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

Camillo Sandri 1, Vittoria Vallarin 2, Carolina Sammarini 1, Barbara Regaiolli Corresp. 3, Alessandra Piccirillo 4, Caterina Spiezio 3

1 Department of Animal Health Care and Management, Parco Natura Viva - Garda Zoological Park, Verona, Italy
2 Department of Neurosciences, University of Parma, Parma, Italy
3 Research and Conservation Department, Parco Natura Viva - Garda Zoological Park, Verona, Italy
4 Department of Comparative Biomedicine and Food Science (BCA), University of Padua, Padua, Italy

Corresponding Author: Barbara Regaiolli
Email address: barbara.regaiolli@parconaturaviva.it

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

Camillo Sandri¹; Vittoria Vallarin²; Carolina Sammarini¹; Barbara Regaiolli³*; Alessandra Piccirillo⁴; Caterina Spiezio³

¹ Department of Animal Health Care and Management, Parco Natura Viva - Garda Zoological Park, Bussolengo, Italy

² Department of Neurosciences, University of Parma, Parma, Italy

³ Research and Conservation Department, Parco Natura Viva - Garda Zoological Park, Bussolengo, Italy

⁴ Department of Comparative Biomedicine and Food Science, University of Padua, Padua, Italy

*Corresponding author: Barbara Regaiolli, PhD, Parco Natura Viva – Garda Zoological Park, Bussolengo (VR), Loc. Figara, 40, 37010, Italy.

Abstract
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 \textit{ex-situ} and \textit{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.

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

Word count: 2 701
INTRODUCTION

In the wild, greater flamingos (*Phoenicopterus roseus*) are threatened by phenomena such as habitat loss and change, human activities and pollution (Ogilvie & Ogilvie, 1986; Nasirwa, 2000; Yosef, 2000; Hockey *et al*., 2005; Miltiadou, 2005; IUCN, 2015). Therefore, *ex-situ* conservation programs are essential. However, the ethical imperative to save threatened species 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 method to assess the welfare of the animals is to verify the performance of natural behaviour, even in captive settings (Hill & Broom, 2009). Flamingos are highly gregarious birds that live and breed in large dense flocks (Pickering *et al*., 1992), often including thousands of pairs. Obtaining information and data on their behaviour in the wild is therefore difficult due to constraints such as individual identification and approach to the birds (Studer-Thiersch, 1975; 2000; King, 2000). For this reason, together with long-term studies on wild flamingo flocks, research on captive colonies might be valuable and complementary to improve the knowledge on the ethology, morphology, physiology and endocrinology of these species (King, 2000). Studying the behaviour of flamingos in the wild and in controlled environment is important for 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 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
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).

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, in the first period, the female is reported to spend more time on the nest than the male (Studer-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, 1975). In addition, they take care of the egg, moving it with the bill. Flamingos could either stand or sit on the egg and the time spent standing up depends on the weather condition. When one partner leaves the egg, the time taken to the other one to climb on the nest and incubate the 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 disturbing the incubation (Studer-Thiersch, 1975).

The aims of the present study was 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, 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 the
wild, to suggest strategies to improve the husbandry of this species in captivity.

MATERIALS AND METHODS

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).
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 male, recording the position of the bird in relation to the nest and the behavioural category performed. Regarding the position of the bird, we recorded whether each flamingo parent was near the nest (less than 150 cm, which is approximately the higher flamingo body length; del Hoyo et al., 1992), on the nest or away from the nest (>150 cm). When the flamingos were on the nest, we recorded whether they were sitting (incubating) or standing. In particular, the behavioural categories collected in the study were agonistic behaviour, including aggressive 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-directed comfort behaviour (preening, stretching and scratching) and sleeping (resting the head 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”.

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.
RESULTS

Position of female and male flamingos in relation to the nest

Among female and male flamingos, significant differences were found in the time spent in different position relatively to the nest. The mean ± SD duration (seconds) spent near the nest (< 150 cm) was 1,049.86 ± 994.80 for females and 3,088.77 ± 1,539.68 for males. Regarding the time spent on the nest, the mean ± SD duration (seconds) was 5,359.51 ± 1,835.92 for females and 6,636.20 ± 1,835.95 for males. Finally, the mean ± SD duration (seconds) spent away from the nest (> 150 cm) was 5,590.63 ± 1,958.91 for females and 2,275.03 ± 1,651.74 for males (Fig. 1). Mann-Whitney tests revealed that males were near the nest and on the nest significantly more than females (Z-score = -5.544, \( P < 0.0001 \), and Z-score = -2.572, \( P = 0.010 \), \( N_1=N_2=35 \), respectively). On the contrary, males were away from the nest significantly less than females (Z-score = 5.761, \( P < 0.0001 \), \( N_1=N_2=35 \)) (Fig. 1).

When flamingo partners were on the nest, we compared the time spent standing and incubating the egg between female and male flamingos. The mean ± SD duration (seconds) spent standing was 295.17 ± 297 for females and 259.54 ± 201.45 for males. On the other hand, the mean ± SD duration (seconds) of incubation was 5,064.34 ± 1,719.51 for females and 6,376.66 ± 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, \( P = 0.005 \), \( N_1=N_2=35 \)), whereas no significant differences were found in the time spent standing on the nest (Z-score = -0.117, \( P = 0.905 \)) (Fig.2).

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, $N_1$=$N_2$=35), self-directed comfort behaviour ($Z$-score = -3.436, $P$ = 0.0006, $N_1$=$N_2$=35), sleeping ($Z$-score = -4.499, $P$ < 0.0001, $N_1$=$N_2$=35) and “other” ($Z$-score = -4.857, $P$ < 0.0001, $N_1$=$N_2$=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, $N_1$=$N_2$=35), whereas no significant differences were found for agonistic behaviour ($Z$-score = -0.141, $P$ = 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, $N_1$=$N_2$=35), whereas no significant differences were found for attentive behaviour ($Z$-score = -1.785, $P$ = 0.073), nest-building ($Z$-score = -1.621, $P$ = 0.105, $N_1$=$N_2$=35), self-directed comfort behaviour ($Z$-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).

**DISCUSSION & CONCLUSION**

Research on flamingo breeding behaviour is needed to improve the knowledge on these species in order to find strategies to increase their welfare and reproductive success in captivity.
The aim of this study was to assess the welfare of a captive colony of greater flamingos, based on ethological parameters. Firstly, greater flamingos of this study were found to perform species-specific behavioural repertoire (Brown & King, 2005) and no abnormal behaviour was observed. Both parents were involved in parental care and displayed all the activities reported in the wild during incubation, such as moving and rotating the egg, nest-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 and shows a good reproductive success, as the number of flamingos rises from 88 in 2012 to 177 in 2016. Therefore, our findings seem to underline that the study flock of greater flamingos is in a good welfare (Hosey et al., 2013; Hill & Broom, 2009).

Results from the current study highlight differences in parental care behaviour between female and male greater flamingos. Firstly, male flamingos of a breeding pair spent significantly more time on the nest and near it than females. These finding are in agreement with previous studies reporting a greater effort of male greater flamingos in incubation (Rendôn-Martos et al., 2000; Rendôn, Garrido, Rendôn-Martos, Ramirez & Amat, 2014). On the contrary, females remained away from the nest, without caring about the egg and the nest, longer than males. On 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 greater for females than males, due to the costs of egg-laying (Cezilly, 1993; Johnson & Cezilly, 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 and replenish her reserves in case a new egg-laying would be necessary (Jenni, 1974; Lenington, 1984; Reynolds & Székely, 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 (Rendón-Martos et al., 2000; Rendón 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 the egg, moving or rotating it to improve the incubation effort. According to our results, females and males spend the same amount of time in the egg care, confirming previous findings on parental care in greater flamingos (Studer-Tiersch, 1975; Brown et al., 1983; Elphick, 2014).

Finally, when flamingos were incubating the egg, males spent significantly more time 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 previously found to increase during the breeding season in flamingos (Farrell et al., 2000) and 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, both female and male flamingos displayed agonistic behaviour. However, male flamingos were 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 birds. Results from our study seem to support some previous hypotheses linking male parental care and sexual monomorphism. Indeed, it has been suggested that in bird taxa, in which males incubate the egg, a reduced sexual dimorphism should be expected (Ketterson & Nolan, 1994).

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

In conclusion the present study assessed a good welfare of the colony of greater flamingos, 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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Figure 1: Mean time spent by flamingo partners in different positions. Mean duration of time spent by flamingo females and males near the nest, on the nest and out of the nest. Error bars stand for standard deviation. Asterisks indicate a significant difference between females and males (Mann-Whitney test: $p < 0.05$).
**Figure 2: Standing and incubating.** Mean duration of time spent by flamingo females and males standing on the nest and incubating (sitting on the nest). Error bars stand for standard
deviation. Asterisks indicate a significant difference between females and males (Mann-Whitney test: $p < 0.05$).
Table 1: Behavioural categories performed by flamingos near the nest and on the nest (standing and incubating). The table reports the mean ± 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.
**Table 1:** Behavioural categories performed by flamingos near the nest and on the nest (standing and incubating). The table reports the mean ± 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.

| Behavioural Category      | Near the nest |                     | On the nest (standing) |                     | On the nest (incubating) |
|---------------------------|---------------|---------------------|------------------------|---------------------|--------------------------|
|                           | F             | M                   | F                      | M                   | F                        |
| Agonistic behaviour       | 82.26 ± 118.99| 233.91 ± 222.63     | 19.91 ± 35.22          | 14.57 ± 19.02       | 636.17 ± 378.00          |
| Attentive behaviour       | -             | -                   | -                      | -                   | -                        |
| Comfort behaviour         | 263.91 ± 271.67| 662.40 ± 569.73     | 83.11 ± 136.36         | 21.09 ± 42.79       | 157.46 ± 266.87          |
| Egg care                  | -             | -                   | 192.14 ± 187.04        | 223.89 ± 167.49     | -                        |
| Nest-bulding              | -             | -                   | -                      | -                   | 2306.31 ± 919.58         |
| Other                     | 384.77 ± 439.51| 1232.97 ± 859.03    | -                      | -                   | 2766.91 ± 1259.69       |
| Sleeping                  | 318.91 ± 564.98| 959.49 ± 733.02     | -                      | -                   | 387.06 ± 485.95         |

The nest or incubating the egg.