

A peer-reviewed version of this preprint was published in PeerJ on 30 May 2017.

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Sandri C, Vallarin V, Sammarini C, Regaiolli B, Piccirillo A, Spiezio C. 2017. How to be a great dad: parental care in a flock of greater flamingo (*Phoenicopterus roseus*) PeerJ 5:e3404
<https://doi.org/10.7717/peerj.3404>

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.

1 RUNNING HEAD: PARENTAL CARE IN GREATER FLAMINGO

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

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21 **Abstract**

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

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44 INTRODUCTION

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

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

67 concave centre. It is generally built on an island or on the coastline of a lake (Studer-Thiersch,
68 1975; Beletsky, 2006; Cezilly, 1993; Elphick, 2014). Before egg laying, the male is primarily
69 involved in nest building, but the female takes over as the laying time approaches. The nest
70 building activity of both partners proceeds also during the first two weeks of incubation, leading
71 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
73 incubation, lasting from 27 to 31 days (Beletsky, 2006; Cezilly, 1993; Elphick, 2014). However,
74 in the first period, the female is reported to spend more time on the nest than the male (Studer-
75 Thiersch, 1975). When incubating the egg, flamingos display different behaviours, such as
76 standing, stretching the wings, preening, self-scratching and looking at the nest (Studer-Thiersch,
77 1975). In addition, they take care of the egg, moving it with the bill. Flamingos could either
78 stand or sit on the egg and the time spent standing up depends on the weather condition. When
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
81 and the vacant one outside the nest can perform aggressive behaviour toward other flamingos
82 disturbing the incubation (Studer-Thiersch, 1975).

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

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

91 **MATERIALS AND METHODS**

92 *Study subjects and area*

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

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

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

111 *Procedure and data collection*

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

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

129 *Statistical analysis*

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

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

136 Among female and male flamingos, significant differences were found in the time spent
137 in different position relatively to the nest. The mean \pm SD duration (seconds) spent near the nest
138 (< 150 cm) was $1,049.86 \pm 994.80$ for females and $3,088.77 \pm 1,539.68$ for males. Regarding the
139 time spent on the nest, the mean \pm SD duration (seconds) was $5,359.51 \pm 1,835.92$ for females
140 and $6,636.20 \pm 1,835.95$ for males. Finally, the mean \pm SD duration (seconds) spent away from
141 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.
142 1). Mann-Whitney tests revealed that males were near the nest and on the nest significantly more
143 than females (Z -score = -5.544 , $P < 0.0001$, and Z -score = -2.572 , $P = 0.010$, $N_1=N_2=35$,
144 respectively). On the contrary, males were away from the nest significantly less than females (Z -
145 score = 5.761 , $P < 0.0001$, $N_1=N_2=35$) (Fig. 1).

146 When flamingo partners were on the nest, we compared the time spent standing and
147 incubating the egg between female and male flamingos. The mean \pm SD duration (seconds) spent
148 standing was 295.17 ± 297 for females and 259.54 ± 201.45 for males. On the other hand, the
149 mean \pm SD duration (seconds) of incubation was $5,064.34 \pm 1,719.51$ for females and $6,376.66 \pm$
150 $1,757.92$ for males (Fig.2). Mann-Whitney tests revealed that males spent significantly more
151 time than females incubating the egg (Z -score = -2.783 , $P = 0.005$, $N_1=N_2=35$), whereas no
152 significant differences were found in the time spent standing on the nest (Z -score = -0.117 , $P =$
153 0.905) (Fig.2).

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

155 When flamingo partners were near the nest, the behavioural categories observed were
156 agonistic behaviour, self-directed comfort behaviour, sleeping and other activities not directly
157 associated with parental care (“other”) (Tab. 1). Mann-Whitney tests revealed that males spent
158 significantly more time than females performing all the behavioural categories mentioned above:
159 agonistic behaviour (Z -score = -3.659, $P = 0.0003$, $N_1=N_2=35$), self-directed comfort behaviour
160 (Z -score = -3.436, $P = 0.0006$, $N_1=N_2=35$), sleeping (Z -score = -4.499, $P < 0.0001$, $N_1=N_2=35$)
161 and “other” (Z -score = -4.857, $P < 0.0001$, $N_1=N_2=35$).

162 When flamingo partners were standing on the nest, the behavioural categories that we
163 observed were agonistic behaviour, egg-care related behaviour (egg-care) and self-directed
164 comfort behaviour (Tab. 1). Mann-Whitney tests revealed that males spent significantly less time
165 than females in self-directed comfort behaviour (Z -score = 2.296, $P = 0.021$, $N_1=N_2=35$),
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, $P = 0.254$, $N_1=N_2=35$).

168 When flamingo partners were incubating the egg, the behavioural categories observed
169 were agonistic behaviour, attentive behaviour, nest-building, self-directed comfort behaviour
170 (preening) and sleeping (Tab. 1). Mann-Whitney tests revealed that males spent significantly
171 more time than females in agonistic behaviour (Z -score = -2.978, $P = 0.003$, $N_1=N_2=35$),
172 whereas no significant differences were found for attentive behaviour (Z -score = -1.785, $P =$
173 0.073), nest-building (Z -score = -1.621, $P = 0.105$, $N_1=N_2=35$), self-directed comfort behaviour
174 (Z -score = -0.482, $P = 0.631$, $N_1=N_2=35$) and sleeping (Z -score = -0.711, $P = 0.477$, $N_1=N_2=35$).

175 **DISCUSSION & CONCLUSION**

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

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

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

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

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

212 When flamingos were standing on the nest the most important behaviour was caring for
213 the egg, moving or rotating it to improve the incubation effort. According to our results, females
214 and males spend the same amount of time in the egg care, confirming previous findings on
215 parental care in greater flamingos (Studer-Tiersch, 1975; Brown *et al.*, 1983; Elphick, 2014).

216 Finally, when flamingos were incubating the egg, males spent significantly more time
217 than females in agonistic behaviour, suggesting a greater effort in nest and egg protection, as
218 suggested by previous research (Johnson & Cèzilly, 2007). Aggressive behaviour has been
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
221 nest/chick protection (Johnson & Cèzilly, 2007; Hinton *et al.*, 2013). According to our results,
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

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

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

230 Occurrence and patterns of different parental care modes have been largely studied in
231 birds. Results from our study seem to support some previous hypotheses linking male parental
232 care and sexual monomorphism. Indeed, it has been suggested that in bird taxa, in which males
233 incubate the egg, a reduced sexual dimorphism should be expected (Ketterson & Nolan, 1994).

234 In greater flamingos, males are slightly larger than females but both sexes look similar in
235 appearance and physiology, showing a reduced sexual dimorphism (Studer-Thiersch, 1975;
236 Beletsky, 2006; Cezilly, 1993; Elphick, 2014). Therefore, our findings add consistency to the
237 hypothesis that male parental care, specifically incubation, is more common in sexual
238 monomorphic species, with a less intense sexual selection (Ketterson & Nolan, 1994).

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

246

247 **Acknowledgement**

248 The study was carried out using non-invasive techniques, through live observation of the
249 birds. The research procedure was in accordance with the EU Directive 2010/63/EU for animal
250 research. We would like to thank Dr. Cesare Avesani Zaborra for allowing this study to take
251 place in Parco Natura Viva. Furthermore, special thanks should be given to Giuseppe Alampi,
252 Parco Natura Viva flamingo zoo-keepers, for his hard work and for their precious help with the
253 flamingo census and monitoring.

254

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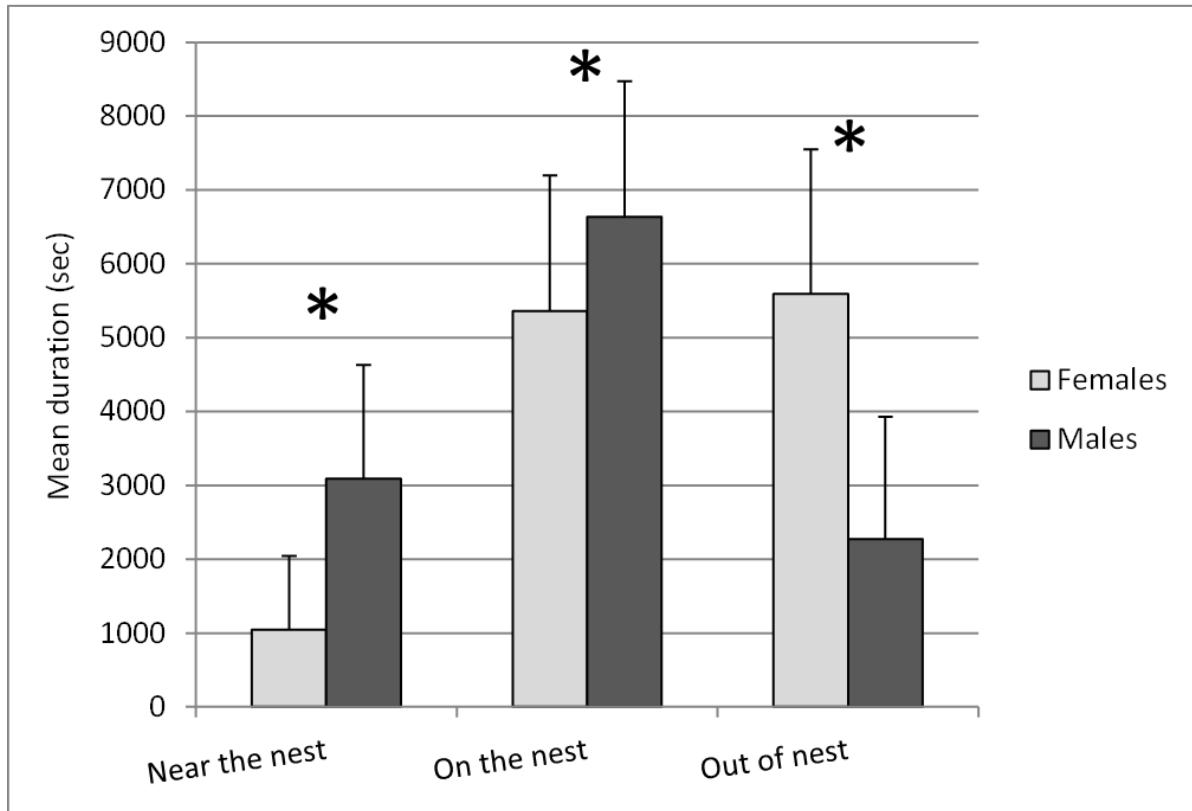
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378 **Figure 1: Mean time spent by flamingo partners in different positions.** Mean duration of
379 time spent by flamingo females and males near the nest, on the nest and out of the nest. Error
380 bars stand for standard deviation. Asterisks indicate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



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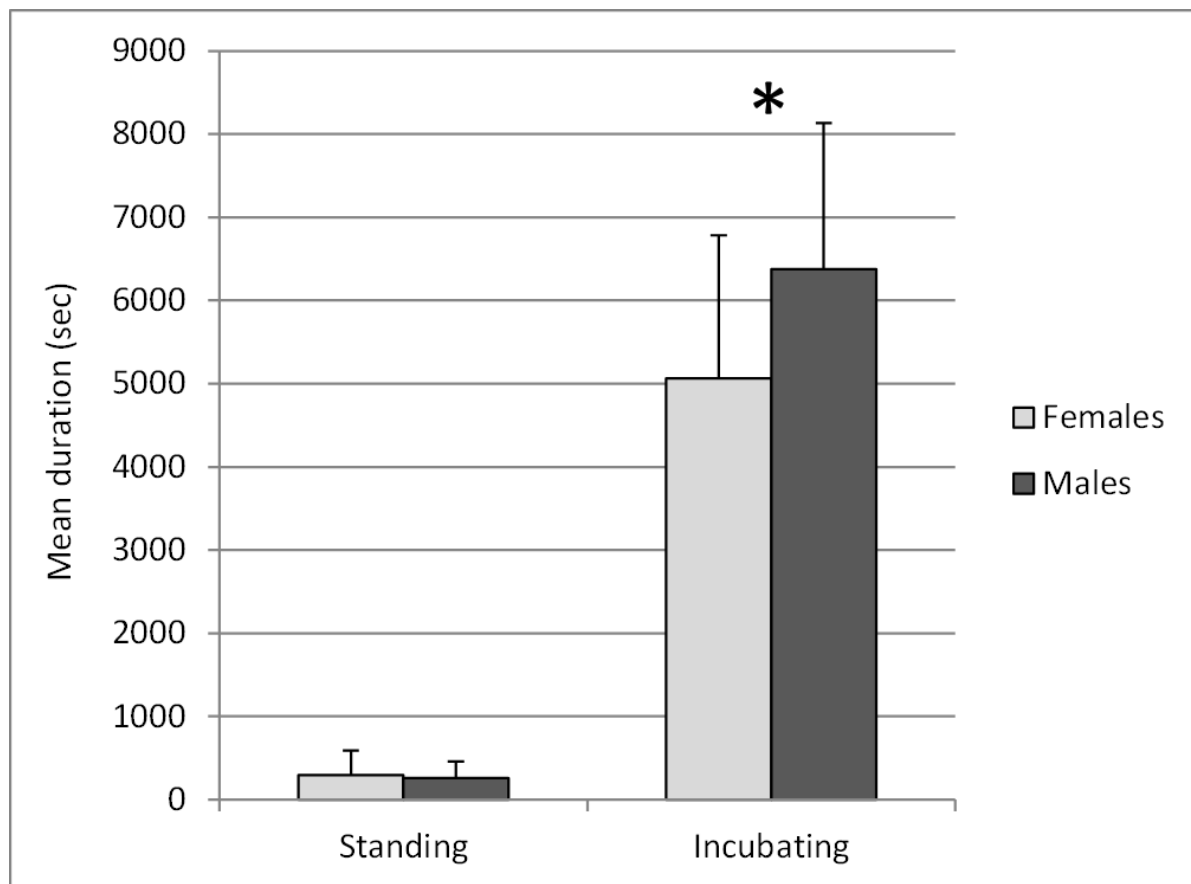
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393 **Figure 2: Standing and incubating.** Mean duration of time spent by flamingo females and

394 males standing on the nest and incubating (sitting on the nest). Error bars stand for standard

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

- 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	M	F	M	F	M
Agonistic behaviour	82.26 \pm 118.99	233.91 \pm 222.63	19.91 \pm 35.22	14.57 \pm 19.02	636.17 \pm 378.00	940.57 \pm 444.17
Attentive behaviour	-	-	-	-	1577.34 \pm 821.43	1949.89 \pm 903.34
Comfort behaviour	263.91 \pm 271.67	662.40 \pm 569.73	83.11 \pm 136.36	21.09 \pm 42.79	157.46 \pm 266.87	191.69 \pm 296.26
Egg care	-	-	192.14 \pm 187.04	223.89 \pm 167.49	-	-
Nest-bulding	-	-	-	-	2306.31 \pm 919.58	2766.91 \pm 1259.69
Other	384.77 \pm 439.51	1232.97 \pm 859.03	-	-	-	-
Sleeping	318.91 \pm 564.98	959.49 \pm 733.02	-	-	387.06 \pm 485.95	527.60 \pm 669.19

- 3 the nest or incubating the egg.