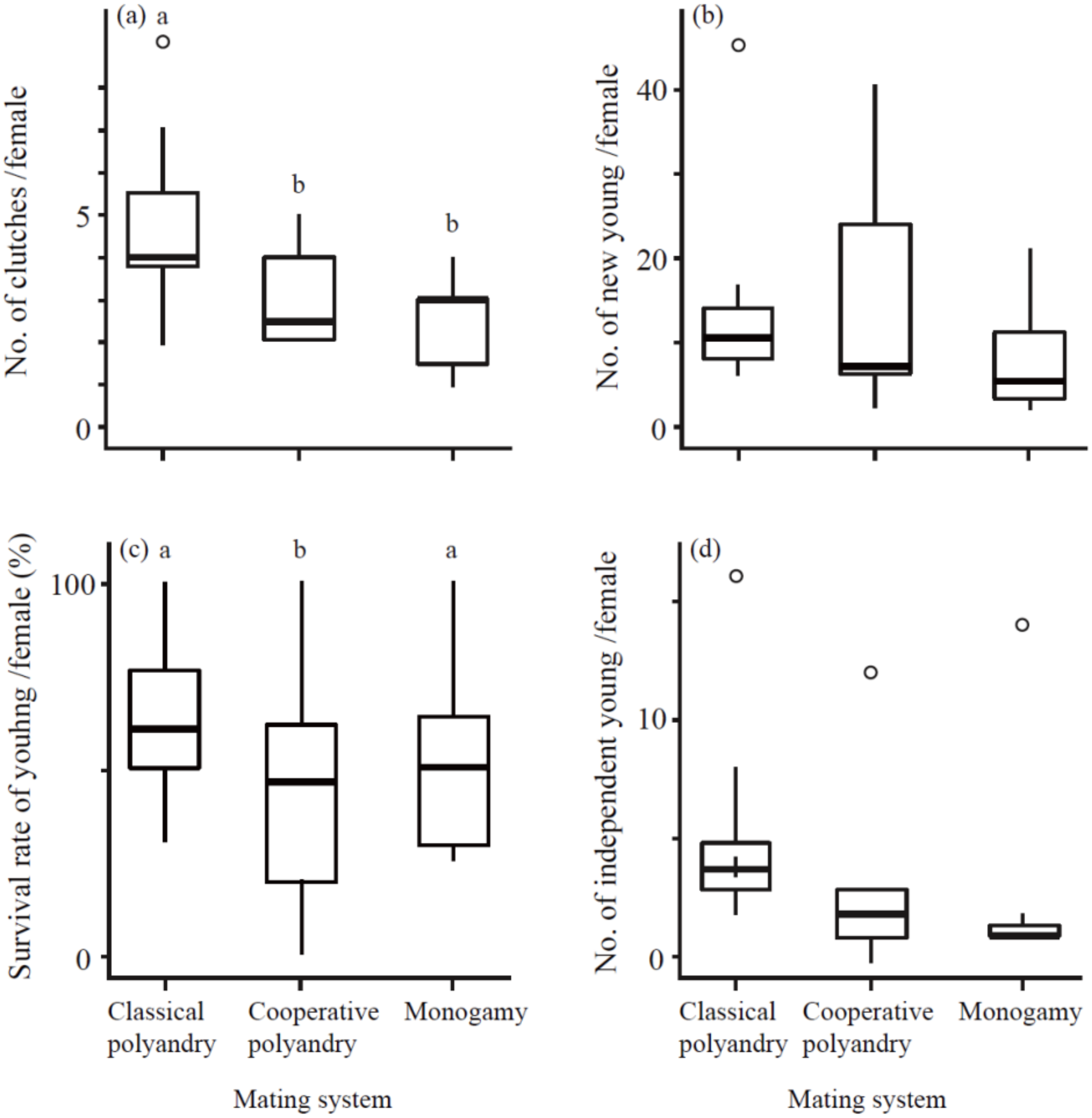


Figure 6. Number of clutches (a), total number of small young or clutch sizes (b) and the survival rates of small young (c) and total number of independent young (d) until the period of 8 weeks later of a female *J. marlieri* in monogamy, cooperative - and classical polyandry mating systems. Box plots show median (central thick lines), 25% and 75% quartile ranges of the median (bottom and top of the box) and upper and lower limits. The lower and upper ends of the whiskers represent minimum and maximum values, respectively, and circles represent outliers. The letter a and b indicate the significant difference.

The number of small young cared by classical polyandrous females were 4.21 ± 2.85 (n = 53), cooperatively polyandrous females were 6.04 ± 4.51 (n = 25) and monogamous females were 4.00 ± 3.96 (n = 13). These values were not significantly different among mating systems (GLM, $\chi^2 = 4.08$, $p = 0.130$) (Fig. 6b). The survival rates of the small young during 8 weeks periods were different among groups of the three mating systems (GLM, $\chi^2 = 15.68$, $p = 0.0004$) (Fig. 6c). As a result, the number of large young of independent size, about 30 mm TL, were 5.00 ± 3.87 in classical polyandry, 2.58 ± 3.17 in cooperative polyandry and 3.00 ± 4.38 in monogamy, with no differences among the three mating systems (negative binomial GLM, $\chi^2 = 3.695$, $p = 0.158$). However, if the three outliers observed were omitted, there was found to be a significant difference in the number of independent young per female (Classic: 4.00 ± 1.73 > cooperative: 1.72 ± 1.19 = monogamy: 1.16 ± 0.41) ($\chi^2 = 11.66$, $p = 0.000$; Fig. 6d).

Guarding young in classical polyandry

If females of classical polyandry spawn different nests, they will guard their clutch at respective nests against predators. Twelve of the 14 classical polyandrous females, having a total of 39 clutches of small young, were observed. The females visited all of these nests and attacked unknown conspecifics (74 attacks in total, 37/39 cases) and potential predators of young such as *Lepidiolamprologus elongates, Neolamprologus pulcher, Telmatochromis temporalis* (66 attacks, 37/39 cases). In 9 of the 13 classical polyandry females, the clutches of young were observed in two different nests during the study period, and the young were defended by the owner females as well as males that were attending the nests. These results indicate the egg-clutches in both nests were probably deposited by the polyandrous females.

Genetic analyses

From the 14 classical polyandries and two additional ones outside of the study area, young from two nests were collected from five polyandries (Table 2). Of the five classical polyandries, the young of both nests were deposited by the female in three cases. The distances between the nests were 4 to 4.5 m. Of 8 polyandries from which young of one nest were captured, 7 nests contained young of the females. Paternity analyses revealed that the alpha males and beta males, if present, were the father of the offspring (Table 2). These molecular date indicate their mating system is truly classical polyandry. In monogamous and cooperative polyandrous nests, most of the eggs were deposited by the owner females and the attending males sired the offspring (Table 2; see also Tables S1, 2, 3 and 4).

Table 2. Summary of offspring caught at each nest and their parentage according to the 3 mating systems of *J. marlieri* at the study area. Total number of nest is 54, 19 and 13 respectively for classical polyandry, cooperative polyandry and monogamy. Data are the number of offspring (the numbers in parentheses represent groups in which young were caught).

		No. of young by female		No. of young sired by male			
Mating system	Total no. of young	Owner	Unknown	Alpha	Beta	Gamma	Unknown
Classical polyandry	80 (16)	48	32	23	27	1	29
Cooperative polyandry	76 (19)	63	13	24	14	3	35
Monogamy	34 (13)	30	4	24	_	_	10
Total	190	141	49	71	41	4	74

Discussion

Many examples of classical polyandry have been reported from bird species, where females spawn eggs in multiple nests at which males are attending and mainly or exclusively care for his eggs and young (Oring 1986; Andersson 2005). The present study shows the evidence of such a typical classical polyandry in fish that take care of young at nests. Field observations and molecular data indicate that large-sized females of *Julidochromis marlieri* have a large territory enclosing two or three nests at which small males are attending and guard their young of egg clutches deposited by the females until their independence. Thus, there are large similarities in this mating system between fish and bird classical polyandry, both performing nest spawning and care of broods (Oring 1986; Andersson 2004, 2005; Goymann et al. 2015, 2016). This is the first documentation of classical polyandry in fish with nest breeding (Yamagishi and Kohda 1996; Berglund and Rosenqvist 2003; Andersson 2004, 2005).

Evidence for classical polyandry

Molecular data indicate young of three individual females of classical polyandry were found into two different nests, which were related and sired by males in respective nest. These nests were in distance so far that the migration of the young between these nests is unlikely. Transportation of young by parental fish was not observed in this fish (Mushagalusa personal observation), and such transportation has not been reported in fish of the genus *Julidochromis*. These indicate these females spawn eggs into two distinctly different nests in her territories.

When females guard broods, their behaviour will be strong evidence indicating the genetic maternity of the brood as revealed both my field observations and genetic figures (see results and supplementary tables S1, 2, 3 and 4). Of a total of 57 broods observed in classical polyandry, all of the females attacked the potential egg predators during guarding their young at own nests, and 9 females that had broods at two nests were attending nests and defended these clutches respectively from potential predators together with the males, suggesting these clutches at the two different nests were her own young. These behavioural observations of parental care also indicate the females are likely to mate with males of the each nest in classical polyandry. In each of the three mating types, female-female aggression for territory defense was prominent in this species (see results).

Advantage of classical polyandry for female

To consider the benefit of the classical polyandry for females of *J. marlieri*, direct comparison between classical polyandry and cooperative polyandry will be reasonable because of their similarity in body size, egg number in ovaries, and territory size, whereas those values were small in monogamy. The main difference of the two mating systems is likely to be the number of nests. Total clutch numbers of a female was higher in classical polyandry, and the numbers of newly appearing small young were not different among the three. However, the survival rates from small to large young during 8 weeks were higher in females of classical polyandry than those of cooperative polyandry, and as a result, the number of large independent young were significantly more than cooperative polyandry, if the outliers were omitted. The small number of the independent young in monogamous females will be due to small number of small young, although its survival rates were higher. Thus, in my study population of high predation pressure on young, classical polyandrous females produce more number of clutches, and receive more parental care from males, and are likely to enjoy higher reproductive success, as theoretical models and empirical study on birds models predicted (Oring 1986; Andersson 2004, 2005; Kvarnemo and Simmons 2013). Classical polyandrous females spawned more number of clutches than females of two other types of breeding systems. In my field research, the mean observation period of classical polyandry was significantly shorter than cooperative polyandry and monogamy, indicating the difference in clutch numbers and total number of small and independent size young will be more than my observations.

How the classical plyandrous females achieved higher reproductive outcome? The large number of clutches by classical polyandrous females would occur due mainly to two ecological factors. One will be the more foraging of females of classical polyandry, which will induce large numbers of clutch. However, the time staying at nests and foraging frequency of females were not different between the classical and cooperative polyandries, and this will not be the factor. I could not count the egg numbers deposited inside nests directly, but data of ripe eggs in ovaries suggest large *J. marlieri* female will spawn more than 100 eggs in one spawning event. If females deposit a clutch of eggs into two nests, the clutch size will be largely reduced. The young of this fish stay in and around nest under parental care, and forage mainly benthic animals (Yamagishi and Kohda 1996; Hori unpublished data). If large numbers of young survive until the middle or large size, the young of the clutch will spread in wide ranges of area, and will be exposed to higher predation risk (Yamagishi and Kohda 1996; Satoh et al. personal communications). If so, clutch of reduced egg number, e.g. 50 - 60 will have higher survival rate than single clutch of 100-150 eggs (Optimal clutch size hypothesis). The large number of clutches of classical polyandry suggests the classical polyandrous females might split the ovarian eggs into two nests each as single clutch.

Body size difference and reversed sex role

The average body size differences between the members of breeding units were 1.3 or more in *J. marlieri*. This value is similar to the cases of *J. ornatus* (Awata et al. 2005) (see also Fig. S1a,b). In *J. ornatus*, the fish combinations with this ratio are of less aggressive and probably of less sexual conflict (Awata and Kohda 2004; Awata et al. 2005). Furthermore, this ratio will be general for the stability of the size dependent dominance hierarchy within a fish group, reducing the aggression or conflict between the participants (Buston 2003; Kohda et al. 2008).

J. marlieri mating system is also organized in this role of size ratio of 1.3. When one female was exchanged, the body size ratio between the new female and alpha males became to be similar and female rejected the alpha males, and then the male left the nest, probably not being fitted to the size ratio.

About 30 years ago, *J. marlieri* in this study area were smaller than those in the present study (Yamagishi and Kohda 1996). In that year, the largest two females observed (91 mm and ca. 80 mm TL) might try to occupy and visit two nests and would be classical polyandrous, while the other smaller two females (81, 79 and 72) were cooperative polyandrous, and two smallest (62 and 61) were monogamous. The occurrence rate of classical polyandry was 2/7, and it is not different from the rates of 14/42 in the present study (Fisher probability test, $p = 1.0$). In all mating units, females are larger than males (Yamagishi and Kohda 1996), being same to the case of the present study. The two large females of classical polyandry and cooperative polyandry in the previous study will be equivalent to cooperative polyandry or monogamy in the present study, respectively, indicating that the relative size will be crucial rather than absolute body size for the determination of their social status.

In the classical polyandry, the large females were mated with two or more males at respective nest site, and these males took care more than the females. The large females took advantage of free from brood guarding and foraging in comparison with the monogamous females, and conducted the defense of their own territories more intensively than males. If males and females of *J. marlieri* are the same size, females will win the physical fight (Barlow and Lee 2005). These indicate the sex-role of this fish is reversal as documented from bird species of classical polyandry (Oring 1986; Andersson 2005).

Social evolution of classical polyandry

The evolutionary step to classical polyandry is proposed based on examples of bird polyandry (Andersson 2005): 1) Male parental care evolved to be main, 2) Female ability to lay more eggs than a male can accommodate, and 3) Female competition to obtain more numbers of males. At the third stage, the bird classical polyandry will occur. In avian species, before the evolution of sexual size dimorphism, the evolution of paternal care is assumed to occur. Then females that produce more eggs than number of eggs of the care capacity of a male and mate with additional male will be advantageous. Here the female become larger and have higher competitive ability, and then the size reversal occurs. The care taking sex is smaller than other sex, and it is the case of female in harem-polygynous species of traditional sex-role where males compete over mates with rival males, whereas it is males in classical polyandry where reversed sex-role is observed. In polygynous bird species, males are larger than females, whereas in classical polyandrous birds females are larger than males. In the former, sex-role is conventional and males compete over mate, whereas in the latter sex role is reversed and females compete over mates. This is also the case of cichlids in Lake Tanganyika: in almost all harem polygynous species, males are larger than females that take care of brood, they show conventional sex-role (Kuwamura 1997).

Classical polyandry observed in the cichlids of the genus *Julidochromis* and harem polygyny in *Chalinochromis brichardi* are likely to be good subjects to consider the social evolution of classical polyandry and cooperative polyandry in fish, which are closely related species (e.g., Tanaka et al. 2018b). The social systems of *J. ornatus* and *J. transcriptus* are unique (Awata et al. 2005; Heg and Bachar 2006; Kohda et al. 2009), where body size of the largest individuals are not different between sexes, and both sexes have large territories that contain several nests with mates (Awata et al. 2005; Awata et al. 2006b) (Fig. 7). Cooperative polyandry of *J. ornatus* consists of large alpha males, middle size female and small-beta males, or the large female, middle alpha males and the smallest beta male (Awata et al. 2005;

see also Fig. S1a,b for comparison). Monogamous pair consists either of large male and small female or of large females and small males, where small and subordinate fish perform parental care independent of sex (Awata and Kohda 2004). Interestingly, similar sized pair is hardly present in these mating units (See Fig. 1 in Awata and Kohda 2004). Some large territorial females control two nests, which the females visit, but they are not evidenced to spawn clutches at multiple nests (Awata et al. 2005).

In *J. marlieri*, females are larger than alpha-males, and the subordinate alpha males care for young. By contrast, *C. brichardi* alpha-males are larger than females, and these males have large territories containing several nests polygynously (Kohda et al. unpublished data) (Fig. 7). In cooperative polyandry of *C. brichardi*, the trio is consistent of large alpha male, middle-sized female and small beta-male, and almost all of monogamous pair consists of large alpha male and smaller females. Thus, sex-role of dominant sexual pair is conventional in *C. brichardi,* but reversed in *J. marlieri*. I assume that from the ancestral 'neutral' species *J. ornatus* or *J. transcriptus* where large male and large female are of similar size, the large female evolve in *J. marlieri* and large male evolve in *C. brichardi* (Kohda et al. unpublished data) (Fig. 7). This evolution that occurs within a closely related species will be unique, but this sexual difference in growth rate will induce the unique mating systems, although their phylogenic relationship still not has general agreement in principle (Tanaka et al. 2018a, 2018b).

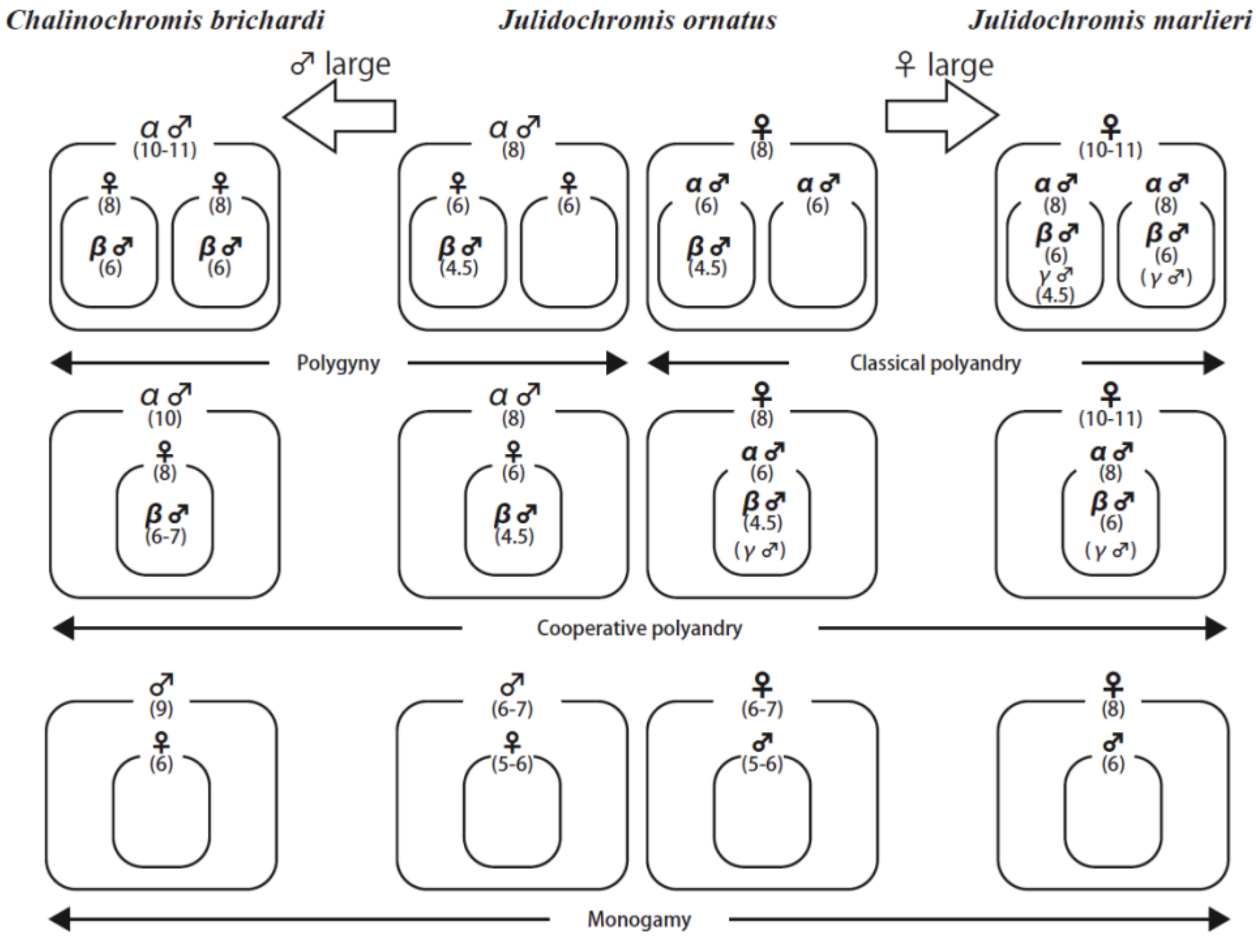


Figure 7. Schematic model of social evolution of cooperative polyandry among species of the genera *Julidochromis* and *Chalinochromis.* The sizes of the symbols ♂ and ♀ show the relative size of the individuals. Arrows are the hypothetical directions of evolution of the mating systems. The references are in *J. ornatus* (Awata and Kohda 2004; Awata et al. 2005), *C. brichardi* (Kohda et al. unpublished data) and *J. marlieri* (the present study).

The sex that performs parental care is not determined sex-specifically in the group of *Julidochromis* and *Chalinochromis,* and parental caretaker is flexible depending on the relative body size of combination of a sexual pair: the smaller members are caretakers (Awata and Kohda 2004; Kohda et al. unpublished data). This will be largely different from the hypothesis of parental care pattern of birds where male paternal care evolves initially (Andersson 2005), whereas the male parental care will not evolve initially in *J. marlieri* as in the other species of this genus. This is also the case of *C. brichardi*, where beta males smaller than females, that become alpha males after growth, perform parental care more than females (Kohda unpublished data). The growth rate or growth pattern will induce faster growing in the large females, and then classical polyandry associated with reversed sex role will be formed. This can happen, for example, by evolution of male smaller body size, or by female production of more or larger eggs, made possible by larger female body size or more food. A favourable food situation to novel specialised foraging methods may be crucial for evolution of classical polyandry (Andersson 2004, 2005). Thus, for the evolution of classical polyandry in *J. marlieri* large size of female will play an important role in the formation of classical polyandry, but initiation of evolution of male parental care will not be always needed.

Chapter 3

Egg-clutch deposition patterns of classical polyandrous females in the nest breeding cichlid *Julidochromis marlieri* in Lake Tanganyika

Abstract

Recently, classical polyandry is documented in the nest breeding cichlid, *Julidochromis marlieri* in Lake Tanganyika, where a territorial female mates with two or three males at the different nest in her territory. In classical polyandrous birds such as shorebirds and coucals with exclusive paternal care, females lay a limited number of eggs that a male can accommodate for care, and they lay a new clutch soon after obtaining new mates. The nests of *J. marlieri* often have unlimited space for spawning, and eggs are spawned at nests during paternal care of elder young. I studied how polyandrous females of *J. marlieri* spawn egg clutches, and here I show the spawning patterns of the classical polyandrous females in *J. marlieri*. Large females of classical polyandry that had many ripe eggs in ovary, ca. > 100, often spawned eggs at two different nests in their territories within several days (split-spawning), with interval of about one month to the next spawning. Intervals were not different between the split-spawning and single spawning whereby one clutch was laid at a single nest, suggesting the clutch size from split-spawning may be reduced, but number of total spawning increased. In the two clutches considered as a split-spawning, clutch size was larger in nest of the larger alpha male than the smaller alpha-male probably associated with parental care abilities. The reduced clutch size might be optimal, which are supported by observations of higher survival of young in these clutches in split-spawning than in single-spawning. I propose the optimum clutch size hypothesis in *J. marlieri* wherein two nests are required, and the process of formation of polyandry is likely to be different between this fish and birds.

Keywords: classical polyandry, reversed sex-role, optimal clutch-size hypothesis, paternal-care investment

Introduction

In classical polyandrous birds, females compete over mates to obtain more number of nests/males, and they mate with more than one male, each of which raises his offspring with little or no support from his mate (Oring 1986; Kotaka 1998; Goymann et al. 2004, 2015, 2016; Andersson 2005; Michalek et al. 2006; Maurer 2008; Muck et al. 2009). Since long ago, this mating system has been of great interest because the traditional sex roles are often reversed: females compete over males who take care of the offspring (Clutton-Brock 2009; Kvarnemo and Simmons 2013). Classical polyandry is rare in fishes, but it has been documented in several pipefish species where belly-plate eggs are spawned and paternal care is conducted (Berglund et al. 1986a, 1986b, 1989; Berglund and Rosenqvist 2003; Jones et al. 2001a; Avice and Liu 2010), and in cardinal fish species where males alone mouth-brood the clutch (Okuda 1999). Recently, I have first reported classical polyandry in a cichlid fish *Julidochromis marlieri*, wherein females spawn eggs at nests where attending males care for their brood (Chapter 2). In *J. marlieri*, larger and dominant females enclose more than one mates at the different nests, where females use as spawning sites. These females likely spawned more number of clutches, and survival rates of young from early stage to the stage of independent size were higher than that of non-classical polyandrous females (Chapter 2). Consequently, numbers of independent young are likely to be more than those of females that have one nest. Thus, classical polyandrous females may obtain higher reproductive success than other females (Chapter 2).

This fish has similarities to birds with classical polyandry mating system and sex-roles reversal (Barlow and Lee 2005; Wood et al. 2014; Chapter 2), but also has differences to bird polyandry (Oring 1986; Goymann et al. 2004, 2015, 2016; Andersson 2005; Michalek et al. 2006). Different from polyandrous birds and pipefishes, where males have the limited space for accommodating eggs, the space of substrate nests of *J. marlieri* are unlikely to be limited (Mushagalusa and Kohda personal observations). In classical polyandrous birds such as shorebirds and coucals, females spawn with a limited number of eggs that a male can accommodate, and after finishing care by the current male or obtaining additional mates, she can spawn a new clutch simultaneously. In contrast, *J. marlieri* spawn eggs into nests during the parental care of elder/larger young by parental males, and this is largely different from polyandrous birds of which paternal males do not accept the next clutch (Szekely and Cuthill 2000; Szekely et al. 2007). How *J. marlieri* females deposit their eggs into two nests? There may be a species-specific spawning pattern probably different from spawning of females in classical polyandrous birds reported hitherto (Andersson 2005).

There has been reported an optimal clutch size in many birds, e.g. major tits and snow geese (Lack 1966; Bêty et al. 2003). Lack's optimal clutch size hypothesis is also applicable to fish brood care of offspring at nests under parental care (Tinbergen and Both 1999; Kasimatis and Riginos 2015; Satoh et al personal communication). Among the cichlids of the tribe Lamprologini in Lake Tanganyika, young that take foods of benthic animals experience sibling competition, and are distributed in wider area of substrate as their foraging sites (Kohda et al. unpublished data; Sato et al. unpublished data). Such a large clutch will not be efficiently protected by parents well, and due to this, clutch will necessarily receive the higher predation pressure likely leading to higher mortality of young. Female fecundity is correlated with the body size in general, and large polyandrous females of *J. marlieri* have 100-150 ripe eggs in their ovary (Chapter 2). If these eggs will be spawned as one clutch, the young would be so large for efficient parental care by one male at the single nest than when spawned into separate nesting sites, inducing higher survival rates (Satoh et al. personal communication).

It is predicted that the classical polyandrous females of *J. marlieri* would divide their eggs into two or more nests, and would induce the ‘optimal clutch size’: by spawning into multiple nests with small clutch size. Due to this spawning pattern, females would enjoy more number of independent young, i.e. higher reproductive success. If eggs are spawned into two nests, the clutch size should be reduced and may approach optimal clutch size. Consequently, the clutch number will increase. Understanding of why and how females of *J. marlieri* can produce more number of clutches may help our elucidation of the evolution of classical polyandry in this fish.

The major hypothesis of evolution of classical polyandry in avian species is that the number of eggs that males can simultaneously care for are limited, but that females have a potential to produce more than the potential of males bringing their offspring up (Reynolds and Szekely 1996; Owens 2002; Thomas and Szekely 2005; Wojczulanis et al. 2012). Thus, polyandrous females will have an advantage, and female competitive ability will increase (Andersson 2005). Here I show how the large females gain benefits by mating multiple males at the different nests in *J. marlieri*. Then, I discuss the social evolution of classical polyandry, by considering the similarity and differences in polyandry between nesting fish and birds. In this study I tested Lack’s optimal clutch size hypothesis, by examining the predictions from the hypothesis using the field data on reproduction in *J. marlieri*.

Materials and methods

Subject animals and field observations

I conducted field researches at Pemba Point (3˚61’S, 29˚15’E), 25 km south from Uvira City, Democratic Republic of the Congo. The genus *Julidochromis* consisting of 6 species have complex mating systems (Awata and Kohda 2004; Awata et al. 2005, 2006b, 2010; Heg and

Bachar 2006; Wood et al. 2014; Ito et al. 2017, 2018), and their phylogenetic positions have been well studied (Sturmbauer et al. 2010; Tanaka et al. 2018a, 2018b). It has been suggested that *J. marlieri* have a classical polyandrous mating system, based on field observations and genetic parentage analyses at Pemba Point (Chapter 2). In the present study, I made a study quadrat of 20 m x 22 m with 2 m x 2 m mesh grid by a thin thread on a rocky bottom. In this quadrat of 3-10 m water depth, there lived a plenty of *J. marlieri*. I conducted field observations in and around the quadrat from the beginning of April to the end of August 2018, three days a week always including every Wednesday. I observed behaviours of individuals throughout the quadrat and individually specific color pattern on head was described on underwater note of which each fish was easily identified (see Yamagishi and Kohda 1996; Sunobe 2000; Awata and Kohda 2004; Awata et al. 2005; Chapter 2). Almost all individuals and their nests sites in and around the quadrat were identified in April. Sex of a fish was estimated based on their body size and finally identified by the examination of gonads of fish captured after the observation period. Nests of this fish were crevices on rocks, interstice between rocks and narrow space beneath a rock. The nest sites were easily located like other congenerics (Awata and Kohda 2004; Awata et al. 2005, 2010). I checked the size and number of young inside the nests on every Wednesday during the study periods, and additional observations in the other days at each nest site. I counted the number of three sized young: small-sized (7-15 mm in TL just after hatching), middle-sized (15-25 mm) and large-sized young (25-35 mm) appearing in or around the nest entrance. Eggs inside nests were not visible.

Small young just after york-sac absorption appeared from inside or entrance of the nests and I could count the number. Therefore, when I found the young of about 7–10 mm TL at a nest, I regarded them as newly hatched young (i.e. a new clutch). The number of the small young (< 10 mm) were counted and regarded as an initial brood number although the egg number would be much larger. Thus, I checked the nest deposition and the growth and number of young at each nest at least once a week. When I observed young continuously, and the number of these young after the eight weeks from the first appearance that often grew into large young around 30 mm TL were regarded as successfully grown young of independent size. Young larger than 30 mm TL were often observed to leave their natal nests (Mushagalusa personal observation), and the number of young of 30 mm are regarded as the best indicator of the number of independent young.

Behaviours of identified fish were observed for 10 min 11 times or more per individual with an interval of a week or more. I recorded fish swimming routes, foraging sites and number of foraging bites, time for staying at nest (within 30 cm from nest entrance), numbers of entering nests and attacking rates against conspecific and heterospecific fish on the territory map. From the records of swimming trace, I determined their territories, of which borders corresponded to the point of female-female aggressions. I observed fish 1 m or more apart from the subject fish and did not affect their behaviour.

Fish were collected from 20 to 27 August 2018. I used standing net of 4 m long and 1.5 cm height (7 mm x 7 mm mesh) and hand nets. When capturing young or small adults inside nests, I used clove oil diluted in 70% ethanol. Captured fish were killed with over doze of clove oil on the land of the study site. After fish were sacrificed, whole body of young and a cut pectoral fin of adults were put in 99% ethanol. Bodies of adults were preserved in 10 % formalin solution.

Data analyses

Statistical analyses were performed using R version 3.3.2 (R Core Team 2016). Body size (TL) of females having different number of nests was differentiated using a one way analysis of variance (ANOVA). The number of small young was examined using generalized linear mixed models (GLMMs) with log link (Poisson or Gamma). Kruskal-Wallis test was used to compare territory size among females with different number of nests. To assess whether polyandrous females with two nests frequently laid eggs at the nest with a larger male, the binomial test was used. All reported *P*-values were two tailed, and results were considered statistically significant at *P*-values of < 0.05.

Results

Fourteen classical polyandrous females were observed, and 10 and 4 females had two and three nests in their territories, respectively (36 nests in total; Fig. 1). The body size of females that had two nests (total length, TL ± SD = 91.7 ± 7.9, n = 10) and three nests (96.2 ± 8.4, n = 4), and single nest, that is cooperative polyandrous females, (91.2 ± 10.0, n = 16) were not different in size (ANOVA, F = 0.74, $p = 0.49$). Territory size of females were not different among females with one, two and three nests (Kruskal-Wallis test, H(x^2) = 4.48, $p > 0.05$, n = 29).

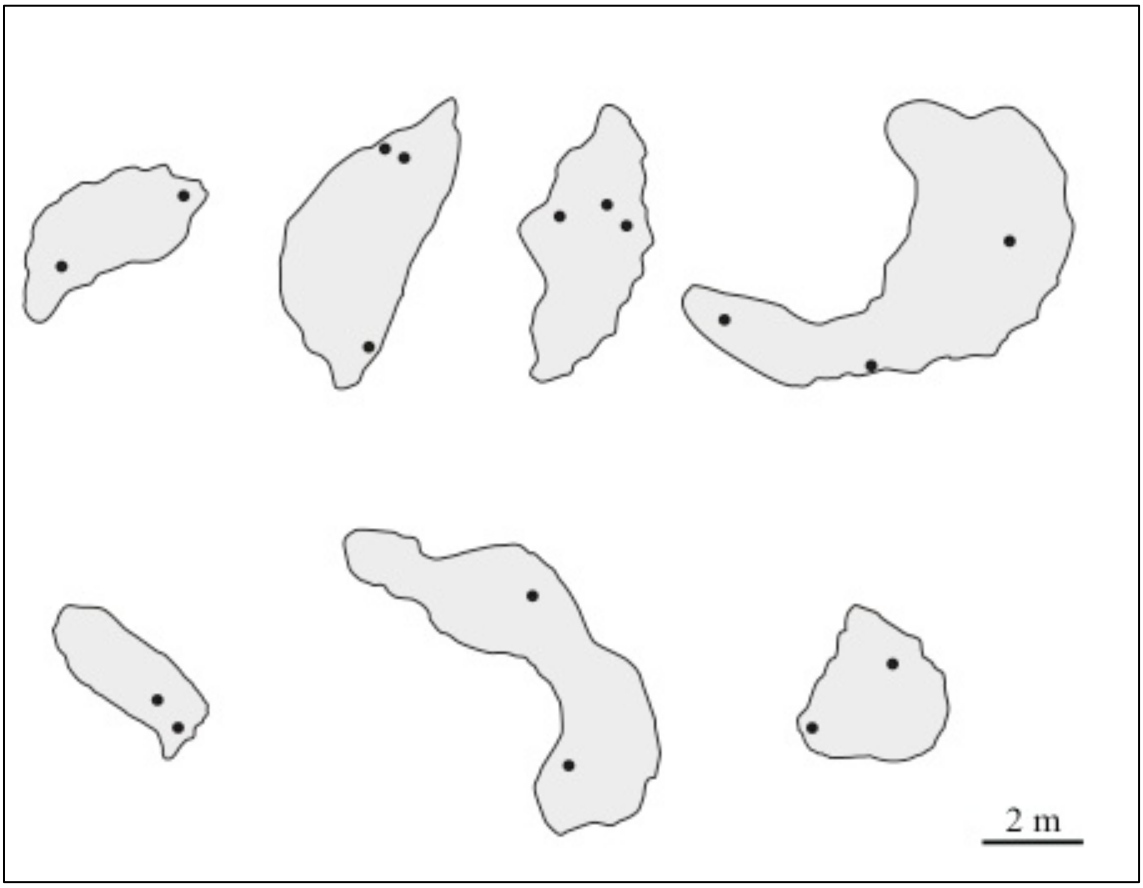


Figure 1. Seven examples of the territory of classical polyandry of *Julidochromis marlieri*, of which females spawn eggs at the two nests within a several days (split spawning). Dots show the nest sites.

Of 10 classically polyandrous females that spawned egg clutches at multiple nests during study period, 9 females spawned egg clutches into two nests within a few days (hereafter called 'split spawning'), a total of 11 times, i.e. 22 clutches (Fig. 1). The 14 females spawned a total of 42 clutches, and thus, about a half of clutches were 'split spawning' and the remaining was regarded as 'single spawning', where females would deposit one egg-clutch at one nest. The interval between single spawning were 3-9 weeks, and the average interval was 5.6 weeks (n = 8). However, the numbers of new young that appeared at the entrance of nests were not different between the split spawning (3.14 ± 2.42, n = 22) and single spawning (4.29 ± 2.89, n = 17). When a set of split spawning is regarded as a single spawning (n = 11), the number of small young in split spawning (6.27 ± 3.47, n = 11) seemed to be larger but was not different from 17 single spawning of these 9 females (Poisson GLMM, $\chi^2 = 1.298$, $p = 0.255$).

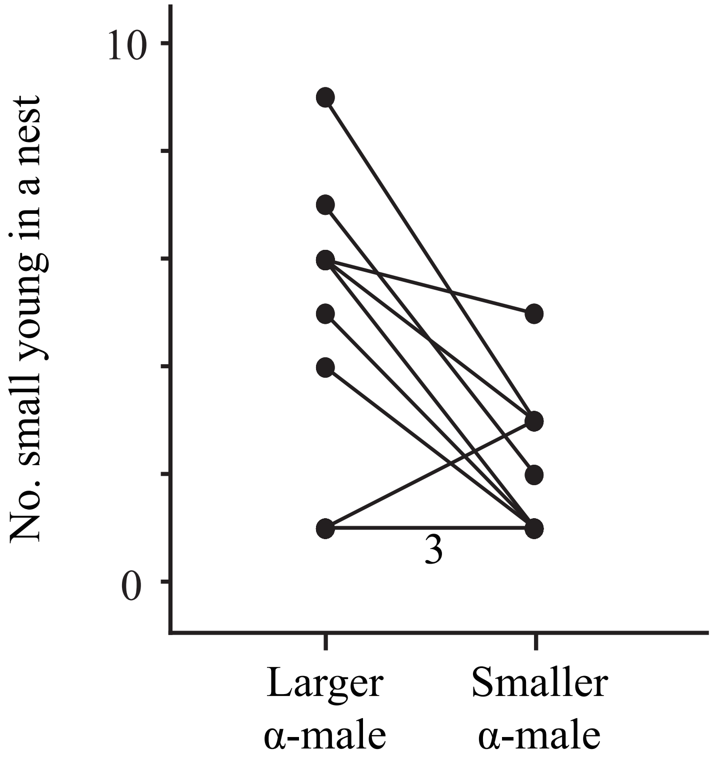


Figure 2. The numbers of small young that appear at the entrance of nests after the split spawning between the large alpha males and the smaller alpha males within groups of classical polyandry of *J. marlieri.*

Of the 11 split spawning, nests of larger alpha males (mean TL ± SD = 79.55 ± 7.27 mm) had more number of small young (4.27 ± 2.73, n = 11) than the nests (2.00 ± 1.28, n = 11; Gamma GLMM, $\chi2 = 8.9403$, $p = 0.0028$) of smaller alpha males (68.29 ± 7.87) (Fig. 2). The smaller males in a classical polyandry received just 2 single spawning clutches additionally to the 11 clutches in the split spawning. In contrast, the larger males and the similar sized alpha males with larger beta males received more numbers of 15 clutches of single spawning additionally to the 11 split spawning clutches (2:15; Binominal test, $p < 0.01$). The number of small young in the clutches deposited at the nest with smaller males was 2.0 (n = 2), whereas that in the clutches deposited at the nest with larger alpha males was 4.44 ± 2.94, range = 2-11, n = 15). Of 11 split spawning, 6 nests of large alpha males were with large young, and 3 nests of small alpha-males were with large young under parental care.

Discussion

The present study shows that classical polyandrous females of *J. marlieri* spawn eggs at two or more nests, often with large young under care, as two clutches within a narrow range of days. Such split-spawning has not been reported from polyandrous birds even if this is likely to induce more number of clutches in classical polyandry of this fish. However, this spawning pattern will be largely different from spawning patterns of classical polyandrous birds such as shorebirds and coucals (Goymann et al. 2004; Michalek et al. 2006; Maurer 2008; Muck et al. 2009), of which females always spawn a whole clutch to one male, and look for another mate.

How many nests do *J. marlieri* females need for this split-spawning pattern? In the cases of classical polyandry in birds, territory size increases as the numbers of males or nests increase (Butchart et al. 1999; Andersson 2005; Goymann et al. 2015). However, territory sizes of polyandrous females in *J. marlieri* were not different among the females with different number of nests. Furthermore, females of this fish that had three nests were not larger than females that had two nests, suggesting that their competition over nests are not so severe, and occupying more than two nests are unlikely to be important for females (Mushagalusa personal observation). Females that had three nests did not spawn eggs at the three nests at the same time in split-spawning, and therefore, two nests seem to be important for classical polyandrous females. If polyandrous females in avian species have more number of nests and/or males, they have more chances for laying a new clutch (Goymann et al. 2004, 2015, 2016; Andersson 2005; Michalek et al. 2006; Maurer 2008; Muck et al. 2009), whereas two nests seem to be effective in *J. marlieri*. This spawning pattern in different nesting sites and male availability may be different between the birds and this fish.

The classical polyandrous females in shorebirds often lay four eggs at a nest and further clutches are deposited either after the current parental male finishes the care or female founds and mates with other males additionally (Maclean 1972; Andersson 1978, 2005). This is due to the efficient incubation by male and effective paternal care and guard of their chicks: If the clutch size was experimentally increased, parents suffered reduced survival and high parental costs (Safriel 1975; Szekely and Cuthill 2000). This limitation is also the case for pipefish and mouth-breeding cardinalfish which are limited by the capacity of their egg pouches and mouth (Berglund et al. 1989; Berglund and Rosenqvist 1990, 2003; Okuda 1999). Thus, females of these fish also require additional mates to increase egg numbers and reproductive success simultaneously (Berglund et al. 1989; Berglund and Rosenqvist 1990). In *J. marlieri*, the area of spawning eggs may not be limited. Furthermore the nests of *J. marlieri* contained several clutches, apparently different sized young at one nest, indicating continuous spawning in the nests and different clutches of young (Chapter 2). This type of spawning is different from parental care for chicks at nests (Andersson, 2005). Thus, these females might not have the spawning restriction like in the case of previous examples of classical polyandry.

Interestingly, the numbers of small young in split-spawning were larger in nests of the large alpha-male than the nests of the smaller alpha-males. The different numbers may be due to two reasons: First, female spawned more number of eggs to the large alpha-male than to small alpha-male, and second, the large alpha males performed parental care better than the small alpha males. At present, both possibilities are not being rejected. Different from the previous examples of classical polyandry, *J. marlieri* females spawn eggs at two nests at the same time, and probably control the number clutches and that of clutch size. The interval of single-spawning and split-spawning were not different, suggesting the possibility that, almost at the same time, in a single-spawning and in split- spawning, females would spawn similar numbers of eggs. If this is the case, the clutch size in single-spawning would be much larger than one clutch of split-spawning, although the numbers of small young were not different between the two. Thus, the large number of new young at the nest with the larger alpha males is due probably to the egg number the female produced. Therefore, split-spawning by females would be advantageous when two nests are available at the same time.

The distribution of eggs at two nests indicates that clutch size may be small. Large females spawn many eggs, which is not a suitable clutch size for effective parental care at the nest (Safriel 1975; Szekely and Cuthill 2000). I propose the hypothesis that the distributed spawning (split-spawning) by *J. marlieri* should induce the optimal size of clutch. Under the possibilities of putting eggs into multiple nests, female may be able to avoid the risk of whole predation (Andersson and Ahlund 2012). However, in some larger females of *J. marlieri*, many clutches are deposited to nest under care of previous broods, and predation to parents was quite rare (Chapter 2). Thus, this possibility may not be the case for *J. marlieri*.

In *J. ornatus*, the egg numbers ranged 20-40, and *J. transcriptus* spawned eggs of 40-50 eggs (80 mm in TL: Awata 2005; Awata et al. 2006b, 2010; Kohda et al. 2009). In contrast, *J. marlieri* females of classical polyandry are much larger than those in *J. ornatus* and *J. transcriptus*, and had up to 150 mature eggs in their ovaries (female size 100-110 mm TL), suggesting that they have a potential to spawn more than 100 eggs at a time (Chapter 2). If they spawn excessively many eggs at one nest, the young might experience high brood competitions and suffer the lack of foods or space at the nesting site (Satoh et al. personal communication). Thus, the consequent expansion of the area young are distributed will not be guarded well by parents, and suffer from high predation pressure.

In some species of polyandrous birds, it was reported that when females take over nests with young of previous females, the female often kill the young to make the parental male sexually acceptable (Emlen et al. 1989). This is because during paternal care, the males that care for the young of previous females cannot accept new females. The infanticide by the female may induce the copulation with male, and this example will be strict example of reversed sex roles (Emlen et al. 1989). Indeed, females copulate with the mated males again, after the finish of the paternal care. However, *J. marlieri* often spawn eggs during the care of elder young, and experimental inducing of takeover of nests with broods of other females would be interesting. It is predicted that the new females in *J. marlieri* should allow young of previous females to continuously stay at the nest. This aspect is largely different from previous examples of classical polyandry in birds.

In conclusion, the classical polyandrous females of *J. marlieri* often spawned many eggs at two different nests by separating a clutch. Such distribution may result in the reduced clutch size and more number of spawning. If large females that produce more than 100 eggs, it is suggested that when the young of the cichlid of lamprologini tribe at Lake Tanganyika will be a substrate type that prey on mainly benthic animals and they disperse on the bottom surface. Thus, they could produce small clutch size. The spawning clutch patterns of classically polyandrous females are largely different between this fish and birds. The fish produces a certain number of eggs, and polyandrous females obtain paternal care from two males for probably optimal clutch size, and achieve higher reproductive success. In contrast, in the case of birds, females produce more number of eggs by mating with more number of mates. In both cases of examples, large females obtain a higher advantage by mating with more numbers of male, and the evolutional process may be different between this fish and birds.

Chapter 4

The parental role of subordinate males can select for sperm competition in a cooperative polyandrous cichlid *Julidochromis marlieri*

Abstract

Polyandry (females multiple mating) across species may increase the testes mass and risk or intensity of sperm competition for fertilizations. In cooperative polyandry (females multiple mating jointly taking care of the brood) sperm competition is expected to be high and absent in monogamy. In these species males will compete for mating with the same female and increase paternity level. Parental effort by males is expected to decrease with decreasing paternity. Here, I tested these hypotheses in the African cichlid fish *Julidochromis marlieri* in the field, in which both monogamy and polyandry (cooperative and classical) occur within a same population. *Julidochromis marlieri* is a reversed sex-role species: small males care mostly for the offspring while females defend a nesting territory containing several males. At a same nesting site, up to three males of different size were found to mate with a single large female, including a large alpha-, a small beta- and an additional smaller gamma male. First, I observed their social and parental behaviours in the field and examined effects of their paternity on parental efforts in comparison to those monogamous pairs using molecular data. Parental efforts considerably varied amongst the male types: most of labour was performed by smaller males which stayed closer to the brood at the nesting site. There was no difference, however, in the mean number of young sired by each male type, suggesting a shared paternity and participation of beta- and gamma males in reproduction. Second, I examined testes mass from all male types to assess sperm competition. A large testes size is presumably advantageous when male–male competition occurs via sperm competition. I found that there was a considerable variation in relative testes mass among male types: cooperating polyandrous males all invested much greater in testes than monogamous males, suggesting a strong relationship between testes investment and sperm competition risks. Neither the foraging frequency nor the time spent at nest did alter variations observed in testes size of male types. These results support the hypothesis on sperm competition in *J. marlieri* which arise as the evolutionary consequence of polyandry. They are discussed and compared to other related *Julidochromis* species at Lake Tanganyika and avian sex-reversed role species.

Keywords: Cooperative polyandry, reversed sex-role, social status, paternity, paternal care, cichlid fish, Lake Tanganyika

Introduction

Many comparative studies have reported that relative testes size is significantly larger in species that breed colonially than in species that breed solitarily, suggesting that higher breeding density is associated with greater sperm competition levels (e.g. Birkhead and Møller 1998; Pitcher et al. 2005; Schradin et al. 2009). It was reported that testes size is affected by the main social mating system and dimorphism effect in avian groups and other animal taxa (Dunn et al. 2001). Cooperative breeding systems are common in a variety of animals where more than two adult individuals provide care to young at a single nest (Emlen 1991; Koenig and Dickinson 2004). In these animals, group members include a pair of breeders and closely related helpers (e.g. Balshine-Earn et al. 1998; Emlen 1991; Stacey and Koenig, 1990).

When females mate with more than one male simultaneously, the potential for sperm competition arises (e.g. Schradin et al. 2009; Koenig et al. 2016). In contrast to birds and mammals, only a few fish species are known to breed cooperatively, despite a wide variety of mating and parental care systems, most of which are cichlid species endemic to Lake Tanganyika (e.g. Taborsky 1994, 2001; Kohler 1998; Wisenden 1999; Awata et al. 2005; Heg et al. 2005; Heg and Bachar 2006; Tanaka et al. 2015). Therefore, cooperatively breeding cichlids may offer to investigate the influence of sperm competition risk on cooperating mates. Thus far, the effects of sperm competition risks on cooperating male social patterns are only reported from *Julidochromis ornatus* (Awata et al. 2006a) and *J. transcriptus* (Awata et al. 2008).

Otherwise, earlier correlational studies in cooperative breeders have reported a variety of different patterns of investment and reproductive effort of mates (e.g. subordinates and dominant pairs). They suggest that the investment by subordinates may cause dominant pairs to invest less (Dunn and Cockburn 1996; Bruintjes and Taborsky 2008, 2011; Bruintjes et al.

2011) or more (Valencia et al. 2006; Awata et al. 2006a, 2008; Li et al. 2015). Furthermore, theoretical and empirical studies have reported that male parental effort may change in relation to paternity (Westneat and Sherman 1993; Whittingham and Dunn 2001; Dunn et al. 2001). Parental care is important to offspring survival as a component of total reproductive effort by individuals (Alexander 1974). In some situations males might have greater fitness if there is a tradeoff between providing parental care and seeking alternatives such as redirected parental efforts into mating opportunities or somatic efforts (Whittingham et al. 1994). Recently, field investigations by Bruintjes et al. (2011) found that dominant males in *Neolamprologus pulcher* provide virtually no help in brood care and might benefit from increased brood care levels provided by male subordinates that have shared in reproduction. To my knowledge, however, few studies have addressed the relationship between testes investment, sperm competition, and parental care variations in relation to paternity levels of males of different social status within cooperatively breeding fish. Testes mass is considered to be a good measure of sperm competition and multiple mating in birds (Møller 1991; Møller and Briskie 1995; Dunn et al. 2001), fishes (Awata et al. 2006a, 2008), and many other taxa (e.g. Hosken and Ward 2001; Byrne et al. 2002; Byrne and Whiting 2011). Nevertheless, most of these studies have focussed on species with the conventional sex roles and only a few studies have investigated sperm competition in female larger species, where the parental care is mainly performed by smaller males with the help or not of the larger mate (Andersson 2004, 2005; Andersson and Ahlund 2012). At present, data on subordinate parentage under natural conditions are lacking and potential effects on subordinate helping effort are unknown (Awata et al. 2005, 2006a; Wong and Balshine 2011; Bruintjes et al. 2011).

In the present study I investigated the social status patterns of males in *J. marlieri*, their testes investment in relation to sperm competition risks and its effects on reproductive and parental roles of each male. *Julidochromis marlieri* is a small benthic cichlid fish that inhabits shallow rocky shores in the northern part of Lake Tanganyika and limited areas of the southern part (Kohda et al. 1996; Yamagishi and Kohda 1996; Konings 1998). Both sexes are similar in color and shape, but exhibit dimorphism in body size: Females (up to 12 cm in total length, TL) are larger than males (up to 9 cm TL) (Yamagishi and Kohda 1996; Sunube 2000). This fish has been found involving in various social breeding systems within a same population; pair breeding, cooperative polyandry and classical polyandry breeding (see Chapter 2). Behavioural observations in the population of Pemba, north of Lake Tanganyika (DR Congo) indicated that females defended large territory enclosing several mates. She was able to monopolize and breed with up to three males at one (cooperative-) or more separate (classical polyandry) nesting sites repeatedly. Breeding takes place inside narrow clefts in or between rocks where all males of a female attended and guarded their young until independence against intruders (conspecifics and heterospecifics). These cooperating males never overlapped in their body size and, due to this, behavioural observations suggest variations in their parental efforts according to social status and breeding system. Here, I hypothesised that males in *J. marlieri* tend: 1) to invest more in testes size particularly, if they engage in cooperative polyandry that might make sperm competition to be high in a group, 2) where sperm competition occurs, average parentage and parental amount to decrease in relation to the social status and mating strategy of males. I suggest that high sperm competition risks will reduce the paternity (proportion of young sired by cooperating males with different social status) and parental care efforts amongst males of different size and social status in *J. marlieri*.

I investigated the social and genetic mating systems and examined testis size of males with different mating systems and social status in *J. marlieri*. First, I directly observed the social and parental behaviours of these fish underwater in their natural habitats. I predicted the amount of parental care should vary following the size and social status, according to previous studies on *J. ornatus* (Awata and Kohda 2004; Awata et al. 2005) and *J. transcriptus* (Awata et al. 2006b; Kohda et al. 2009). I also predicted that larger male in a group would decrease parental efforts and increase testes size if subordinate males participate in reproduction and share parentage of young (Bruintjes et al. 2011). Additionally, I examined whether the parental care of these males related to their paternity level. Second, I examined the testes size as measures of sperm competition within males of different social status (e.g., alpha, beta and gamma) and between males of different mating systems (e.g. monogamy vs. cooperative polyandry). The investment in testes size is presumably advantageous when male–male competition occurs via sperm competition (Møller and Briskie 1995; Awata et al. 2006a, 2008). I predicted that males within cooperative polyandry would have larger testes and experience high sperm competition risks compared to the monogamous males in *J. marlieri*. If these predictions are confirmed and my hypotheses are supported, and this will be the first report of a sperm competition and its effects on parental effort in a sex-role reversed and size dimorphism polyandrous fish. Finally, I discuss these results with previous findings in other cichlids of the same genus that have evolved cooperative polyandry in Lake Tanganyika.

Material and methods

Study subjects and site

Julidochromis species, endemic to Lake Tanganyika, are biparental substrate spawners, retreating to caves or rock crevices (Awata and Kohda 2004; Awata et al. 2006a, 2006b). *Julidochromis marlieri* occurs in the shallow rocky shores mostly at the northern part of Lake Tanganyika (Brichard 1989, Yamagishi and Kohda 1996; Konings 2015). It forages mainly on sponges and algae on rock surfaces (Hori et al. 1993; Yamagishi and Kohda 1996). This study focused on *J. marlieri* population at Pemba (Bemba) about 25 km away from Uvira, northern shore of Lake Tanganyika (DR Congo). In the study area *J. marlieri* fish were stably plenty. Sexes were similar in color and shape, but in breeding groups females (11 cm in max.) were always larger than males (9 cm in max.). The parental care of broods was likely taken by the smaller fishes (i.e., males), leading to reversed sex roles within pairs. Females defended territories containing a single male or multiple males having different nests (Yamagishi and Kohda 1996; Chapters 2 and 3). In this respect, the pair-bonding sex-role-reversed in *J. marlieri* is similar to some polyandrous, sex-role-reversed birds, in which smaller males nest in the territory of a large dominant female (Jenni and Collier 1972; Butchart et al. 1999; Goymann et al. 2004; Andersson 2005). This fish showed flexibility in breeding systems from monogamy, cooperative polyandry to classical polyandry breeding within the same population, and the same breeding members spawned at the nest repeatedly (Chapter 2). The nest was of several shapes and mainly consisted of narrow clefts and holes in or between rocks. In cooperative and classical polyandry breeding groups, females had respectively 1 and 2 (sometimes 3) separate nests (see Chapter 2). At each nest, there were 2 or 3 hierarchically different males in body size, social status and parental duties. Due to these features, I categorized, herein, these males into four following main types: i) the largest polyandrous alpha-, ii) the second largest polyandrous beta-, iii) the third largest polyandrous gamma males in cooperative groups, and iv) monogamous males in monogamous breeding pairs (see Awata et al. 2008; Kohda et al. 2009; Li et al. 2015).

Behavioural observations, fish collections and measurements

Breeding groups of *J marlieri* were observed between April and August 2018 in the field, and they were collected after the observations. I conducted underwater observations and fish sampling using SCUBA in a 20 m x 22 m quadrat squares with 2 m x 2 m mesh sections over the rock and pebble substrates in 4-10 m water. A map of each square was drawn on waterproof papers on which behaviours and positions of the parental individuals were recorded during focal observations (Yamagishi and Kohda 1996; Awata and Kohda 2004; Awata et al. 2005; Awata et al. 2006a). For each fish a 10-min focal observation was conducted 2 times in the week on different days (Monday and Friday between 9:00 - 16:00). Throughout the study period, the following data were recorded (see Awata and Kohda 2004 for further details): (1) swimming tracks and number of nest attended (see Chapter 2), (2) time spent in and around the nest (within 30 cm from the nest entrance): which allowed to estimate the parental care amount of each individual for the young as the eggs were difficult to observe from outside of the nest, (3) total number of visits to the nest, (3) frequency of foraging (i.e. picking on benthic algae and sponges) and (4) attacks against unknown conspecifics and heterospecifics within 30 cm from the nest entrance. The threatening posture with open mouth and appeasement with quivering body were also reported for each male (Awata et al. 2005; Barlow and Lee 2005; Ito et al. 2017, 2018). All fish were easily identified by their individually distinct head color patterns, body size and social behaviour (Yamagishi and Kohda 1996; Awata and Kohda 2004; Awata et al. 2006a, 2006b).

After behavioural observations, 79 males and 167 offspring (0-17 per nest) belonging to 61 nests were captured with gillnets or with hand nets after application of the anaesthetic (30% clove oil diluted in ethanol; Awata et al. 2005, 2006a, 2006b) in the study area. Five specimens and 29 offspring without behavioural observations were also collected around the quadrat area. Some males failed to be caught, either because of nest shape or inconvenient habitat topography. Within and around the quadrat area, the number of observed and caught males were as follows: monogamous males: 15 observed and 11 caught (73.3 %), polyandrous alpha-: 40 and 25 (62.5 %), polyandrous beta-: 40 and 23 (57.5 %) and polyandrous gamma males: 14 and 10 (71.4 %). Of 25 nests visited by large classical polyandrous females, four contained a single male breeder, fourteen had alpha and beta males and seven had three males, i.e. alpha, beta and gamma (Chapter 2). Of a total of sixteen nest occupied by cooperative polyandrous females, nine were sheltered by alpha and beta males and seven had additional smaller gamma males. Four nesting males remained alone in their nests and were regarded as monogamous mates; although these were each visited by large polyandrous females with additional separate nests in the study area (see Chapter 2). Only captured samples were used in gonad and parentage analyses. In the laboratory, all fish sampled from the field were sexed, measured in TL (to 0.1 mm), body and gonad were weighed (to 0.001 g).

Analyses of genetic relatedness and paternity

DNA extractions, PCR and fragment analyses are described in Chapter 2. Dyadic estimates of genetic relatedness were calculated using ML-Relate software (Kalinowski et al. 2006) using background allele frequencies of 130 unrelated individuals (see Chapter 2 for details). I used pairwise relatedness to establish whether unrelated fish bred cooperatively by comparison of the relatedness among group members (i.e. female breeder, alpha-, beta- and gamma males). Relatedness was analyzed with a Kruskal-Wallist test using a data set of 6 combinations of pairwise relatedness.

The potential father and mother for each offspring were the fish that visited nests where the offspring was present. Paternity and maternity of young at each nest were assigned using the CERVUS 3.0.7 (Marshall et al. 1998; Wang 2004). Offspring with two or more alleles that did not match the alleles of putative fathers and mothers were considered to be young sired by unknown (uncaptured) males and females, respectively.

Data analysis

Data analyses were performed using the PAST 3.21 (Hammer et al. 2001) and SPSS (ver. 16.0; Chicago, IL, USA) for Windows statistical software package. All data sets were tested for normality (Shapiro-Wilk test and homogeneity of variances) before statistical analyses (Schradin et al. 2009). All tests were two-tailed, and statistical significance was accepted at $p < 0.05$. Values are given as the mean ± SD throughout. Tukey post hoc tests were likely used to identify specific differences.

There were no differences between males belonging to cooperative- and classical polyandry for all studied parameters (Tukey post hoc tests, all $p > 0.05$), so I combined them into one polyandrous group with 3 social male types (see below) which were compared to monogamous. Kendall's rank correlation tests were run to determine the relationship between time spent at the nest, foraging frequency and body size amongst all male types. The gonad somatic index GSI (gonad weight/body weight x 100) was calculated as an index of gonadal investment (see Awata 2005). For all specimens caught, the body and testis masses were log-transformed to make a normal distribution of data and to minimize the allometric scaling of the gonad weight on the body weight (Awata et al. 2006a, 2008; Tanaka et al. 2015).

Two general linear models (GLM 1 and 2) with a multivariate design were used to compare the log gonad weight (dependent variable) and log soma weight among the four male types (both independent variables) of *J. marlieri*. Log soma weight was included as continuous predictors (covariate) in the analysis to account for its influence on log gonad weight. The model 1 tested whether male types different in their log gonad weights and whether log gonad weights depended on log soma weights (see Awata et al. 2006a, 2008). Parallel to model 1, the model 2 combined only the log gonad weights and male types to pin the differences between all males each other by multiple comparison analyzes. In these analyses, significance was determined using Tukey contrast statistics, and estimate coefficients were used to assess the strength of each independent factor on the dependent variable when the Tukey statistic was significant. Then, the residuals from the regression of log testes weight on log soma weight were considered as the relative testes mass. These residuals were a continuous predictor (independent variable) in the model and were regressed on the behavioural parameters including the time spent at the nest and the frequency of foraging of the four male types. The total numbers of offspring sired by each male type were confirmed by paternity analyses. They were only compared amongst male types as they indicated any clear predictions when plotted either on log gonad weights or log soma weights, mainly due to unequal distribution and outliers in values or the failure in catching all group members.

Results

Social status and behavioural parameters

Males observed in this study differed significantly in their body size (total length, TL, mm) according to their social status (Table 1): cooperative polyandrous alpha males were larger, followed by monogamous males, beta males and gamma males, although the size was not different between the monogamous and beta males (Tukey post hoc test, $p > 0.05$). The time spent at nest, the foraging frequency and the number of entrance/visit to the nest were significantly lower for polyandrous alpha males compared to the three other male types (Table 1). All three parameters together did not vary significantly between monogamous, polyandrous beta and gamma males, except for the number of visits to the nest which differed for monogamous and polyandrous gamma males (Tukey test, $p = 0.03$). I did not observe aggressions or threatening behaviours between group members for all types. Aggressive attacks against unknown conspecifics and heterospecific intruders to the nest were lower by numbers for all male types with no statistically significant differences (Table 1).

Overall, the time spent at the nest decreased with the body size (Kendall's rank correlation test, $\tau = -0.57$, $p < 0.0001$, n = 109) while the frequency of foraging increased ($\tau = -0.45$, $p < 0.0001$), suggesting that large polyandrous alpha males spent more time to forage than monogamous and small polyandrous beta and gamma males which were likely close to the brood and nest shelter (Figure 1, Table 1). There was a negative relationship between time spent at the nest and the foraging frequency (Negative binomial GLM, $x^2 = 48.44$, $p < 0.0001$).

Table 1. Comparisons of behavioural parameters (mean ± SD) observed in all male types of *J. marlieri* at the study area. Data in parentheses are the number of individuals observed for each male type (observation time is 10 min. x 11 times for each fish). F- and *p*-values are based on ANOVAs.

	Monogamy	Cooperative polyandrous males				
Parameter	male (15)	Alpha (40)	Beta (40)	Gamma (14)	F stat	*p*-value
Body size (TL mm)	57.3 ± 14.5	73.9 ± 8.5	53.9 ± 8.7	44.6 ± 8.4	F= 46.1	p < 0.001
Time at nest (/10 min.)	7.7 ± 2.2	4.6 ± 1.9	8.3 ± 1.9	9.6 ± 0.8	F= 40.0	p < 0.001
Foraging frequency	36.3 ± 13.3	52.3 ± 12.8	40.2 ± 10.7	33.7 ± 5.3	F= 14.5	p < 0.001
No. of entrance to the nest	6.2 ± 2.7	2.9 ± 1.1	6.3 ± 2.6	8.6 ± 1.9	F= 32.6	p < 0.001
Heterospecific attack	0.5 ± 0.5	0.4 ± 0.5	0.3 ± 0.5	0.1 ± 0.2	F= 2.1	p = 0.10
Conspecific attack	0.2 ± 0.2	0.3 ± 0.3	0.2 ± 0.3	0.2 ± 0.3	F= 1.2	p = 0.30
Threat between males	0.1 ± 0.2	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.2	F= 0.2	p = 0.87

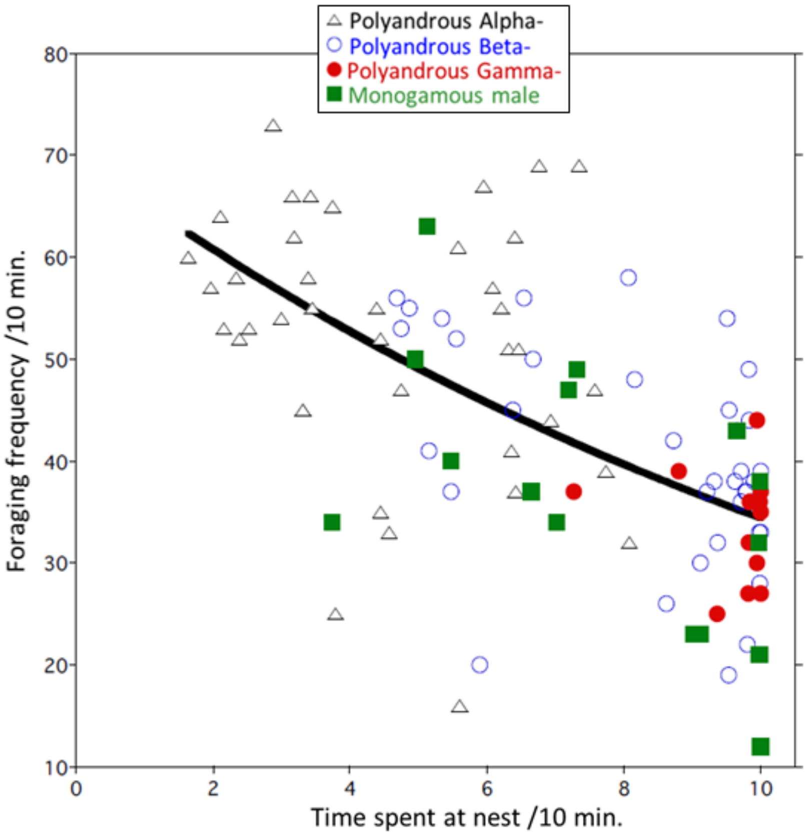


Figure 1. Relationship between the time spent at the nest and the frequency of foraging within types of males in *J. marlieri*.

Body and testis masses with the paternity

The body mass differed among the male types but widely overlapped (One-way ANOVA, F = 36.96, $p < 0.0001$, n = 79). Polyandrous alpha males were heavier than monogamous males and respective subordinates beta- and gamma males (Table 2). The gonado-somatic index (GSI), however, was almost similar between monogamous and polyandrous alpha males; and both had lower GSI compared to subordinate beta- and gamma males (Table 2).

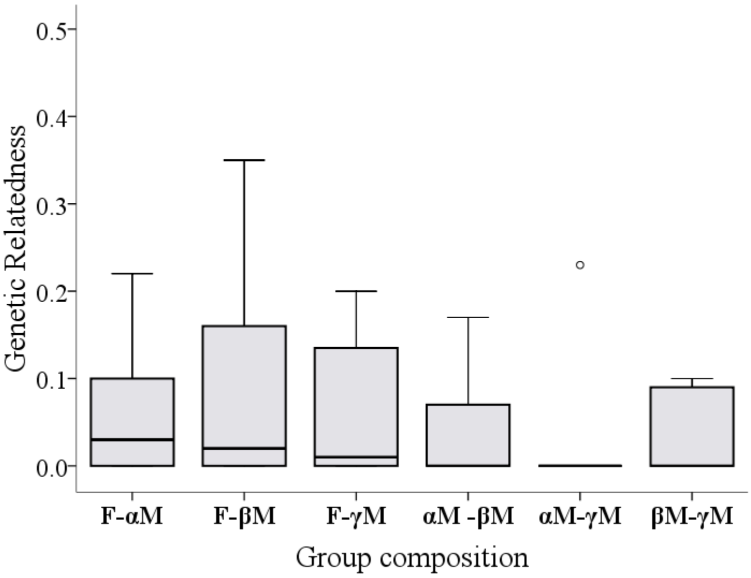


Figure 2. Combinations and pairwise genetic relatedness between breeding females, alpha-, beta- and gamma males from the same group of *J. marlieri* at the study area.

Pairwise genetic relatedness of combinations between breeding females, alpha-, beta- and gamma males from the same group was almost zero, and no significant difference in genetic relatedness was found among the six combinations (Kruskal-Wallis test, $x^2 = 2.51$, $p = 0.88$; Fig. 2). These results suggest that beta and gamma males are unrelated to dominant alpha and females of the same group. Genetic paternity analyses revealed a high percentage of multiple paternities within cooperative polyandrous male types. Of a total of 167 young caught at the study area, 43 (25.7 %,) were fathered by polyandrous alpha males (median: 2, n = 25), 22 (13.2 %) by monogamous males (median: 2.5, n = 11), 39 (23.4 %) by polyandrous beta- (median: 2, n = 23) and 4 (2.4 %) by gamma males (median: 1, n = 10). The average number of young sired by the four male types was not significantly different (Table 2). The remaining 59 (35.3 %) young were sired by unknown males (median: 3, n = 18), e.g., mainly due to the failure to capture one or another male type in some nests. Relative number of young sired by each male type was not related to body mass (Kendall's rank correlation, $\tau = 0.10$, $p > 0.053$, n = 69) and GSI ($\tau = - 0.19$, $p = 0.077$).

Table 2. Variations (mean ± SD) in body mass, GSI and number of young sired by each male type of *J. marlieri*. Different alphabets denote statistically significant differences by Tukey post hoc tests, and data in parentheses are the sample sizes for each male type.

	Monogamy	Cooperative polyandrous males				
Parameter	male (11)	Alpha (25)	Beta (23)	Gamma (10)	*F* stat	*p*-value
Body weight (g)	3.99 ± 2.13^{a}	6.80 ± 2.16^{b}	2.48 ± 1.41^{a}	0.90 ± 0.34^{c}	36.96	P < .0001
GSI	0.76 ± 0.56^{a}	0.89 ± 0.41^{ab}	1.69 ± 0.69^{b}	2.39 ± 0.79^{c}	20.9	P < .0001
No. of young	5.5 ± 7.1	2.9 ± 2.1	4.3 ± 4.3	1.3 ± 0.6	1.10	P > .05

Interactions between body and testes masses with different male types

The first GLM (model 1) showed a significant relational effect of log soma weight and male types on log gonad weight, but not in their interactions (Table 3). The model 2, therefore, was run with multiple comparisons amongst the four male types. In particular, these comparisons clearly showed that the log gonad weight of monogamous males were smaller compared to those of polyandrous alpha-, beta- and gamma males, suggesting the lack of sperm competition risk for monogamous males. Apparently, polyandrous beta- and gamma males showed large log gonad weights than polyandrous alpha males, but no statistically differences appeared (Table 3, Figure 3).

Table 3. Comparisons from GLMs of the log gonad weights on male types, with the covariate log soma weights. The superscripted letters represent statistically significantly differences among the four male types.

	Model 1				Model 2			
Independent variable	Coefficient ± SE	*df*	*F*	*p*	Coefficient ± SE	*df*	*F*	*p*
Male type		3	14.95	<0.0001		3	14.73	<0.0001
Intercept	-1.98 ± 0.24				-1.78 ± 0.10			
Monogamous male	-0.15 ± 0.27				-0.21 ± 0.08^{a}			
Polyandrous Beta-	0.31 ± 0.25				0.10 ± 0.10^{b}			
Polyandrous Gamma-	0.34 ± 0.25				0.11 ± 0.13^{b}			
Polyandrous Alpha-	0				0^{ab}			
Log soma weight	0.85 ± 0.30	1	25.75	<0.0001	0.60 ± 0.12	1	25.38	<0.0001
Male type x log soma weight		3	1.31	0.28				
Monogamous male	-0.56 ± 0.36							
Polyandrous Beta-	-0.25 ± 0.35							
Polyandrous Gamma-	0.09 ± 0.42							
Polyandrous Alpha-	0							

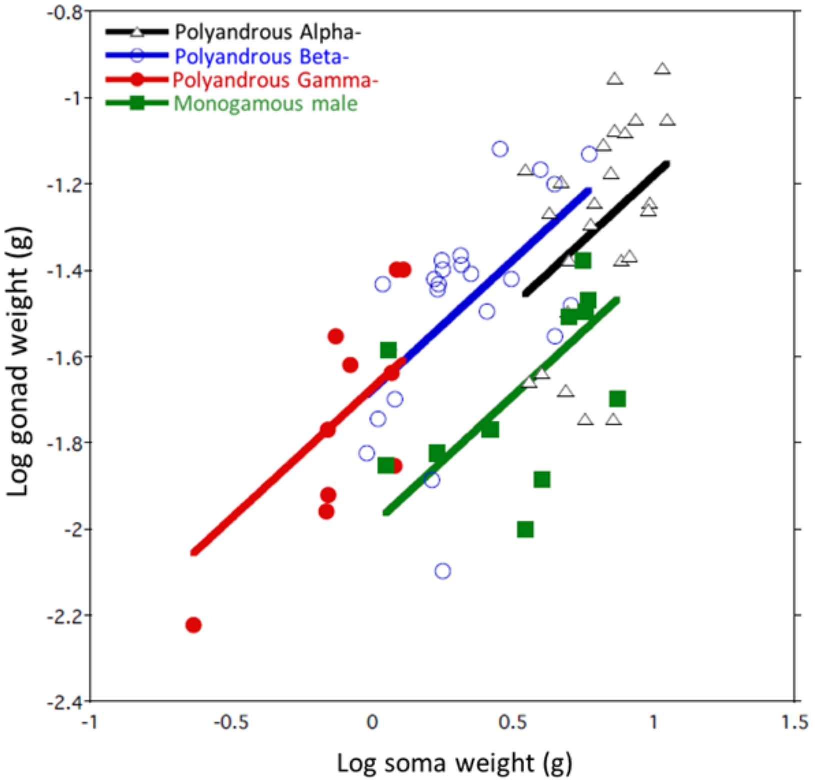


Figure 3. Relationship (GLM 2) between log gonad weight and log soma weight from the four male types of *J. marlieri*. See Table 3 and text for statistics.

Relationship between behavioural and testis parameters

Contrary to my expectation, relative testes weight (estimated as the residuals from the regression of log testes weights on log body weights) did not depend neither on the foraging frequency or the time spent at the nest of the male types (Figure 4).

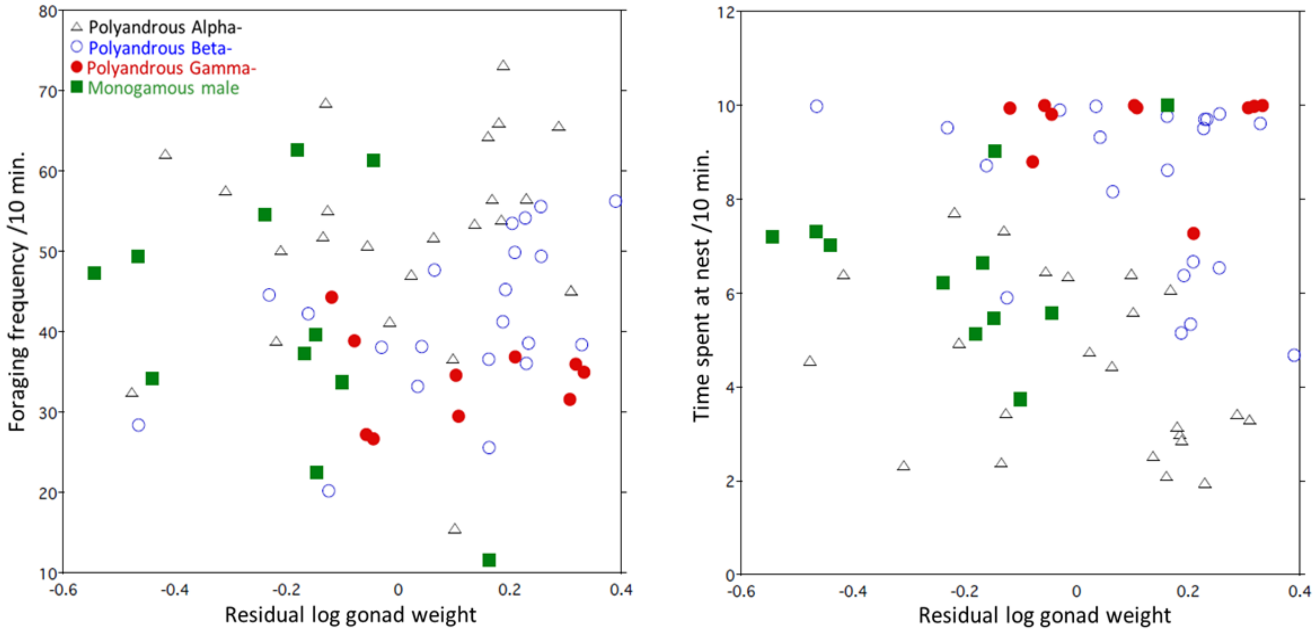


Figure 4. Effects of residual log gonad weights (relative testes mass) on the frequency of foraging (left) and the time spent at the nest (right) by the different male types of *J. marlieri*.

Discussion

Mating with multiple partners (polygamy) is common reproductive strategy observed across a wide range of animal taxa (Firman 2011). In many animals, females often copulate with multiple males and incite post copulatory sexual selection, i.e. sperm competition (Keil et al. 1999; Kvarnemo and Simmons 2013). Sexual selection hypothesis suggests that the reproductive success of both female and male can largely depend on number of mates, because of direct and indirect benefits (Kvarnemo and Simmons 2013). When females mate multiply and, ejaculates overlap in the reproductive tract, sperm from different males are forced to compete for fertilization of eggs (Parker and Simmons 1996; Firman 2011). However, empirical supports for this hypothesis are limited, particularly in fish, as only a few studies have reported a link between sperm competitiveness and male investment in testes size and social patterns in polyandry. This study performed field and genetic investigations at Lake Tanganyika to understand whether polyandry selects for increased testes size by ensuring sperm competition between males and their offspring in *Julidochromis* species. In particular, I studied the parental care and mating strategy in *J. marlieri*, and compared the findings with other *Julidochromis* species, for which observations in the field still rare.

In *J. marlieri*, females were substantially largest and mated with multiple males at one or multiple nesting sites (see Chapters 2 and 3). This evidence is shared by many polyandrous birds like the wattled jacana (*Jacana jacana*) and bronze-winged jacana (*Metopidius indicus*) where a single female was found to mate with up to four males (see Andersson 2005; Kvarnemo and Simmons 2013). At least, members of this fish encountered three different social breeding systems from monogamy, cooperative- and classical polyandry within the same population at the study area. The size and parental roles were sexually reversed (see Chapter 2). I found that in polyandrous breeders, up to 3 males of different sizes were living at one nesting site and bred cooperatively with a single larger female, which obtained sperm from all males repeatedly. Likewise, the smaller males (e.g., polyandrous beta- and gamma males) within a family exhibited most parental care, expressed as spending more time closer to the nest and brood. Polyandrous beta- and gamma-, as well as monogamous males, had reduced foraging time and space than larger polyandrous alpha males, frequently found foraging out of the nesting site with considerably reduced parental care. Previous studies have found similar results in *J. ornatus* (Awata and Kohda, 2004; Awata et al. 2005; Awata et al. 2008) and in *J. transcriptus* (Awata et al. 2006b), where the small partners in a pair care more independent of their sex. These authors indicated, however, no evidence for mate guarding as a way to ensure paternity. Indeed, the variation in parental division of labour observed amongst *J. marlieri* male types can be expected as trade-offs between polyandrous alpha-, which accepted the risk of sharing paternity with both beta- and gamma males for parental care of young. This evidence is supported by my data and those of Dierkes et al. (1999) and Bruintjes et al. (2011), which predicted that the higher the costs caused by subordinates, the more they should help in *Neolamprologus pulcher*. They also indicated that dominant males may benefit more by increased male subordinate aid than what they lose by sharing part of reproduction. Subsequently, this evidence will favour increased testes mass between polyandrous male types arising in sperm competition selects, which has been supported by my data in *J. marlieri* and those of Pitcher et al (2005) in cooperatively breeding birds. Theoretical and experimental studies in fish, insects, birds and mammals have concluded that males reduce their expenditure on parental care when the risk of lost paternity through sperm competition is elevated (reviewed in Wright et al. 1998; Kokko and Jennions 2008; Alonzo 2009; Kvarnemo and Simmons 2013). And so, a male can select against parental care if he expects to gain greater paternity in future breeding attempts (Kvarnemo and Simmons 2013), particularly in *J. marlieri* where the females can spawn each 2-3 weeks (Mushagalusa personal observation). The sperm depletion hypothesis proposes that males of taxa that copulate often have relatively larger testes (Klemme et al. 2006a, 2006b). Males must trade-off between costly sperm production and investment in other life-history traits, such as parental care (Avise and Liu 2010) or energy invested into searching for a mate (Whittingham and Dunn 2001). In monogamous pairs, small males alone ensured much of the parental care than females did (see Chapter 2), and were not under risks of sharing or lost paternity. Because of this, my results and those by Awata et al. (2006a, 2008) showed that monogamous males invested less in testes size when compared to cooperative polyandrous males. Moreover, beyond these considerations in parental duties, I found a high percentage of multiple paternities in *J. marlieri* (by both observations and DNA based parentage analysis). A similar finding has been described only in the cooperative breeding species like *J. ornatus* (Awata et al. 2006a) and *N. pulcher* (Bruintjes et al. 2011) in the southern part of the lake. At many nests involved in cooperative polyandry, the number of young found was paternally shared by all males (i.e. polyandrous alpha-, beta- and sometimes gamma males) and that female mated with all males present in her territory (or her nests). This was not the case for monogamous males which alone sired all of eggs deposited by females in the nest.

Males were smaller than females in the same breeding group of all mating systems and overlapped in their body size and mass according to social rank in polyandry groups. This evidence, however, led to the competition between cooperative polyandrous males to fertilize the eggs deposited by female in the same nest and to guard young. The testes size, however, were high for polyandrous males than monogamous males, although there were no major statistic differences found within polyandrous males. The fact that polyandrous males had relatively large testes; even these males seemed to pair with the same female repeatedly can arise from the sperm competition in *J. marlieri*. This indicated that males will compete for mating with the female by improving their testes size and reproductive success throughout sperm competition (Awata et al. 2006a). This suggested also, as already discussed above, that monogamous males had low risks of sperm competition than polyandrous male types did. My result is not far different from that of Awata et al. (2006a) and Awata et al. (2008), which showed that testis investment varies in relation to the level of sperm competition in *J. ornatus* and *J. transcriptus* where male breeders (alpha males) with male helpers (beta males) invested more in testes than all other male breeders (i.e., monogamous and cooperative polygynous males). The ability to produce frequent ejaculates with a high volume of sperm is determined in large part by testes size and is presumably advantageous when male–male competition occurs via sperm competition (Birkhead 1995; Birkhead and Møller 1998; Byrne et al. 2002; Pitcher et al. 2005). Apparently, polyandrous beta males and, at some level gamma males, invested more in testes than alpha males, as their testes and bodies mass were allometrically associated. This result showed that testes mass is not related to body conditions, but to sperm competition and paternity improvement by cooperating males of *J. marlieri*. Furthermore, these results on testes mass of male types were not in connection with the frequency of foraging or time spent at the nest. Although these two variables did differ between male types, they did not provide some interesting insights into the evolution of intrasexual variation found in testes size. Awata et al. (2008) have shown that testes investments are traded off against growth in beta males, which had the greatest testis investment among all male types. Although this study did not examine growth in *J. marlieri*, it is highly possible that testis investment affect growth especially for beta- and gamma males. Yet, sperm competition according to male social status has been reported in *J. ornatus* and *J. transcriptus*, a substrate-brooding cichlid species sister of *J. marlieri* in which pairs with either larger females or larger males were reported cooperatively breeding with subordinates (Awata et al. 2005, 2006a, 2008; Kohda et al. 2009). Examples of sperm competition in cooperating and polyandrous vertebrates have been also known in the common English backyard dunnocks (*Prunella modularis*) (Davies 1983, Davies et al. 1992) and acorn woodpeckers (*Melanerpes formicivorus*) (Møller 1991; Møller and Briskie 1995; Koenig and Dickinson 2004; Koenig et al. 2016; Koenig and Walters 2018).

In conclusion, my results show that testes investment varies in relation to the level of sperm competition in *J. marlieri*. The monogamous males do not invest in testes or undergo this competition at all than the three polyandrous males which invested more in testes. This suggests that polyandrous males increased their investment in testes only when they share in paternity and cooperate to care young and maintain the female at the nest. This also suggests that polyandrous alpha males seem to be not significantly affected by this competition as they accepted subordinate beta males and compensated their participation in reproduction by the parental care of their young. But, this benefit of parental care can vary to the extent to which the female may also compensate for reduction in alpha male care. Apparently, beta- and gamma males tended to invest more in testes than alpha males. These evidence are consistent with those by Awata et al. (2006a) and Awata et al. (2008) and indicate that investment in testes due to sperm competition likely evolved in all species of the genus *Julidochromis* (S. Awata, personal communication). Thus, the larger testes could be a response to sperm competition for both polygynous and polyandrous groups at Lake Tanganyika.

References

Alexander RD (1974) The evolution of social behavior. *Ann Rev Ecol Syst* 5: 325-383.

Alonzo SH (2009) Social and coevolutionary feedbacks between mating and parental investment. *Trends Ecol Evol* 25: 99–108.

Andersson M, M Ahlund (2012) Don't put all your eggs in one nest: Spread them and cut time at risk. *Am Nat* 180: 354–363.

Andersson M (2005) Evolution of classical polyandry: Three steps to female emancipation. *Ethol* 111: 1-23.

Andersson M (2004) Social polyandry, parental investment, sexual selection, and revolution of reduced female gamete size. *Evol* 58: 24-34.

Andersson M (1994) Sexual Selection. Princeton Univ. Press, Princeton, NJ, USA.

Andersson M (1978) Optimal egg shape in waders. *Ornis Fenn* 55, 105-109.

Arnold KE, IPF Owens (1999) Cooperative breeding in birds: The role of ecology. *Behav Ecol* 10(1): 465–471.

Arnold KE, IPF Owens (1998) Cooperative breeding in birds: a comparative analysis of the life history hypothesis. *Proc R Soc Lond B* 265: 739–45.

Arnold SJ, D Duvall (1994) Animal mating systems: a synthesis based on selection theory. *Am Nat* 143:317–348.

Avise JC, JX Liu (2010) Multiple mating and its relationship to alternative modes of gestation in male-pregnant versus female-pregnant fish species. *Proc Nat Acad Sci* 107: 18915-18920

Awata S, M Kohda, JY Shibata, M Hori, D Heg (2010) Group structure, nest size and reproductive success in the cooperatively breeding cichlid *Julidochromis ornatus*: A correlation study. *Ethol* 116: 316–328.

Awata S, T Takeyama, Y Makino, Y Kitamura, M Kohda (2008) Cooperatively breeding cichlid fish adjust their testis size but not sperm traits in relation to sperm competition risk. *Behav Ecol Sociobiol* 62: 1701–1710.

Awata S, D Heg, H Munehara, M Kohda (2006a) Testis size depends on social status and the presence of male helpers in the cooperatively breeding cichlid *Julidochromis ornatus*. *Behav Ecol* 17: 372–379.

Awata S, H Takeuchi, M Kohda (2006b) The effect of body size on mating system and parental roles in a biparental cichlid fish (*Julidochromis transcriptus*): a preliminary laboratory experiment. *J Ethol* 24: 125–132.

Awata S, H Munehara, M Kohda (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.

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, M Kohda (2004) Parental roles and the amount of care in a bi-parental substrate brooding cichlid: the effect of size differences within pairs. *Behav* 141:1135–1149.

Balshine-Earn S, FC Neat, H Reid, M Taborsky (1998) Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behav Ecol* 9: 432–438.

Bennett PB, IPF Owens (2002) Evolutionary ecology of birds. Life histories, mating systems and extinction. Oxford Univ. Press, Oxford, UK.

Barlow GW, JSF Lee (2005) Sex-reversed dominance and aggression in the cichlid fish *Julidochromis marlieri*. *Ann Zool Fennici* 42: 477-483.

Bateman AJ (1948) Intra-sexual selection in Drosophila. *Her* 2: 349–368.

Berglund A, MS Widemo, G Rosenqvist (2005) Sex-role reversal revisited: choosy females and ornamented, competitive males in a pipefish. *Behav Ecol* 16: 649-655.

Berglund A, G Rosenqvist (2003) Sex role reversal in pipefish. *Adv. Study Behav* 32: 131–167.

Berglund A, G Rosenqvist (1990) Male limitation of female reproductive success in a pipefish: effects of body size differences. *Behav Ecol & Sociobiol* 27: 129-133.

Berglund A, G Rosenqvist, I Svensson (1989) Reproductive success of females limited by males in two pipefish species. *Am Nat* 133: 506–516.

Berglund A, G Rosenqvist, I Svensson (1986a) Mate choice, fecundity and sexual dimorphism in two pipefish species (Syngnathidae). *Behav Ecol & Sociobiol* 19: 301–307.

Berglund A, G Rosenqvist, I Svensson (1986b) Reversed sex roles and parental energy investment in zygotes of two pipefish (Syngnathidae) species. *Marine Ecol Prog Ser* 29: 209-215.

Betts BJ, DA Jenni (1991) Time budgets and the adaptiveness of polyandry in northern jacanas. *Wilson Bul* 103, 578-597.

Bêty J, G Gauthier, JF Giroux (2003) Body condition, migration, and timing of reproduction in snow geese: A test of the condition□dependent model of optimal clutch size. *Am Nat* 162: 110-121.

Birkhead TR (1995) Sperm competition: evolutionary causes and consequences. *Reprod Fertil Dev* 1995; 7:755-75.

Birkhead TR, AP Møller (1998) Sperm competition and sexual selection. Academic Press, London.

Breder CM, DE Rosen (1966) Modes of reproduction in fishes. New York: Natural History Press.

Brichard P (1989) Cichlids and All the Other Fishes of Lake Tanganyika. T.H.F. Publications, Neptune City.

Bruintjes R, D Bonfils, D Heg, M Taborsky (2011) Paternity of subordinates raises cooperative effort in cichlids. *PLoS ONE* 6: e25673.

Bruintjes R, M Taborsky (2011) Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *An Behav* 81:387-394.

Bruintjes R, M Taborsky (2008) Helpers in a cooperative breeder pay a high price to stay: effects of demand, helper size and sex. *An Behav* 75:1843-1850.

Buston P (2003) Size and growth modification in clownfish. *Nature* 424: 145-146.

Butchart SHM (2000) Population structure and breeding system of the sex-role reversed, polyandrous bronze-winged jacana *Metopidius indicus*. *Ibis* 142: 93-102.

Butchart SHM, N Seddon, JMM Ekstrom (1999) Polyandry and competition for territories in bronze-winged jacanas. *J An Ecol* 68: 928-939.

Byrne PG, JD Roberts, LW Simmons (2002) Sperm competition selects for increased testes mass in Australian frogs. *J Evol Biol* 15: 347–355.

Byrne PG, MJ Whiting (2011) Effects of simultaneous polyandry on offspring fitness in an African tree frog. *Behav Ecol* 22:385–391.

Clutton-Brock TH (2009) Sexual selection in females. *An Behav* 77: 3-11.

Clutton-Brock TH (2002) Breeding together: kin selection and mutualism in cooperative vertebrates. *Science* 296, 69–72.

Clutton-Brock TH, AF Russell, LL Sharpe, AJ Young, Z Balmforth, GM McIlrath (2002) Evolution and development of sex differences in cooperative behavior in meerkats. *Science* 297:55-79.

Clutton-Brock TH (1991) The Evolution of Parental Care. Princeton Univ. Press, Princeton, NJ, USA.

Colwell MA, LW Oring (1988) Habitat use by breeding and migrating shorebirds in southcentral Saskatchewan. *Wilson Bul* 100: 554-566.

Darwin C (1871) The Descent of Man, and Selection in Relation to Sex. London: John Murray.

Davies NB, BJ Hatchwell, T Robson, T Burke (1992) Paternity and parental effort in Dunnocks *Prunella modularis* : how good are male chick-feeding rules? *An Behav* 43:729–746.

Davies NB (1991) Mating systems. In: Krebs JR, NB Davies (eds.) Behavioural ecology: An evolutionary approach, 3^{rd} edition, pp. 263–294. Oxford, Blackwell Scientific Publications.

Davies NB (1983) Polyandry, cloaca-pecking and sperm competition in dunnocks. *Nature* 302: 334–336.

Dawson CE (1985) Indo-Pacific pipefishes (Red Sea to the Americas), III + 230 pp; Ocean Springs, Mississippi, USA. (Gulf Coast Research Laboratory).

Dey CJ, CM O'Connel, H Wilkinson, S Shultz, S Balshine, JL Fitzpatrick (2017) Direct benefits and evolutionary transitions to complex societies. *Nat Ecol Evol* 1: 137.

Dierkes P, M Taborsky, U Kohlerb (1999) Reproductive parasitism of broodcare helpers in a cooperatively breeding fish. *Behav Ecol* 10:510–515.

Dunn PO, LA Whittingham, TE Pitcher (2001) Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. *Evol* 55: 161–175.

Dunn PO, A Cockburn (1996) Evolution of male parental care in a bird with almost complete cuckoldry. *Evol* 50: 2542–2548.

Edwards SV, S Naeem (1993) The phylogenetic component of cooperative breeding in perching birds. *Am Nat* 141: 754–789.

Eens M, R Pinxten (2000) Sex-role Reversal in vertebrates: behavioural and endocrinological accounts. *Behav Proc* 51: 135–147.

Emlen ST, PH Wrege (2004) Size dimorphism, intrasexual competition, and sexual selection in wattled jacana (*Jacana jacana*), a sex-role reversed shorebird in Panama. *Auk* 121: 391–403

Emlen ST (1991) Evolution of cooperative breeding in birds and mammals. In: Behavioural ecology: an evolutionary approach, 3^{rd} edition (Krebs JR, NB Davies, eds.). Oxford: Blackwell Scientific Publications, pp 301–337.

Emlen ST, Demong, DJ Emlen (1989) Experimental induction of infanticide in female Wattled Jacanas. *Auk* 106: 1-7.

Emlen ST, LW Oring (1977) Ecology, Sexual selection, and the evolution of mating systems. Science 197: 215–223.

Erckmann WJ (1983) The evolution of polyandry in shorebirds: an evaluation of hypotheses. In: Social behavior of female vertebrates (Wasser SK, ed.). New York, Academic Press: 113-168.

Firman RC (2011) Polyandrous females benefit by producing sons that achieve high reproductive success in a competitive environment. *Proc R Soc Lond B* 278:2823–2831.

Goldizen AW (1990) A comparative perspective on the evolution of tamarin and marmoset social systems. *Inter J Primatol* 11(1): 63–83.

Goymann W, I Safari, C Muck, I Schwabl (2016) Sex roles, parental care and offspring growth in two contrasting coucal species. *R Soc Open Sci* 3: 160463.

Goymann W, M Makomba, F Urasa, I Scwabl (2015) Social monogamy vs. polyandry: ecological factors associated with sex roles in two closely related birds within the same habitat. *J Evol Biol* 28: 1335-1353.

Goymann W, A Wittenzellner, JC Wingfield (2004) Competing females and caring males. Polyandry and sex-role reversal in African black coucals, *Centropus grillii. Ethol* 110: 807-823.

Hammer O, DAT Harper, PD Ryan (2001) PAST: Paleontological statistics software package for education and data analysis. *Paleont Electr* 4, 9.

Haresign TW, SE Shumway (1981) Permeability of the marsupium of the pipefish *Syngnathus fuscus* to [^{14}C]-alpha amino isobutyric acid. *Comp Biochem Physiol* 69A:603–604.

Hays H (1972) Polyandry in the spotted sandpiper. *Living Bird* 11: 43-57.

Heg D, Z Bachar (2006) Cooperative breeding in the Lake Tanganyika cichlid *Julidochromis ornatus. Environ Biol Fish* 76:265–281.

Heg D, Z Bachar, M Taborsky (2005) Cooperative breeding and group structure in the Lake Tanganyika cichlids *Neolamprologus savory*. *Ethol* 111: 1017-1043.

Heinsohn R, D Ebert, S Legge, R Peakal (2007) Genetic evidence for cooperative polyandry in reverse dichromatic *Eclectus* parrots. *An Behav* 74: 1047-1054.

Herald ES (1959) From pipefish to seahorse – a study of phylogenetic relationships. *Proc Calif Acad Sci* 29: 465–473.

Hori M, MM Gashagaza, M Nashombo, H Kawanabe (1993) Littoral fish communities in Lake Tanganyika: irreplaceable diversity supported by intricate interactions among species. *Conserv Biol* 7:657-666.

Hosken DJ, PI Ward (2001) Experimental evidence for testis size evolution via sperm competition. *Ecol Let* 4(1): 10-13.

Hrdy SB (2009) Mothers and others. Cambridge: Harvard University Press.

Ito MH, M Yamaguchi, N Kutsukake (2018) Redirected aggression as a conflict management tactic in the social cichlid fish *Julidochromis regain*. *Proc R Soc B* 285: 20172681.

Ito MH, M Yamaguchi, N Kutsukake (2017) Sex differences in intrasexual aggression among sex-role-reversed, cooperatively breeding cichlid fish *Julidochromis regain. J Ethol* 35: 137–144.

Jenni DA (1974) Evolution of Polyandry in Birds. *Am Zool* 14 (01): 129-144.

Jenni DA, G Collier (1972) Polyandry in the American jacana (Jacana spinosa). *Auk* 89: 743-765.

Jones AG, JC Avise (2001) Mating systems and sexual selection in male-pregnant pipefishes and seahorses: Insights from microsatellite-based studies of maternity. *J Her* 92(2).

Jones AG, D Walker, C Kvarnemo, K Lindström, JC Avise (2001a). How cuckoldry can decrease the opportunity for sexual selection: data and theory from a genetic parentage analysis of the sand goby, *Pomatoschistus minutus. Proc Natl Acad Sci* 98: 9151–9156.

Jones AG, D Walker, JC Avise (2001b) Genetic evidence for extreme polyandry and extraordinary sex-role reversal in a pipefish. *Proc R Soc Lond B* 268: 2531-2535.

Jones AG, G Rosenqvist, A Berglund, SJ Arnold, JC Avise (2000). The Bateman gradient and the cause of sexual selection in a sex-role-reversed pipefish. *Proc R Soc Lond B* 267: 677–680.

Kalinowski ST, AP Wagner, ML Taper (2006) ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Mol Ecol Notes* 6: 576–579.

Kasimatis K, C Riginos (2015) A phylogenetic analysis of egg size, clutch size, spawning mode, adult body size, and latitude in reef fishes. *Coral Reefs* 35(2).

Keil A, JT Epplen, N Sachser (1999) Reproductive success of males in the promiscuous mating yellow-toothed cavy (*Galea musteloides*). *J Mam* 80(4): 1257-1263.

Klemme I, JA Eccard, H Ylönen (2006a) Do female bank voles (*Clethrionomys glareolus*) mate multiply to improve on previous mates? *Behav Ecol Sociobiol* 60: 415–421.

Klemme I, JA Eccard, G Gerlach, TJ Horne, H Ylönen (2006b) Does it pay to be a dominant male in a promiscuous species? *Ann Zool Fennici* 43: 248–257.

Klug, H, MB Bonsall, SH Alonzo (2013) The origin of parental care in relation to male and female life-history. *Ecol Evol* 3:779–791.

Klug H, J Heuschele, MD Jennions et al. (2010) The mismeasurement of sexual selection. *J Evol Biol* 23(3): 447–462.

Koenig WD, EL Walters (2018) Causes of seasonal decline in reproduction of the cooperatively-breeding acorn woodpecker. *J Avian Biol* 49(11): e01784.

Koenig WD, EL Walters, J Haydock (2016) Acorn woodpeckers: Helping at the nest, polygynandry, and dependence on a variable acorn crop. In: Koenig WD, JL Dickinson (eds.) Cooperative Breeding in Vertebrates. *St Ecol, Evol and Behev*, Cambridge University Press.

Koenig WD, JL Dickinson (2004) Ecology and evolution of cooperative breeding in birds. Cambridge, U K, Cambridge University Press.

Kohda M, D Heg, Y Makino, T Takeyama, J Shibata, K Watanabe, H Munehara, M Hori, S Awata (2009) Living on the wedge: female control of paternity in a cooperatively polyandrous cichlid. *Proc R Soc B* 276: 4207–4214.

Kohda M, J Shibata, S Awata, D Gomagano, T Takeyama, M Hori, D Heg (2008) Niche differentiation depends on body size in a cichlid fish: a model system of a community structured according to size regularities. *J Anim Ecol* 77: 859–68.

Kohda M, Y Yanagisawa, T Sato, K Nakaya, Y Niimura, K Matsumoto, H Ochi (1996) Geographical colour variation in cichlid fishes at the southern end of Lake Tanganyika. *Environ Biol Fish* 45:237–248.

Kohler U (1998) Zur Struktur und Evolution des Sozialsystems von Neolamprologus multifasciatus (Cichlidae, Pisces), dem kleinsten Schneckenbuntbarsch des Tanganjikasees. Aachen, Germany: Shaker Verlag.

Kokko H, MD Jennions (2008) Parental investment, sexual selection and sex ratios. *J Evol Biol* 21 (2008) 919–948.

Konings A (2015) Tanganyika cichlids in their natural habitat. 3rd Edition, Cichlid Press, El Paso.

Konings A (1998) Tanganyika cichlids in their natural habitat. El Paso: Cichlid Press.

Kotaka N (1998) Classical polyandry in the great spotted woodpecker *Dendrocopos major*. *Ibis* 140: 335-336.

Kuwamura T (1997) The evolution of parental care and mating systems among Tanganyikan cichlids. In: (Kawanabe H, M Hori, M Nagoshi, eds.) Fish Communities in Lake Tanganyika, pp. 57-86, Kyoto University Press, Kyoto.

Kvarnemo C, LW Simmons (2013) Polyandry as a mediator of sexual selection before and after mating. *Philos Trans R Soc Lond B Biol Sci* 368(1613):20120042.

Lack D (1966) Population studies of birds. Clarendon Press, Oxford.

Lank DB, LW Oring, SJ Maxson (1985) Mate and Nutrient Limitation of Egg□laying in a Polyandrous Shorebird. *Ecol* 66 (5): 1513-1524.

Lenington S (1984) The evolution of polyandry in shorebirds. pp. 149–167. In: Shorebirds: Breeding behavior and populations (Burger J, BL Olla, eds.). Plenum Press, NY.

Li N, T Takeyama, JL Alexander, M Kohda (2015) Female control of paternity by spawning site choice in a cooperatively polyandrous cichlid. *Behav* 152(2): 231 – 245.

Ligon DJ, DB Burt (2004) Evolutionary origins. In: Cooperative breeding in birds (Koenig WD, JL Dickinson, eds.), pp. 5–34. Cambridge, UK: Cambridge University Press.

Ligon DJ (1999) The Evolution of Avian Breeding Systems. New York: Oxford University Press.

Lourie SA, ACJ Vincent, HJ Hall (1999) Seahorses: An Identification Guide to the World's Species. Project Seahorse, London. 214pp.

Maclean GL (1972) Clutch size and evolution in the Charadrii. *Auk* 89: 299—324.

Marshall TC, J Slate, LEB Kruuk, JM Pemberton (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol* 7: 639–655.

Maurer G (2008) Who cares? Males provide most parental care in a monogamous nesting cuckoo. *Ethol* 114: 540-547.

Michalek KG, H Winkler, D Blomqvist, P Pechacek (2006) Classical polyandry found in the three-toed woodpecker *Picoides tridactylus. J. Ornithol* 147: 112-114.

Møller AR, J V Briskie (1995) Extra-pair paternity, sperm competition and the evolution of testis size in birds. *Behav Ecol & Sociobiol* 36: 35-365.

Møller AP (1991) Sperm competition, sperm depletion, paternal care, and relative testis size in birds. *Am Nat* 137:882–906.

Muck C, B Kempenaers, S Kuhn, M Valcu, W Goymann (2009) Paternity in the classical polyandrous black coucal (*Centropus grillii*) – a cuckoo accepting cuckoldry? *Behav Ecol* 20: 1185–1193.

Munehara H, S Awata, R Katoh, M Kohda, T Sunobe (2001) Primer sequences and cross-species amplification for parentage discrimination of Tanganyikan cichlid fishes. *Bull Fish Sci Hokkaido Univ* 52: 131–133.

Okuda N (1999) Female mating strategy and male brood cannibalism in a sand-dwelling cardinal fish. *An Beh* 58: 273–279.

Ota K, S Awata, M Morita, R Yokoyama, M Kohda (2014) Territorial males can sire more offspring in nests with smaller doors in the cichlid *Lamprologus lemairii*. *J Her* 105(3): 416–422.

Oring LW, DB Lank (1986) Polyandry in spotted sandpipers: the impact of environment and experience. In: Ecological aspects of social evolution (Rubenstein DR, RW Wrangham, eds.). Princeton, NJ: Princeton University Press; 21–42.

Oring LW (1986). Avian polyandry. *Curr Ornithol* 3:309-351.

Oring LW (1982). Avian mating systems. *Avian Biol* 6:1-92.

Oring LW, ML Knudson (1972) Monogamy and polyandry in the spotted sandpiper. *Living Bird* 11: 59–73.

Owens IPF (2002) Male-only care and classical polyandry in birds: phylogeny, ecology and sex differences in remating opportunities. *Phil Trans Roy Soc B* 357: 283-293.

Owens IPF, T Burke, DBA Thompson (1994) Extraordinary sex roles in the Eurasian dotterel: female mating arenas, female–female competition and female mate choice. *Am Nat* 144: 76–100.

Parker A, I Kornfield (1996) Polygynandry in *Psuedotropheus zebra*, a cichlid fish from Lake Malawi. *Environ Biol Fish*. 47:345–352.

Parker GA, LW Simmons (1996) Parental investment and the control of sexual selection: predicting the direction of sexual competition. *Proc R Soc Lond B* 263: 315–321.

Partridge C, A Boettcher, AG Jones (2013) The role of courtship behavior and size in mate preference in the sex-role-reversed Gulf Pipefish, *Syngnathus scovelli*. *Ethol* 119 (8): 692-701.

Pitcher TE, PO Dunn, LA Whittingham (2005) Sperm competition and the evolution of testes size in birds. *J Evol Biol* 18: 557–567.

R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reynolds JD, NB Goodwin, RP Freckleton (2002) Evolutionary transitions in parental care and live bearing in vertebrates. *Phil Trans R Soc Lond B* 357: 269–281.

Reynolds JD, T Szekely (1996) The evolution of parental care in shorebirds: life histories, ecology, and sexual selection. *Behav Ecol* 8: 126-134.

Reynolds JD (1996) Animal breeding systems. *T Ecol & Evol* 11: 68–72.

Ridley M (1978) Paternal care. *An Behav* 26: 904-932.

Rosenqvist G (1990) Male mate choice and female-female competition for mates in the pipefish *Nerophis ophidion*. *An Behav* 39: 1110–1115.

Safriel UN (1975) On the significance of clutch size in nidifugous birds. *Ecol* 56: 703-708.

Schaffner CM, JA French, (2004) Behavioral and endocrine responses in male marmosets to the establishment of multimale breeding groups: Evidence for non-monopolizing facultative polyandry. *Inter J Primatol* 25(3): 709-732.

Schradin C, AA Kinahan, N Pillay (2009) Cooperative breeding in groups of synchronously mating females and evolution of large testes to avoid sperm depletion in African Striped Mice. *Biol Repr* 81: 111–117.

Sefc MK (2011) Mating and parental care in Lake Tanganyika's cichlids. *Inter J Evol Biol* 2011: ID 470875, 20 pp.

Stacey PB, WD Koenig (1990) Cooperative breeding in birds: Long-term studies of ecology and behavior. Cambridge Univ Press, Cambridge.

Sturmbauer C, W Salzburger, N Duftner, R Schelly, S Koblmüller (2010) Evolutionary history of the Lake Tanganyika cichlid tribe Lamprologini (Teleostei: Perciformes) derived from mitochondrial and nuclear DNA data. *Mol Phy Evol* 57: 266-284.

Sunobe M (2000) Social structure, nest guarding and interspecific relationships of the cichlid fish (*Julidochromis marlieri*) in Lake Tanganyika. *Afric St Monograph* 21: 83-89.

Szekely T, A Kosztolanyi, C Kupper, GH Thomas (2007) Sexual conflict over parental care: a case study of shorebirds. *J Ornithol* 148: S211-S217.

Szekely T, IC Cuthill (2000) Trade-off between mating opportunities and parental care: brood desertion by female Kentish plovers. *Proc R Soc Lond B* 267: 2087-2092.

Taborsky M, M Wong (2017) Sociality in fish. In: Rubenstein D, P Abbot (eds.) Comparative social evolution. Cambridge Univ Press, Cambridge, pp 354–389.

Taborsky M (2016). Cichlid fishes: A model for the integrative study of social behavior. In: Koenig W, J Dickinson (eds.) Cooperative breeding in vertebrates: studies of ecology, evolution, and behavior. Cambridge Univ Press, Cambridge, pp 272-293.

Taborsky M (2001) The evolution of parasitic and cooperative reproductive behaviors in fishes. *J Her* 92:100–110.

Taborsky M (1994) Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. *Adv Study Behav* 23: 1–100.

Taborsky M, D Limberger (1981) Helpers in fish. *Behav Ecol & Sociobiol* 8: 143–145.

Tanaka H, M Kohda, JG Frommen (2018a). Helpers increase the reproductive success of breeders in the cooperatively breeding cichlid *Neolamprologus obscurus*. *Behav Ecol & Sociobiol* 72: 152.

Tanaka H, JG Frommen, M Kohda (2018b) Helpers increase food abundance in the territory of a cooperatively breeding fish. *Behav Ecol & Sociobiol* 72:51.

Tanaka H, J G Frommen, T Takahashi, M Kohda (2016) Predation risk promotes delayed dispersal in the cooperatively breeding cichlid *Neolamprologus obscurus*. *An Behav* 117: 51-58.

Tanaka H, D Heg, H Takeshima, T Takeyama, S Awata, M Nishida, M Kohda (2015) Group composition, relatedness, and dispersal in the cooperatively breeding cichlid *Neolamprologus obscurus*. *Behav Ecol & Sociobiol* 69: 169–181.

Thomas GH, T Szekely (2005) Evolutionary pathways in shorebird breeding systems: sexual conflict, parental care, and chick development. *Evol* 59: 2222-2230.

Tinbergen JM, C Both (1999) Is clutch size individually optimized? *Behav Ecol* 10(5): 504–509.

Trivers RL (1972) Parental investment and sexual selection. In: Sexual selection and the descent of man, 1871–1971 (Campbell B, ed). London: Heinemann; 136–179.

Valencia J, E Solis, G Sorci, C de la Cruz (2006) Positive correlation between helpers at nest and nestling immune response in a cooperative breeding bird. *Behav Ecol & Sociobiol* 60: 399–404.

van Oppen MJG, C Rico, TC Deutsch, GF Turner, GM Hewitt (1997) Isolation and characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Mol Ecol* 6: 387–388.

Vernon CJ (1971) Notes on the biology of the black coucal. *Ostrich* 42: 242—258.

Wagner AP, S Creel, LG Frank, ST Kalinowski (2007) Patterns of relatedness and parentage in an asocial, polyandrous Striped *Hyena* population. *Mol Ecol* 16: 4356–4369.

Wang J (2004) Sibship reconstruction from genetic data with typing errors. *Genetics* 166: 1963–1979.

Westneat DE, PW Sherman (1993) Parentage and the evolution of parental behavior. *Behav. Ecol.*4:66–77.

Whittingham LA, PO Dunn (2001) Male Parental Care and Paternity in Birds. *Curr Ornithol* 16: 257–298.

Whittingham LA, PO Dunn, RJ Robertson (1994) Female response to reduced male parental care in birds: an experiment in Tree Swallows. *Ethol* 96: 260–269.

Wiebe KL, B Kempenaers (2008) The social and genetic mating system in flickers linked to partially reversed sex roles. *Behav Ecol* 20: 453-458.

Williams GC (1975) Sex and Evolution. Princeton Univ Press, Princeton, NJ, USA.

Wisenden BD (1999) Alloparental care in fishes. *Rev Fish Biol Fisheries* 9:45-70.

Wojczulanis-Jakubas K, D Jakubas, D Kidawa, A Kosmicka (2012) Is the transition from biparental to male-only care in a monogamous seabird related to changes in body mass and stress level? *J Ornithol* 153:793–800.

Wong M, S Balshine (2011) The evolution of cooperative breeding in the African cichlid fish, Neolamprologus pulcher. *Biol Rev* 86: 511–530.

Wood KJ, VH Zero, Jones A, SP Renn SP (2014) Social reversal of sex□biased aggression and dominance in a biparental cichlid fish (*Julidochromis marlieri*). *Ethol* 120(6): 540-550.

Wright J (1998) Paternity and paternal care. In: Sperm competition and sexual selection (Birkhead T, ed.), Academic Press, New York, pp. 117–145.

Yamagishi S, M Kohda (1996) Is the cichlid fish *Julidochromis marlieri* polyandrous? *Ichthyol Res* 43: 469-471.

Zardoya R, DM Vollmer, C Craddock et al. (1996) Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (Pisces: Perciformes). *Proc R Soc Lond Series B, Biol Sc* 263: 1589–1598.

Appendix

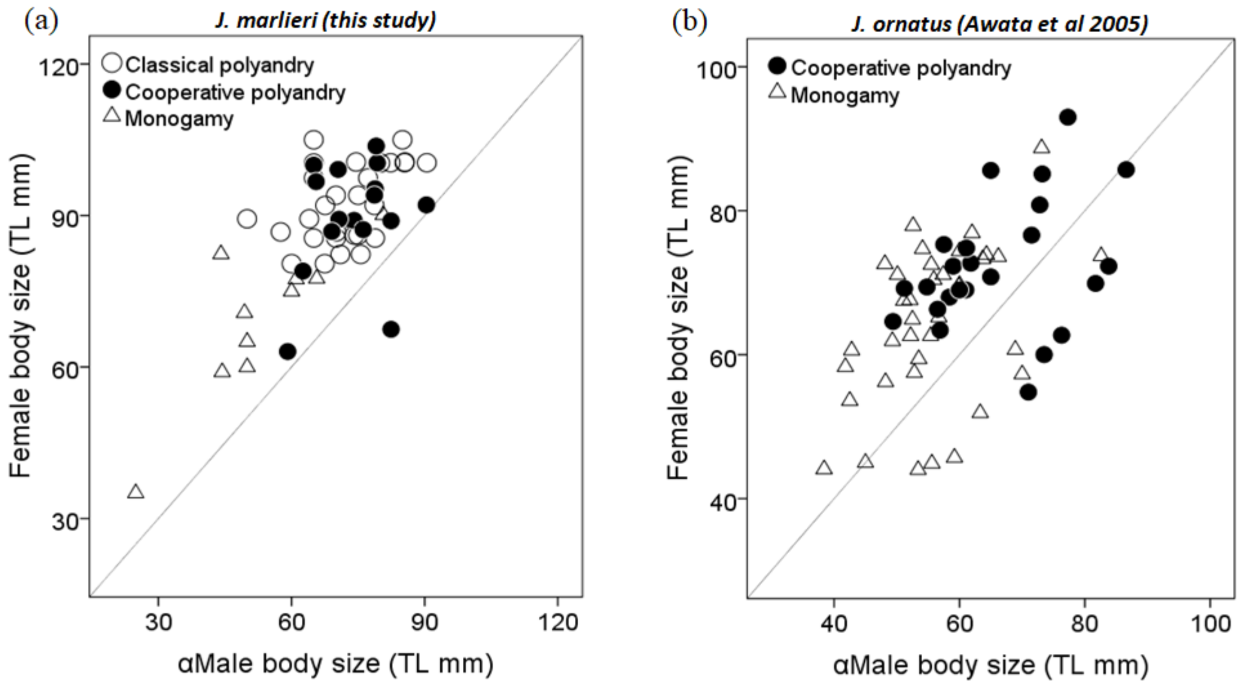


Fig. S1. Size relationship between alpha male and female in classical- (n =14) and cooperative polyandry (n = 16), and monogamy (n =12) of *J. marlieri* (a) and in cooperative (n = 24) and monogamy (n = 37) breeding pairs of *J. ornatus* (b). A line is y = x, separating the female-largest pairs and male-largest pairs observed in both species.

Supplementary Table S1 Polymorphism among 8 microsatellite loci used for parentage analyses in the cichlid *Julidochromis marlieri*. The data are based on the CERVUS analysis of 130 fish including 1 young having individual specific alleles.

Locus	N_a	Size	H_{obs}	H_{exp}	PIC	NE_{1p}	NE_{2p}	F_{null}
Chb1	15	92–132	0,892	0,876	0,861	0,403	0,251	-0,011
Adew8	2	88, 97	0,485	0,489	0,368	0,881	0,816	0,0024
Pzeb1	28	120-206	0,862	0,913	0,904	0,298	0,176	0,0288
UME003	15	207-257	0,808	0,848	0,83	0,461	0,297	0,023
Pzeb3	24	346-404	0,954	0,913	0,903	0,307	0,182	-0,025
Pzeb4	3	138-150	0,154	0,194	0,178	0,981	0,909	0,1124
UME002	6	315-341	0,654	0,712	0,661	0,71	0,537	0,0305
TmoM25	6	388-416	0,646	0,622	0,575	0,786	0,615	-0,029

N_a: number of different alleles; Size: range of allele sizes in base-pairs; H_{obs}: observed heterozygosity; H_{exp}: expected heterozygosity; PIC: the polymorphic information content; NE_{1p}: probability of non-exclusion assuming no parents known; NE_{2p}: probability of non-exclusion assuming one parent known; F_{null}: estimated null allele

Supplementary Table S2 Fragment sizes at 8 microsatellite loci of 130 unrelated *Julidochromis marlieri* individuals inculding 1 young having individual specific alleles.

IndID	Status	Chb1A	Chb1B	Adew8A	Adew8B	Pzeb1A	Pzeb1B	UME003A	UME003B	Pzeb3A	Pzeb3B	Pzeb4A	Pzeb4B	UME002A	UME002B	TmoM25A	TmoM25B
1-F-N-B4(n2)	F	112	120	88	97	148	158	215	215	368	384	143	143	321	333	388	416
2-M-N2-4B	M1	98	106	88	88	154	158	215	227	372	386	143	143	333	333	388	416
6-F-New-B4	NewComerF1	94	126	88	88	152	172	227	233	374	390	143	143	317	333	392	416
7-F-New-B4-C4	NewComerF2	126	126	97	97	156	176	215	227	370	374	143	150	319	333	388	392
8-F-N3-B4	F	116	126	88	97	166	174	233	233	370	390	143	143	321	321	416	416
9-M-N3-B4	M1	102	112	88	88	166	180	215	233	370	374	138	138	333	333	416	416
11-Unk-M-caught-N3-4B	NewComerF	112	120	97	97	172	176	227	231	370	394	138	138	317	333	392	416
12-F-N-1Ga	F	102	120	88	88	152	172	215	227	370	382	143	143	321	333	416	416
13-M-N3-1Ga	M1	106	110	88	97	134	134	207	215	374	382	138	143	319	321	416	416
14-M2-N1-1Ga	M2	120	126	88	97	156	156	215	215	370	394	143	143	321	333	388	400
21-F-N-1Gb	F	120	126	88	88	138	170	215	219	374	376	143	143	321	321	416	416
22-M1-N-1Gab	M1	124	126	97	97	152	152	215	215	368	384	143	143	321	333	392	416
28-M2-NewC-N-1Gb	NewComerF	92	104	88	88	152	152	215	233	372	384	143	143	317	333	392	416
29-M-NewC-N-1Gb	NewComerM	112	120	88	97	134	180	215	227	370	384	143	143	317	333	392	416
30-Unk-N-1Gab	NewComerF	98	120	88	97	124	172	227	235	370	394	138	143	317	333	416	416
31-F-N-8D-6E	F	94	120	88	88	152	154	215	223	368	392	143	143	321	333	388	416
32-M1-N-8D	M1	116	120	97	97	170	182	215	257	368	368	143	143	317	321	414	416
49-F-New-N-8D	NewComerF	120	126	88	97	154	154	215	231	362	382	143	143	333	333	392	392
50-Unk-New-n-8D	NewComerM	126	126	88	97	152	180	233	245	376	384	138	143	319	321	416	416
51-F?M?-N-E5-D8	NewComerF	120	126	88	88	134	170	215	229	374	392	143	143	317	317	392	416
52-M1-N-E6	F	98	126	88	88	134	172	215	215	374	384	143	143	321	333	392	416
53-M2-N-E6	M1	104	120	88	97	152	192	215	227	374	386	143	143	321	333	414	416
55-M1-N-E5	M1	112	126	88	88	154	166	215	223	374	398	138	143	333	333	392	416
60-F-New-E5	NewComerF	110	112	88	88	138	152	215	231	370	392	143	143	317	333	392	416
61-YG-N-4Em-cght-Ne5	M	104	126	88	88	138	162	207	231	370	372	143	143	333	333	388	392
64-F-N-B9	F	120	120	88	88	168	168	227	227	372	380	138	143	319	333	416	416
65-M1-N-B9	M1	104	120	88	97	124	162	215	257	370	372	143	143	317	333	392	414
72-M-caught-C8(cfr-F-B9)	NewComerF	92	98	88	97	120	166	215	231	374	392	143	143	333	333	388	416
73-F-New-N-B9 (cfr F-C9)	F1	104	124	88	97	124	138	217	257	376	384	143	143	317	333	416	416
74-M1-N-C9	M1	104	116	97	97	138	166	227	231	386	390	143	143	317	333	414	416
77-F-N-4H	F	98	120	88	88	152	152	217	257	366	382	143	143	321	333	414	416
78-M-N-4H	M1	120	120	88	88	136	168	227	227	372	384	138	143	319	319	416	416
80-M-New-N-H4	NewComerF	112	124	97	97	154	168	227	251	374	392	143	143	317	317	416	416
81-F-N--A3	F	110	116	88	97	152	152	215	227	370	374	143	143	319	319	416	416
82-M-N1--A3	M1	104	116	88	97	124	166	227	231	386	390	143	143	317	333	414	416
84-M-N2-A3	M1	112	126	88	97	134	134	231	233	384	384	143	143	321	333	392	416
86-F-New-N2--A3	NewComerF	112	126	88	88	134	156	225	229	368	378	143	143	319	319	416	416
87-F-New-N1--A3	NewComerF	120	126	88	97	120	124	245	255	372	378	143	143	321	321	416	416
88-F-N-6Dd	F	106	126	88	88	124	152	207	215	374	384	143	143	319	319	392	416
89-M-N-6Dd	M1	106	120	88	97	138	156	231	233	374	378	143	143	321	333	414	416
94-F-N-6Du	F	104	106	88	97	152	158	215	215	350	376	143	143	333	333	388	392
95-M-N-6Du	M1	106	126	88	88	152	156	225	257	370	374	143	143	321	333	388	416
105-F-New-N-6Du	NewComerF	98	106	88	97	152	156	227	257	370	384	143	143	317	333	414	416
106-M-New-N-6Du	NewComerM	116	124	88	97	156	166	223	245	382	390	143	143	317	321	416	416
107-F-N-4Eu	F	98	126	88	97	152	178	215	233	370	392	138	143	321	333	392	416
108-F-N-3Eu	F	94	120	88	97	156	158	219	225	374	384	143	143	319	319	416	416
109-M1-N-3Eu	M1	108	126	88	88	170	186	215	231	384	388	138	143	319	333	392	416
111-F-N-2A	F	106	116	88	97	134	166	233	257	350	374	143	143	321	333	416	416
112-M1-N-2A	M1	112	120	88	97	152	170	207	235	370	386	143	143	321	333	416	416
129-F?M?-N-C1-B1	F	110	120	88	88	134	164	215	233	374	384	143	143	321	321	416	416
130-H-N-B1-C1	NewComerM	112	124	88	97	154	172	215	227	382	384	143	143	319	333	392	414
131-M/F?-N-B1-C1-New	M1	98	112	97	97	162	162	215	257	384	386	138	143	317	321	414	416
132-F-N-2D	F	98	112	88	97	138	174	217	231	378	386	143	143	317	333	414	416
133-M-N-2D	M1	98	126	88	97	152	156	231	233	374	392	143	143	333	333	392	416
146-F-New-N-2D	NewComerF	92	106	88	88	176	192	217	217	372	390	143	143	319	333	416	416
147-M-New-N-2D	NewComerM	98	106	97	97	138	152	217	235	374	386	143	143	321	333	414	416

Continued …

148-F-N-6B	F	120	126	97	97	156	156	217	217	364	368	143	143	319	319	388	416
149-M-N-6B	M1	106	110	88	88	156	158	233	233	374	380	138	138	333	333	392	392
150-F-N-6C	F	112	120	88	97	124	162	215	229	366	404	143	143	319	321	416	416
151-M-N-6C	M1	106	124	97	97	138	206	231	233	374	378	143	143	333	333	414	416
168-F-N-I8	F	124	126	88	88	124	162	217	231	374	386	143	143	333	333	416	416
169-M1-N-I8	M1	120	126	88	88	138	152	215	227	374	386	138	138	319	319	392	392
173-F-N-H8	F	98	98	97	97	124	134	215	217	370	384	143	143	321	333	392	416
174-M1-N1-H8	M1	102	106	88	97	152	180	207	217	372	374	143	143	319	319	392	416
180-F-Newc-N1-8H	NewComerF	112	124	88	97	154	206	215	257	372	402	143	143	321	333	388	416
181-F-N-Ent3	F	120	120	97	97	166	168	215	235	372	390	143	143	321	333	392	416
182-M-N1-Ent3	M1	120	126	97	97	124	152	231	231	376	384	138	143	317	317	392	416
187-F-NewC-N1-Ent3 (F-N-0C)	NewComerF	110	126	88	97	152	166	207	231	364	402	138	143	317	333	388	416
188-F-N-9Gb	F	108	126	88	88	152	154	217	233	374	384	143	143	333	333	392	414
189-M1-N-9Gb	M1	106	124	97	97	152	152	215	233	370	402	143	143	321	321	392	414
192-F?-N(2)-0C	F	92	94	88	88	130	134	215	223	370	374	138	143	333	333	414	416
195-M1-N2-0C	M1	120	124	97	97	152	162	207	223	390	394	143	143	319	333	392	416
203-M-N3-0C	SolitaryF	120	126	97	97	152	156	215	215	370	384	143	143	317	317	400	416
204-M1-N2-0C	SolitaryM	106	126	88	97	134	154	231	235	384	384	143	143	321	333	414	416
205-F-N-I6	F	98	102	88	88	124	152	207	233	372	372	143	143	315	321	392	416
206-M1-N1-I6	M1	102	120	88	97	156	166	215	231	372	384	138	143	321	333	388	416
207-M-N2-6I	M1	98	104	88	88	134	148	215	215	368	372	143	143	319	333	414	416
208-F-N-H9	F	126	126	88	97	152	182	215	233	370	380	143	143	333	333	416	416
209-M-N1-H9	M1	106	126	88	88	158	174	215	227	368	374	143	143	317	321	392	392
212-M1-N2-H9	M1	104	120	88	97	152	158	217	231	374	384	143	143	319	321	416	416
216-M-N3-H9	SolitaryF	120	126	88	88	156	156	215	231	370	380	143	143	321	333	388	414
217-F-N-0E	F	102	120	88	97	120	138	217	225	368	396	143	143	317	333	414	416
218-M-N-0E	M1	124	126	88	88	152	172	217	233	374	392	138	143	321	333	416	416
224-F-N-3Em	F	116	126	88	97	138	158	215	231	368	370	143	143	317	333	392	416
225-M1-N-3Em	M1	112	112	88	97	124	172	223	235	372	394	143	143	319	333	414	416
229-F-N-9Gc	M1	106	112	88	88	156	172	215	227	372	392	138	143	321	333	392	416
230-M-N-9Gc	F	120	132	88	88	152	152	215	233	382	384	143	143	317	333	416	416
236-F-N-3Ed	F	98	126	88	88	128	172	215	231	380	382	143	143	319	333	392	416
237-M-N-3Ed	M1	112	126	97	97	124	138	231	235	368	372	143	143	319	319	392	416
241-F-N-E10	F	92	124	88	97	166	180	231	231	346	372	143	143	319	333	388	414
242-M-N-E10	M1	116	116	88	97	152	186	215	257	368	390	143	143	321	333	414	416
252-F-N-F10	F1	120	124	88	97	138	162	207	235	368	370	143	143	321	333	392	416
253-M-N-F10	NewComerF	98	126	97	97	134	166	215	235	372	386	143	143	319	321	414	414
257-F-N-J2	F	120	120	88	97	134	136	227	233	372	392	138	143	317	333	392	416
259-H-N2-J2	M	106	126	88	88	162	168	233	233	372	392	143	143	333	333	416	416
260-F-Newc-J2	VisitF	92	112	88	97	152	152	219	227	374	392	143	143	321	333	414	416
261-F-N-J7	F	120	122	88	97	124	172	207	217	374	382	143	143	333	333	416	416
266-F-N-9Ga	F	110	126	97	97	138	174	215	257	372	392	143	143	321	333	388	416
268-F-N-Ent1	F	92	126	88	97	124	152	227	227	384	390	143	143	317	333	414	416
269-M1-N1-N2-Ent1	M1	98	124	97	97	128	158	215	215	374	384	143	150	317	321	388	416
270-M2-N1-Ent1	NewComerF	120	126	88	97	152	152	215	233	372	376	143	143	333	333	416	416
275-F-N-Ent2	F	112	120	88	88	152	172	215	215	376	386	143	143	321	333	392	416
276-M1-N-Ent2	M1	92	94	97	97	170	180	215	217	350	374	143	143	319	333	392	414
278-F-N-LQ2	F	98	112	88	88	152	154	223	233	364	374	138	143	333	333	388	416
279-M1-N-LQ2	NewComerF	120	120	88	97	134	158	215	215	384	384	143	143	321	321	416	416
280-M2-N-LQ2	M1	106	126	88	97	152	156	207	227	384	390	143	143	317	333	414	416
287-F-N-H7	F	98	120	88	88	156	164	215	255	368	370	143	143	317	321	388	392
288-F-N-6Jd	F	98	126	88	97	152	166	217	223	368	390	143	143	321	321	414	416
289-M-N-6Jd	M	106	106	88	88	134	134	227	231	372	374	143	143	321	321	388	388
291-F-N-NewN	F	126	126	88	97	128	166	231	233	380	384	143	143	333	333	388	416
293-M1-N1-NewN	VisitF	98	106	88	97	158	176	215	235	370	384	143	143	321	333	392	416
320-KazakiriF	F	112	124	88	88	152	168	215	233	368	382	143	143	317	333	392	416
321-KazakiriF	F	116	120	88	97	130	134	215	233	368	386	143	143	317	333	388	416
323-F-A2	F	106	124	88	97	134	152	215	231	374	384	143	143	317	333	392	416

Continued …

324-M-A2	M1	124	126	88	97	152	184	207	215	364	380	143	143	319	319	416	416
326-A2-Hside	NewComerM	112	126	97	97	152	166	217	227	378	380	143	143	317	317	392	416
335-DangoF	F	120	120	88	97	154	172	227	245	380	384	143	143	317	321	392	414
336-DangoM	F	112	120	88	97	152	152	215	215	370	404	143	143	319	321	416	416
337-D3E3-F	F	106	124	88	97	158	186	227	233	382	384	143	143	315	321	414	416
338-D3E3-M	M	98	126	88	88	136	154	215	235	366	382	143	143	317	321	416	416
343-D3E3-NA-M	M	106	120	88	97	152	154	215	215	374	384	143	143	317	333	388	388
345-hanawaF-II-T-N-F	F	104	106	88	97	134	154	227	231	384	392	143	143	317	321	416	416
346-HanawaM-II-T-N-M	M1	120	126	97	97	124	168	217	227	380	384	143	143	317	321	414	416
351-KusabiF	F	104	126	88	88	134	154	231	231	374	392	143	143	321	333	416	416
353-F-T	F	102	120	88	88	156	166	215	231	372	384	138	143	333	333	392	414
354-M-T	M	104	106	88	97	152	180	215	215	390	390	143	143	321	333	416	416
75-M2-N-C9	F2	120	126	88	97	152	170	207	227	378	384	143	143	321	341	416	416
136-YG-N-2D	Y2	122	126	88	88	138	152	231	231	378	392	143	143	333	333	394	416
170-H-N-I8	M2	108	110	88	97	154	194	215	233	374	382	143	143	333	333	416	416
177-M2-N2-H8	M2	98	120	88	97	152	158	217	229	368	400	143	143	317	321	414	414

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
120	88	88	172	184	207	227	374	382	138	143	321	333	416	416
120	88	97	172	184	215	215	374	382	138	143	319	321	416	416
126	88	88	134	152	215	215	384	392	143	143	321	321	388	392
126	88	88	138	170	215	219	374	376	143	143	321	321	416	416
126	97	97	152	152	215	215	368	384	143	143	321	333	392	416
120	88	88	136	162	219	233	366	376	138	143	319	333	416	416
120	97	97	154	156	215	227	350	370	143	143	317	317	400	416
126	88	88	136	170	215	219	366	376	143	143	321	333	416	416
120	88	88	162	170	215	219	374	376	138	143	321	333	416	416
126	88	97	138	152	215	219	376	384	143	143	321	321	416	416
104	88	88	152	152	215	233	372	384	143	143	317	333	392	416
120	88	97	134	180	215	227	370	384	143	143	317	333	392	416
120	88	97	124	172	227	235	370	394	138	143	317	333	416	416
120	88	88	152	154	215	223	368	392	143	143	321	333	388	416
120	97	97	170	182	215	257	368	368	143	143	317	321	414	416
120	88	97	134	162	215	257	374	384	143	143	321	333	388	416
126	88	97	138	152	215	231	374	384	143	143	317	321	392	414
94	88	97	154	162	215	215	384	392	143	143	321	333	388	416
120	88	88	134	154	215	223	374	392	143	143	321	321	416	416
120	88	97	154	162	223	257	384	392	143	143	321	333	388	416

inued …

126	88	97	134	152	215	233	384	392	143	143	321	321	392	416
110	88	97	134	154	207	215	370	386	143	143	333	333	392	416
124	88	97	134	134	215	231	380	390	143	143	319	333	416	416
116	88	97	152	154	207	235	372	400	143	143	321	333	392	416
112	88	88	138	152	215	231	370	392	143	143	317	333	392	416
126	88	88	138	162	207	231	370	372	143	143	333	333	388	392
124	88	97	138	152	215	215	370	374	143	143	317	333	392	416
126	88	97	156	178	229	233	372	376	143	143	321	333	416	416
120	88	88	168	168	227	227	372	380	138	143	319	333	416	416
120	88	97	124	162	215	257	370	372	143	143	317	333	392	414
112	97	97	158	166	231	233	374	374	143	143	319	319	414	414
132	88	88	166	174	215	227	350	370	138	143	321	321	414	416
120	88	97	166	168	227	233	372	374	138	143	319	319	414	416
120	88	88	162	168	227	257	370	372	138	143	319	319	392	416
120	88	88	162	168	215	227	372	380	143	143	317	333	414	416
120	88	97	166	168	227	231	374	380	143	143	317	333	414	416
98	88	97	120	166	215	231	374	392	143	143	333	333	388	416
124	88	97	124	138	217	257	376	384	143	143	317	333	416	416
116	97	97	138	166	227	231	386	390	143	143	317	333	414	416
126	88	97	152	170	207	227	378	384	143	143	321	341	416	416
126	88	88	124	138	215	257	372	372	143	143	319	321	416	416

inued …

106	88	97	152	158	215	215	350	376	143	143	333	333	388	392
126	88	88	152	156	225	257	370	374	143	143	321	333	388	416
126	88	97	138	152	207	233	374	384	143	143	319	333	416	416
126	88	97	152	152	215	257	374	376	143	143	333	333	388	388
108	88	88	152	156	215	257	370	376	143	143	333	333	388	388
106	88	97	152	156	215	225	350	370	143	143	321	333	388	416
106	88	88	156	158	215	257	350	370	143	143	321	333	388	388
106	88	88	152	156	215	257	350	370	143	143	321	333	388	392
108	88	88	152	156	215	257	370	376	143	143	333	333	388	388
126	88	97	152	152	215	225	350	374	143	143	321	333	388	416
126	88	97	156	158	215	225	374	376	143	143	321	333	392	416
106	88	97	152	156	227	257	370	384	143	143	317	333	414	416
124	88	97	156	166	223	245	382	390	143	143	317	321	416	416
126	88	97	152	178	215	233	370	392	138	143	321	333	392	416
120	88	97	156	158	219	225	374	384	143	143	319	319	416	416
126	88	88	170	186	215	231	384	388	138	143	319	333	392	416
132	97	97	152	152	231	231	392	392	143	143	333	333	392	416
116	88	97	134	166	233	257	350	374	143	143	321	333	416	416
120	88	97	152	170	207	235	370	386	143	143	321	333	416	416
126	88	97	156	158	207	217	374	382	143	143	321	321	414	416
126	97	97	134	152	219	227	362	374	143	143	319	333	414	416
126	88	88	134	156	217	257	374	374	143	143	321	321	416	416

inued …

112	88	97	138	174	217	231	378	386	143	143	317	333	414	416
126	88	97	152	156	231	233	374	392	143	143	333	333	392	416
126	88	97	152	174	217	233	370	372	143	143	319	333	388	416
126	88	97	156	174	231	231	386	392	143	143	319	333	392	416
126	88	88	138	152	231	231	378	392	143	143	333	333	394	416
126	88	88	138	152	217	233	386	392	143	143	319	333	414	416
112	97	97	154	170	231	231	384	386	138	138	321	333	392	392
120	88	88	172	174	217	217	384	386	143	143	333	333	414	416
98	88	88	152	174	231	233	374	386	143	143	333	333	416	416
126	97	97	138	152	217	231	386	392	143	143	317	333	392	414
122	88	97	156	174	217	231	374	386	143	143	317	333	392	414
126	97	97	152	174	231	231	386	392	143	143	317	333	392	416
126	97	97	138	152	217	231	378	392	143	143	333	333	392	416
126	88	97	138	152	217	231	386	392	143	143	317	333	392	414
106	88	88	176	192	217	217	372	390	143	143	319	333	416	416
106	97	97	138	152	217	235	374	386	143	143	321	333	414	416
126	97	97	156	156	217	217	364	368	143	143	319	319	388	416
110	88	88	156	158	233	233	374	380	138	138	333	333	392	392
120	88	97	124	162	215	229	366	404	143	143	319	321	416	416
124	97	97	138	206	231	233	374	378	143	143	333	333	414	416
112	88	97	162	206	229	233	366	378	143	143	321	333	416	416
112	88	97	138	162	215	233	378	404	143	143	319	333	416	416
124	97	97	124	138	229	231	366	374	143	143	319	333	414	416

inued …

126	88	88	138	162	217	227	386	386	138	143	319	333	392	416
126	88	97	162	194	231	233	374	382	143	143	333	333	416	416
98	97	97	124	134	215	217	370	384	143	143	321	333	392	416
106	88	97	152	180	207	217	372	374	143	143	319	319	392	416
120	97	97	136	152	215	215	384	392	143	143	333	333	392	392
106	88	97	134	152	215	217	372	384	143	143	319	321	392	416
120	88	97	152	158	217	229	368	400	143	143	317	321	414	414
120	97	97	154	154	215	231	362	374	143	143	317	333	392	416
120	97	97	124	154	217	231	370	374	143	143	333	333	392	416
124	88	97	154	206	215	257	372	402	143	143	321	333	388	416
120	97	97	166	168	215	235	372	390	143	143	321	333	392	416
126	97	97	124	152	231	231	376	384	138	143	317	317	392	416
120	97	97	152	166	215	231	372	376	143	143	317	321	392	416
120	97	97	152	168	215	231	372	376	143	143	317	333	416	416
120	97	97	152	166	215	231	376	390	143	143	317	321	392	416
126	97	97	152	168	215	231	384	390	138	143	317	321	392	416
126	88	97	152	166	207	231	364	402	138	143	317	333	388	416
126	88	88	152	154	217	233	374	384	143	143	333	333	392	414
124	97	97	152	152	215	233	370	402	143	143	321	321	392	414
132	88	97	154	166	215	217	350	386	138	143	321	333	392	416
126	88	97	152	174	215	233	370	372	143	143	319	321	416	416
94	88	88	130	134	215	223	370	374	138	143	333	333	414	416
126	88	97	158	174	215	233	368	370	143	143	319	333	392	416

nued …

120	88	88	138	158	231	231	368	370	143	143	317	333	392	414
126	88	88	130	176	215	231	368	370	143	143	317	333	392	414
120	88	97	152	158	217	231	374	384	143	143	319	321	416	416
126	88	97	138	158	231	255	368	370	143	143	333	333	414	416
120	88	97	158	206	223	231	370	380	143	143	317	317	392	392
102	88	88	152	156	215	257	372	374	143	143	321	333	392	416
126	88	88	156	156	215	231	370	380	143	143	321	333	388	414
120	88	97	120	138	217	225	368	396	143	143	317	333	414	416
126	88	88	152	172	217	233	374	392	138	143	321	333	416	416
132	88	97	138	184	225	251	350	396	143	143	317	333	416	416
132	97	97	138	166	225	251	350	368	143	143	317	317	416	416
108	88	97	138	166	225	251	368	382	143	143	333	333	416	416
132	88	97	120	184	217	251	350	368	143	143	317	317	414	416
132	88	97	120	184	215	217	350	396	143	143	317	333	392	414
126	88	97	138	158	215	231	368	370	143	143	317	333	392	416
112	88	97	124	172	223	235	372	394	143	143	319	333	414	416
126	88	97	134	166	215	223	374	398	143	143	319	333	392	416
126	97	97	152	186	215	215	374	384	143	143	317	321	416	416
126	88	97	138	152	215	215	368	374	143	143	317	321	416	416
112	88	88	156	172	215	227	372	392	138	143	321	333	392	416
132	88	88	152	152	215	233	382	384	143	143	317	333	416	416
120	88	88	152	168	215	215	384	384	143	143	317	333	416	416
132	88	88	152	156	215	227	382	392	138	143	317	321	392	416

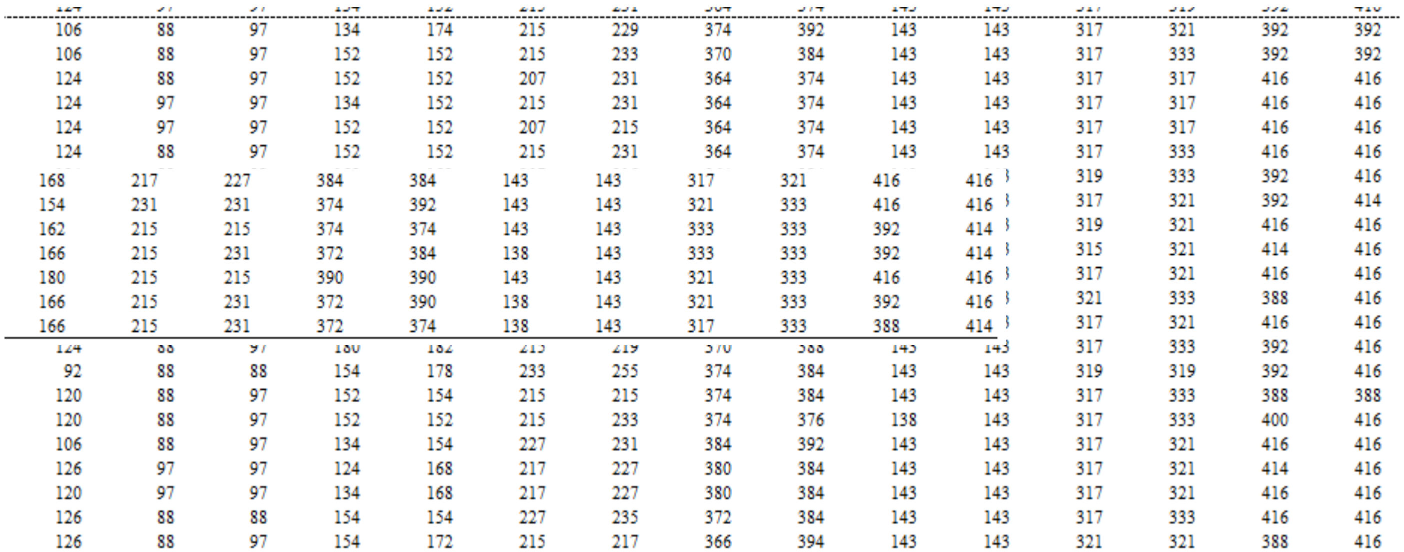
inued …

92	88	97	180	186	215	231	346	368	143	143	321	333	388	416
124	97	97	152	180	215	231	372	390	143	143	333	333	414	416
110	88	97	134	180	215	231	346	374	143	143	321	333	414	416
124	88	97	138	162	207	235	368	370	143	143	321	333	392	416
126	97	97	134	166	215	235	372	386	143	143	319	321	414	414
120	97	97	134	174	229	235	386	390	143	143	319	321	414	414
126	97	97	134	174	229	235	372	390	143	143	319	321	414	414
120	97	97	134	152	215	229	372	384	143	143	319	319	414	414
120	88	97	134	136	227	233	372	392	138	143	317	333	392	416
126	88	97	170	172	233	235	374	384	143	143	333	333	414	416
126	88	88	162	168	233	233	372	392	143	143	333	333	416	416
112	88	97	152	152	219	227	374	392	143	143	321	333	414	416
122	88	97	124	172	207	217	374	382	143	143	333	333	416	416
98	88	88	152	156	215	231	374	384	143	143	319	321	392	416
120	88	97	152	206	215	229	380	392	143	143	321	333	388	414
120	88	97	124	152	217	231	374	384	143	143	319	333	392	416
120	88	97	124	156	207	231	374	384	143	143	319	333	392	416
126	97	97	138	174	215	257	372	392	143	143	321	333	388	416
124	88	97	152	154	233	235	370	394	143	150	333	333	388	416
126	88	97	124	152	227	227	384	390	143	143	317	333	414	416
124	97	97	128	158	215	215	374	384	143	150	317	321	388	416
126	88	97	152	152	215	233	372	376	143	143	333	333	416	416
126	97	97	128	152	215	215	372	384	143	150	321	333	416	416

inued …

106	88	88	134	134	227	231	372	374	143	143	321	321	388	388
106	88	97	134	166	223	231	368	372	143	143	321	321	388	414
126	88	97	128	166	231	233	380	384	143	143	333	333	388	416
120	88	88	158	172	233	235	374	382	143	143	317	317	392	416
106	88	97	158	176	215	235	370	384	143	143	321	333	392	416
112	97	97	138	138	215	215	384	404	143	143	317	321	416	416
126	97	97	128	138	215	231	380	384	143	143	321	333	388	416
126	88	97	134	166	215	233	378	380	143	143	333	333	416	416
126	88	88	158	166	231	233	380	382	143	143	317	333	388	392
126	88	97	128	158	233	233	374	380	143	143	317	333	392	416
126	88	88	128	172	231	235	380	382	143	143	317	333	388	416
126	88	88	128	158	233	235	382	384	143	143	317	333	416	416
126	88	88	128	158	233	235	380	382	143	143	317	333	416	416
126	88	97	128	158	233	235	380	382	143	143	317	333	416	416
126	88	88	152	166	231	245	370	384	143	143	321	333	388	416
126	88	88	152	166	231	235	370	380	143	143	333	333	416	416
126	97	97	138	166	215	233	380	384	143	143	321	333	416	416
126	97	97	128	138	215	233	384	384	143	143	321	333	416	416
126	97	97	138	166	215	231	380	384	143	143	321	333	388	416
126	88	97	128	138	215	231	384	384	143	143	321	333	416	416
126	88	97	128	154	233	235	370	380	143	143	321	333	416	416
126	88	97	128	138	215	231	384	384	143	143	321	333	388	416

inued …



106	88	97	134	174	215	229	374	392	143	143	317	321	392	392
106	88	97	152	152	215	233	370	384	143	143	317	333	392	392
124	88	97	152	152	207	231	364	374	143	143	317	317	416	416
124	97	97	134	152	215	231	364	374	143	143	317	317	416	416
124	97	97	152	152	207	215	364	374	143	143	317	317	416	416
124	88	97	152	152	215	231	364	374	143	143	317	333	416	416
168	217	227	384	384	143	143	317	321	416	416	319	333	392	416
154	231	231	374	392	143	143	321	333	416	416	317	321	392	414
162	215	215	374	374	143	143	333	333	392	414	319	321	416	416
166	215	231	372	384	138	143	333	333	392	414	315	321	414	416
180	215	215	390	390	143	143	321	333	416	416	317	321	416	416
166	215	231	372	390	138	143	321	333	392	416	321	333	388	416
166	215	231	372	374	138	143	317	333	388	414	317	321	416	416
											317	333	392	416
92	88	88	154	178	233	255	374	384	143	143	319	319	392	416
120	88	97	152	154	215	215	374	384	143	143	317	333	388	388
120	88	97	152	152	215	233	374	376	138	143	317	333	400	416
106	88	97	134	154	227	231	384	392	143	143	317	321	416	416
126	97	97	124	168	217	227	380	384	143	143	317	321	414	416
120	97	97	134	168	217	227	380	384	143	143	317	321	416	416
126	88	88	154	154	227	235	372	384	143	143	317	333	416	416
126	88	97	154	172	215	217	366	394	143	143	321	321	388	416

Supplementary Table S4. Total number of offspring caught at each nest of *J. marlieri* in the study area and their relatedness to parents (females, alpha-, beta and gamma males). Female and nest identities are shown for classical-, cooperative polyandry and monogamy. * indicates the fry were found and related to females into separate nests in classical polyandry groups. Unknown males and females mean one another of the parents was not caught during sampling. The data are based on the CERVUS analysis.

				Male size TL (mm)				No. of young of female		No. of young sired by male			
Mating system	Female Id.	Female TL (mm)	Nest Id.	α	β	γ	Total no. of young	Owner	Unknown	α	β	γ	Unknown
Classical polyandry	F2-n-4B	89.34	n1-4B	50									
			n2-4B	64			3	2	1	2			1
Classical polyandry *	F-n-8H	100.58	n1-8Ha	85.57	51.25		1	1		1			
			n2-8Ha	74.55	53.04	41.35	1	1				1	
Classical polyandry	F-n-9Gb	80.46	n1-9Gb	67.49	51.08	39.28							
			n2-9Gb	60	45								
Classical polyandry	F-n-3_A	82.28	n1-_3A	71			1		1				1
			n2-_3A	75.58	44.2								
Classical polyandry	F-n-9H	86.74	n1-9H	57.55	36.11		2		2				2
			n2-9H	70.1	45.77		1		1				1
Classical polyandry	F-n-7J	100.38	n1-J7	80	51.09	30.36	2	2			2		
			n2-J7	65	50								
Classical polyandry	F-n-6I	92	n1-6I	78.7	55								
			n2-6I	67.58	50								
Classical polyandry	F-n-0C	86.1	n1-0C	75	50.8								
			n2-0C	74.2	44.1		6		6	1			5
Classical polyandry	F-n-Ent1	101.92	n1-Ent1	89.25	75		4		4	4			
			n2-Ent1	89.25	45								
Classical polyandry	F-n-Ent3	97.42	n1-Ent3	77.24	50		4		4	4			
			n2-Ent3	65	45								
Classical polyandry	F1-n-4B	105	n1-4B	85	50								
			n2-4B	85	64								
			n3-4B	65	60								
Classical polyandry *	F-n-1Ga	85.53	n1-1Ga	78.82	54.38		3	2	1	2	1		
			n2-1Ga	65	54.38								
			n3-1Ga	70			3	2	1				3
Classical polyandry	F-n-8D	100.44	n1-8D	85.47	60.3	30.13	15	13	2	8	5		2
			n2-6E	82.34	67.4	61.98							
			n3-5E	90.48	77.2	60	5		5				5
Classical polyandry	F-n-2J	94	n1-2J	75	60	45							
			n2-2J	75	65	44.1							
			n3-2J	70									
Classical polyandry *	F-n-NN	102	n1-N	70a	43.11		8	8			6		2
			n2-N	80	50.6		17	17			13		4
Classical polyandry	F-D3E3	105.53	B	64.24			4		4	1			3
Cooperative polyandry	F-n-2A	94.01	n-2A	78.69	65.87	44.16	14	14		3	9	2	
Cooperative polyandry	F-n-2D	100.4	n-2D	79.3	51.18		11	8	3	5			6
Cooperative polyandry	F-n-6Du	89.25	n-6Du	70.69	45		9	7	2	6			3
Cooperative polyandry	F-n-1Gb	95.28	n-1Gb	78.82	68.96	46.16	3	3		2	1		
Cooperative polyandry	F-n-9C	86.84	n-9C	69.09	50	40.31							
Cooperative polyandry	F-n-9B	103.76	n-9B	79.08	56.05	39.05	4	4		1	1		2
Cooperative polyandry	F-n-3Em	79	n-3Em	62.58	49.4	44.45	1	1				1	
Cooperative polyandry	F-n-3Eu	89.04	n-3Eu	74.1	58.84	50							
Cooperative polyandry	F-n-4Er	63.07	n-4Er	59.12	45								
Cooperative polyandry	F-n-10E	99.12	n1-10E	70.5	50		9	9		1			8
Cooperative polyandry	F-n-4H	96.7	n-4H	65.55	50.72								
Cooperative polyandry	F-n-9Ga	88.95	n1-9Ga	82.44	65	38.4							
Cooperative polyandry	F-n-9Gc	67.46	n-9Gc	82.44	50		5	5		2			3
Cooperative polyandry	F-n-10F	99.98	n-10F	65	50		3	0	3				3

Continued …

Cooperative polyandry	F-n-8I	87.21	n1-8I	76.14	48.9	2	2		1	1		
Cooperative polyandry	F-n-Ent2	92.09	n-Ent2	90.4	69.3							
Cooperative polyandry	F-A2	101.94	A	73.74	54.46	8	8		2	1		5
Cooperative polyandry	F-T	103.75	D	84.34		2	2		1			1
Cooperative polyandry	F-n-LQ	90	n-LQ	65	47.53	5		5		1		4
Monogamy	F-n-6B	90.14	n-6B	80.67								
Monogamy	F-n-6C	77.4	n-6C	61.05		16	16		16			
Monogamy	F-N-6Dd	70.73	n-6Dd	49.4		4	2	2	4			
Monogamy	F-n3Ed	35.01	n-3Ed	24.9		3	2	1				3
Monogamy	F-n-4Ed	95	n-4Ed	65								
Monogamy	F-n-4Em	65	n-4Em	50								
Monogamy	F-n-4Eu	85.73	n-4Eu	60								
Monogamy	F-n-8Hu	60	n-8Hu	50								
Monogamy	F-n-0E	88.62	n-0E	74.22		5	5					5
Monogamy	F-n-B1C1	82.36	n-1C1B	44.1								
Monogamy	F-n-6Jd	59.01	n-6Jd	44.38		1	1		1			
Monogamy	F-C3B4	77.52	n-C3B4	65.68		1	1		1			
Monogamy	Hanawa	88.74	C	72.65		4	3	1	2			2
Total						190	141	49	71	41	4	74