How to be a great dad: Parental care in a flock of greater flamingo (*Phoenicopterus roseus*) (#16887)

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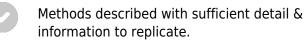
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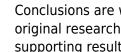
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How to be a great dad: Parental care in a flock of greater flamingo (*Phoenicopterus roseus*)

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The zoo-science literature on flamingos, and avian species in general, is lacking. However, this kind of research is important to improve the knowledge on these species and to improve their *ex-situ* and *in-situ* conservation. The aims of the present study were to assess the welfare of a captive colony of greater flamingo hosted at Parco Natura Viva, an Italian zoological garden, through ethological parameters and to improve the knowledge on this species in zoological gardens. In particular, the present study investigated and compared the parental care of females and males in 35 breeding pairs of greater flamingos. For each pair, we collected data on the parental care behaviour of both females and males, recording their position in relation to the nest (near the nest, on the nest, away from the nest) and the behavioural category that was performed. The main results were that males spent more time than females on the nest and near it and were more aggressive toward other flamingos. Therefore, male flamingos seem to be more involved in incubation duties and nest protection than females. Greater flamingos of this study performed species-specific behaviours. Both parents were involved in parental care and displayed all the activities reported in the wild. Therefore, the study flock of greater flamingos seems to be in a good welfare. This kind of research is important not only to expand the knowledge on bird species such as flamingos, but also to improve their husbandry and breeding in controlled environment.



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21	Abstract

22 The zoo-science literature on flamingos, and avian species in general, is lacking. However, this kind of research is important to improve the knowledge on these species and to 23 improve their *ex-situ* and *in-situ* conservation. The aims of the present study were to assess the 24 welfare of a captive colony of greater flamingo hosted at Parco Natura Viva, an Italian 25 zoological garden, through ethological parameters and to improve the knowledge on this species 26 27 in zoological gardens. In particular, the present study investigated and compared the parental care of females and males in 35 breeding pairs of greater flamingos. For each pair, we collected 28 data on the parental care behaviour of both females and males, recording their position in relation 29 to the nest (near the nest, on the nest, away from the nest) and the behavioural category that was 30 performed. The main results were that males spent more time than females on the nest and near it 31 and were more aggressive toward other flamingos. Therefore, male flamingos seem to be more 32 involved in incubation duties and nest protection than females. Greater flamingos of this study 33 performed species-specific behaviours. Both parents were involved in parental care and 34 displayed all the activities reported in the wild. Therefore, the study flock of greater flamingos 35 seems to be in a good welfare. This kind of research is important not only to expand the 36 knowledge on bird species such as flamingos, but also to improve their husbandry and breeding 37 38 in controlled environment.

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40 Keywords: parental investment; sex differences; incubation; animal welfare

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42 Word count: 2 701

44 INTRODUCTION

In the wild, greater flamingos (Phoenicopterus roseus) are threatened by phenomena such 45 as habitat loss and change, human activities and pollution (Ogilvie & Ogilvie, 1986; Nasirwa, 46 2000; Yosef, 2000; Hockey et al., 2005; Miltiadou, 2005; IUCN, 2015). Therefore, ex-situ 47 conservation programs are essential. However, the ethical imperative to save threatened species 48 49 from further decline and extinction in the wild has for them a priority over concerns regarding individual animal welfare (Minteer & Collins, 2013). A scientific non-invasive ethological 50 method to assess the welfare of the animals is to verify the performance of natural behaviour, 51 even in captive settings (Hill & Broom, 2009). Flamingos are highly gregarious birds that live 52 and breed in large dense flocks (Pickering et al., 1992), often including thousands of pairs. 53 Obtaining information and data on their behaviour in the wild is therefore difficult due to 54 constraints such as individual identification and approach to the birds (Studer-Thiersch, 1975; 55 2000; King, 2000). For this reason, together with long-term studies on wild flamingo flocks, 56 research on captive colonies might be valuable and complementary to improve the knowledge on 57 the ethology, morphology, physiology and endocrinology of these species (King, 2000). 58 Studying the behaviour of flamingos in the wild and in controlled environment is important for 59 60 the implementation of the husbandry and the breeding of this species (Melfi, 2009; Rose et al., 2014). However, the zoo-science literature on flamingos, and avian species in general, is still 61 62 under-represented (Rose et al., 2014).

Greater flamingos display a reduced sexual dimorphism as the only difference between sexes is that males are larger than females. Greater flamingos are monogamous birds and can form long-term pair bonds. Both partners work together to build a nest, in which the female lays a single egg. The nest is usually a mound made of mud, ground or other materials, with a

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concave centre. It is generally built on an island or on the coastline of a lake (Studer-Thiersch,
1975; Beletsky, 2006; Cezilly, 1993; Elphick, 2014). Before egg laying, the male is primarily
involved in nest building, but the female takes over as the laying time approaches. The nest
building activity of both partners proceeds also during the first two weeks of incubation, leading
to an increase in the nest height (Studer-Thiersch, 1975).

72 After mating, the female lays one egg in the nest. Both females and males take part in the incubation, lasting from 27 to 31 days (Beletsky, 2006; Cezilly, 1993; Elphick, 2014). However, 73 in the first period, the female is reported to spend more time on the nest than the male (Studer-74 75 Thiersch, 1975). When incubating the egg, flamingos display different behaviours, such as standing, stretching the wings, preening, self-scratching and looking at the nest (Studer-Thiersch, 76 1975). In addition, they take care of the egg, moving it with the bill. Flamingos could either 77 stand or sit on the egg and the time spent standing up depends on the weather condition. When 78 79 one partner leaves the egg, the time taken to the other one to climb on the nest and incubate the 80 egg is generally short or even simultaneous (Studer-Thiersch, 1975). Both the incubating partner and the vacant one outside the nest can perform aggressive behaviour toward other flamingos 81 disturbing the incubation uder-Thiersch, 1975). 82

aims of the present study was to assess the welfare of a captive colony of greate
flamingo hosted at Parco Natura Viva, an Italian zoological garden, through ethological
parameters and to improve the knowledge on this species in zoological gardens, especially
during the breeding season. In particular, the present study investigated and compared the
parental care of females and males in a flock of greater flamingos. For each breeding pair, the
behaviour of both the female and the male during the egg incubation period was recorded. The

results of the study are discussed with the behavioural pattern shown by greater flamingos in thewild, to suggest strategies to improve the husbandry of this species in captivity.

91 MATERIALS AND METHODS

92 Study subjects and area

The study was carried out in a flock of 147 greater flamingos of different age, 70 females and 77 males, housed at Parco Natura Viva – Garda Zoological Park in Italy, in a 1,100 m² enclosure. The study subjects were 35 breeding pairs, during the peak of their breeding activity. The enclosure was composed by a muddy area and a grassy area. The muddy area surrounded a water pool with two islands, used by flamingos to build their nest mounds and rear the chicks. The basal structure of the nest mound was built by humans, whereas flamingo pairs completed the nest construction properly.

Trees, bushes and rocks were present in the enclosure, together with a wooden house to provide the flamingos with protection from weather conditions and a long feeding station. To minimize human disturbance, food was administered to the flamingos once a day in the feeding point. No interactions between humans and flamingos were allowed. The flamingo diet was composed by a specific pellet, containing cereals, vegetables, oils and fats, algae, shellfish, vitamins and mineral salts.

Flamingos were identified through a ring on one leg. The ring differed in colour and letters (three-letter combination). At the time of the study, the density of the flamingos in the enclosure was 0.13 individuals/m². In the wild, a density of 0.2 individuals/m² is usually found, corresponding to 180 flamingos/km² (Ramesh & Ramachandran, 2005), Subjects of the study were pairs that incubated an egg in the 2016 breeding season (N = 35).

111 Procedure and data collection

Subjects of the study were breeding pairs in which the female laid the egg. For each pair, a total of twenty 10-minute sessions were carried out during the incubation period. In particular, two sessions per day were done, one in the morning and one in the afternoon. Thus, the data collection for each breeding pair lasted for ten days. Data were collected using a continuous focal animal sampling method (Altmann, 1974).

For each pair, we conducted observations of parental care behaviour of both female and 117 male, recording the position of the bird in relation to the nest and the behavioural category 118 performed. Regarding the position of the bird, we recorded whether each flamingo parent was 119 near the nest (less than 150 cm, which is approximately the higher flamingo body length; del 120 Hoyo et al., 1992), on the nest or away from the nest (>150 cm). When the flamingos were on 121 the nest, we recorded whether they were sitting (incubating) or standing. In particular, the 122 behavioural categories collected in the study were agonistic behaviour, including aggressive 123 124 interactions, such as extending the neck and beak at another bird (Stevens et al., 1992; Farrell et al., 2000), egg-care related behaviour (egg-rolling and moving), nest-building behaviour, self-125 directed comfort behaviour (preening, stretching and scratching) and sleeping (resting the head 126 127 in the back). In addition, when flamingos were near the nest, all the other behaviours not directly associated with parental care were grouped in the behavioural category "Other". 128

129 *Statistical analysis*

Kolmogorov-Smirnov goodness-of-fit tests revealed that not all data were normally
distributed. Therefore, non-parametric statistic tests were used. In particular, Mann-Whitney tests
were run to compare the duration of different positions and behaviours between females and
males.

134 **RESULTS**

135 *Position of female and male flamingos in relation to the nest*

Among female and male flamingos, significant differences were found in the time spent 136 in different position relatively to the nest. The mean \pm SD duration (seconds) spent near the nest 137 (< 150 cm) was 1,049.86 ± 994.80 for females and 3,088.77 ± 1,539.68 for males. Regarding the 138 time spent on the nest, the mean \pm SD duration (seconds) was 5,359.51 \pm 1,835.92 for females 139 and $6,636.20 \pm 1,835.95$ for males. Finally, the mean \pm SD duration (seconds) spent away from 140 the nest (> 150 cm) was $5.590.63 \pm 1.958.91$ for females and $2.275.03 \pm 1.651.74$ for males (Fig. 141 1). Mann-Whitney tests revealed that males were near the nest and on the nest significantly more 142 than females (Z-score = -5.544, P < 0.0001, and Z-score = -2.572, P = 0.010, N₁=N₂=35, 143 respectively). On the contrary, males were away from the nest significantly less than females (Z-144 score = 5.761, P < 0.0001, N₁=N₂=35) (Fig. 1). 145 When flamingo partners were on the nest, we compared the time spent standing and 146 incubating the egg between female and male flamingos. The mean \pm SD duration (seconds) spent 147 standing was 295.17 ± 297 for females and 259.54 ± 201.45 for males. On the other hand, the 148 mean \pm SD duration (seconds) of incubation was 5,064.34 \pm 1,719.51 for females and 6,376.66 \pm 149

150 1,757.92 for males (Fig.2). Mann-Whitney tests revealed that males spent significantly more

- time than females incubating the egg (Z-score = -2.783, P = 0.005, N₁=N₂=35), whereas no
- significant differences were found in the time spent standing on the nest (*Z*-score = -0.117, P = 0.905) (Fig.2).

154 *Female and male activity near the nest and on the nest*

When flamingo partners were near the nest, the behavioural categories observed were agonistic behaviour, self-directed comfort behaviour, sleeping and other activities not directly associated with parental care ("other") (Tab. 1). Mann-Whitney tests revealed that males spent significantly more time than females performing all the behavioural categories mentioned above: agonistic behaviour (*Z*-score = -3.659, *P* = 0.0003, N₁=N₂=35), self-directed comfort behaviour (*Z*-score = -3.436, *P* = 0.0006, N₁=N₂=35), sleeping (*Z*-score = -4.499, *P* < 0.0001, N₁=N₂=35) and "other" (*Z*-score = -4.857, *P* < 0.0001, N₁=N₂=35).

When flamingo partners were standing on the nest, the behavioural categories that we observed were agonistic behaviour, egg-care related behaviour (egg-care) and self-directed comfort behaviour (Tab. 1). Mann-Whitney tests revealed that males spent significantly less time than females in self-directed comfort behaviour (*Z*-score = 2.296, P = 0.021, N₁=N₂=35),

166 whereas no significant differences were found for agonistic behaviour (Z-score = -0.141, P =

167 0.889, $N_1 = N_2 = 35$) and egg-care (Z-score = -1.139, P = 0.254, $N_1 = N_2 = 35$).

When flamingo partners were incubating the egg, the behavioural categories observed were agonistic behaviour, attentive behaviour, nest-building, self-directed comfort behaviour (preening) and sleeping (Tab. 1). Mann-Whitney tests revealed that males spent significantly more time than females in agonistic behaviour (*Z*-score = -2.978, P = 0.003, N₁=N₂=35), whereas no significant differences were found for attentive behaviour (*Z*-score = -1.785, P =

173 0.073), nest-building (Z-score = -1.621, P = 0.105, N₁=N₂=35), self-directed comfort behaviour

174 (Z-score = -0.482, P = 0.631, N₁=N₂=35) and sleeping (Z-score = -0.711, P = 0.477, N₁=N₂=35).

175 DISCUSSION & CONCLUSION

Research on flamingo breeding behaviour is needed to improve the knowledge on thesespecies in order to find strategies to increase their welfare and reproductive success in captivity

(Ogilvie & Ogilvie, 1986). The aim of this study was to assess the welfare of a captive colony of 178 greater flamingos, based on ethological parameters. Firstly, greater flamingos of this study were 179 found to perform species-specific behavioural repertoire (Brown & King, 2005) and no abnormal 180 behaviour was observed. Both parents were involved in parental care and displayed all the 181 activities reported in the wild during incubation, such as moving and rotating the egg, nest-182 183 building, self-preening and stretching, nest protection and resting (Studer-Thiersch, 1975; Pickering et al., 1992; Beletsky, 2006; Elphick, 2014). Moreover, the study flock breeds yearly 184 and shows a good reproductive success, as the number of flamingos rises from 88 in 2012 to 177 185 in 2016. Therefore, our findings seem to underline that the study flock of greater flamingos is in 186 a good welfare (Hosey et al., 2013; Hill & Broom, 2009). 187

Results from the current study highlight differences in parental care behaviour between 188 female and male greater flamingos. Firstly, male flamingos of a breeding pair spent significantly 189 more time on the nest and near it than females. These finding are in agreement with previous 190 studies reporting a greater effort of male greater flamingos in incubation (Rendòn-Martos et al., 191 2000; Rendòn, Garrido, Rèndon-Martos, Ramirez & Amat, 2014). On the contrary, females 192 remained away from the nest, without caring about the egg and the nest, longer than males. On 193 194 the basis of previous studies, male flamingos take care of the egg but do not feed their partner during the incubation process. Moreover, at least in the early stages, the parental investment is 195 196 greater for females than males, due to the costs of egg-laying (Cezilly, 1993; Johnson & Cezilly, 197 2007). Therefore, it is possible that female flamingos remained less time in proximity of the nest, caring for the egg, and spent more time looking for food, to recover from the egg-laying effort 198 199 and replenish her reserves in case a new egg-laying would be necessary (Jenni, 1974; Lenington, 200 1984; Reynolds & Szèkely, 1997).

When flamingos were on the nest, they could either be standing or sitting to incubate the egg. Our findings suggest that male flamingos spend more time incubating the egg than females, although no differences between sexes for standing on the egg were reported. Together with previous results on nest attendance (being near the nest or on the nest), these findings suggest a greater involvement of male flamingos in the incubation process (Rendòn-Martos *et al.*, 2000; Rendòn *et al.*, 2014).

When flamingos were near the nest, males were significantly more aggressive to defend the nest from other individuals than females and performed more self-comfort behaviour, sleeping and other behavioural categories. It is possible that, since males were more involved in nest defence, they remained near the nest for a longer time when their partner was on the nest, instead of going away similarly to females.

When flamingos were standing on the nest the most important behaviour was caring for 212 the egg, moving or rotating it to improve the incubation effort. According to our results, females 213 and males spend the same amount of time in the egg care, confirming previous findings on 214 parental care in greater flamingos (Studer-Tiersch, 1975; Brown et al., 1983; Elphick, 2014). 215 Finally, when flamingos were incubating the egg, males spent significantly more time 216 217 than females in agonistic behaviour, suggesting a greater effort in nest and egg protection, as suggested by previous research (Johnson & Cèzilly, 2007). Aggressive behaviour has been 218 219 previously found to increase during the breeding season in flamingos (Farrell et al., 2000) and 220 might be due to competition over mates and over nest sites and food resources, as well as for nest/chick protection (Johnson & Cèzilly, 2007; Hinton et al., 2013). According to our results, 221 222 both female and male flamingos displayed agonistic behaviour. However, male flamingos were 223 more aggressive than females when they were either near the nest and incubating the egg. These

findings suggest that male flamingos are also largely involved in nest/chick protection (Johnson
& Cèzilly, 2007).

In the current study, we focused on the parental care behaviour of the partners after the egg was laid and the nest was almost completed. The lack of differences in nest building between sexes reported in the current study seems to confirm that during incubation, after the egg is laid, nest building duties are equally shared by both partners (Studer-Tiersch, 1975).

Occurrence and patterns of different parental care modes have been largely studied in 230 birds. Results from our study seem to support some previous hypotheses linking male parental 231 care and sexual monomorphism. Indeed, it has been suggested that in bird taxa, in which males 232 incubate the egg, a reduced sexual dimorphism should be expected (Ketterson & Nolan, 1994). 233 In greater flamingos, males are slightly larger than females but both sexes look similar in 234 appearance and physiology, showing a reduced sexual dimorphism (Studer-Thiersch, 1975; 235 Beletsky, 2006; Cezilly, 1993; Elphick, 2014). Therefore, our findings add consistency to the 236 237 hypothesis that male parental care, specifically incubation, is more common in sexual monomorphic species, with a less intense sexual selection (Ketterson & Nolan, 1994). 238

In conclusion the present study assessed a good welfare of the colony of greater flaming \bigcirc as regards to the attempt to cope with its environment, in particular during the breeding season. Moreover, data on the parental care of females and males of greater flamingos can be added to the previous literature and used to improve the husbandry of this species in captivity. This kind of research is important not only to expand the knowledge on bird species such as flamingos, but also to improve the situation for captive animals and have a greater scientific understanding of issues important to modern zoos and *ex-situ* conservation.

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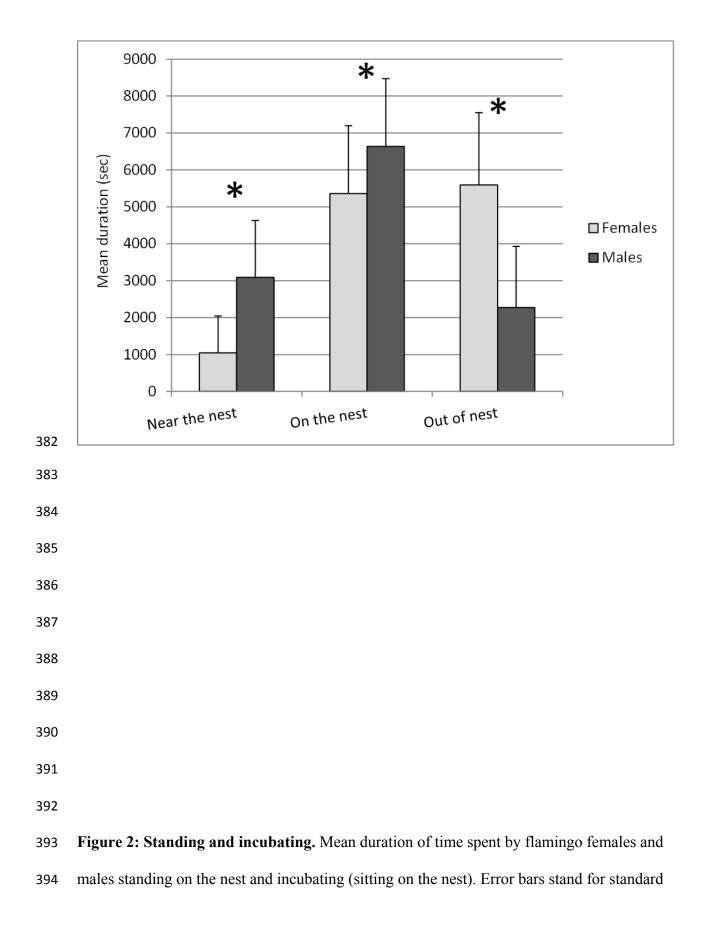
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378	Figure 1: Mean time spent by flamingo partners in different positions. Mean duration of
379	time spent by flamingo females and males near the nest, on the nest and out of the nest. Error
380	bars stand for standard deviation. Asterisks indicate a significant difference between females and
381	males (Mann-Whitney test: $p < 0.05$).



deviation. Asterisks indicate a significant difference between females and males (Mann-Whitney

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396 test: p < 0.05).
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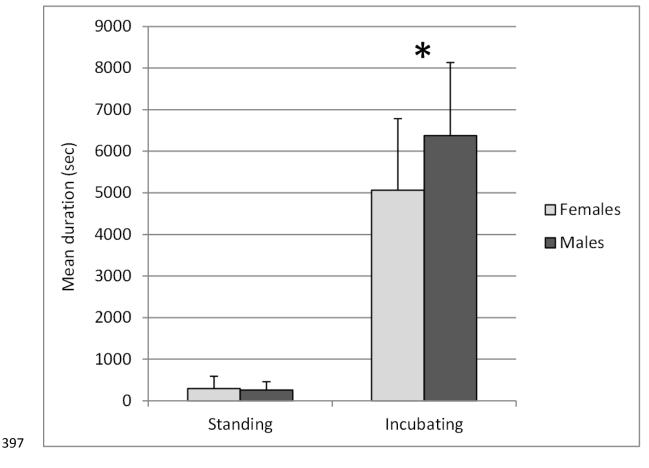


Table 1(on next page)

Behavioural categories performed by flamingos near the nest and on the nest.

Table 1: behavioural categories performed by flamingos near the nest and on the nest (standing and incubating). The table reports the mean \pm SD duration of each behavioural category performed by females (F) and males (M) when they are near the nest, standing on the nest or incubating the egg.

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1 Table 1: behavioural categories performed by flamingos near the nest and on the nest (standing and incubating). The table reports the

2 mean \pm SD duration of each behavioural category performed by females (F) and males (M) when they are near the nest, standing on

Near the nest		On the nest (standing)		On the nest (incubating)	
F	М	F	М	F	Μ
82.26 ± 118.99	233.91 ± 222.63	19.91 ± 35.22	14.57 ± 19.02	636.17 ± 378.00	940.57 ± 444.17
-	-	-	-	1577.34 ± 821.43	1949.89 ± 903.34
263.91 ± 271.67	662.40 ± 569.73	83.11 ± 136.36	21.09 ± 42.79	157.46 ± 266.87	191.69 ± 296.26
-	-	192.14 ± 187.04	223.89 ± 167.49	-	-
-	-	-	-	2306.31 ± 919.58	2766.91 ± 1259.69
384.77 ± 439.51	1232.97 ± 859.03	-	-	-	-
318.91 ± 564.98	959.49 ± 733.02	-	-	387.06 ± 485.95	527.60 ± 669.19
	F 82.26 ± 118.99 - 263.91 ± 271.67 - 384.77 ± 439.51	FM 82.26 ± 118.99 233.91 ± 222.63 263.91 ± 271.67 662.40 ± 569.73 384.77 ± 439.51 1232.97 ± 859.03	FMF 82.26 ± 118.99 233.91 ± 222.63 19.91 ± 35.22 263.91 ± 271.67 662.40 ± 569.73 83.11 ± 136.36 192.14 \pm 187.04 384.77 ± 439.51 1232.97 ± 859.03 -	FMFM 82.26 ± 118.99 233.91 ± 222.63 19.91 ± 35.22 14.57 ± 19.02 263.91 ± 271.67 662.40 ± 569.73 83.11 ± 136.36 21.09 ± 42.79 192.14 \pm 187.04 223.89 ± 167.49 384.77 ± 439.51 1232.97 ± 859.03	FMFMF 82.26 ± 118.99 233.91 ± 222.63 19.91 ± 35.22 14.57 ± 19.02 636.17 ± 378.00 1577.34 \pm 821.43 263.91 ± 271.67 662.40 ± 569.73 83.11 ± 136.36 21.09 ± 42.79 157.46 ± 266.87 192.14 \pm 187.04 223.89 ± 167.49 2306.31 \pm 919.58 384.77 ± 439.51 1232.97 ± 859.03

3 the nest or incubating the egg.