

**Using behavior and social associations to assess the welfare of a captive flock
of Caribbean flamingos (*Phoenicopterus ruber*)**

Caitlin Finlay

*Department of Biology
Concordia University
Montreal, Québec, Canada*

A Thesis in the Department of Biology

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science at
Concordia University

Montreal, Québec, Canada

April 2017

© Caitlin Finlay, 2017

CONCORDIA UNIVERSITY

School of Graduate Studies

This is to certify that the thesis prepared

By: Caitlin Finlay

Entitled: Using behavior and social associations to assess the welfare of a captive flock of Caribbean flamingos (*Phoenicopterus ruber*)

and submitted in partial fulfillment of the requirements for the degree of

Master of Science (Biology)

Complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final examining committee:

_____ Chair

Dr. Ian Ferguson

_____ Examiner

Dr. Dylan Fraser

_____ Examiner

Dr. James Grant

_____ External Examiner

Dr. Grant Brown

_____ Supervisor

Dr. Robert Weladji

Approved by

Chair of Department or Graduate Program Director

_____ 2017

Dean of Faculty

Abstract

Using behavior and social associations to assess the welfare of a captive flock of Caribbean flamingos (*Phoenicopterus ruber*)

Caitlin Finlay

While the four main roles of zoos are education, research, conservation, and entertainment, the most important goal of zoos is to ensure good animal welfare. Animal welfare is generally assessed by observing the animals for indicators of poor welfare in three categories: health, physiological condition, and behavior. A common indicator of poor welfare is decreased reproduction, which is a problem in many captive flamingo flocks. We assessed the welfare of the flamingo flock at Zoo de Granby using behavior patterns, environmental effects, visitor effects, and social associations. Our results suggest that the behavior patterns differ between a wild population and the Zoo de Granby flock. We found an increased frequency of preening during periods of high humidity, and increased aggressive behaviors during periods of increased heat and humidity. Visitor variables had an effect on most behaviors but did not affect vigilance frequency. Contrary to previous literature, several of the Zoo de Granby pairings were not maintained over time. Association strengths were not different from their previous pairings, or between the nonbreeding and breeding season. Our findings suggest that flamingo behavior is affected by captivity although we do not believe it is a negative effect. We recommend that Zoo de Granby investigate the effect of animal keeper presence and the effect of wing clipping on copulation. Our association findings allow us to provide Zoo de Granby with information regarding strongly associated pairings who should not be separated in the event of a transaction between institutions.

Acknowledgements

I would like to thank NSERC and Zoo de Granby for providing the funding to make this research possible. Thank you for Dr. Robert Weladji for his support and guidance throughout this project. Thank you to my committee members, Dr. Dylan Fraser and Dr. James Grant, for their feedback and advice. Within Zoo de Granby I would like to thank Patrick Paré and Louis Lazure for their guidance and for acting as liaisons with the zoo staff. I would also like to thank the animal care staff in the Africa section for their help, cooperation, and keeping me updated on any changes with the flock. Additionally, I would like to thank Lauren Finlay and Ashley Watt for their indispensable help with data entry. Thank you to my wonderful family, friends, and boyfriend for their unwavering support and belief in me. Last but not least, thank you to the past and current members of the Weladji lab for their help and support throughout the last two years.

Contribution of authors

As the first author of this thesis, my responsibilities included the design of the project, data collection and analysis, and writing of the manuscripts. Both chapters were co-authored by Robert Weladji and Patrick Paré, who provided the concept for the project, provided advice, and acted as supervisors. Patrick Paré also provided background information on the Zoo de Granby flamingo flock and acted as a liaison to coordinate with zoo staff. The statistical analyses were conducted with the guidance of Dr. Weladji. The chapter manuscripts were reviewed and edited by both Dr. Weladji and Patrick Paré.

Table of Contents

List of Tables	viii
List of Figures	x
General Introduction	1
Chapter 1:	4
The effects of a captive environment on the behavior of Caribbean flamingos (<i>Phoenicopterus ruber</i>) and the implications for welfare	4
Abstract	5
Introduction	6
Methods	9
Study subjects	9
Study area	10
Data collection	10
Data analysis	12
Results	12
Activity budget comparison	12
Time of day, habitat, and activity patterns	13
Effect of weather on behavior	13
Effect of visitors on behavior	13
Effect of weather and habitat on aggression	14
Effect of visitors on aggression	15
Discussion	15
Conclusions	19
Tables and Figures	21
Chapter 2:	31
Social associations in captive Caribbean flamingos (<i>Phoenicopterus ruber</i>) and their implications for flock management	31
Abstract	32
Introduction	33
Methods	37
Study area and subjects	37
Data collection	38

Data analysis	39
Results	39
Association strength in 2015 and 2016	39
Association strength of maintained pairings	40
Association strength of new pairings in 2016	40
Age and likelihood to change partners	41
Partner age and sex preference	41
Discussion	42
Conclusions	46
Figures	47
General Conclusions	51
References	52
Appendix A	59
Appendix B	60
Appendix C	61
Appendix D	62
Appendix E	63
Appendix F	64
Appendix G	65

List of Tables

Table 1.1: Protocol determined by Zoo de Granby as the acceptable temperature range for each month the flamingos are in the indoor habitat.

Table 1.2: Protocol at Zoo de Granby to gradually increase the number of daylight hours in the flamingo indoor habitat.

Table 1.3. Ethogram used to define various flamingo behaviors for the focal sampling, adapted from King et al. (2005). Several behaviors were added to the original ethogram and are individually cited in the definitions.

Table 3.1. Individual flamingos can be identified using their unique leg bands. The left and right leg columns denote which leg the specific band is found on. When there is a number and a color, it is a colored band with a number on it. When there is no number listed, it means the band is a plain color. The ZIMS number is the individual's number in the Species360 Zoological Information Management System (ZIMS) database.

Table 3.2. Model selection based on AIC to explain the variability in the frequency of behaviors (resting, preening, foraging, locomotion, vigilance) based on outdoor weather conditions (maximum temperature, maximum humidity, cloud cover). We present all models within $\Delta AIC < 2$. The bold terms correspond with the most parsimonious model.

Table 3.3. Model selection based on AIC to explain the variability in the frequency of behaviors (resting, preening, foraging, locomotion, vigilance) based on indoor weather conditions (maximum temperature, maximum humidity). We present all models within $\Delta AIC < 2$. The bold terms correspond with the most parsimonious model.

Table 3.4. Model selection based on AIC to explain the variability in the frequency of aggressive behaviors (aggressive encounters, neck swaying, sparring, pecking, hooking, chasing) based on outdoor weather conditions (maximum temperature, maximum humidity, cloud cover). We present all models within $\Delta AIC < 2$. The bold terms correspond with the most parsimonious model.

Table 3.5. Model selection based on AIC to explain the variability in the frequency of aggressive behaviors (aggressive encounters, neck swaying, sparring, pecking, hooking, chasing) based on indoor weather conditions (maximum temperature, maximum humidity). We present all models within $\Delta AIC < 2$ or the two models with the lowest AIC if there was only one model within $\Delta AIC < 2$. The bold terms correspond with the most parsimonious model.

List of Figures

Figure 1.1. Comparison of captive and wild activity budgets in the nonbreeding season. The activity budget for the Zoo de Granby flamingo flock was compared budget to the activity budget means of a wild population (Espino-Barros and Baldassarre, 1989a). The dark gray bars represent the mean behavior focal percentages for Zoo de Granby during the nonbreeding season, and the light gray bars represent the activity means of the wild population during the nonbreeding season. The lines represent the 95% confidence intervals. * for $p < 0.05$, *** for $p < 0.001$.

Figure 1.2. Comparison of captive and wild activity budgets in the breeding season. The activity budget for the Zoo de Granby flamingo flock was compared budget to the activity budget means of a wild population (Espino-Barros and Baldassarre, 1989a). The dark gray bars represent the mean behavior focal percentages for Zoo de Granby during the nonbreeding season, and the light gray bars represent the activity means of the wild population during the nonbreeding season. The lines represent the 95% confidence intervals. *** for $p < 0.001$.

Figure 1.3. Comparison of activity budget focal count means from the morning (AM) and afternoon (PM). The dark gray bars represent the mean behavior focal counts for the morning period, and the light gray bars represent the mean behavior focal counts for the afternoon period. The lines represent the 95% confidence intervals. ** for $p < 0.01$, *** for $p < 0.001$.

Figure 1.4. Effect of habitat on activity budget percent means. The dark gray bars represent the mean behavior focal percent for the outdoor habitat, which can also be considered the summer period, and nonbreeding season. The light gray bars represent the mean behavior focal percent for the indoor habitat, which can also be considered the winter period, and breeding season. The lines represent the 95% confidence intervals. *** for $p < 0.001$.

Figure 1.5. Boxplot of the relationship between cloud cover and the frequency of preening behavior in the outdoor habitat. The x-axis features the four categories of the cloud cover variable: cloudy (C), mostly cloudy (MC), mostly sunny (MS), and sunny (S). The boxes represent the 1st to 3rd quartiles with the darkest line representing the median value, and the dashed lines representing the minimum and maximum values. * for $p < 0.05$.

Figure 1.6. Comparison of aggressive behaviors in the indoor and outdoor habitats. The lines represent the 95% confidence intervals. The behaviors include neck swaying (NS), sparring (SP), pecking (PECK), chasing (CH), hooking (HOOK), and bumping (BUMP). The dark gray bars represent the data collected while the flamingos were indoors, and the light gray bars represent the data collected while the flamingos were in their outdoor habitat.

Figure 2.1. Distribution of the number of social interactions between groupings of flamingos during the 2015 time period (July - December 2015). There is a pattern of steadily decreasing interactions which suggest close associations on the left side of the x-axis and little to no association on the right side. For clarity, the interacting individuals are named using only their leg band numbers, except in the case of B02048 and B03020 as they did not have numbered bands.

Figure 2.2. Distribution of the number of social interactions between groupings of flamingos during the 2016 time period (January - June 2016). There is a sudden decrease in interaction frequency between 51+B03020 and 52+62; the interaction frequency decreased from 13 to 4. We considered this sudden decrease to be a division between possibly associated pairs and flamingos that occasionally interacted. For this reason, we only used groupings of flamingos that had been recorded as interacting a minimum of five times during the study in our analysis. For clarity, the interacting individuals are named using only their leg band numbers, except in the case of B02048 and B03020 as they did not have numbered bands.

Figure 2.3. Flowchart depicting the pair changes that occurred in late December or early January. The adjacent boxes are pairings; the solid boxes represent the original pairings from 2015 and the outlined boxes are the new pairings from 2016. The arrows show the movement of the individuals from their 2015 to 2016 pairings. The individual 37-B01035♀ had equal associations (AI = 0.625) to both 10-B00026♂ and 50-B02046♂ in 2015 which is why she is shown twice in this figure.

Figure 2.4. Distribution of the change in partner age from 2015 to 2016. On the x-axis there is a list of individuals who changed partners in 2016; these individuals are arranged in order of increasing age (range = 8-38). The y-axis shows the age of the partner in years. The light gray

bars show the data from the 2015 associations, and the dark gray bars show the data from the 2016 associations.

Figure 3.1. The indoor habitat is $\sim 66\text{m}^2$ consisting of a large nesting area and a shallow water basin. The habitat is broken down into zones for ease of recording locations. The zones are divided into an entrance area (E) which the flamingos did not have access to during observations, a nesting area (N), indoor feeding area (IF), and a water basin (WB). The habitat features mirrors spanning halfway up all of the walls other than the entrance area. The solid black rectangles near the visitor path represent the two-way mirrors which allow visitors to view the flamingos. The curved, dashed line represents the hiding wall behind which the observations are made, and the observer spot is denoted by the “X”. The circles represent the placement of food trays in an attempt to minimize disturbance to the flamingos.

Figure 3.2. The photo in the top right is an aerial view of the habitat, while the center image is a schematic of the habitat. The outdoor habitat consists of $\sim 286\text{m}^2$ of accessible ground cover (grass) and a large pond ($\sim 686\text{m}^2$) covering approximately 71% of the habitat. The habitat is broken down into zones for ease of recording locations. The zones are divided into an outdoor feeding area (OF), land cover (L), section of the pond near the viewing area (W1), section of the pond near the raised boardwalk (W2), and a section of the pond with dense foliage where visibility is low (W3). The “X” represents the observer spot on the public boardwalk.

General Introduction

Entertainment, education, conservation, and research are the four main roles of zoos, however their top priority is to ensure the welfare of animals in their care (Hosey et al., 2013; World Association of Zoos and Aquariums, 2005). Welfare is defined as the ability of an animal to cope with changes to its environment, and is a spectrum ranging from very good to very poor (Barber and Mellen, 2013; Broom, 2001). The study of animal welfare began in an agricultural setting to ensure the needs of the animals were being met while maximizing production (Melfi, 2009). The methods used to assess the welfare of agricultural animals have since been adapted to assess the welfare of animals in zoos (Melfi, 2009). There are three criteria used to assess animal welfare: health, behavior, and physiological condition of the animal (Melfi, 2009; World Association of Zoos and Aquariums, 2005). Most commonly, welfare is studied by looking for indicators of poor welfare such as a shift in behavior patterns compare to their wild counterparts, high frequency of disease or injury, lack of exercise, stereotypical behaviors such as pacing, and reduced reproduction or lifespan (Frumkin et al., 2016; Melfi, 2009; Shannon, 2000; World Association of Zoos and Aquariums, 2005). Zoos aim to reproduce the natural habitat of the animals to the best of their abilities to ensure their physiological, health, and behavioral needs are being met (Hosey et al., 2013; World Association of Zoos and Aquariums, 2005). While these indicators are important, we must also study the effects of the captive environment which can include weather and visitor effects (Melfi, 2009).

Zoo studies have recently become popular to assess animal welfare but they also allow the opportunity for experimental studies that may not be possible in the field (Bildstein et al., 1993). Studies in captivity tend to be biased towards charismatic megafauna, although they are usually less abundant in captivity than other animals (Melfi, 2009). Flamingos are one of the most abundant animals in captivity however there are not many studies conducted on captive flamingos (Bildstein et al., 1993; King, 2000; Melfi, 2009; Rose et al., 2014). Not all species adapt to captivity in the same way or have the same needs so it is important to study a wide range of species (Melfi, 2009). Captive flamingos have inconsistent or reduced reproduction, which can be an indicator of poor welfare, so they are an important species to study (Farrell et al., 2000; Melfi, 2009).

A well-known method of assessing welfare in captivity is the comparison of a captive and wild activity budget (Rose et al., 2014). Large differences between activity patterns could be an indicator of poor welfare in captivity (Melfi, 2009; Rose et al., 2014). Environmental variables such as temperature, humidity, light availability, and intensity of UV radiation can also affect the activity patterns of flamingos. Caribbean flamingos have been found to be more active when there is more sunlight and are more aggressive during warmer weather, and when there is a higher UV index (Peluso et al., 2013).

Visitor effect in both captive and wild populations has been studied. Wild flamingos were found to become vigilant but continue foraging when people walked past (Yosef, 2000). On the other hand, when tourists approached on foot after leaving a vehicle, the flamingos decreased the distance between individuals (Yosef, 2000). This decreased distance between individuals is known as seeking protection in numbers and is a behavior commonly done in the presence of a threat such as a predator (Hamilton, 1971; Yosef, 2000). As in the wild, when captive flamingos are stressed, they will huddle together in a small space to seek safety in numbers (Hamilton, 1971; King, 2008b; Yosef, 2000).

Another potential source of stress in captivity due to visitors is exposure to noise levels which are louder than the noises of a natural environment (Morgan and Tromborg, 2007). As the number of visitors increases, the level of visitor noise will also increase (Morgan and Tromborg, 2007). While visitor presence in zoos is expected and animals have the potential to habituate to visitor presence, not all species will adapt and there is always the possibility of stress in response to visitors (Mason, 2010; Studer-Thiersch, 2000).

Decreased or unpredictable reproduction is a common problem with captive flamingo populations although the causes are unknown (Farrell et al., 2000). Successful reproduction is an important component of animal welfare and is vital to the success of captive breeding programs (Melfi, 2009; Tetley and O'Hara, 2012). Captive breeding programs exist in order to maintain a viable, reproducing population but many species have trouble reproducing in captivity (Tetley and O'Hara, 2012). There are many possible causes of low reproductive success in captivity including behavioral problems, stressful reactions to their environment, high levels of aggression, asynchronous courtship rituals, motor inadequacies such as improper mounting, and incompatible pairings (Lindburg and Fitch-Snyder, 1994; Zhang et al., 2004).

As reduced reproduction can be an indicator of poor welfare, it is important to study the environmental and social needs of captive flamingos to ensure good welfare (Melfi, 2009). Flamingo reproduction is often erratic because it is dependent on unpredictable social and environmental cues such as sex ratio, age structure, size of the colony, food availability, rainfall, and photoperiod (King, 2008b; Pickering et al., 1992).

Flamingos are gregarious birds that form preferential associations within their flocks (King et al., 2005; Rose et al., 2014). Wild pairings do not tend to be maintained, however captive pairings have exhibited mate fidelity (Johnson and Cézilly, 2009; King, 2008a; Pickering, 1992; Rose et al., 2014; Shannon, 2000; Studer-Thiersch, 2000). Through studying their associations, we are able to assess their welfare and provide flock management suggestions to ensure strongly bonded pairs are kept together since the separation of bonded pairs could lead to decreased welfare and stress (Melfi, 2009; Rose and Croft, 2015a; Shannon, 2000).

This study assessed the activity patterns, environmental factors, visitor effects, and social associations within the Zoo de Granby flamingo flock to provide the zoo with methods to improve flamingo welfare, flock management, and increase reproductive success.

Chapter 1

The effects of a captive environment on the behavior of Caribbean flamingos (*Phoenicopterus ruber*) and the implications for welfare

Caitlin A. Finlay¹, Robert B. Weladji¹, Patrick Paré²

1. Biology Department, Concordia University, 7141 Sherbrooke St. W, Montreal, Québec, Canada
2. Zoo de Granby, 1050 Boulevard David Bouchard N, Granby, Québec, Canada

Abstract:

Zoos play an important role in conservation, research, and education but their primary goal is to ensure the welfare of the animals in their care. Captive research tends to be biased towards charismatic megafauna and often ignores the lesser known but abundant animals in captivity. Flamingos are one of the most abundant animals in captivity yet little captive research has been done. The flamingo flock at Zoo de Granby was studied to provide the zoo with methods to improve flamingo welfare. To do this we assessed the activity patterns, as well as how they are affected by environmental factors and visitor effects. We found that the frequency of resting, preening, foraging, locomotion, and vigilance differed between the Zoo de Granby population and a wild population. Flamingos rested most in the morning and foraged in the afternoon. Vigilance did not differ between the indoor and outdoor habitat, and they were more active in the outdoor habitat. Maximum humidity played a role in the frequency of resting outdoors, locomotion outdoors, and preening indoors. The frequency of preening while in the outdoor habitat was affected by the availability of sunlight. While the variation in visitor number between consecutive days did not have an effect on behavior, the number of visitors affected resting, preening, and foraging. Noise levels were found to affect resting and locomotion. Surprisingly, vigilance was not affected by any of the visitor variables. Aggression was affected by temperature and humidity, but habitat itself did not have an effect. The various visitor variables were found to affect all aggressive behaviors. The environmental and visitor effects are challenging to manage due to the habitats location and high visitor traffic within the zoo however we did not find significant effects on vigilance which suggest the flamingos are not distressed by visitors.

Keywords: activity budget, zoo, captive breeding, welfare, Caribbean flamingo, flamingo, *Phoenicopterus ruber*, environment, weather, visitor effect

Introduction:

Entertainment is the best known role of zoos however they also play important roles in education, conservation, and research (Hosey et al., 2013). Zoos are constantly seeking to learn more about the animals in their care to ensure their general and species-specific needs are being met (Hosey et al., 2013). Welfare has been defined as the ability of an animal to cope with changes to its environment, and is a spectrum ranging from very good to very poor (Barber and Mellen, 2013; Broom, 2001). The study of animal welfare is subjective but there have been methods put in place to study welfare more objectively in agricultural animals and these methods have been expanded to include captive environments (Melfi, 2009). Welfare is generally assessed using three categories: behavior, physiological condition, and health (Melfi, 2009). The most common method of studying welfare is to look for indicators of poor welfare such as high frequency of disease or injury, reduced reproduction or lifespan, stereotypical behaviors such as pacing, and changes in behavior when compared to their wild counterparts (Broom, 1991; Frumkin et al., 2016; Melfi, 2009; Shannon, 2000). While these indicators are important, we must also study the effects of the captive environment which can include weather and visitor effects (Melfi, 2009).

Studies in captivity are biased towards the more charismatic animals, such as apes and big cats, even though they are not necessarily the most abundant animals in captivity (Melfi, 2009). Flamingos are one of the most popular and abundant animals in captivity however there have been few studies conducted on captive flamingos (Bildstein et al., 1993; King, 2000; Melfi, 2009; Rose et al., 2014). Not all species will adapt to captivity in the same way or have the same needs so it is important to study a wide range of species (Melfi, 2009). Captive flamingos often have inconsistent or reduced reproduction, which can be an indicator of poor welfare, so they are an important species to study (Broom, 1991; Farrell et al., 2000; Melfi, 2009).

Wild flamingos have erratic reproduction that depends on unpredictable environmental and social cues such as food availability, rainfall, photoperiod, and the sex ratio, age structure, and size of the colony (King, 2008b; Pickering et al., 1992). While zoos can control many of these factors, captive breeding of flamingos remains a challenge (Farrell et al., 2000). As reduced reproduction can be an indicator of poor welfare, it is important to study the potential causes of reduced reproduction and the needs of captive flamingos to ensure good welfare (Melfi, 2009).

One way to study welfare in captivity is to create an activity budget to determine the amount of time spent on specific behaviors and observe for stereotypical behaviors (Rose et al., 2014). Captive activity budgets can then be compared to the activity budgets of their wild counterparts to assess welfare; if there are significant differences between activity patterns, namely an increase in stereotypies or abnormal behaviors, it could be an indicator of poor welfare in captivity (Melfi, 2009; Rose et al., 2014). Wild and captive flamingos have been found to spend the majority of their time foraging, preening, and resting (Bildstein et al., 1991; Espino-Barros and Baldassarre, 1989a; Rose et al., 2014). Researchers have shown that flamingos spend a similar amount of time feeding in both wild and captive populations (Bildstein et al., 1993). We can also compare their activity patterns with time of day and time of year. The resting and preening behaviors observed in captivity were found to occur most during the middle of the day, which is consistent with wild flamingo activity patterns (Rose et al., 2014). Wild flamingos appear to forage during the late afternoon and night, and are vigilant most often during sunrise and sunset (Bildstein et al., 1991; Britton et al., 1986). Activity patterns can differ throughout the year due to weather, the nonbreeding or breeding season, and in captivity it can depend on whether the flamingos are kept indoors or outdoors or if they are moved between habitats depending on the weather. Activity budgets of wild Caribbean flamingos in Yucatán, Mexico, have been studied during both the breeding and nonbreeding season (Espino-Barros and Baldassarre, 1989a; Espino-Barros and Baldassarre, 1989b). They were found to spend the majority of their time feeding, resting, and preening during both seasons however the proportion of time spent on each activity varied by season (Espino-Barros and Baldassarre, 1989a; Espino-Barros and Baldassarre, 1989b).

Environmental variables such as temperature, humidity, light availability, and intensity of UV radiation can also affect the activity patterns of flamingos. Caribbean flamingos have been found to be more active when there is more sunlight (Peluso et al., 2013). They were also found to be more aggressive during warmer weather, and when there is a higher intensity of UV radiation which was measured using a UV index (Peluso et al., 2013). Warmer weather can also affect the resting behavior of flamingos through determining whether they rest on one leg or two; in warmer weather they will rest on one leg for shorter time periods because they are not trying to conserve body heat (Bouchard and Anderson, 2011). Researchers also found that flamingos

are more likely to rest in a bipedal stance when there is higher average wind speed which they suggest is done to increase stance stability (Bouchard and Anderson, 2011).

Wild populations of flamingos have been studied to determine if flamingos are stressed by the presence of tourists. Stress has been defined by Morgan and Tromborg (2007) as experiencing demands to which an animal is unable to adequately respond based on their current resources. Flamingos were found to become alert, or vigilant, when people walked or jogged past but continued to forage (Yosef, 2000). On the other hand, when tourist vehicles approached and the tour group exited the vehicle, the flamingos decreased distance between conspecifics (Yosef, 2000). This decreased distance between individuals is known as seeking protection in numbers and is a behavior commonly done in the presence of a threat such as a predator (Hamilton, 1971; Yosef, 2000). As in the wild, when captive flamingos are stressed, they will huddle together in a small space to seek safety in numbers (Hamilton, 1971; King, 2008b; Yosef, 2000). This huddling behavior could lead to decreased reproduction through egg loss if the flamingos were to congregate near the nesting sites where they could step on the eggs or knock them off the nests (King, 1994). There is also the possibility of the flamingos abandoning their nests when visitors or animal keepers are nearby (Stevens et al., 1992). Should the pair abandon their nest, another pair may choose to take over the nest and displace the egg of the original pair (Stevens et al., 1992). Egg loss is one of the most common causes of low reproductive success in captive flamingos (King, 1994).

Another potential source of stress in captivity is exposure to noise levels which were found to be significantly higher than the noises of a natural environment (Morgan and Tromborg, 2007). Distress responses have been observed following loud, sudden, and unpredictable noises in captivity including caretaker sounds and visitor noise (Morgan and Tromborg, 2007; Ogden et al., 1994). As the number of visitors increases, the level of visitor noise will also increase (Morgan and Tromborg, 2007). Increased visitor noise has been found to increase vigilance behavior in koalas (*Phascolarctos cinereus*) (Larsen et al., 2014), harbor seals (*Phoca vitulina*) (Stevens et al., 2013), and little penguins (*Eudyptula minor*) (Sherwen et al., 2015). While visitor presence in zoos is expected and animals have the potential to habituate to visitor presence, not all species will adapt and there is always the possibility of visitor caused stress (Mason, 2010; Studer-Thiersch, 2000).

This study assessed the activity patterns, environmental factors, and visitor effects to provide Zoo de Granby with methods to improve flamingo welfare and increase reproductive success. Based on previous research, we predicted that (1) the Zoo de Granby flamingo flock activity budget would differ from that of a wild population, (2) the flamingos would be more active in the afternoon compared to the morning, (3) we predicted that they will be more active in the outdoor habitat and rest more in the indoor habitat, (4) they would preen and forage more when it is sunny and warm, (5) they would exhibit increased vigilance, and locomotion with increased visitor number, visitor variation, and noise levels (6) they would be more aggressive when it is warmer, and (7) they would be more aggressive with increased visitor number variation, and noise levels.

Methods:

Study subjects:

A flock of captive Caribbean flamingos (*Phoenicopterus ruber*) was studied at Zoo de Granby which is located in Southern Québec. The flock consists of 28 individuals (♂=15, ♀=13) between the age of 7 and 38 years old (mean = 20.2). Individual flamingos could be identified from the unique leg bands with color and number combinations (Appendix A). Each individual was also entered in the Species360 Zoological Information Management System (ZIMS) database which provided information from zoo records regarding age, sex, and history of the individuals. Unfortunately the history of individual flamingos is often unknown; from the Zoo de Granby flock there are 16 flamingos wild-caught from Cuba, 1 was of unknown origin, and 11 were captive-born. For the purpose of this study, flamingos will be referred to using their band number followed by their ZIMS number and sex (ex: 27-B98004♂).

The flamingos were fed a regular diet of Mazuri Flamingo Complete (#5644), and during a week at the start of the breeding season (December 2015) they were gradually switched to the Mazuri Breeder Reproduction (#5645). They were fed once a day by the zookeepers between 10:30am and 1:30pm. They had multiple types of feeders in their outdoor habitat including a large feeding trough at the edge of the water, seven artificial nests along the shore, and a duck-proof standing feeder in the water near the shoreline. Metal food trays were used in the indoor habitat and were strategically placed to decrease the disturbance to the flock from the presence of an animal caretaker in the habitat (Appendix B).

Study area:

Data were collected at both the outdoor and indoor habitats, depending on time of year. Observations from the outdoor habitat were done from a public boardwalk along the edge of the habitat. The outdoor habitat features a large pond (~686m²) which covers approximately 71% of the habitat, and ~286m² of ground cover accessible to the flamingos (Appendix C). Indoor habitat observations were done from inside the habitat using a blind to minimize the presence of the observer. The indoor habitat is a building at the edge of the outdoor habitat which is about 66m². It consists of a shallow water basin, feeding area, and nesting area (Appendix B). There are mirrored panels halfway up the walls to encourage breeding through the illusion of a larger flock (O'Connell-Rodwell et al., 2004). The indoor habitat was a controlled environment in which there were monitored protocols for temperature (Table 1.1), and photoperiod (Table 1.2). The nesting substrate was maintained through the addition of peat moss, hay, clay, and water every 3 to 10 days. The flamingos shared the indoor habitat with six cattle egrets (*Bubulcus ibis*). To simplify the recording of locations, the habitats were split into functional zones including land cover, foraging areas, nesting areas, and water areas which were subdivided in the outdoor habitat since it covered such a large area (Appendix B, C).

Data Collection:

Data collection took place between July 2015 and June 2016. During the warmer months (July-October 2015, May-June 2016) the flamingos were in their outdoor habitat and during the colder months (October 2015 – May 2016) the flamingos were in their indoor habitat. Outdoor observations were conducted daily, and indoor observations were conducted 1-2 days per week. Based on the timing of the breeding season according to Shannon (2000), the study was divided into two time periods of “2015” also considered the nonbreeding season (July - December 2015), and “2016” (January - June 2016) which could also be considered the breeding season.

Behavioral data were collected using focal sampling where the focal individual was chosen based on visibility and ease of identification. Data was recorded every 15 seconds during a 10 minute focal period. The observer was notified every 15 seconds using a cellphone application called Interval Timer AD (halmi.sk, 2015). An ethogram adapted from King et al. (2005)(Table 1.3) was used to define common behaviors (resting, preening, foraging,

locomotion, vigilance) as well as aggressive behaviors (neck swaying, sparring, etc.). Agonistic behaviors were collected opportunistically during focal periods. If an agonistic encounter (AE) involved more than two flamingos, it was counted as separate encounters between the individuals directly interacting. Behavioral data was used to create an activity budget which we compared to a wild activity budget published by Espino-Barros and Baldassarre (1989a) to determine if the flamingos are behaving differently than their wild counterparts. Our activity budget also allowed us to analyze the flamingos' activity patterns throughout the day and in the different habitats.

Environmental variables were collected in both the outdoor and indoor habitats. Outdoor environmental variables included cloud cover, maximum temperature, and maximum humidity. Cloud cover was collected using a ranking system of “cloudy”, “mostly cloudy”, “mostly sunny”, and “sunny”. Maximum temperature and maximum humidity were collected from the Weather Network (The Weather Network, 2016). Indoor environmental variables included maximum temperature and maximum humidity, which were recorded from a digital thermometer in the habitat.

The effect of noise level on the frequency of behaviors (rest, preening, foraging, locomotion, vigilance) and aggression was studied to assess possible visitor effects. While it was not possible to collect the number of visitors at the flamingo habitat itself due to the sheer number of visitors and the design of the habitat, we were able to collect the total number of zoo visitors per day which were recorded by the zoo admission staff. We also calculated the variation in visitor number between days using the visitor data. The flamingo habitat is in a high visitor traffic location within Zoo de Granby which means the habitat is subject to various noise levels from visitors. The noise level was collected before each focal sample. The ambient noise level was determined using a Reed Model ST-805 Sound Level Meter. The meter was set in the “slow” position to collect the average sound level, and was “A” weighted to collect the general sound level and frequencies in the area most sensitive to the human ear (500 to 10,000Hz). While research has yet to determine the auditory range for the Caribbean flamingo, birds in general are sensitive to sounds between 1,000 and 5,000Hz (Beason, 2004; Martin, 2012). The sound level meter was set to low (LO)(30-100dB) as it would automatically switch to high (HI)(60-130dB) if the audio was outside the original measurement range.

Data Analysis:

All data analyses were done using R statistical software (R Core Team, 2016). The effects of habitat and time of day on mean activity patterns were analyzed using two-tailed Welch's t-tests (also known as an unequal variances t-test). The comparison of the captive and wild activity budgets during the nonbreeding and breeding season required a one sample student's t-test. The mean behavior frequencies for the captive activity budget data were compared to the mean behavior frequencies of a wild population obtained from a study by Espino-Barros and Baldassarre (1989a). Unfortunately we were unable to find a captive activity budget to compare with ours. Bar plots were created using the aggregate and bar plot functions as described on the R-bloggers website (Wetherill, 2015). Behavior and aggression data was analyzed to see if there was an effect from visitor number, visitor variation, or noise level. Chasing and bumping were removed from the indoor analyses because there were no chasing or bumping behaviors done while the flamingos were indoors. The behavior and aggression analyses were done using generalized linear models (GLM) with a log link using the negative binomial distribution function from the MASS package (Venables and Ripley, 2002). Generally count data requires a Poisson distribution however our data was over dispersed and required a negative binomial distribution. GLMs were done for individual behaviors (rest, preen, forage, locomotion, vigilance), and aggressive behaviors (AE, neck swaying, sparring, pecking, hooking, chasing) in which the daily frequencies of the behaviors were the response variable and the environmental variables (maximum temperature, maximum humidity, cloud cover) were the predictor variables. To choose the most parsimonious model, we used backward selection using the Akaike information criterion (AIC) as a criterion for model fit. We ran generalized linear models with all possible environmental variable combinations and used the model with the lowest AIC value (Burnham and Anderson, 2002). Models with a ΔAIC of less than 2 were also analyzed using GLMs to check for potentially significant variables that were not included in the most parsimonious model (Burnham and Anderson, 2002)(Appendix D, E, F, G).

Results:

During the nonbreeding season, we found that the Zoo de Granby flamingos rested ($t(698) = 10.73, p < 0.001$), preened ($t(698) = 10.03, p < 0.001$), and moved around their habitat ($t(698) = 2.18, p = 0.03$) significantly more than the wild population (Figure 1.1). They also

foraged less ($t(698) = -11.345, p < 0.001$) and were vigilant less often ($t(698) = -2.22, p = 0.03$) in captivity (Figure 1.1). During the breeding season, the Zoo de Granby flock rested significantly more ($t(314) = 13.93, p < 0.001$) and foraged significantly less ($t(314) = -19.65, p < 0.001$) than the wild population (Figure 1.2). Preening, locomotion, and vigilance were not significantly different between the captive and wild populations during the breeding season (all $p > 0.05$; Figure 1.2).

We found that when comparing the morning and afternoon time periods, time spent preening, moving, and vigilant were not significantly different (all $p > 0.05$). We also found that the flamingos rested significantly more in the morning ($t(707) = 2.98, p = 0.003$) and foraged significantly more in the afternoon ($t(803) = -3.46, p < 0.001$) (Figure 1.3). When we compared the indoor and outdoor habitat, it coincided with summer and winter, as well as the nonbreeding and breeding seasons respectively. We found that the flamingos' locomotion and vigilance was not significantly different between the indoor and outdoor habitats (all $p > 0.05$). There was a significant difference in the amount of time they spent resting, preening, and foraging depending on the habitat (all $p < 0.05$). They rested significantly more ($t(539) = -6.59, p < 0.001$) in the indoor habitat, while they preened ($t(730) = 4.87, p < 0.001$) and foraged ($t(785) = 7.15, p < 0.001$) significantly more in the outdoor habitat (Figure 1.4).

When looking at the effect of environmental variables on frequency of behavior in the outdoor habitat using GLMs we found percent time resting to decrease ($\beta \pm SE; -0.014 \pm 0.004, N = 62, p = 0.0018$) with the maximum percent humidity. The flamingos preened significantly less when it was sunny than when it was cloudy (estimated difference $\pm SE; -0.38 \pm 0.15, N = 62, p = 0.015$; Figure 1.5). Locomotion increased with the maximum percent humidity ($\beta \pm SE; 0.017 \pm 0.004, N = 62, p < 0.001$). The maximum percent humidity did not affect time spent in foraging ($p = 0.13$) or vigilance ($p = 0.08$). Indoor maximum temperature was the variable which best explained the variation in foraging behavior however the relationship was not significant ($p = 0.82$). Maximum humidity was not significantly related to time spent in resting, locomotion, or vigilance (all $p > 0.05$). We did find a significant relationship between preening ($\beta \pm SE; 0.02 \pm 0.008, N = 30, p = 0.048$) and maximum humidity.

The number of visitors per day negatively affected time spent resting ($\beta \pm SE; -4.33e-5 \pm 1.70e-5, N = 95, p = 0.011$), positively affected time spent preening ($\beta \pm SE; 2.52e-5 \pm 1.11e-5,$

N = 95, $p = 0.023$), and foraging ($\beta \pm SE$; $4.99e-5 \pm 2.06e-5$, N = 95, $p = 0.015$), but did not affect the frequency of locomotion ($p = 0.13$) or vigilance ($p = 0.27$). The variation in number of visitors between consecutive days did not affect any of the behavioral variables (all $p > 0.05$). We found that resting ($\beta \pm SE$; -0.03 ± 0.16 , N = 42, $p = 0.04$) and locomotion ($\beta \pm SE$; 0.04 ± 0.01 , N = 42, $p = 0.005$) were significantly affected by noise level (mean = 56.4dB, SD = 6.6), but not the frequency of preening, foraging, or vigilance (all $p > 0.05$).

Habitat did not have a significant effect on the frequency of AE, or the individual behaviors of neck swaying, sparring, pecking, and hooking (all $p > 0.05$; Figure 1.6).

We found that for the frequency of AE, the most parsimonious GLM based on AIC selection for outdoor environmental variables included cloud cover and maximum humidity. The frequency of AE was significantly less when it was sunny than when it was cloudy (estimated difference $\pm SE$; -0.46 ± 0.17 , N = 62, $p = 0.0058$) and AE was significantly lower with increased maximum humidity ($\beta \pm SE$; -0.02 ± 0.003 , N = 62, $p < 0.001$). The second most parsimonious model, which was within 2 ΔAIC , found the frequency of AE to significantly decrease with temperature ($\beta \pm SE$; -0.02 ± 0.01 , N = 62, $p < 0.032$). The frequency of neck swaying increased ($\beta \pm SE$; 0.07 ± 0.03 , N = 62, $p = 0.008$) with an increase in maximum temperature. Temperature did not affect sparring, pecking, or chasing behavior (all $p > 0.05$). Maximum humidity was the most parsimonious model as predicted by the lower AIC for hooking behavior, and outdoor hooking was shown to decrease significantly with increasing maximum humidity ($\beta \pm SE$; -0.03 ± 0.01 , N = 62, $p = 0.001$). Another model within 2 ΔAIC found that cloud cover had a significant effect on the frequency of hooking behavior; the flamingos were less likely to hook when it was mostly cloudy (estimated difference $\pm SE$; -1.08 ± 0.53 , $p = 0.04$) or sunny (estimated difference $\pm SE$; -0.95 ± 0.48 , $p = 0.005$) compared to when it was cloudy.

In the indoor habitat, maximum temperature was found to significantly increase AE frequency ($\beta \pm SE$; 0.20 ± 0.09 , N = 33, $p = 0.02$). The most parsimonious model as predicted by lower AIC for neck swaying included maximum temperature ($\beta \pm SE$; 1.36 ± 0.61 , N = 33, $p = 0.03$) and maximum humidity ($\beta \pm SE$; -0.05 ± 0.03 , N = 33, $p = 0.14$), although only maximum temperature showed a significant effect. We did not find maximum temperature or maximum humidity to have a significant effect on sparring or pecking behavior in the indoor habitat (all $p >$

0.05). The most parsimonious model for hooking behavior as predicted by the lower AIC included maximum temperature which showed a significant effect ($\beta \pm SE$; 0.54 ± 0.0254 , $N = 33$, $p = 0.03$).

Visitor effect on aggression was studied using daily visitor numbers, visitor variation between consecutive days, and noise level prior to a focal. All data analyzed for this portion is from the summer when the flamingos were outdoors. The number of visitors did not affect the number of AE, neck swaying, or sparring (all $p > 0.05$). Frequency of pecking ($\beta \pm SE$; $7.47e-5 \pm 3.53e-5$, $N = 62$, $p = 0.03$), chasing ($\beta \pm SE$; $2.09e-4 \pm 7.16e-5$, $N = 62$, $p = 0.004$), and bumping ($\beta \pm SE$; $2.87e-4 \pm 9.16e-5$, $N = 62$, $p = 0.002$) increased with the number of visitors, while hooking decreased ($\beta \pm SE$; $-1.06e-4 \pm 3.57e-5$, $N = 62$, $p = 0.003$). The variation in number of visitors between consecutive days did not significantly affect the number of AE, nor the frequency of neck swaying, pecking, chasing, hooking, or bumping (all $p > 0.05$). The frequency of sparring increased with visitor variation ($\beta \pm SE$; $6.62e-5 \pm 3.12e-5$, $N = 62$, $p = 0.034$).

The total number of AE ($\beta \pm SE$; -0.02 ± 0.009 , $N = 42$, $p = 0.015$) was negatively affected by noise level. In terms of individual aggressive behaviors, we found that sparring, chasing, hooking and bumping (all $p > 0.05$) were not affected by noise level (mean = 56.4 dB, SD = 6.6). Neck swaying ($\beta \pm SE$; 0.01 ± 0.03 , $N = 42$, $p = 0.69$) and pecking ($\beta \pm SE$; 0.08 ± 0.04 , $N = 42$, $p = 0.04$) were positively affected by noise level.

Discussion:

We predicted that the activity budgets of the Zoo de Granby captive population and a wild population would differ during both the nonbreeding and breeding seasons. In support of this prediction, we found several significant differences between the populations. During the nonbreeding season, the captive population rested, preened, and moved around more than their wild counterparts. On the other hand, the captive population foraged and was vigilant less than the wild population. These findings could possibly be explained by the different levels of predation pressure on the captive and wild populations, provided the captive flamingos are not viewing visitors as predators. The captive population likely had lower occurrences of vigilance behavior because they are not exposed to predators whereas wild flamingos are at risk of predation and therefore must remain vigilant in order to detect nearby predators (Beauchamp and McNeil, 2003; Huntingford, 2004; Kelley et al., 2006). This would also mean that the flamingos

are free to spend more time doing low-energy behaviors, such as resting and preening, and exploring their habitat without the risk of predation (Huntingford, 2004). The decreased frequency of foraging behavior in captivity is not surprising considering captive animals are consistently provided with high quality food sources and do not need to spend time searching for food unlike wild animals (Huntingford, 2004; Kelley et al., 2006).

Similar results were found during the breeding season; the captive population continued to rest more and forage less than their wild counterparts. However, we found that preening, locomotion, and vigilance were not significantly different between the populations. The lack of differences between preening and locomotion behaviors could be because both captive and wild populations switch to reproductive behaviors during the breeding season. The finding that vigilance is no longer significantly less than the wild population during the breeding season suggests the flamingos are more susceptible to disturbances in their indoor habitat or that wild flamingos are less vigilant during the breeding season due to the congregation of flamingos at breeding grounds (Cézilly and Johnson, 1995; King, 1994).

Our second prediction was that the flamingos would be more active in the afternoon relative to the morning. The flamingos were found to rest more in the morning and forage more in the afternoon, which is likely connected to their feeding schedule which ranged from 10:30am and 1:30pm. This finding is consistent with both captive and wild populations which have been found to spend the majority of their day resting and preening, and forage most during the afternoon and night (Bildstein et al., 1991; Britton et al., 1986; Rose et al., 2014).

We predicted that habitat would have an effect on behavior in that the flamingos would be more active in the outdoor habitat. The time spent in the indoor habitat coincides with their breeding season (Shannon, 2000) so it is reasonable to expect that they would shift from primarily regular behaviors outdoors to include reproductive behaviors indoors. Vigilance was not found to be significantly different between the indoor and outdoor habitat which suggests that the presence of cattle egrets and an observer in the indoor habitat were no more disturbing the flamingos than visitors around the outdoor habitat. We found that they were more active in preening and foraging behaviors in the outdoor habitat, and they spent more time resting indoors. A possible explanation for this is the exposure to more sunlight in the outdoor habitat; the indoor

habitat features skylights however there is supplemental lighting required. Caribbean flamingos have been shown to increase activity when exposed to sunlight (Peluso et al., 2013).

Following the information that flamingos are more active in sunlight, we predicted that flamingos would preen and forage more when it is sunny and warm outside. Contrary to our prediction, we found that flamingos preened less when it was sunny compared to when it was cloudy, and rested more when it was humid. We also found no significant change in foraging behavior although the foraging patterns are likely explained by the feeding schedule and time of day rather than environmental variables. Perhaps the unexpected behaviors are due to the lack of predation in captivity which allows the flamingos to adjust their activity patterns to rest during the humid time of day and become active during more favorable environmental conditions later in the day (Bildstein et al., 1991; Huntingford, 2004). While the flamingos were indoors, most behaviors were not significantly affected by temperature or humidity however preening behavior increased with humidity. A possible explanation is the increased moisture in the air would lead to the flamingos preening more (Henson et al., 2007). One reason for this increase due to moisture is that in humid environments, there is a higher risk of ectoparasites which leads to anti-parasite defense behaviors such as preening (Moyer et al., 2002).

We predicted that if there was a visitor effect, the visitor variables (number, variation, noise level) would cause increased locomotion and vigilance. Variation in visitor attendance between consecutive days did not affect any behavior frequencies. Locomotion was not affected by visitor count but did increase with noise level. Resting behavior was negatively affected by visitor count and noise level. Preening and foraging increased with visitor count but were not affected by noise level. The most surprising result was that vigilance was not affected by any of the visitor variables. Vigilance is a behavior commonly used to interpret whether there is a stressful effect of visitors on animals in captivity. Several studies have used changes in vigilance behavior to assess visitor effects in many species including captive cheetahs (*Acinonyx jubatus*) (O'Donovan et al., 1993), ungulates (Thompson, 1989), orangutans (*Pongo pygmaeus*) (Birke, 2002), and many other primate species (Hosey and Druck, 1987). Visitor effects are not consistently found between species and the results can sometimes be difficult to interpret. Researchers did not find negative visitor effects in cheetahs (O'Donovan et al., 1993) but did find minor changes in vigilance were found in ungulates although it was unclear if these changes

were signs of stressful reactions to the visitors (Thompson, 1989). Orangutans were found to increase vigilance and avoidance behaviors with increased visitor number but particularly with increased visitor noise (Birke, 2002). Twelve primate species were studied and found to increase locomotion and other active behaviors, including those directed towards the visitors, with increased visitor presence suggesting a visitor effect but it is difficult to say whether it is a positive or negative effect (Hosey and Druck, 1987). The observed shift from resting to active behaviors could suggest the flamingos are negatively affected by noise level or visitor presence and are actively moving away from visitor noise (Anderson et al., 2002; Chamove et al., 1988). The flamingos do have a retreat area in the habitat should they wish to be further away from the visitors, however this area was rarely used so we do not believe the flamingos were actively avoiding visitor noise. A study on oldfield mice (*Peromyscus polionotus subgriseus*) found that an increased number of generations in captivity decreased the likelihood of an animal seeking refuge from a predator (McPhee, 2004). The majority of the Zoo de Granby flock is wild-caught or their pedigree is unknown so it is difficult to assess this theory. It is possible that learned irrelevance has occurred and they have learned that visitors are a neutral stimulus which does not directly affect them so they do not feel the need to seek refuge from visitors (Baker, 1976).

Habitat did not have a significant effect on any aggressive behaviors, nor the frequency of aggression however the environmental variables did affect the occurrence of aggression. Based on previous research about aggression and environmental conditions, we predicted that the flamingos would be more aggressive during warmer weather (Peluso et al., 2013). We found that the frequency of neck swaying outdoors increased with warmer temperatures; however the frequency of AE decreased with warmer temperature. We also found increasing temperature to cause an increase in the frequency of AE, neck swaying, and hooking indoors. The effect of hot temperatures on behavior, and specifically aggression, has been observed for centuries in both humans and animals (Anderson et al., 2000; Peluso et al., 2013; Reifman et al., 1991). Our findings show that certain aggressive behaviors can be positively affected by temperature (Anderson et al., 2000). We also found that hooking behavior decreased when it was mostly cloudy or sunny, and the number of AE while outdoors was significantly lower during sunny periods with high humidity. Previous research has shown that high humidity has been associated with increased resting or inactive behaviors, which supports our findings of decreased aggressive behaviors during periods of high humidity (Howarth and Hoffman, 1984).

Our last prediction was that flamingos would be more aggressive with increased visitor number variation, and noise levels. We found that the number of visitors per day did not have an effect on some aggressive behaviors however the frequency of pecking, chasing, and bumping increased with visitor number while hooking decreased. The frequency of sparring increased with visitor variation between consecutive days. The number of AE decreased with increasing noise level, while neck swaying and pecking behaviors increased. Our findings show that while some aggressive behaviors decrease based on visitor variables, most show an increase in aggression. These results are consistent with previous research in which petting zoo animals exposed to high visitor density exhibited increased frequency of aggression if they were unable to move away from the visitors (Anderson et al., 2002).

Conclusions:

While we predicted that captive and wild populations would have similar activity budgets, we found that many behaviors differed which is likely due to the different levels of predation and availability of food. We found the flamingos were more likely to rest in the morning and forage in the afternoon. They were more active in the outdoor habitat, but did not differ in vigilance. We found several effects of weather on the frequency of behaviors, and found that most behaviors were affected by visitor number or noise levels. Contrary to our expectation, vigilance was not affected by any of the visitor variables. Habitat did not affect aggression but several aggressive behaviors were affected by temperature and humidity. Visitor number caused an increase in most aggressive behaviors, while visitor variation only affected sparring. Noise level caused an increase in both neck swaying and pecking behavior although the overall frequency of AE decreased. Based on our findings, we believe there is an effect of captivity on flamingo behavior; however we do not believe that our results suggest a welfare issue at Zoo de Granby. There was no effect of visitor variables on vigilance, which could suggest the flamingos have habituated to the presence of visitors. While we did see an increase in locomotion frequency with noise level, we do not believe this is an indicator of poor welfare as the flamingos have a retreat area within their habitat that was not used to avoid visitors. We recommend that Zoo de Granby continue to investigate the Caribbean flamingo welfare with future research on the effect of animal caretaker presence in the habitat, and the effect of wing clipping on the population mechanisms. We believe these two aspects could be affecting the reproductive

success of the flock and should be investigated in order to stabilize and increase reproductive success at Zoo de Granby.

Tables:

Table 1.1: Protocol determined by Zoo de Granby as the acceptable temperature range for each month the flamingos are in the indoor habitat.

Month	Temperature (°C)
October	14.0
November	19.0
December	22.4 – 24.1
January	21.9 – 23.1
February	22.1 – 23.5
March	22.1 – 26.1
April	24.7 – 25.9
May	23.7 – 26.1

Table 1.2: Protocol at Zoo de Granby to gradually increase the number of daylight hours in the flamingo indoor habitat.

Dates	AM	PM	Daylight Hours
November 7 – November 13	6:30	6:45	12 h 15 min.
November 14 – November 20	6:30	7:00	12 h 30 min.
November 21 – November 27	6:30	7:15	12 h 45 min.
November 28 – December 4	6:15	7:15	13 h
December 5 – December 11	6:15	7:30	13 h 15 min.
December 12 – December 18	6:15	7:45	13 h 30 min.
December 19 – December 25	6:00	7:45	13 h 45 min.
December 26 – January 1	6:00	8:00	14 h 00

Table 1.3. Ethogram used to define various flamingo behaviors for the focal sampling, adapted from King et al. (2005). Several behaviors were added to the original ethogram and are individually cited in the definitions.

Behavior	Definition
General Behaviors	
Resting/Sleeping	Flamingos can rest standing up or sitting with their legs tucked under themselves. When standing, they tend to rest on one leg with the other tucked under them, and their neck is twisted to rest in their back feathers.
Preening	A grooming behavior in which a flamingo will run its bill over or through its feathers.
Foraging/Feeding	The process of searching for or consuming food or water. Behaviors include: Mud-dredging: running their bill along a muddy substrate in order to filter nutrients from the mud. Filter-feeding: Flamingos feed with their bills upside down in the water or mud, using their tongue to push water through the lamellae which filter food particles. Ground feeding: the flamingo picks up food from the ground rather than filter feeding. Treading/stamp feeding: the flamingos alternate lifting and placing their feet on the substrate to stir up nutrients in the water. They may slowly spin in a circle while treading or may tread in place. (Schmitz and Baldassarre, 1992)
Locomotion	Any form of locomotion (walking, running, flying, and swimming) that is not motivated by aggression or fear.
Vigilance	Performed when there is a disturbance or unknown occurrence nearby causing the flamingos to become alert. They stand straight and extend their neck vertically to survey their surroundings. This posture is generally done as a group, and can be joined with honking vocalizations.
Standing	An inactive behavior in which a flamingo stands still but is not resting or vigilant.
Stretching	An extension of a leg or wing(s) while standing or resting. A sighing vocalization can sometimes accompany a stretch.
Attempted flight	Flamingos would stand in the water or on land facing into the wind and run while flapping their wings in an attempt to fly. The flamingos were pinioned or wing clipped so they were unable to fly. (Lindgren and Pickering, 1997)
Agonistic Behaviors	
Agonistic encounter	An interaction between two individuals which features aggressive behaviors or displays

Unresolved interaction	Agonistic encounters with not a clear winner or loser (Hughes, 2015)
Resolved interaction	Agonistic encounters with a clear winner or loser (Hughes, 2015)
Winner	The individual which is dominant in the aggressive encounter and continues to be aggressive while the other retreats
Loser	The subordinate individual in the aggressive encounter who retreats and withdraws from the encounter
Neck swaying	The neck is extended horizontally and swayed back and forth while facing another individual. It is often performed before a fight or to deter an approaching individual. (Lindgren and Pickering, 1997)
Sparring	Two or more flamingos perform neck swaying behaviors towards each other while barely avoiding contact between their bills
Pecking	A flamingo pecks at another individual
Chasing	One flamingo chases after another individual
Fleeing	One flamingo runs away from another individual who is displaying aggressive behaviors
Bumping	A flamingo purposefully bumps chest first into the flank of another individual. The bumped individual usually leaves without retaliating. (Lindgren and Pickering, 1997)
Hooking	The neck is extended fully toward another flamingo while the scapular and the back feathers are raised. It is often performed before a fight.

Figures:

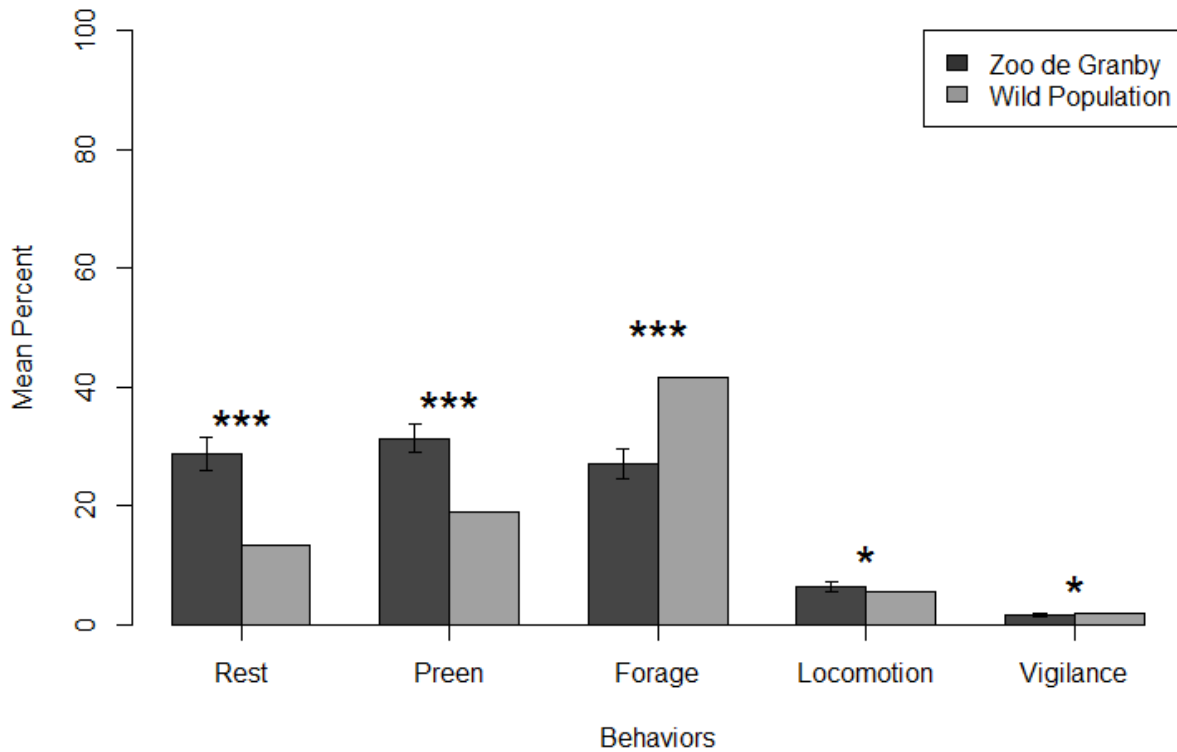


Figure 1.1. Comparison of captive and wild activity budgets in the nonbreeding season. The activity budget for the Zoo de Granby flamingo flock was compared budget to the activity budget means of a wild population (Espino-Barros and Baldassarre, 1989a) using a one sample student's t-test since the variance of the wild population was not available. The dark gray bars represent the mean behavior focal percentages for Zoo de Granby during the nonbreeding season, and the light gray bars represent the activity means of the wild population during the nonbreeding season. The lines represent the 95% confidence intervals. * for $p < 0.05$, *** for $p < 0.001$.

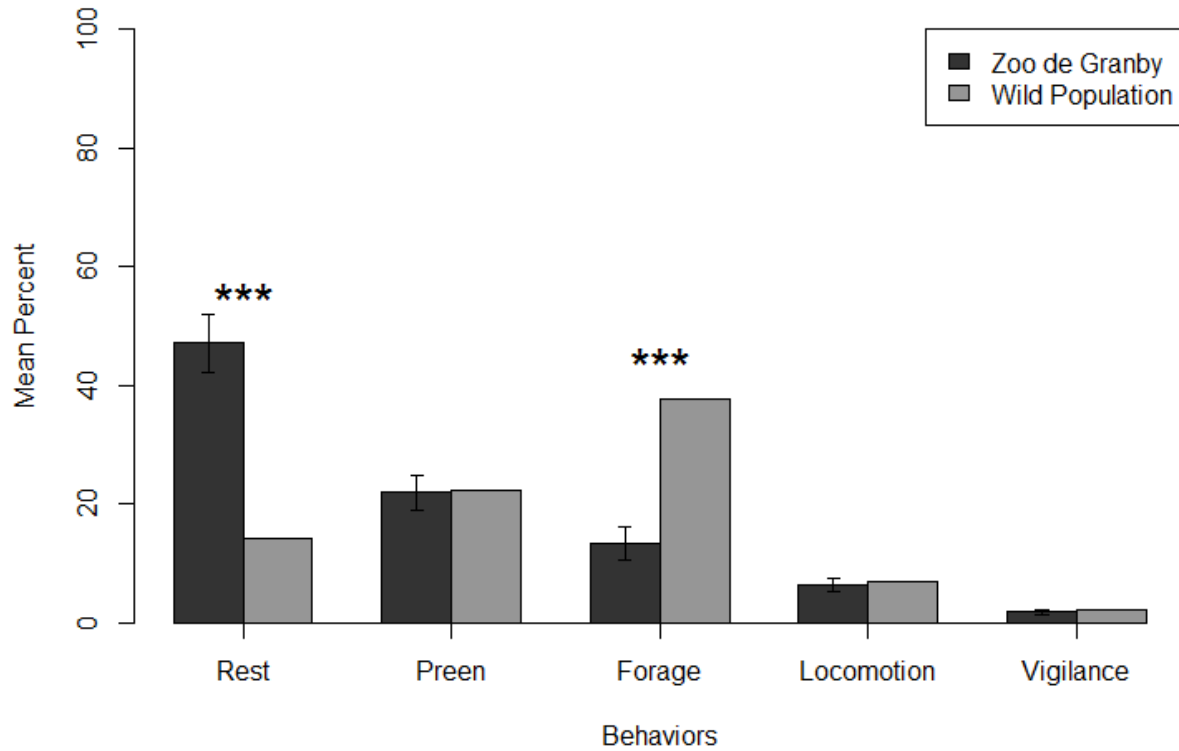


Figure 1.2. Comparison of captive and wild activity budgets in the breeding season. The activity budget for the Zoo de Granby flamingo flock was compared budget to the activity budget means of a wild population (Espino-Barros and Baldassarre, 1989a) using a one sample student's t-test since the variance of the wild population was not available. The dark gray bars represent the mean behavior focal percentages for Zoo de Granby during the nonbreeding season, and the light gray bars represent the activity means of the wild population during the nonbreeding season. The lines represent the 95% confidence intervals. *** for $p < 0.001$.

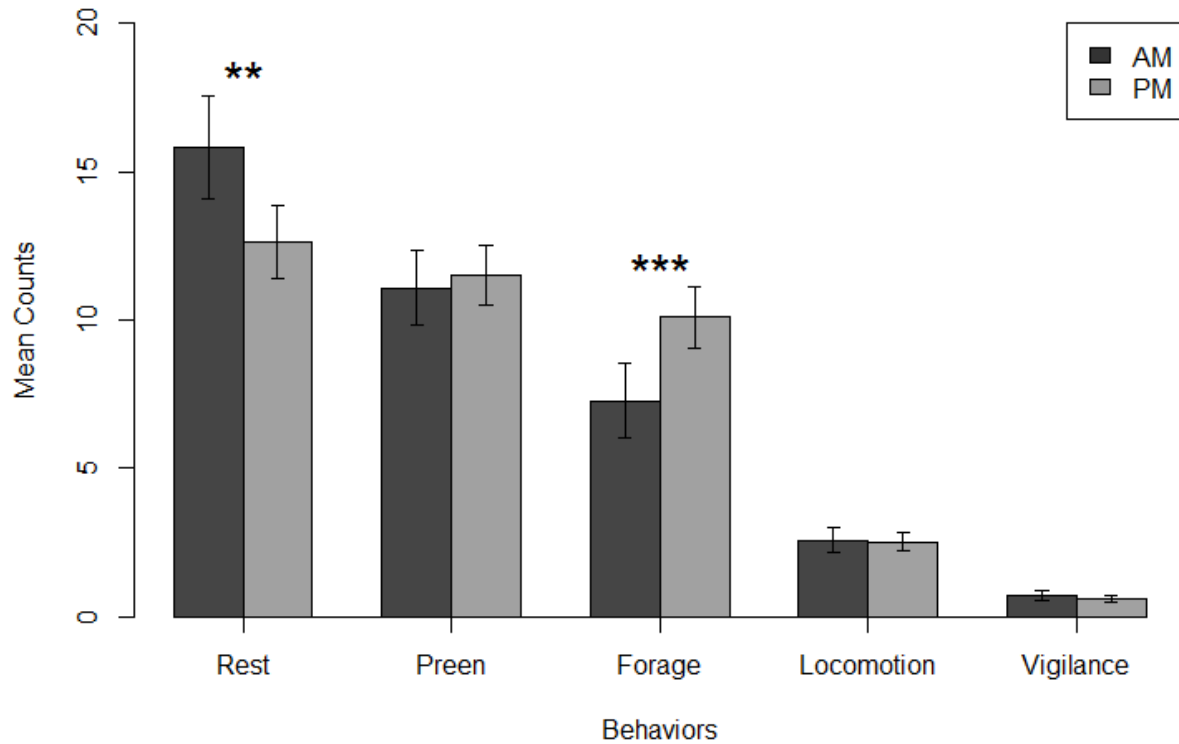


Figure 1.3. Comparison of activity budget focal count means from the morning (AM) and afternoon (PM). Differences between the morning and afternoon in terms of behavior means per focal were analyzed using two-tailed Welch's t-tests. The dark gray bars represent the mean behavior focal counts for the morning period, and the light gray bars represent the mean behavior focal counts for the afternoon period. The lines represent the 95% confidence intervals. ** for $p < 0.01$, *** for $p < 0.001$.

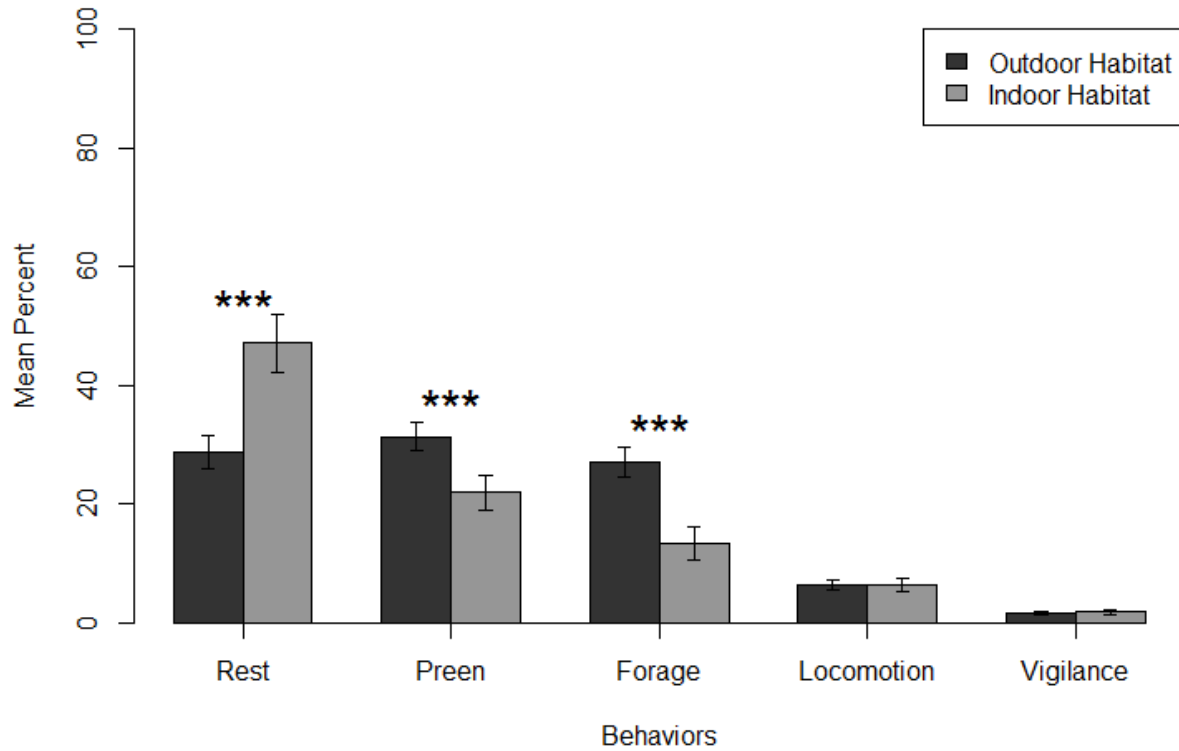


Figure 1.4. Effect of habitat on activity budget percent means. Using two-tailed Welch's t-tests we were able to analyze the effect of habitat on the focal mean percent for each behavior. The dark gray bars represent the mean behavior focal percent for the outdoor habitat, which can also be considered the summer period, and nonbreeding season. The light gray bars represent the mean behavior focal percent for the indoor habitat, which can also be considered the winter period, and breeding season. The lines represent the 95% confidence intervals. *** for $p < 0.001$.

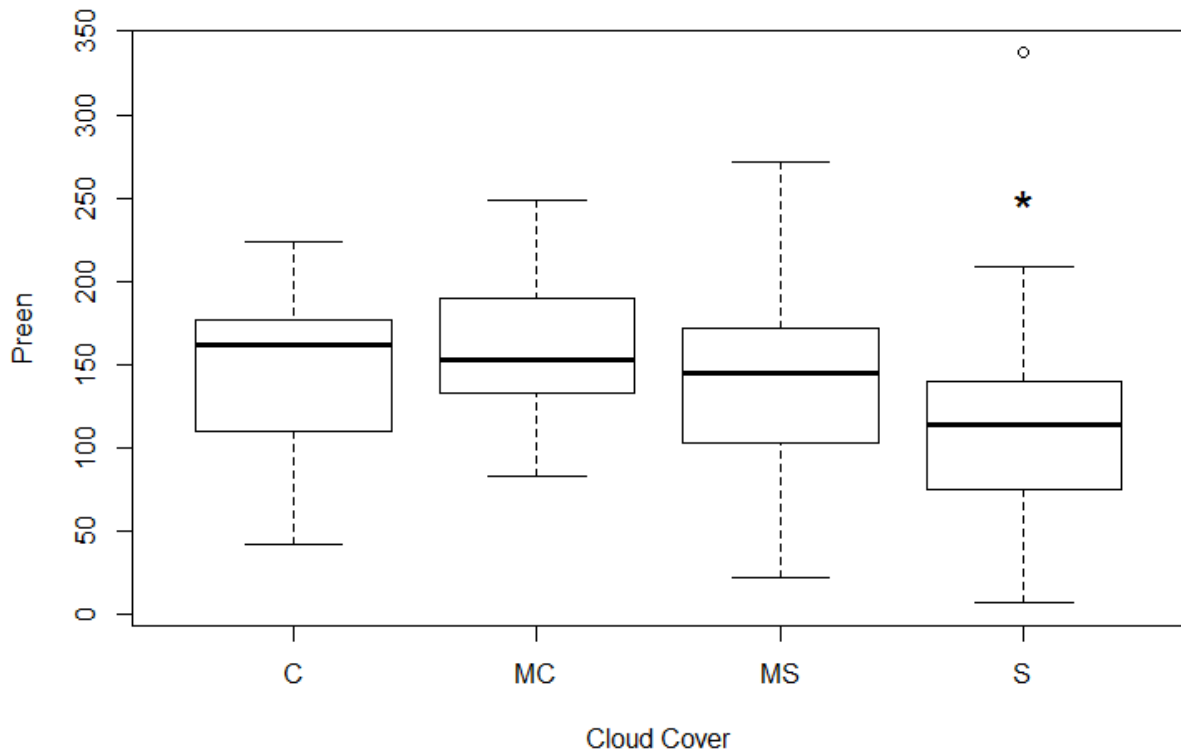


Figure 1.5. Boxplot of the relationship between cloud cover and the frequency of preening behavior in the outdoor habitat. This relationship was tested using a generalized linear model (GLM) with a log link using the negative binomial distribution function from the MASS package (Venables and Ripley, 2002). The x-axis features the four categories of the cloud cover variable: cloudy (C), mostly cloudy (MC), mostly sunny (MS), and sunny (S). The boxes represent the 1st to 3rd quartiles with the darkest line representing the median value, and the dashed lines representing the minimum and maximum values. * for $p < 0.05$.

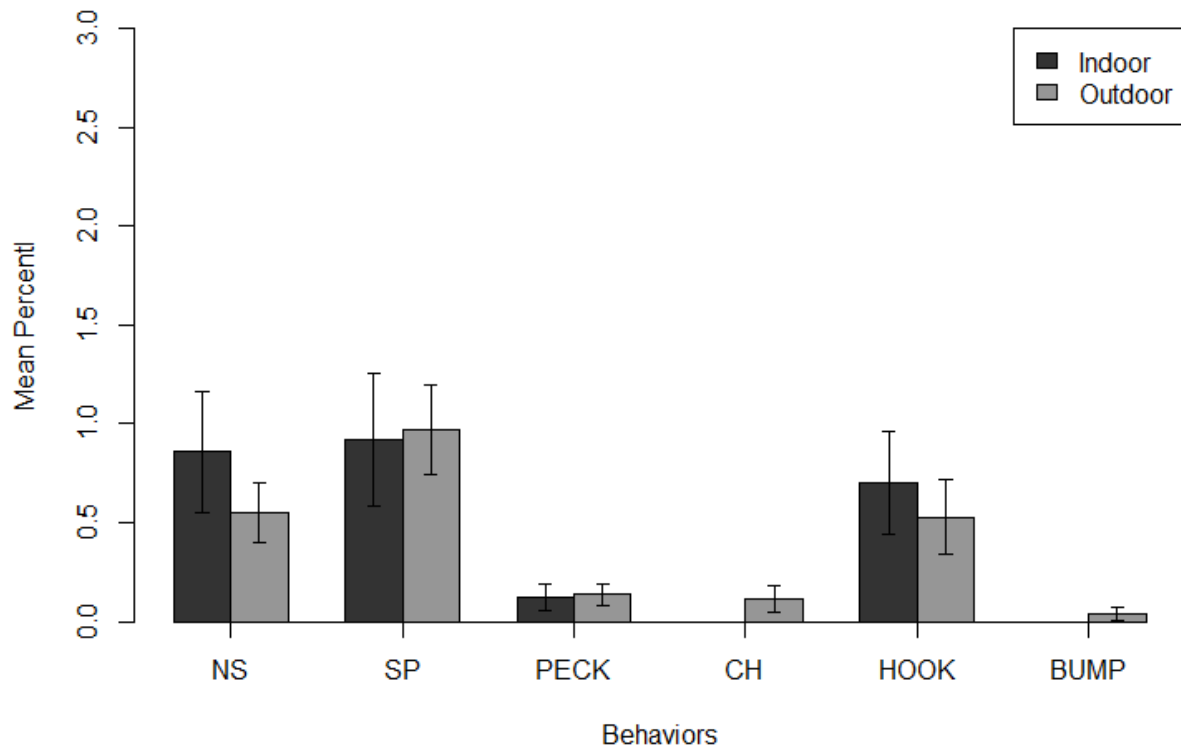


Figure 1.6. Comparison of aggressive behaviors in the indoor and outdoor habitats. Frequency of aggressive behaviors in the two habitats was analyzed using a generalized linear model (GLM) with a log link using the negative binomial distribution function from the MASS package (Venables and Ripley, 2002). The mean percent is the mean percent time of a focal in which each behavior was performed. The lines represent the 95% confidence intervals. The behaviors include neck swaying (NS), sparring (SP), pecking (PECK), chasing (CH), hooking (HOOK), and bumping (BUMP). The dark gray bars represent the data collected while the flamingos were indoors, and the light gray bars represent the data collected while the flamingos were in their outdoor habitat.

Chapter 2

Social associations in captive Caribbean flamingos (*Phoenicopterus ruber*) and their implications for flock management

Caitlin A. Finlay¹, Robert B. Weladji¹, Patrick Paré²

1. Biology Department, Concordia University, 7141 Sherbrooke St. W, Montreal, Québec, Canada
2. Zoo de Granby, 1050 Boulevard David Bouchard N, Granby, Québec, Canada

Abstract:

Successful reproduction is an important component of animal welfare and is vital to captive breeding programs. Regardless of management efforts, many species in captivity have trouble reproducing. Inconsistent reproduction in captivity is common in flamingos. Studying the flamingo's preferential associations provides information on welfare indicators such as aggression and reproduction. We studied the associations within the Caribbean flamingo (*Phoenicopterus ruber*) flock at Zoo de Granby using an association index (AI) to measure the strength of associations. We predicted that (1) pairs would remain stable, (2) associations would become stronger during the breeding season, (3) if pair changes occurred, they would be more common in younger individuals, and that (4) individuals would prefer older mates. Contrary to our prediction, we found that pairs were not stable throughout the study, and the new pairings association strengths were not significantly different from their previous pairings. Although we saw a slight increase in association strength during the breeding season, it was not a significant increase. Lastly, we did not see an increased likelihood for younger individuals to change partners nor did we see a significant preference for partner age regardless of sex. Our findings will allow us to provide Zoo de Granby with information regarding strongly associated pairings who should not be separated in the event of a transaction between institutions.

Keywords: social, age, reproduction, zoo, welfare, captive breeding, Caribbean flamingo, *Phoenicopterus ruber*

Introduction:

The most common roles of zoos include conservation, research, education, and entertainment however the underlying goals are animal welfare and increasing knowledge about the needs of the animals in their care (Hosey et al., 2013). There are many ways to assess animal welfare but because it is subjective, the most used methods are indicators of poor welfare (Melfi, 2009). Indicators of poor welfare can include high frequency of disease or injury, stereotypical behaviors such as pacing, changes in behavior when compared to their wild counterparts, and reduced reproduction (Frumkin et al., 2016; Melfi, 2009; Shannon, 2000). The ability to reproduce successfully is an important component of animal welfare and is of vital importance to captive breeding programs (Melfi, 2009; Tetley and O'Hara, 2012). Captive breeding programs exist in order to maintain a viable, healthy, reproducing population; however, many species appear to have trouble reproducing in captivity (Tetley and O'Hara, 2012). A classic example of a species that has a history of trouble reproducing in captivity is the giant panda (Zhang et al., 2004). Captive breeding programs and research studies have been created in an attempt to determine the cause of the giant panda's poor reproductive performance. Zoos put a lot of time and money into providing the best possible environment, nutrition, and veterinary services for the animals in their care (Lindburg and Fitch-Snyder, 1994). Unfortunately, these efforts do not necessarily equate to a successful breeding program which is why more research is necessary to determine the causes of poor reproductive performance in captivity (Lindburg and Fitch-Snyder, 1994). When studying reproduction it is important to analyze each step in the reproduction process as there are various reasons for reproductive failure (Zhang et al., 2004). There could be behavioral problems, stressful reactions to their environment, high levels of aggression, asynchronous courtship rituals, motor inadequacies such as improper mounting, and incompatible pairings (Lindburg and Fitch-Snyder, 1994; Zhang et al., 2004). There are many possible causes to consider and they are often species-specific so zoos must take the species natural history into consideration when designing captive breeding programs (Lindburg and Fitch-Snyder, 1994; Zhang et al., 2004).

Decreased reproduction is a common problem with captive flamingo populations although the reasons are largely unknown (Farrell et al., 2000). Wild flamingos have erratic reproduction that depends on unpredictable environmental and social cues such as food

availability, rainfall, photoperiod, and the sex ratio, age structure, and size of the colony (King, 2008b; Pickering et al., 1992). While zoos can control many of these factors, captive breeding of flamingos remains a challenge (Farrell et al., 2000). Flamingos are long lived, gregarious birds that form associations within their colonies (King and Bračko, 2014; Rose et al., 2014). Through studying their associations, we are able to gain insight on possible causes of aggression and reproductive issues within the flock which in turn allows us to assess their welfare (Melfi, 2009; Shannon, 2000). We can also use this information for flock management to ensure strongly bonded pairs are kept together since social bonds are important for their welfare and the separation of bonded pairs could lead to decreased welfare and stress (Rose and Croft, 2015a).

Flamingos are highly social birds and form preferential associations within the colony (King and Bračko, 2014; Rose et al., 2014). Wild flamingos can form pairs but do not tend to maintain the same pairing through multiple breeding seasons (Johnson and Cézilly, 2009; King, 2008a). However, captive flamingos have been found to form lasting partnerships and exhibit mate fidelity (King, 2008a; Pickering, 1992; Rose et al., 2014; Shannon, 2000; Studer-Thiersch, 2000). Researchers have suggested mate fidelity in captivity is due to the limited number of individuals from which to choose a mate (Rose et al., 2014; Studer-Thiersch, 2000). While high fidelity pairs can be the most productive, these pairs tend to be more aggressive and can decrease the overall reproductive success of the colony (Peluso and Anderson, 2014; Stevens et al., 1992).

Aggression rates can also increase when there is an uneven sex ratio within the colony (King and Bračko, 2014). Operational sex ratio (OSR), the ratio of sexually active males to fertilizable females, is used as a predictor for mating systems and intensity of mate competition within a population (Emlen and Oring, 1977; Mills and Reynolds, 2003; Weir et al., 2011). OSR can also predict which sex will be competing for mates and resources; in general the OSR will be biased towards the sex with the lower parental investment (Kvarnemo and Ahnesjö, 1996). It is predicted that as OSR increases, there will be an increase in mate and resource competition (Mills and Reynolds, 2003; Weir et al., 2011). Competition and aggression will increase with OSR until it reaches a threshold ratio of 1.99, at which point competition and aggression will decrease due to the increasingly high cost of competition (Weir et al., 2011). In flamingos, a male-biased OSR encourages the establishment of same-sex pairings and trios (King and Bračko, 2014; King, 1994). These same-sex pairings can be particularly damaging to overall reproductive

success, as they often attempt to copulate with incubating birds or steal the nest of a pair that have successfully laid an egg (King and Bračko, 2014; King, 1994). While many captive flamingos appear to be monogamous, extra pair copulations are common (Rose et al., 2014). Extra-pair copulations from paired flamingos have been observed both before and during the breeding season (King, 1994; Studer-Thiersch, 1975).

OSR and extra-pair copulations have also been shown to increase mate guarding (Parker, 1974). When OSR is male-biased, males are more likely to guard a female until they are receptive to breeding because females are limited resource and the males want to ensure reproductive success (Kvarnemo and Ahnesjö, 1996; Parker, 1974). The frequency and duration of mate guarding after copulation also increases with a male-biased sex ratio in an attempt to assure paternity (Weir et al., 2011).

The extra-pair paternity hypothesis (Birkhead and Møller, 1992) is one of several hypotheses to explain divorce in birds. The term “divorce” is used when a pairing splits while both partners are still alive (Coulson, 1972; Ens et al., 1993). The general consensus is that divorce is a strategy used to increase the reproductive success of an individual (Choudhury, 1995). The main hypotheses to explain divorce in birds are the extra-pair paternity hypothesis (Birkhead and Møller, 1992), the incompatibility hypothesis (Coulson, 1972), and the better option hypothesis (Ens et al., 1993). The extra-pair paternity hypothesis suggests that males will increase their reproductive success through divorce or rapidly switching between partners in order to mate with multiple females within a single breeding season (Birkhead and Møller, 1992; Choudhury, 1995). When two individuals mutually divorce due to decreased reproductive success, it is considered the incompatibility hypothesis (Choudhury, 1995; Coulson, 1972). Regardless of the quality of each individual, the pairing is not compatible so it is beneficial to both individuals to find a new mate with whom they will likely have better reproductive success (Coulson, 1972; Cézilly and Nager, 1995). The better option hypothesis is when one individual within the pairing initiates the divorce to seek a higher quality partner (Ens et al., 1993). In this case, divorce is based on the likelihood of finding a higher quality partner while the risk of changing mates is low; an individual is likely to change partners if there are high quality, unpaired individuals available within the population (Choudhury, 1995; Ens et al., 1993).

Another important factor to flamingo reproduction and socialization is the age of the flamingos. Flamingos are long-lived species that continue to breed for many years, and captive flamingos have been recorded to continue breeding into their 50s and 60s (King, 2008a; Studer-Thiersch, 1998). Some have suggested that flamingos may have increased reproductive success as they age suggesting that older flamingos could be more successful than younger flamingos (Pradel et al., 2012; Schmaltz et al., 2011). Previous studies have found that the probability of breeding was dependent on the breeding experience of the individuals (Pradel et al., 2012) and that flamingos showed a preference towards older individuals (Cézilly et al., 1997). They hypothesized it was due to the increased experience of the older flamingos and that the older, more experienced individuals would be better at obtaining resources such as food, mates, and nesting sites (Cézilly et al., 1997; Pradel et al., 2012; Schmaltz et al., 2011). Young flamingos are hypothesized to be inexperienced and inefficient at foraging which means that while they are physically able to reproduce, it will be costly (Pradel et al., 2012). Another study provided support for this hypothesis by finding that there is a delayed survival cost for female flamingos that begin breeding before they are seven years old (Tavecchia et al., 2001). Age also plays a role in the likelihood of changing partners; younger individuals are thought to change partners more often than older individuals due to their inexperience (Choudhury, 1995; Ens et al., 1993). Inexperienced individuals are more likely to choose an incompatible mate and would therefore gain more from a pair change (Choudhury, 1995; Ens et al., 1993).

For the purpose of this study, we looked at the social associations within the flock in the hope of providing Zoo de Granby with methods to improve flamingo welfare while increasing and stabilizing reproductive success. Associations were quantified using an association index, from now on referred to as AI, to analyze the relative strengths of the associations. Based on previous literature, we can make predictions about the associations within the Zoo de Granby flock. We predicted that (1) pairs would remain stable throughout the study, (2) the associations would be stronger during the breeding season than the nonbreeding season, (3) younger individuals would be more likely to change partners than older individuals, and (4) individuals would tend to choose an older mate rather than a younger mate.

Methods:

Study area and subjects:

A flock of Caribbean flamingos (*Phoenicopterus ruber*) was studied at Zoo de Granby in Southern Québec. Observations were conducted at both the indoor and outdoor habitats. Outdoor habitat observations were conducted from the public viewing areas, and indoor habitat observations were conducted from inside the habitat using a blind to remain hidden from the flock. The outdoor habitat consists of ~286m² of accessible ground cover (grass) and a large pond (~686m²) covering approximately 71% of the habitat (Appendix C). The indoor habitat is about 66m² consisting of a nesting area and shallow water basin (Appendix B). The habitat features mirrored half walls in an attempt to make flock appear larger to the flamingos which can encourage breeding (O'Connell-Rodwell et al., 2004). It allowed for a stable environment in which temperature and humidity were monitored and controlled by zookeepers. The indoor habitat was shared by the flamingo flock and six cattle egrets (*Bubulcus ibis*). The flock (n=28; ♂=15, ♀=13) ranges in age from 7 to 38 years old (mean=20.2). We were able to identify individual flamingos using leg bands with unique numbers and color combinations (Appendix A). Through the identification of each individual, we could gain further information from zoo records to determine age, sex, Species360 Zoological Information Management System (ZIMS) database number, and history of the individuals. The history of individual flamingos is often unknown; from the Zoo de Granby flock there are 16 flamingos wild-caught from Cuba, 1 was of unknown origin, and 11 were captive-born. For the purpose of this study, mentions of individual flamingos will include their band number followed by their ZIMS number and sex (ex: 27-B98004♂).

The flamingos were fed a regular diet of Mazuri Flamingo Complete (#5644), and Mazuri Breeder Reproduction (#5645) during the breeding season (December - May). They were fed at various times; usually between 10:30am and 1:30pm. When the flamingos were indoors, the food trays were strategically placed throughout the enclosure in an attempt to decrease the disturbance to the flock (Appendix B). Their outdoor feeders included a large trough at the water's edge, a standing duck-proof feeder near the shoreline, and seven fake nests on the shore.

Data Collection:

Focal sampling was conducted to collect individual's behavior and social affiliations. Focal animals were chosen based on the visibility of their leg bands for identification, without a determined order from one day to the next. Focals lasted for 10 minutes with the behavior recorded every 15 seconds. A cellphone application called Interval Timer AD (halmi.sk, 2015) was used as a notification of when to record a behavior; it was set to beep every 15 seconds for the 10-minute period. Social interactions were recorded based on time spent interacting with another individual or frequent proximity to an individual; individuals could be actively feeding with another flamingo or resting side by side and both would be considered a social interaction. Proximity was a challenge to define for our study because the flamingos were always within 1-2 wing lengths of another flamingo and they tended to move about the habitat as a group. Their tendency toward group movement is likely because flamingos are prey animals and rely on the dilution effect, many-eyes effect, and/or confusion effect for protection from predators (Boukhriss et al., 2007; Hamilton, 1971; Pulliam, 1973; Vine, 1973). Their tendency to move as a group meant we could not use distance criteria such as the "nearest neighbor" criteria since multiple flamingos would be within the defined distance (Rose and Croft, 2015a). For the purposes of our study, proximity was defined as the flamingo closest to the focal individual relative to the rest of the flock.

Data were collected between July 2015 and June 2016. The flamingos were in their outdoor habitat between July and October of 2015, and again between May and June of 2016. During these months, data was collected daily. During the colder months, October to May, the flamingos were in the indoor habitat and data was collected 1-2 days per week. While the social pairs seemed to be stable, there was a sudden change in pairings in late December of 2015 or early January of 2016. This change was not observed but it involved two triads splitting into multiple pairs. The new pairs appeared to remain stable after the initial changes. Due to this change in mate pairs, we split the study into two time periods of "2015" (July - December 2015) and "2016" (January - June 2016). Group displays during the breeding season are most frequent between January and May, so we can also say that the "2016" period aligns with the breeding season (Shannon, 2000).

Data from the focal sampling was used to obtain a list of unique pairings, how often each individual was seen within that pairing, how often each individual was seen alone, and if the individual maintained the pairing throughout the study or changed partners. Zoo records allowed us to know the age and sex of the individuals to better examine their social preferences.

Data Analysis:

Data were then compiled and the social interactions were analyzed using an association index (AI) (Martin and Bateson, 2007). The AI used the number of times each individual (a and b) was each seen separately (N_a , N_b), and the number of time the individuals were seen together (N_{ab}). Our flock had two triads (consistent grouping of three individuals, in our case it was two triads consisting of two males and a single female) so we altered the AI to include a third individual (N_c) and the time spent together then included all three individuals (N_{abc}). Using the altered AI equation ($N_{abc} / N_a + N_b + N_c + N_{abc}$), we were able to calculate a value between 0 and 1 representing the ratio of time individuals spend together versus time spent apart. This association value allows us to obtain information on social affiliations within the flock: a value of 0 demonstrates no affiliation between individuals, and a value of 1 suggests a full affiliation. An AI value of 0.5 would mean individuals spend equal time together and apart; it is for this reason we will consider affiliated pairs to be those with AI values of 0.5 or greater. We will consider a pair to be strongly associated when it has an AI value of 0.8 or higher and fully associated with an AI value of 1.0 (Bräger et al., 1994). Groupings were only used in the analysis if they had interacted a minimum of 5 times throughout the study. The reasoning for this minimum is when looking at the distribution of interaction frequency, during the 2015 time period (Figure 2.1) there was a steady decrease in frequency however in the 2016 time period (Figure 2.2) there was a sudden drop from 13 to 5 interactions. We considered this jump to be a division between possibly associated pairings and flamingos that occasionally interacted. The significance of relationships was analyzed by first doing an F-test to determine if the variances were equal followed by a corresponding two-tailed t-test with a confidence level of 0.95.

Results:

In 2015, the AI values ranged from 0.2 to 0.9 with an average value of 0.70 (SD = 0.19) demonstrating a large range of association strengths. The pair with the strongest association in

2015 was 4-900085♂ and 63-B92165♀, a pair of flamingos aged 31 and 24 respectively, who had an association value of 0.90. While they had the strongest association in 2015, this pairing split in 2016 and was later only seen interacting once during a focal (AI = 0.25). Several other pairings from 2015 split in early 2016 (Figure 2.3). Two triads and three pairs split into a total of four pairs and one triad. The number of individuals between the 2015 and 2016 pairings is not equal because one individual (37-B01035♀) was equally associated to two individuals causing two independent pairings in 2015. Of the pairs that formed in 2016, all were male-female pairs. The triad that formed involved a fully associated pair and an additional individual that was seen with that pair 79% of the time.

There was a general increase in AI values between 2015 (mean = 0.70, SD = 0.19) and 2016 (mean = 0.82, SD = 0.28) however the change was not significant ($t(28) = 1.31, p=0.20$). We did see an increase in the frequency of strong associations (AI = 0.80 - 0.99) and the appearance of full associations (AI = 1.0) in 2016 after the pairs changed. In 2015 there were only six strong associations, and in 2016 there were ten pairs considered strongly affiliated. Of these ten strongly associated pairs, five had full affiliations meaning they were always interacting and seen together.

We saw a similar trend in the pairs that were maintained between 2015 and 2016. There were eight pairs that did not change in 2016, all of which were male-female pairs. These maintained pairs also showed an increase in AI values between 2015 (mean = 0.78, SD = 0.09) and 2016 (mean = 0.86, SD = 0.20) but the differences were not significant ($t(14) = 1.03, p = 0.32$).

Of the twelve individuals who changed partners in 2016, only two had weaker association values. The first was 50-B02046♂ who was previously associated with 37-B01035♀ (AI= 0.63) and did not find a new partner (AI= 0) after 37-B01035♀ paired with 58-B02045♂. The second individual with a weaker association in 2016 was 4-900085♂ who switched from being partnered with 63-B92165♀ (AI= 0.90) to 61-B08007♀ (AI= 0.66). From observations, 61-B08007♀ did not appear to be an individual who associated closely with others; in 2015 her closest association was with 6-B00024♂ with an AI value of 0.20. The AI values in 2016 ranged from 0 to 1 (mean = 0.82, SD = 0.28); the reason there is an AI value of 0 in 2016 is because 50-B02046♂ was part of a pairing that split and he did not associate with another individual in 2016. If we do not

consider this individual as part of the average association value, it becomes 0.88 (range = 0.48 – 1.0, SD = 0.17). When comparing the association strengths in 2015 and 2016, we did one analysis where 50-B02046♂ was included and another where he was excluded. In general, there was an increase in association strength from the 2015 (mean = 0.67, SD = 0.21) to 2016 (mean = 0.82 SD = 0.30) pairings however when 50-B02046♂ was included in the analysis, the change was not a significant one ($t(22) = 1.53, p = 0.14$). When we excluded 50-B02046♂ from the analysis, the average increase in association strength was found to be significant ($t(20) = 2.94, p = 0.008$).

In addition to association strength, we also looked at the age of individuals within pairings. We did not find a significant relationship between the age of an individual and its likelihood to change partners ($t(18) = 0.6874, p = 0.50$). The age of the individuals who remained with their original partner ranged from 7 to 38 years old with an average age of 20.3 years old (SD = 10.87). We looked at the age differences between associated individuals and found the average age difference was 13.13 years (SD = 10.86). There was one pair in which both individuals were the same age (15 years), and there were three pairs which had an age difference of 24 years.

When analyzing the age of individuals who had changed partners, 50-B02046♂ was excluded as he did not have a partner in 2016. The age of the individuals who changed partners ranged from 8 to 38 years old, with an average age of 20.17 years old (SD = 9.16). The average age difference increased from 8.5 (SD = 8.64) in 2015 to 11.68 (SD = 9.04) in 2016 but the increase was not significant ($t(22) = 1.13, p = 0.27$). On average they chose a partner 4.08 (SD = 11.51) years older or younger than themselves. The average age of partners in 2015 (mean = 20.95, SD = 7.04) and 2016 (mean = 21.0, SD = 9.34) did not show significant change ($t(20) = 0.01, p = 0.99$). Of the individuals who changed partners, six chose a partner younger than their previous partner, and five chose a partner older than their previous partner (Figure 2.4). There was no apparent trend in preference of partner age depending on the age of the individual ($t(9) = -1.390, p = 0.198$). There did not seem to be a difference between the sexes in terms of changing to an older or younger partner ($t(1) = 1, p = 0.5$); 3 males chose an older partner, 2 females chose an older partner, 3 males chose a younger partner, and 3 females chose a younger partner.

Discussion:

Wild flamingos do not maintain pair bonds between breeding seasons but studies have shown captive populations of flamingos to have high levels of mate fidelity (Farrell et al., 2000; Pickering, 1992; Shannon, 2000; Studer-Thiersch, 2000). Based on this literature, we predicted associations would remain stable throughout this study. Our initial observations in 2015 seemed to support this prediction however the sudden mate changes in early 2016 contradicted our prediction. This contradiction was surprising because many studies have seen mate fidelity in captivity (King, 2008a; Pickering, 1992; Rose et al., 2014; Shannon, 2000; Studer-Thiersch, 2000) and the Zoo de Granby flock is relatively small which suggests there should be higher levels of mate fidelity due to limited mate choices (Farrell et al., 2000; Pickering, 1992; Rose et al., 2014; Shannon, 2000; Studer-Thiersch, 2000). Unfortunately the changes were not observed so the reasons behind the changes are unknown. There are several possible reasons for pair separations including failure to reproduce in the current or previous breeding season, availability of unpaired individuals, location, and age (Choudhury, 1995; Ens et al., 1993). Location refers to the instability of a habitat which is a concern and likely cause of pair changes in wild flamingos but is less likely to affect captive populations due to the regulated environment of the indoor habitat (Choudhury, 1995; Farrell et al., 2000). However, the flock is moved between the indoor and outdoor habitats which could perhaps have an effect on pair stability. Failure to reproduce is a likely reason behind pair changes in the Zoo de Granby flock as they have not successfully reproduced in ten years. Pair changes have been observed following a breeding attempt or season without reproductive success in several species including great tits (*Parus major*) (Linden, 1991), short-tailed shearwaters (*Puffinus tenuirostris*) (Bradley et al., 1990), and Greater flamingos (*Phoenicopterus ruber roseus*) (Cézilly and Johnson, 1995).

The pair changes are also likely to be in connection with the prior unavailability of unpaired individuals; there had not been individuals added to the flock so the flamingos were more likely to remain with their current partner. According to the better option hypothesis by Ens et al. (1993), in order for an individual to leave its partner, there needs to be a high chance of finding a better partner that outweighs the risk of being unpaired. If unpaired flamingos are not available within the population, it is unlikely for pairs to risk separating (Ens et al., 1993). In the wild it would be high risk to leave a partner without having a future partner established because there are risks of mortality from predation, increased competition, and losing social status or territory

(Choudhury, 1995). In captivity the risk of mortality is minimal (depending on the enclosure design and native wildlife) but there is still the risk of losing social status, increased competition, or ending up unpaired (Brown and King, 2005; Choudhury, 1995; Ens et al., 1993). When the triads in our flock split, there were suddenly three unpaired individuals available. These newly available individuals may have been the stimulus for other pairs to switch partners to potentially increase future reproductive success (Choudhury, 1995; Ens et al., 1993). In contrast, another study found that when new individuals were added to a captive flock, no pairings were formed between the established and new individuals even though they had not successfully reproduced the previous breeding season (Frumkin et al., 2016). Their finding is consistent with most research on captive flamingo populations where mate fidelity is common (King, 2008a; Pickering, 1992; Shannon, 2000). Further research on the Zoo de Granby flock would need to be conducted to determine if the switch was a coincidence or if there are consistent pair changes between breeding seasons as there are in wild populations of flamingos. A study by Cézilly and Johnson (1995) showed that Greater flamingos (*Phoenicopterus ruber roseus*) in southern France changed mates between consecutive breeding seasons 98.3% of the time. They also observed mate changes within a breeding season when males would leave a female that had failed to breed that season (Cézilly and Johnson, 1995). This observation is interesting when combined with a study which suggests females are more likely to maintain pairings between breeding seasons (Frumkin et al., 2016). Contrary to this finding, we found that the females in the Zoo de Granby flock were no more likely to maintain their pairing than males. Many other bird species have been found to change pairs between breeding seasons including blue tits (*Parus caeruleus*) (Dhondt and Adriaensen, 1994), collared flycatchers (*Ficedula albicollis*) (Part and Gustafsson, 1989), and oystercatchers (*Haematopus ostralegus*) (Ens et al., 1993).

Our second prediction was that associations would be stronger during the breeding season than during the nonbreeding season. Due to the pairing changes in early January coinciding with the breeding season, we considered the 2016 AI values to represent the breeding season while the 2015 AI values represented the nonbreeding season. While the average AI values appeared to be higher during the breeding season, they were not significantly different from those in 2015. The frequency of strong and full associations was higher during the breeding season when compared to the nonbreeding season. While there does not seem to be much research done on association strengths during the breeding and nonbreeding seasons, a study found that black-capped

chickadee pair attachments became stronger as they got closer to the breeding season and the strongest attachments were those of mating pairs (Ficken et al., 1981). In flamingos, the predicted increase in association strength during the breeding season would likely be due to their synchronized reproduction (Darling, 1938; Shannon, 2000; Stevens and Pickett, 1994). In the wild, flamingo reproduction is largely dependent on food availability (Darling, 1938; Pickering et al., 1992). In response to short-term food availability, flamingos have group displays in order to synchronize breeding so as to take advantage of the often short-term food availability and simultaneously decrease predation risk (Darling, 1938). Group displays are a courtship behavior in which the group can synchronize breeding while forming mating pairs (Shannon, 2000). Once a pair has been formed, these individuals may move away from the group and spend more time with each other than the group (Rose and Croft, 2015b; Shannon, 2000). The pairs will then continue courtship displays, begin nest building, and begin copulating (Hinton et al., 2013; Shannon, 2000). These behaviors mean that paired individuals will spend the majority of their time in close proximity to one another throughout the breeding season (Rose and Croft, 2015b; Shannon, 2000). Research has shown that birds maintaining pair bonds or with longer pair formation periods will have better reproductive success due to increased pair synchronization and coordination (Griggio and Hoi, 2011). The flamingos at Zoo de Granby appeared to be fairly synchronized in their courtship and reproductive behaviors however the group displays never featured the entire flock. While we saw frequent nest building behaviors from the pairs, not all pairs attempted to copulate, and we did not observe any successful copulations attempts. Although it was not significant, the pairs did seem to spend slightly more time in close proximity during the breeding season but it is difficult to say whether that was due to choice or the limited space of the indoor habitat. The pairs that were maintained spent the majority of their time together during the nonbreeding season as well as the breeding season whereas wild flamingo pairings tend to disperse during the nonbreeding season and form pairing when they return to the breeding grounds (Cézilly and Johnson, 1995).

An alternate hypothesis for the increased time pairs spent together during the breeding season, thus increasing their association strength, is mate guarding (Griggio and Hoi, 2011; Rose and Croft, 2015b). Mate guarding is done in order to reinforce pair bonds, and is generally done during the breeding season but can be done year-round in monogamous species (Griggio and

Hoi, 2011). Both Greater flamingos (*Phoenicopterus roseus*) in the wild and Caribbean flamingos (*Phoenicopterus ruber*) in captivity, including those at Zoo de Granby, have been observed mate guarding (Rose and Croft, 2015b). Considering the OSR at Zoo de Granby is male biased ($\delta=15$, $\phi=13$), it is not surprising that we observed mate guarding as male biased OSRs tend to increase the frequency and duration of mate guarding to ensure reproductive success and paternity (Kvarnemo and Ahnesjö, 1996; Parker, 1974; Weir et al., 2011). Mate guarding in flamingos consists of the paired male and female being in close proximity to one another, and the male forming a protective stance by leaning over the female in an attempt to deter the approach of other males (Rose and Croft, 2015b). The flamingos at Zoo de Granby were primarily observed mate guarding in the nesting area when a male would approach a paired female. We suspect the strengthened associations are due to a combination of mate guarding and increased time spent in close proximity in order to synchronize reproduction.

Our third prediction was that younger individuals would be more likely than older individuals to change partners. Research has been suggested that younger birds would change pairs more frequently than older birds because the younger, more inexperienced individuals are more likely to pick an incompatible mate and therefore have the most to gain in terms of future reproductive success by changing mates (Choudhury, 1995; Ens et al., 1993). There is still the possibility of mate changes in older individuals because should a high quality individual become available, it would be advantageous to leave the current partner for the higher quality unpaired individual (Choudhury, 1995). Contrary to the literature, our results did not show a difference in terms of age and likelihood of changing partners. A possible explanation is the distribution of ages within the flock; the mean age is 20.2 with the ages ranging from 7 to 38 years old while flamingos have been known to reproduce as young as 7 years old and can live for over 61 years (King, 2008a; Tavecchia et al., 2001). This age distribution suggests that the Zoo de Granby flock is still relatively young and inexperienced considering their previous lack of reproductive success in the past.

Our final prediction was that individuals would tend to choose an older partner. Research shows that breeding with individuals of a similar age is common in Greater Flamingos (*Phoenicopterus roseus*) (Cézilly et al., 1997) and barnacle geese (*Branta leucopsis*) (Black and Owen, 1995) however long-lived species generally show a preference for older individuals

because they are more experienced and generally have better breeding success (Pradel et al., 2012; Schmaltz et al., 2011). Contrary to the literature, we found no significant preference for either an older or younger partner. Similar to our third prediction, we suspect that the limited mate options within the flock, younger age distribution, and previous lack of reproductive success are likely explanations for the lack of preference in partner age.

Conclusions:

While we did not predict pair changes within the flock, it did provide an interesting counterpoint to much of the previous research done on captive flamingo populations showing stability in pairs. It goes to show that more research is required to understand the complex social dynamics and reproduction of captive populations. Our research will allow us to provide Zoo de Granby with suggestions for flock management, namely which individuals are strongly associated and should therefore not be separated in the event of a transaction between institutions. Although we saw a slight increase in association strength, it was not significant therefore we were unable to provide support for our prediction that the associations would be stronger during the breeding season. Age turned out to be an interesting factor because we did not see the predicted preference for older partners nor did we see an increased likelihood for younger individuals to change partners. Our findings provide a contrast to much of the literature and identify areas of study that require further research.

Figures:

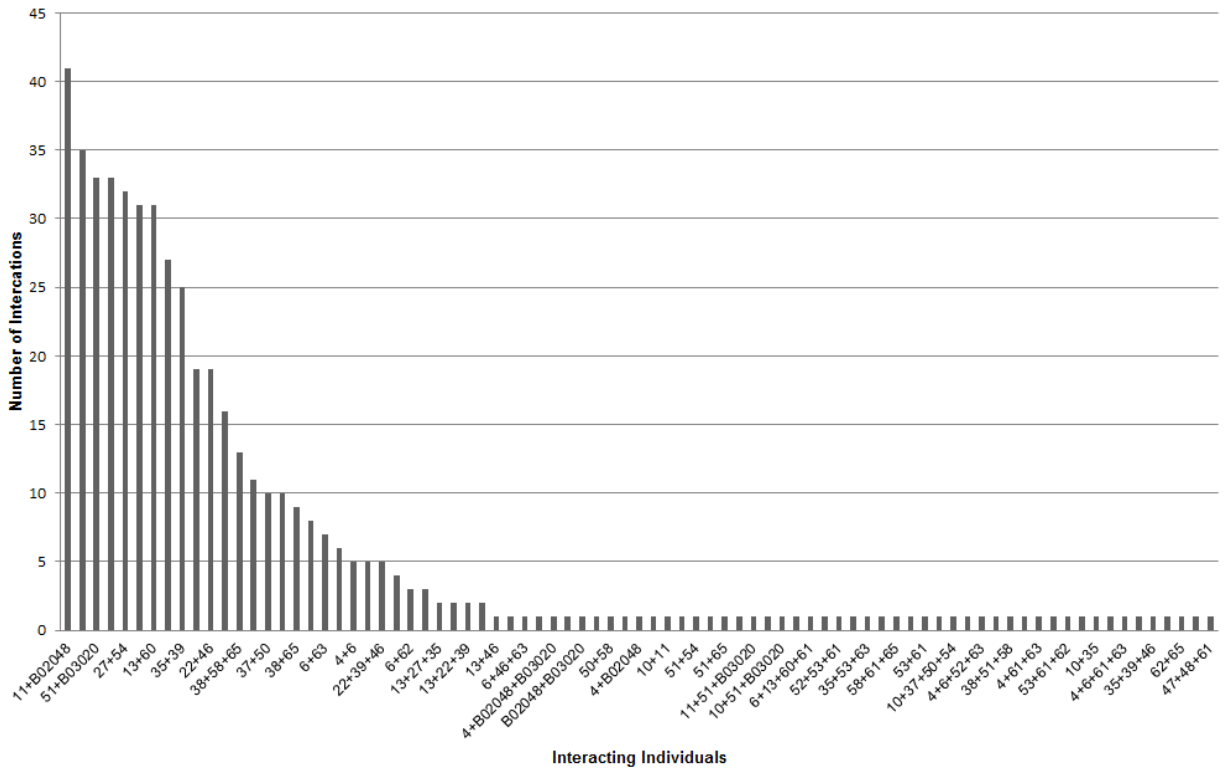


Figure 2.1. Distribution of the number of social interactions between groupings of flamingos during the 2015 time period (July - December 2015). There is a pattern of steadily decreasing interactions which suggest close associations on the left side of the x-axis and little to no association on the right side. For clarity, the interacting individuals are named using only their leg band numbers, except in the case of B02048 and B03020 as they did not have numbered bands.

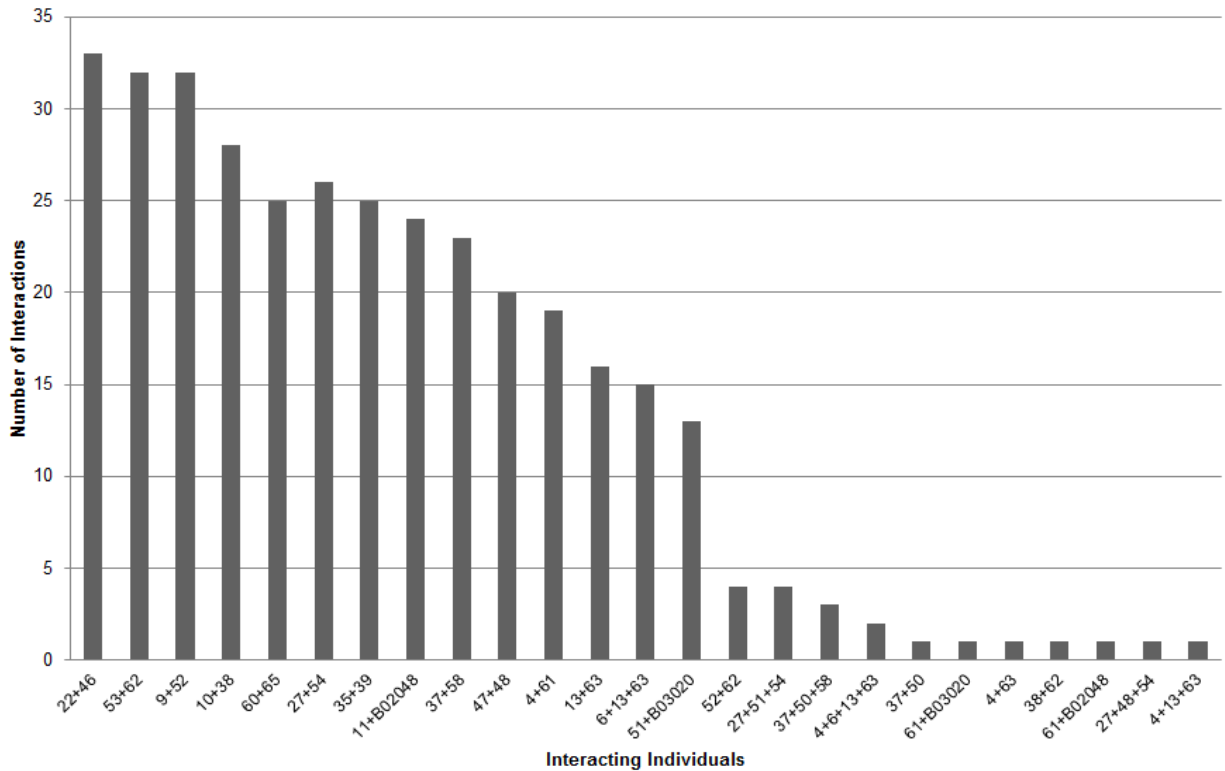


Figure 2.2. Distribution of the number of social interactions between groupings of flamingos during the 2016 time period (January - June 2016). There is a sudden decrease in interaction frequency between 51+B03020 and 52+62; the interaction frequency decreased from 13 to 4. We considered this sudden decrease to be a division between possibly associated pairs and flamingos that occasionally interacted. For this reason, we only used groupings of flamingos that had been recorded as interacting a minimum of five times during the study in our analysis. For clarity, the interacting individuals are named using only their leg band numbers, except in the case of B02048 and B03020 as they did not have numbered bands.

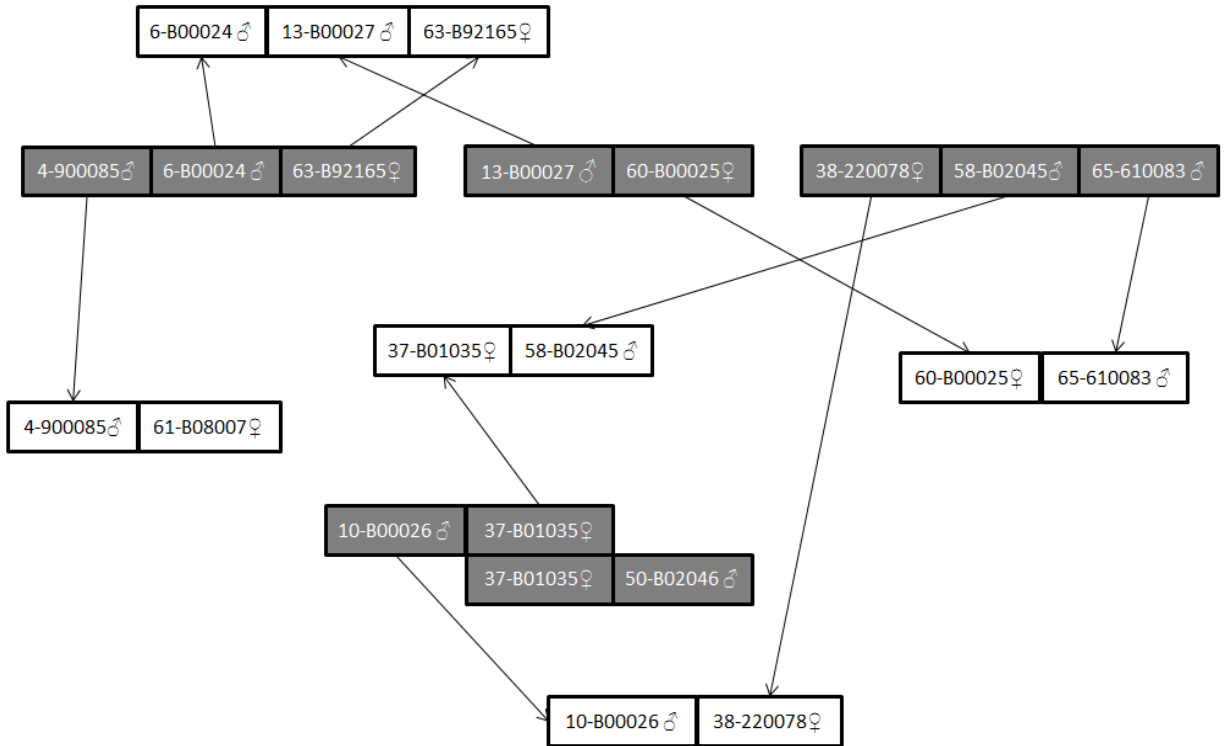


Figure 2.3. Flowchart depicting the pair changes that occurred in late December or early January. The adjacent boxes are pairings; the solid boxes represent the original pairings from 2015 and the outlined boxes are the new pairings from 2016. The arrows show the movement of the individuals from their 2015 to 2016 pairings. The individual 37-B01035♀ had equal associations (AI = 0.625) to both 10-B00026♂ and 50-B02046♂ in 2015 which is why she is shown twice in this figure.

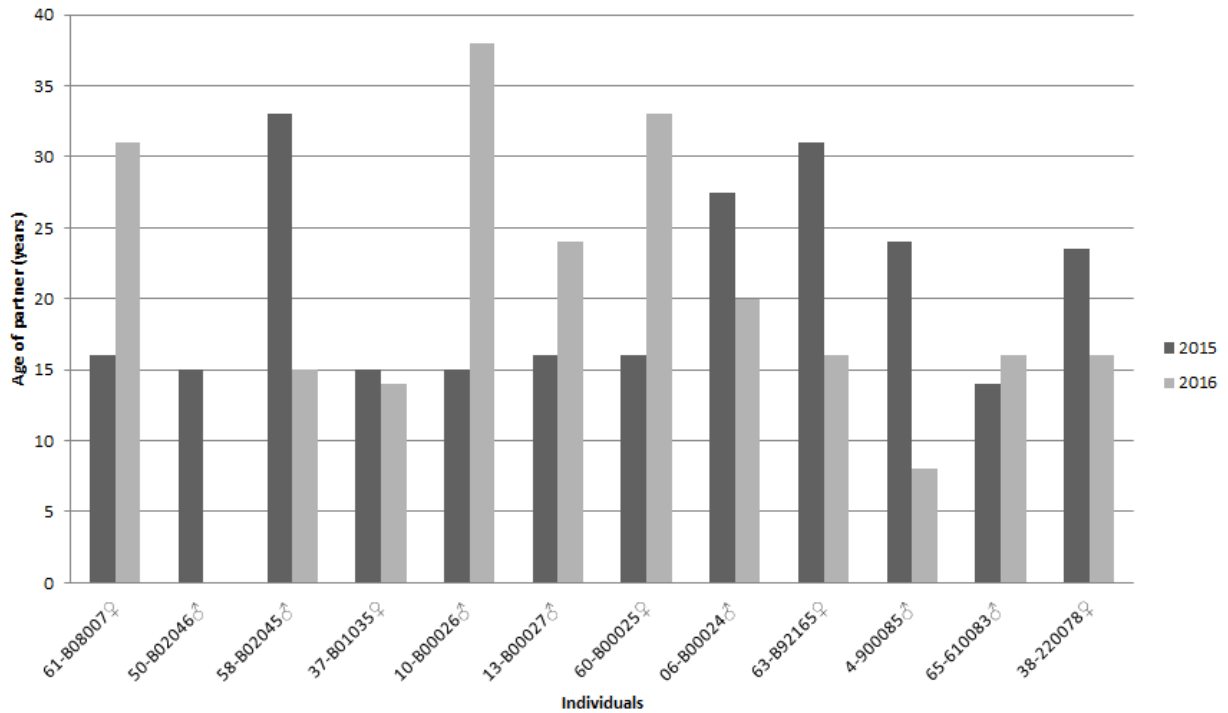


Figure 2.4. Distribution of the change in partner age from 2015 to 2016. On the x-axis there is a list of individuals who changed partners in 2016; these individuals are arranged in order of increasing age (range = 8-38). The y-axis shows the age of the partner in years. The light gray bars show the data from the 2015 associations, and the dark gray bars show the data from the 2016 associations.

General Conclusions

The results of our study show that the activity budgets of the Zoo de Granby flamingo flock and that of a wild population differ, which is most likely explained by the differences in food availability and predation pressure. The environment and visitors were shown to have varying levels of effects on behaviors and aggression. Aggressive behaviors in particular increased with temperature, humidity, and the number of visitors. The most surprising result was the lack of a visitor effect on the frequency of vigilance which is a behavior often used to identify distress in response to visitors. While previous research suggested captive pairs are maintained, the Zoo de Granby flock has several pair changes. We did not find a difference in the strength of associations between previous and new pairings or between the nonbreeding and breeding season. We did not observe a preference for older partners nor were flamingos more likely to change partners based on their age or sex. These results demonstrate the need for more research to understand the complex social dynamics of captive flocks. The environmental and visitor effects are challenging to manage due to the habitat being outdoors and its high visitor traffic location within the zoo. We do not believe our results suggest a welfare issue in the flock as there were no visitor effects on vigilance. While the increased locomotion due to noise level could be an indicator of agitation, the flamingos have a retreat area within the habitat where they could go to avoid visitors. This retreat area was rarely used suggesting they are not trying to avoid visitor presence or noise. We recommend Zoo de Granby continue their research efforts through investigating the effects of animal caretaker presence on behavior, and the effect of wing clipping on copulation behavior as we suspect these factors could have impacts on reproductive success. We are able to make suggestions for flock management through the identification of strongly associated pairs who should not be separated in the event of a transaction between institutions.

References

- Anderson CA, Anderson KB, Dorr N, DeNeve KM, Flanagan M. 2000. Temperature and aggression. *Advances in Experimental Social Psychology* 32:63-133.
- Anderson US, Benne M, Bloomsmith MA, Maple TL. 2002. Retreat space and human visitor density moderate undesirable behavior in petting zoo animals. *Journal of Applied Animal Welfare Science* 5(2):125-137.
- Baker A. 1976. Learned irrelevance and learned helplessness: Rats learn that stimuli, reinforcers, and responses are uncorrelated. *Journal of Experimental Psychology: Animal Behavior Processes* 2(2):130-141.
- Barber JC, Mellen JD. 2013. *Zookeeping: An Introduction to the Science and Technology*. University of Chicago Press. p 53-61.
- Beason RC. 2004. What can birds hear? USDA National Wildlife Research Center-Staff Publications:78.
- Beauchamp G, McNeil R. 2003. Vigilance in greater flamingos foraging at night. *Ethology* 109(6):511-520.
- Bildstein KL, Frederick PC, Spalding MG. 1991. Feeding Patterns and Aggressive Behavior in Juvenile and Adult American Flamingos. *The Condor* 93(4):916.
- Bildstein KL, Golden CB, McCraith BJ, Bohmke BW. 1993. Feeding behavior, aggression, and the conservation biology of flamingos: integrating studies of captive and free-ranging birds. *American Zoologist* 33(2):117-125.
- Birke L. 2002. Effects of browse, human visitors and noise on the behaviour of captive orangutans. *Animal Welfare* 11(2):189-202.
- Birkhead TR, Møller AP. 1992. *Sperm competition in birds: evolutionary causes and consequences*. Academic Press London.
- Black JM, Owen M. 1995. Reproductive performance and assortative pairing in relation to age in barnacle geese. *Journal of Animal Ecology*:234-244.
- Bouchard LC, Anderson MJ. 2011. Caribbean Flamingo resting behavior and the influence of weather variables. *Journal of Ornithology* 152(2):307-312.
- Boukhriss J, Selmi S, Bechet A, Nourira S. 2007. Vigilance in Greater Flamingos Wintering in Southern Tunisia: Age-Dependent Flock Size Effect. *Ethology* 113(4):377-385.
- Bradley J, Wooller R, Skira I, Serventy D. 1990. The influence of mate retention and divorce upon reproductive success in short-tailed shearwaters *Puffinus tenuirostris*. *The Journal of Animal Ecology*:487-496.

- Britton RH, de Groot ER, Johnson AR. 1986. The daily cycle of feeding activity of the Greater Flamingo in relation to the dispersion of the prey *Artemia*. *Wildfowl* 37(37):151-155.
- Broom D. 1991. Assessing welfare and suffering. *Behavioural Processes* 25(2-3):117-123.
- Broom D. 2001. Coping, stress and welfare. *Coping with challenge: Welfare in animals including humans*:1-9.
- Brown C, King C. 2005. Flamingo husbandry guidelines: A Joint Effort of the AZA and EAZA in cooperation with Wildfowl and Wetlands Trust. Dallas Zoo Dallas, TX.
- Bräger S, Würsig B, Acevedo A, Henningsen T. 1994. Association Patterns of Bottlenose Dolphins (*Tursiops truncatus*) in Galveston Bay, Texas. *Journal of Mammalogy* 75(2):431-437.
- Burnham KP, Anderson DR. 2002. Model selection and multimodel inference: a practical information-theoretic approach. 2nd Edition ed. New York, New York, USA: Springer-Verlag.
- Chamove AS, Hosey GR, Schaetzel P. 1988. Visitors excite primates in zoos. *Zoo Biology* 7(4):359-369.
- Choudhury S. 1995. Divorce in birds: a review of the hypotheses. *Animal Behaviour* 50(2):413-429.
- Coulson J. The significance of the pair-bond in the kittiwake; 1972. p 424-433.
- Cézilly F, Boy V, Tourenq CJ, Johnson AR. 1997. Age-assortative pairing in the Greater Flamingo *Phoenicopterus ruber roseus*. *Ibis* 139(2):331-336.
- Cézilly F, Johnson AR. 1995. Re-mating between and within breeding seasons in the Greater Flamingo *Phoenicopterus ruber roseus*. *Ibis* 137(4):543-546.
- Cézilly F, Nager RG. 1995. Comparative evidence for a positive association between divorce and extra-pair paternity in birds. *Proceedings of the Royal Society of London B: Biological Sciences* 262(1363):7-12.
- Darling FF. 1938. Bird flocks and the breeding cycle: a contribution to the study of avian sociality. University Press.
- Dhondt AA, Adriaensen F. 1994. Causes and effects of divorce in the blue tit *Parus caeruleus*. *Journal of Animal Ecology*:979-987.
- Emlen ST, Oring LW. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* 197:215-223.
- Ens BJ, Safriel UN, Harris MP. 1993. Divorce in the long-lived and monogamous oystercatcher, *Haematopus ostralegus*: incompatibility or choosing the better option? *Animal Behaviour* 45(6):1199-1217.
- Espino-Barros R, Baldassarre GA. 1989a. Activity and Habitat-Use Patterns of Breeding Caribbean Flamingos in Yucatan, Mexico. *The Condor* 91(3):585.
- Espino-Barros R, Baldassarre GA. 1989b. Numbers, migration chronology, and activity patterns of nonbreeding Caribbean Flamingos in Yucatan, Mexico. *Condor*:592-597.

- Farrell MA, Barry E, Marples N. 2000. Breeding behavior in a flock of Chilean flamingos (*Phoenicopterus chilensis*) at Dublin zoo. *Zoo Biology* 19(4):227-237.
- Ficken MS, Witkin SR, Weise CM. 1981. Associations among members of a black-capped chickadee flock. *Behavioral Ecology and Sociobiology* 8(4):245-249.
- Frumkin NB, Wey TW, Exnicios M, Benham C, Hinton MG, Lantz S, Atherton C, Forde D, Karubian J. 2016. Inter-annual patterns of aggression and pair bonding in captive American flamingos (*Phoenicopterus ruber*). *Zoo biology* 35(2):111-119.
- Griggio M, Hoi H. 2011. An experiment on the function of the long-term pair bond period in the socially monogamous bearded reedling. *Animal Behaviour* 82(6):1329-1335.
- halmi.sk. 2015. Interval Timer 4 HIIT Training (2.4.1) [Mobile application software].
- Hamilton WD. 1971. Geometry for the selfish herd. *Journal of theoretical Biology* 31(2):295-311.
- Henson SM, Galusha JG, Hayward JL, Cushing JM. 2007. Modeling territory attendance and preening behavior in a seabird colony as functions of environmental conditions. *Journal of Biological Dynamics* 1(1):95-107.
- Hinton MG, Bendelow A, Lantz S, Wey TW, Schoen L, Brockett R, Karubian J. 2013. Patterns of aggression among captive american flamingos (*Phoenicopterus ruber*). *Zoo biology* 32(4):445-453.
- Hosey G, Melfi V, Pankhurst S. 2013. *Zoo animals: behaviour, management, and welfare*. OUP Oxford.
- Hosey GR, Druck PL. 1987. The influence of zoo visitors on the behavior of captive primates. *Applied Animal Behaviour Science* 18(1):19-29.
- Howarth E, Hoffman MS. 1984. A multidimensional approach to the relationship between mood and weather. *British Journal of Psychology* 75(1):15-23.
- Hughes AL. 2015. Stability of social behavior in captive American Flamingos (*Phoenicopterus ruber*): A quantitative case study. *Zoo biology* 34(4):305-313.
- Huntingford FA. 2004. Implications of domestication and rearing conditions for the behaviour of cultivated fishes. *Journal of Fish Biology* 65(s1):122-142.
- Johnson A, Cézilly F. 2009. *The greater flamingo*. A&C Black.
- Kelley J, Magurran A, García CM. 2006. Captive breeding promotes aggression in an endangered Mexican fish. *Biological Conservation* 133(2):169-177.
- King C. 2008a. The potential contribution of captive flamingos to research. *Flamingo, Bulletin of the IUCN-SSC/Wetlands International Flamingo Specialist Group*(16):61-64.
- King C, Bračko A. 2014. Nineteen years of management for *Phoenicopteriformes* in European Association of Zoos and Aquaria institutions: the Fabulous Flamingo Surveys and strategies to increase reproduction in captivity. *International Zoo Yearbook* 48(1):184-198.

- King CE. 1994. Management and research implications of selected behaviours in a mixed colony of flamingos at Rotterdam Zoo. *International Zoo Yearbook* 33(1):103-113.
- King CE. 2000. Captive flamingo populations and opportunities for research in zoos. *Waterbirds*:142-149.
- King CE. 2008b. A hypothetical husbandry point system for breeding flamingos in captivity. *Flamingo* 16:57-61.
- King CE, Maher S, Stevens E. 2005. Flamingo husbandry guidelines: A Joint Effort of the AZA and EAZA in cooperation with Wildfowl and Wetlands Trust. p 164-167.
- Kvarnemo C, Ahnesjö I. 1996. The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution* 11(10):404-408.
- Larsen MJ, Sherwen SL, Rault J-L. 2014. Number of nearby visitors and noise level affect vigilance in captive koalas. *Applied Animal Behaviour Science* 154:76-82.
- Lindburg DG, Fitch-Snyder H. 1994. Use of behavior to evaluate reproductive problems in captive mammals. *Zoo Biology* 13(5):433-445.
- Linden M. 1991. Divorce in great tits--chance or choice? An experimental approach. *The American Naturalist* 138(4):1039-1048.
- Lindgren CJ, Pickering S. 1997. Ritualised displays and display frequencies of Andean Flamingos *Phoenicoparrus andinus*. *Wildfowl* 48(48):194-201.
- Martin GR. 2012. Through birds' eyes: insights into avian sensory ecology. *Journal of Ornithology* 153(1):23-48.
- Martin P, Bateson P. 2007. *Measuring behaviour: an introductory guide*. Third ed. Cambridge, UK: Cambridge University Press.
- Mason GJ. 2010. Species differences in responses to captivity: stress, welfare and the comparative method. *Trends in Ecology & Evolution* 25(12):713-721.
- McPhee ME. 2004. Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. *Biological Conservation* 115(1):71-77.
- Melfi V. 2009. There are big gaps in our knowledge, and thus approach, to zoo animal welfare: a case for evidence-based zoo animal management. *Zoo Biology* 28(6):574-588.
- Mills SC, Reynolds JD. 2003. Operational sex ratio and alternative reproductive behaviours in the European bitterling, *Rhodeus sericeus*. *Behavioral Ecology and Sociobiology* 54(2):98-104.
- Morgan KN, Tromborg CT. 2007. Sources of stress in captivity. *Applied Animal Behaviour Science* 102(3/4):262-302.
- Moyer BR, Drown DM, Clayton DH. 2002. Low humidity reduces ectoparasite pressure: implications for host life history evolution. *Oikos* 97(2):223-228.

- O'Connell-Rodwell CE, Rojek N, Rodwell TC, Shannon PW. 2004. Artificially induced group display and nesting behaviour in a reintroduced population of Caribbean Flamingo *Phoenicopterus ruber ruber*. *Bird Conservation International* 14(01).
- O'Donovan D, Hindle JE, Sean M, Sean OD. 1993. Effect of visitors on the behaviour of female cheetahs. *International Zoo Yearbook* 32:238-244.
- Ogden JJ, Lindburg DG, Maple TL. 1994. A preliminary study of the effects of ecologically relevant sounds on the behaviour of captive lowland gorillas. *Applied Animal Behaviour Science* 39(2):163-176.
- Parker G. 1974. Courtship persistence and female-guarding as male time investment strategies. *Behaviour* 48(1):157-183.
- Part T, Gustafsson L. 1989. Breeding dispersal in the collared flycatcher (*Ficedula albicollis*): possible causes and reproductive consequences. *The Journal of Animal Ecology*:305-320.
- Peluso A, Anderson M. 2014. The role of lateralization in feeding behavior and scratching preference in relation to social behavior in captive Caribbean flamingos (*Phoenicopterus ruber*). *Anim Behav Cogn* 1:51-65.
- Peluso AI, Royer EA, Wall MJ, Anderson MJ. 2013. The relationship between environmental factors and flamingo aggression examined via internet resources. *Avian Biology Research* 6(3):215-220.
- Pickering S, Creighton E, Stevens-Wood B. 1992. Flock size and breeding success in flamingos. *Zoo Biology* 11(4):229-234.
- Pickering SPC. 1992. The comparative breeding biology of flamingos *Phoenicopteridae* at The Wildfowl and Wetlands Trust Centre, Slimbridge. *International Zoo Yearbook* 31(1):139-146.
- Pradel R, Choquet R, Bechet A. 2012. Breeding experience might be a major determinant of breeding probability in long-lived species: the case of the greater flamingo. *PloS one* 7(12):e51016.
- Pulliam HR. 1973. On the advantages of flocking. *Journal of theoretical Biology* 38(2):419-422.
- R Core Team. 2016. R: A language and environment for statistical computing. Version 3.3.1. Vienna, Austria: R Foundation for Statistical Computing.
- Reifman AS, Larrick RP, Fein S. 1991. Temper and temperature on the diamond: The heat-aggression relationship in major league baseball. *Personality and Social Psychology Bulletin* 17(5):580-585.
- Rose P, Croft D. 2015a. The potential of Social Network Analysis as a tool for the management of zoo animals. *Animal Welfare* 24(2):123-138.
- Rose PE, Croft DP. 2015b. Evidence of directed interactions between individuals in captive flamingo flocks. *Wildfowl* 65(65):121-132.
- Rose PE, Croft DP, Lee R. 2014. A review of captive flamingo (*Phoenicopteridae*) welfare: a synthesis of current knowledge and future directions. *International Zoo Yearbook* 48(1):139-155.

- Schmaltz L, Cézilly F, Béchet A. 2011. Using multistate recapture modelling to assess age-specific bottlenecks in breeding success: a case study in the greater flamingo *Phoenicopterus roseus*. *Journal of Avian Biology* 42(2):178-186.
- Schmitz RA, Baldassarre GA. 1992. Correlates of flock size and behavior of foraging American flamingos following hurricane Gilbert in Yucatan, Mexico. *Condor*:260-264.
- Shannon PW. 2000. Social and reproductive relationships of captive Caribbean flamingos. *Waterbirds: The International Journal of Waterbird Biology* 23:173.
- Sherwen SL, Magrath MJ, Butler KL, Hemsworth PH. 2015. Little penguins, *Eudyptula minor*, show increased avoidance, aggression and vigilance in response to zoo visitors. *Applied Animal Behaviour Science* 168:71-76.
- Stevens EF, Beaumont JH, Cusson EW, Fowler J. 1992. Nesting behavior in a flock of Chilean flamingos. *Zoo Biology* 11(3):209-214.
- Stevens EF, Pickett C. 1994. Managing the social environments of flamingos for reproductive success. *Zoo Biology* 13(5):501-507.
- Stevens J, Thyssen A, Laevens H, Vervaecke H. 2013. The influence of zoo visitor numbers on the behaviour of harbour seals (*Phoca vitulina*). *Journal of Zoo and Aquarium Research* 1(1):31-34.
- Studer-Thiersch A. 1975. Basle zoo. Flamingos Berkhamsted, England: Poyser p:120-130.
- Studer-Thiersch A. 1998. Basle Zoo's veteran flamingos. *Flamingo Specialist Group Newsletter* 8:33.
- Studer-Thiersch A. 2000. What 19 years of observation on captive greater flamingos suggests about adaptations to breeding under irregular conditions. *Waterbirds*:150-159.
- Tavecchia G, Pradel R, Boy V, Johnson AR, Cézilly F. 2001. Sex-and age-related variation in survival and cost of first reproduction in greater flamingos. *Ecology* 82(1):165-174.
- Tetley CL, O'Hara SJ. 2012. Ratings of animal personality as a tool for improving the breeding, management and welfare of zoo mammals. *Animal Welfare* 21(4):463-476.
- The Weather Network. 2016. Granby, Quebec Hourly Weather Forecast. The Weather Network.
- Thompson VD. 1989. Behavioral response of 12 ungulate species in captivity to the presence of humans. *Zoo Biology* 8(3):275-297.
- Venables W, Ripley B. 2002. *Modern Applied Statistics with S*. Fourth ed. New York: Springer.
- Vine I. 1973. Detection of prey flocks by predators. *Journal of Theoretical Biology* 40(2):207-210.
- Weir LK, Grant JW, Hutchings JA. 2011. The influence of operational sex ratio on the intensity of competition for mates. *The American Naturalist* 177(2):167-176.
- Wetherill C. 2015. Building barplots with error bars. R-bloggers.
- World Association of Zoos and Aquariums. 2005. Building a future for wildlife: The world zoo and aquarium conservation strategy. Waza Executive Office.

- Yosef R. 2000. Individual Distances among Greater Flamingos as Indicators of Tourism Pressure. *Waterbirds: The International Journal of Waterbird Biology* 23:26.
- Zhang G, Swaisgood RR, Zhang H. 2004. Evaluation of behavioral factors influencing reproductive success and failure in captive giant pandas. *Zoo Biology* 23(1):15-31.

Appendix A

Table 3.1. Individual flamingos can be identified using their unique leg bands. The left and right leg columns denote which leg the specific band is found on. When there is a number and a color, it is a colored band with a number on it. When there is no number listed, it means the band is a plain color. The ZIMS number is the individual's number in the Species360 Zoological Information Management System (ZIMS) database.

Left Leg	Right Leg	#ZIMS	Sex	Birth Year
47 Yellow	Blue / 018 Metal	800078	Female	1978
11 Yellow / Blue / 05 Metal		210078	Male	1978
38 White	Red / 58 Metal	220078	Female	1978
9 Yellow / Red / 61 Metal		240078	Male	1978
54 Yellow	Green / 01 Metal	700078	Female	1978
65 Yellow / Green		610083	Male	1983
4 White / Yellow / 22 Metal		900085	Male	1985
56 Metal	63 Yellow / Yellow/ 430 Metal	B92165	Female	1992
22 White / Orange		B96014	Male	1996
27 Yellow / Blue	Blue	B98004	Male	1998
	06 Orange / Orange	B00024	Male	2000
Green	60 Yellow / Green	B00025	Female	2000
10 Orange	Yellow	B00026	Male	2000
13 Orange / White	White	B00027	Male	2000
39 White / Orange	Orange	B02036	Male	2001
Red / Red	46 Yellow / Blue	B01032	Female	2001
	35 White / Green	B01033	Female	2001
Red	37 White / Yellow	B01035	Female	2001
Blue	52 Yellow / Yellow / 083 Metal	B02042	Female	2002
Blue / 087 Metal	53 Yellow / White	B02043	Female	2002
48 Yellow / Blue	Orange / 85 Metal	B02044	Male	2002
58 Yellow / Green	Yellow / 095 Metal	B02045	Male	2002
50 Yellow / Green / White	99 Metal	B02046	Male	2002
51 Yellow / Green	Orange / Metal	B02047	Male	2002
Yellow / 160 Metal	Orange / 69 Metal	B02048	Female	2002
Red / White	Orange / 200 Metal	B03020	Female	2003
61 Yellow / Blue	Green / 0867 Metal	B08007	Female	2008
62 Yellow / Yellow	White / 155 Metal	B09007	Male	2009

Appendix B

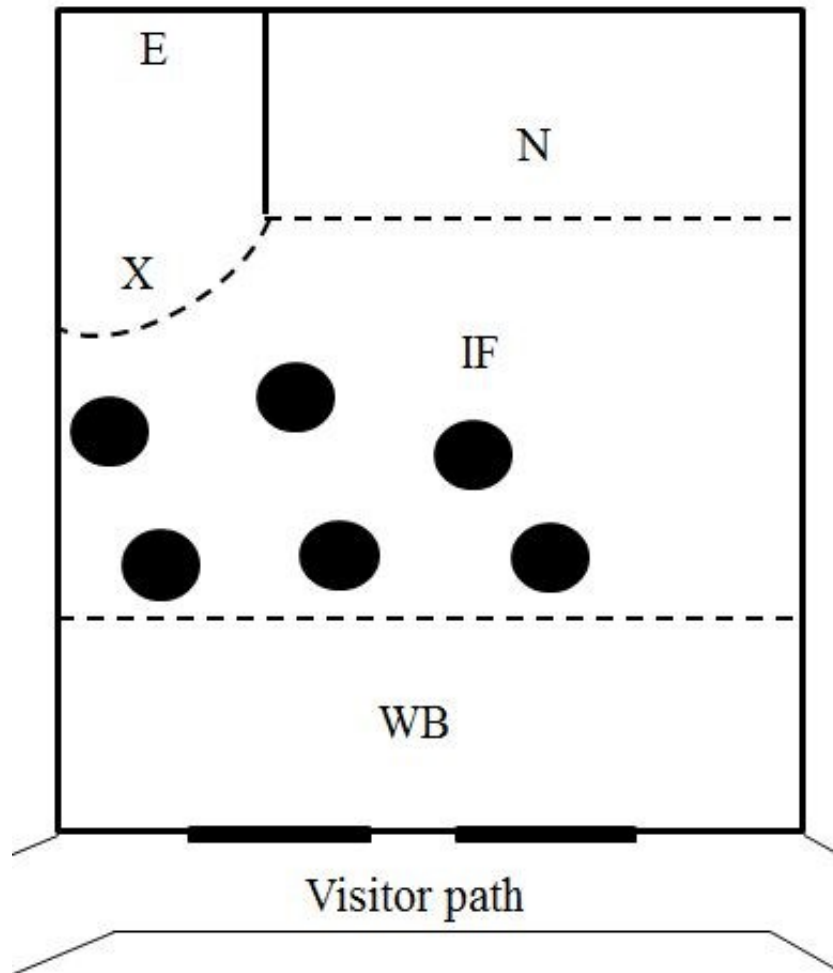


Figure 3.1. The indoor habitat is $\sim 66\text{m}^2$ consisting of a large nesting area and a shallow water basin. The habitat is broken down into zones for ease of recording locations. The zones are divided into an entrance area (E) which the flamingos did not have access to during observations, a nesting area (N), indoor feeding area (IF), and a water basin (WB). The habitat features mirrors spanning halfway up all of the walls other than the entrance area. The solid black rectangles near the visitor path represent the two-way mirrors which allow visitors to view the flamingos. The curved, dashed line represents the hiding wall behind which the observations are made, and the observer spot is denoted by the “X”. The circles represent the placement of food trays in an attempt to minimize disturbance to the flamingos.

Appendix C

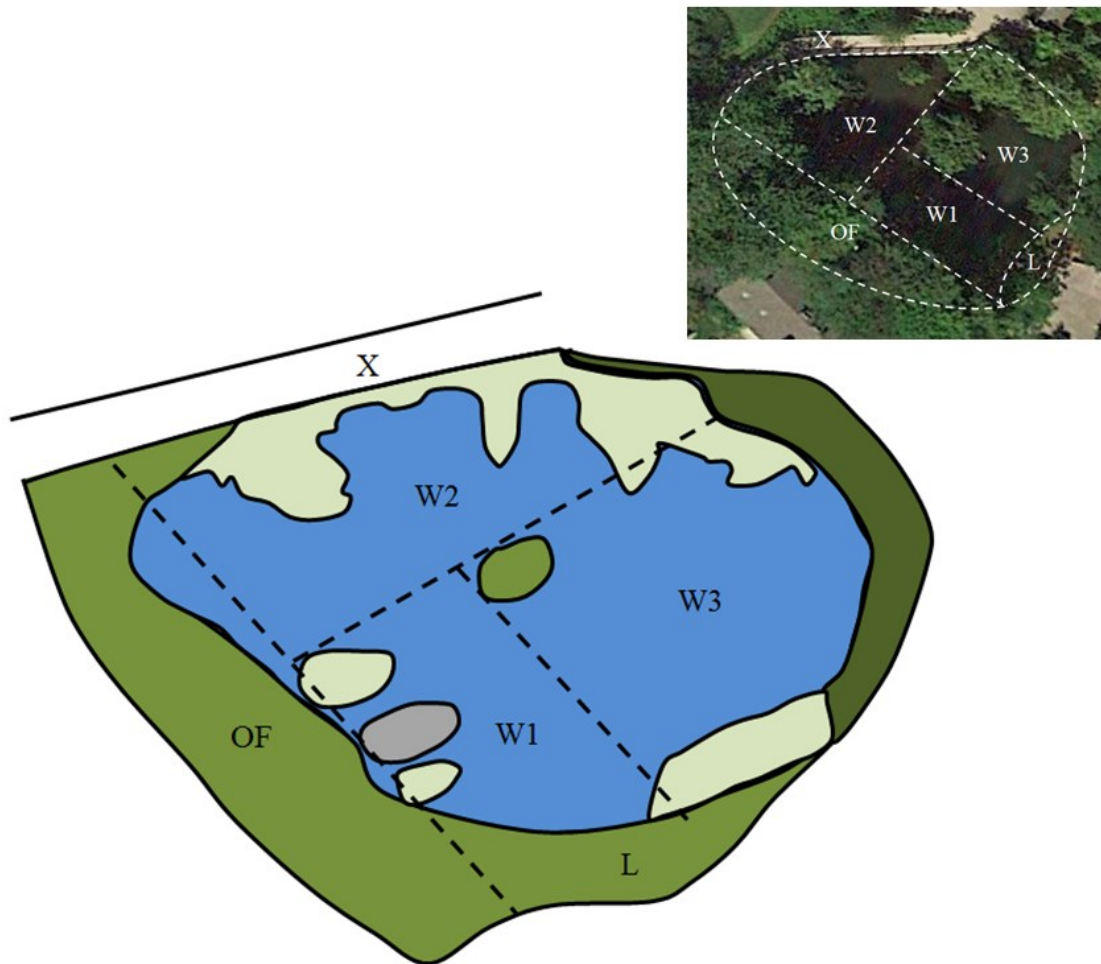


Figure 3.2. The photo in the top right is an aerial view of the habitat, while the center image is a schematic of the habitat. The outdoor habitat consists of $\sim 286\text{m}^2$ of accessible ground cover (grass) and a large pond ($\sim 686\text{m}^2$) covering approximately 71% of the habitat. The habitat is broken down into zones for ease of recording locations. The zones are divided into an outdoor feeding area (OF), land cover (L), section of the pond near the viewing area (W1), section of the pond near the raised boardwalk (W2), and a section of the pond with dense foliage where visibility is low (W3). The “X” represents the observer spot on the public boardwalk.

Appendix D

Table 3.2. Model selection based on AIC to explain the variability in the frequency of behaviors (resting, preening, foraging, locomotion, vigilance) based on outdoor weather conditions (maximum temperature, maximum humidity, cloud cover). We present all models within $\Delta AIC < 2$. The bold terms correspond with the most parsimonious model.

Model	Temperature	Humidity	Cloud Cover	AIC	ΔAIC
Rest					
1	X	X		707.876	1.9892
2		X		705.8868	0
Preen					
1	X		X	677.4019	1.4223
2		X	X	677.4307	1.4511
3	X	X		677.8813	1.9017
4			X	675.9796	0
5		X		676.2636	0.284
Forage					
1	X	X		696.7794	0.9782
2	X			696.3594	0.5582
3		X		695.8012	0
Locomotion					
1	X	X		495.9396	1.6695
2		X		494.2701	0
Vigilance					
1	X	X		362.0861	1.9787
2	X			360.1074	0

Appendix E

Table 3.3. Model selection based on AIC to explain the variability in the frequency of behaviors (resting, preening, foraging, locomotion, vigilance) based on indoor weather conditions (maximum temperature, maximum humidity). We present all models within $\Delta AIC < 2$. The bold terms correspond with the most parsimonious model.

Model	Temperature	Humidity	AIC	ΔAIC
Rest				
1	X	X	373.0131	1.9982
2	X		371.7368	0.7219
3		X	371.0149	0
Preen				
1	X	X	286.3617	0.0473
2	X		286.4432	0.1288
3		X	286.3144	0
Forage				
1	X	X	303.2519	1.953
2	X		301.2989	0
3		X	301.3479	0.049
Locomotion				
1	X	X	254.9227	1.5813
2	X		254.8778	1.5364
3		X	253.3414	0
Vigilance				
1	X	X	188.1998	1.5237
2	X		188.1968	1.5207
3		X	186.6761	0

Appendix F

Table 3.4. Model selection based on AIC to explain the variability in the frequency of aggressive behaviors (aggressive encounters, neck swaying, sparring, pecking, hooking, chasing) based on outdoor weather conditions (maximum temperature, maximum humidity, cloud cover). We present all models within $\Delta AIC < 2$. The bold terms correspond with the most parsimonious model.

Model	Temperature	Humidity	Cloud Cover	AIC	ΔAIC
Aggressive Encounters					
1	X	X	X	727.1205	0.4158
2	X	X		726.7957	0.091
3		X	X	726.7047	0
Neck Swaying					
1	X	X		255.4413	1.9996
2	X			253.4417	0
Sparring					
1	X	X		312.1872	1.6738
2	X			310.8335	0.3201
3		X		310.5134	0
Pecking					
1	X	X		135.7892	1.596
2	X			134.1932	0
Hooking					
1	X	X	X	242.8577	0.7199
2	X	X		242.1652	0.0274
3		X	X	242.4536	0.3158
4		X		242.1378	0
Chasing					
1	X	X		86.3957	1.7152
2	X			84.78382	0.10332
3		X		84.6805	0

Appendix G

Table 3.5. Model selection based on AIC to explain the variability in the frequency of aggressive behaviors (aggressive encounters, neck swaying, sparring, pecking, hooking, chasing) based on indoor weather conditions (maximum temperature, maximum humidity). We present all models within $\Delta AIC < 2$ or the two models with the lowest AIC if there was only one model within $\Delta AIC < 2$. The bold terms correspond with the most parsimonious model.

Model	Temperature	Humidity	AIC	ΔAIC
Aggressive Encounters				
1	X	X	344.0251	0
2		X	346.7275	2.7024
Neck Swaying				
1	X	X	255.4413	1.9996
2	X		253.4417	0
Sparring				
1	X	X	312.1872	1.6738
2	X		310.8335	0.3201
3		X	310.5134	0
Pecking				
1	X	X	135.7892	1.596
2	X		134.1932	0
Hooking				
1	X	X	242.1652	0.0274
2		X	242.1378	0