

# Effect of antipredator training on spatial behaviour of male and female Greater Rheas *Rhea americana* reintroduced into the wild

Marilina VERA CORTEZ<sup>1,2</sup>, Joaquín Luis NAVARRO<sup>1,2\*</sup> & Mónica Beatriz MARTELLA<sup>1,2,3</sup>

<sup>1</sup>Universidad Nacional de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Centro de Zoología Aplicada, ARGENTINA

<sup>2</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) — Instituto de Diversidad y Ecología Animal (IDEA), ARGENTINA

<sup>3</sup>Universidad Nacional de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Departamento de Diversidad Biológica y Ecología, Cátedra de Ecología y Conservación, ARGENTINA

\*Corresponding author, e-mail: joaquin.navarro@unc.edu.ar

Vera Cortez M., Navarro J. L., Martella M. B. 2018. Effect of antipredator training on spatial behaviour of male and female Greater Rheas *Rhea americana* reintroduced into the wild. *Acta Ornithol.* 53: 00–00. DOI 10.3161/00016454AO2018.53.1.008

**Abstract.** The Greater Rhea *Rhea americana* is a South-American flightless bird whose populations have decreased due to poaching and fragmentation of suitable habitats, and there has been an urgent need to carry out management practices to prevent local or regional extinctions. Several studies revealed that reintroduction might be an appropriate action for increasing the viability of wild populations of this species. Nevertheless, although this is a popular and useful conservation strategy, the animals to be released should be prepared against risks like predation and dispersion that could influence the post-release success. We evaluated the effect of an antipredator training applied to captive-bred Greater Rheas before they were released into the wild, on their dispersal and home range size and overlap, supposing that the trained animals would avoid dispersing into places with predators and, consequently, they would reduce their home range. We also studied the habitat use by the released rheas. Eleven trained and nine control (untrained) animals were marked and monitored two to four times per day during the first week, and from 4 to 19 consecutive days per month during four months, throughout the breeding and post-breeding seasons. The antipredator training affected the home range in different ways according to sex. Trained females exhibited smaller home ranges (mean  $\pm$  S.E.:  $0.54 \pm 0.58$  km<sup>2</sup>) than the rest of the individuals (control females  $5.8 \pm 0.75$  km<sup>2</sup>, control and trained males:  $2.11 \pm 0.65$  km<sup>2</sup> and  $2.96 \pm 0.65$  km<sup>2</sup>, respectively), whereas their overlap was greater ( $63.83 \pm 16.95\%$ ) than that of the untrained females ( $24.04 \pm 21.88\%$ ). Males of both experimental groups showed similar distances travelled ( $3.21 \pm 0.29$  km), which were also greater than those of females ( $2.21 \pm 0.32$  km). The home ranges of males were not influenced by the training and they showed a high degree of overlapping (among control males  $29.96 \pm 18.95\%$ , and among trained males:  $35.81 \pm 18.95\%$ ). Both groups similarly used crops and in lesser extent open areas and grassland. Only the trained females reduced their movements and wandering as a result of the previous conditioning. They moved to crop or open areas, avoiding tall vegetation such as that present in grasslands, which may make difficult to detect predators. On the other hand, in the males, the influence of the reproductive season and the complex mating system exhibited by this species prevailed over the possible effects of training. Our work shows new and useful data about the spatial behaviour and reintroduction for conservation of the Greater Rhea.

**Key words:** radio telemetry, conservation, wildlife management, captive management, reintroduction, Rhea

Received — Nov. 2017, accepted — June 2018

## INTRODUCTION

Reintroduction is a widely used conservation tool (Armstrong & Seddon 2008, Seddon 2010), but it frequently fails due to different reasons, such as stress, or the lack of a suitable habitat (Dickens et al. 2010). Also dispersion (Le Gouar et al. 2012) and predation should be taken as possible causes of failure. Particularly the latter is an important factor when reintroduced animals came from captivity (Dickens et al. 2010), because they can

exhibit deficiencies in behaviours such as anti-predator strategies, foraging, locomotion, or reproduction (Rabin 2003). Because these can be disadvantageous to the birds when they are reintroduced into the wild (McPhee & Silverman 2004), the candidates to be released should be subjected to previous conditioning experiences, particularly those focused on the recognition of predators (Kleiman 1989, Beck et al. 1994). After release, they often perform extensive movements, either to identify appropriate resources (Kesler et

al. 2012) or to cope with the stress generated by the new habitat (Dickens et al. 2010). This high dispersion can be harmful to the animals because it can lead to their isolation, making difficult their finding of mates (Armstrong & Wittmer 2011), and to their passing through unsuitable areas, increasing their probability of being predated (Yoder et al. 2004), while it consumes time at the expense of other activities, like foraging, reproduction and/or vigilance (Le Gouar et al. 2012).

Habitat quality, age, sex and other factors (Börger et al. 2008) can affect the home range within a species, because of the different ways in which the animals may gather their food or avoid potential predators (Powell & Mitchell 2012). In vertebrates, sex differences in home range can emerge from a combination of the reproductive strategy, and the social organization of the species and both aspects are related to spatial behaviour (Bonatto et al. 2012, Campioni et al. 2013).

Wild populations of Greater Rheas *Rhea americana* in Argentina are distributed from the north of the country to the Río Negro, in steppes, savannas, shrublands and woodlands, where they select different sites throughout the year, based upon the quality and availability of proper food, and the predation risk (Martella et al. 1996). The species prefers open habitats, where predators can be more easily detected (Bellis et al. 2004a, Herrera et al. 2004, Carro & Fernández 2009) and where there is low or absent human disturbance (Bellis et al. 2004a, Herrera et al. 2004). Its natural predators are the Puma *Puma concolor* and the Jaguar *Panthera onca* (Del Hoyo et al. 1992), but the actual pressure they exert on rhea populations is still unknown. The main threats to rheas are related to human activities: poaching and agriculture intensification (Giordano et al. 2010, Navarro & Martella 2011). Consequently, the species is categorised as Near Threatened by IUCN (BirdLife International 2017). A conservation tool that might be used as to reverse the current situation of this species in central Argentina is reintroduction (Navarro & Martella 2011, Bazzano et al. 2014). Related studies carried out under captivity have shown that the Greater Rheas changed their spatial behaviour, increasing the time devoted to running and walking, either as a stress response generated by transportation (Della Costa et al. 2013), or after an antipredator training (Azevedo & Young 2006a,b, Vera Cortez et al. 2015).

The relationship between the home range and the release of captive-bred animals has been studied using different approaches in the Greater

Rhea (Bellis et al. 2004a,b, Bazzano et al. 2010), in the closely related Lesser Rhea *Rhea pennata* (Bellis et al. 2004b) and even in other ratite species with distinct life-history characteristics different from those of rheas, such as the Great Spotted Kiwi *Apteryx haastii* (Keye et al. 2011, Jahn et al. 2013) and the Ostrich *Struthio camelus* (Islam et al. 2008). However, the combined influence of the gender of released animals and the antipredator training on home range remains unexplored.

We studied the effect of antipredator training on spatial behaviour, particularly the size of home range and intra and intersexual overlap, and the dispersal distance of Greater Rheas of both sexes released into the wild. Given that the habitat and the surrounding matrix could influence the success of reintroductions, we selected a site that we considered highly suitable, where the requirements of rheas were mostly covered (Bellis et al. 2004a). This reintroduction place was an extensive area, with low vegetation and with high density of the preferred food (Martella et al. 1996). Although the training was oriented to the recognition of the main predator in the release area, and was not related to habitat or vegetation height preferences, we worked under the assumption that dispersion towards surrounding sites with taller shrubby vegetation, where Pumas were present, would be low. For validating this assumption, we studied the habitat use of the rheas in the release area. Accordingly, we expected that the trained animals would avoid those risky places, reducing their home range.

## MATERIALS AND METHODS

### Animals

Twenty adult Greater Rheas, between two and three years old, bred under captive conditions at the experimental farm at Córdoba Zoo, Argentina (31°25'31.79"S, 64°10'29.92"W), were individually identified with Velcro® leg-bands and were randomly separated into two groups. Six males and five females constituted the antipredator trained group (ATG), while five males and four females composed the control group (CG, untrained). These animals were kept grouped in two separate pens where food (pelleted feed and dehydrated alfalfa) and water were provided *ad libitum*, until reintroduction.

Antipredator training was carried out during captivity at the Zoo, from May to July 2011, and consisted of exposing individual Greater Rheas to

a stimulus (a taxidermized Puma in a moving platform) along with an aversive experience (a simulated chase conducted by a person under a costume). Also, the animals were exposed to an innocuous stimulus (a chair) that was used to detect if they responded to the predator model and not simply to the movement of the platform. Each training session was filmed and lasted 18–19 min. Training with both models (Puma and chair) was performed in a similar way, but only the appearance of the Puma was paired to the presentation of the aversive stimulus (Vera Cortez et al. 2015). Each individual received five sessions with each model. Taking into account that training sessions might be considered a stressful situation, and that Della Costa et al. (2013) reported that individuals undergoing a stressful experience show stress-related behaviors for up to 3 days, we decided to conduct training sessions at 3 to 5-day intervals. Then a memory test was carried out individually on control and trained animals with the aim of comparing behavioral responses between groups (according to Azevedo & Young 2006a,b). This test was performed twice: 30 and 60 days before the last day of training. In this case, the predator model was exhibited to the birds after training, but not associated with any aversive event. The animals actually learn to react against the predator and we observed that they modified the time they devoted to antipredator behaviours (for more details, see Vera Cortez et al. 2015).

### Release area

The birds were released 400 km away from the Zoo, at Las Dos Hermanas ranch (33°40'S, 63°19'W), in the southeast of Córdoba province (Argentina). This 4189-ha ranch comprises different habitats devoted to cattle raising and wildlife conservation, which result suitable for establishing Greater Rhea populations (Bellis et al. 2004a). Vegetation in this area included grasslands, implanted species as Alfalfa *Medicago sativa*, shrubs, salt flats and saline marshes (Cantero et al. 1994). Main herbivores were cattle, horses, and hares. A wild Greater Rhea population was present originally in this area, but it declined probably due to illegal poaching until 2006, when it disappeared.

### Reintroduction procedure

In December 2011 (breeding season), all the rheas were transported, maintaining their original group composition in two conditioned vehicles,

according to the procedure suggested by Navarro & Martella (2011) and to IUCN (1998) guidelines for reintroduction. Before being transported, eight trained individuals and six from the control group were equipped with CB-5 expansion collars with transmitters (Telonics, Arizona, USA) (Table 1).

Upon arrival to the release area, the birds were housed in two temporary pens built with plastic shade cloth and they were provided with water and food, following a soft-release strategy (Bellis et al. 2004b). Both pens were located at a distance of 1.36 km from each other, in Alfalfa paddocks, as this is the preferred food of Greater Rheas in agroecosystems (Martella et al. 1996). The animals were maintained in the pens for three days because Greater Rheas recover their basal levels of fecal glucocorticoids metabolites 72 h after transportation (Lêche 2012) and then their behavioural activities are stabilized (Della Costa et al. 2013). After this period, the pens were completely removed and no further water or feed was provided, allowing animals to leave the site and move away freely to obtain food and water by themselves from the environment.

Table 1. Characteristics of the released individuals: identification, group, sex, 95% Minimum Convex Polygon (MCP), and maximum linear distance traveled (from the releasing site to the farthest localization). \* — Individuals with radio-collar. † — Individuals excluded from all analyses (located only a few times, as they died during the first weeks after being released: C5 — euthanized because it was found with a broken leg; E3 — killed by Puma; E4 — disappeared, probably poached).

| Rhea ID                           | Sex     | 95% MCP<br>(km <sup>2</sup> ) | Distance<br>travelled (km) |
|-----------------------------------|---------|-------------------------------|----------------------------|
| Control group (CG)                |         |                               |                            |
| C1                                | Male*   | 0.81                          | 2.82                       |
| C2                                | Male*   | 0.91                          | 4.02                       |
| C3                                | Male    | 4.99                          | 3.57                       |
| C4                                | Male    | 1.72                          | 4.72                       |
| C5                                | Male†   | ---                           | ---                        |
| C7                                | Female† | ---                           | ---                        |
| C8                                | Female* | 5.59                          | 3.62                       |
| C9                                | Female* | 7.01                          | 3.85                       |
| C10                               | Female* | 3.84                          | 4.58                       |
| Antipredator training group (ATG) |         |                               |                            |
| E2                                | Male*   | 1.5                           | 3.60                       |
| E3                                | Male†   | ---                           | ---                        |
| E4                                | Male†   | ---                           | ---                        |
| E5                                | Male*   | 2.8                           | 3.67                       |
| E6                                | Male*   | 3.85                          | 3.71                       |
| E7                                | Female* | 0.99                          | 1.38                       |
| E8                                | Female* | 0.54                          | 0.62                       |
| E9                                | Female  | 0.37                          | 0.66                       |
| E10                               | Female  | 0.49                          | 0.68                       |
| E11                               | Male    | 3.65                          | 2.73                       |
| E12                               | Female* | 0.32                          | 1.53                       |

### Data collection and analysis

Each individual was located either by direct radio-tracking, triangulation or by observation two to four times per day at  $\geq 2$  h intervals, to minimize dependency between successive locations, following the method used by Bellis et al. (2004b). This tracking was carried out during the first week and for 4–19 consecutive days per month for four months, using a Telonics TR4 (168–172 MHz) portable receiver with a Telonics two-element Yagi antenna. This post-release interval was selected because it provided the maximum number of localizations for all individuals (after that there occurred deaths caused by poaching and by dog attacks). As the animals remained in groups during the sampling period, it was feasible to obtain localizations for all of them, even those that did not have radio-collars, allowing us to include them also in the home range analysis.

Following the criteria of Bellis et al. (2004b), we calculated the individual home range by means of the 95% Minimum Convex Polygon method, and the maximum linear distance travelled was taken as that measured from the releasing site to the farthest location. Besides, we estimated the degree of overlap of individual home range, according to Steinmann et al. (2005). To estimate the individual average value of overlap, we included all birds of both genders, even those whose home ranges did not exhibit any overlap at all. It was assumed that when the home range overlap is  $\leq 10\%$ , it indicates an avoiding behaviour by those animals, which in turn is taken as an evidence of territorial behaviour (Steinmann & Bonatto 2015).

We calculated home ranges by means of the 95% Minimum Convex Polygon because this method has been already used in rhea studies (Bellis et al. 2004a,b, Juan et al. 2013). Also, it is easier to calculate and it is not conditioned on an underlying statistics distribution, then it has been widely used for comparisons in the literature (Laver & Kelly 2008).

Software Biotas TM 2.0 2005 was employed to conduct exploratory data analysis and to estimate the linear distances, and the home range size and overlap.

Spatial data from each group (trained and control) were evaluated using a mixed model analysis. The response variables were the home range size, home range overlap, and linear distance. For home range and linear distance analysis the predictor variables were the treatment (training and control), sex (male and female), and the possible interactions that may have occurred between

treatment and sex. The variable individual was included as a random factor.

For the home range overlap analysis, the fixed factors were the type of overlap (male-male, male-female, or female-female), the experimental group (trained and control), and the interactions. The variable individual was included as a random factor. Each gender was analysed separately and the levels of the type of overlap were assessed.

Additionally, taking into account that the trained animals would avoid areas with high vegetation that impedes the view of possible predator attacks, and this could exert influence on their home range, we analyzed the use of different habitats by the rheas in the study area. Following the methods of Bellis et al. (2004a), the localizations of each individual of both experimental groups were counted as frequencies of use and assigned to each habitat type present in the study area when the study was conducted.

The habitat types considered here were: crops (34% including Alfalfa, wheat, oats, barley, and combinations of the tree latter in the same plot), open areas (12%, "fallow areas"), grasslands (40%, *Stipa* sp., *Spartina densiflora*, *Distichlis spicata*, *Cyperus* sp., *Juncus* sp., and *Eleocharis* sp.), shrublands (6.1%), and salted flats (6.9%). The habitat use throughout the study period was evaluated by means of mixed generalized linear models for each group and sex, because data did not fulfill the requirements for parametric statistics. The response variable was the frequency given by each localization transformed to ranks and, as it was a count, we used the Poisson distribution, and the predictor variables were the habitat types. The individual was included as a random factor. Lastly, an *a posteriori* Fisher LSD test was carried out.

Two control individuals, a male and a female, and two trained males were excluded from all analysis because they were located only a few times, as they died during the first weeks after being released (Table 1). Statistical analyses were performed using the Infostat statistical software package (Di Rienzo et al. 2012).

## RESULTS

The antipredator training affected the home range of Greater Rheas, and its size was dependent on gender ( $F_{1,12} = 18.72$ ,  $p < 0.005$ ). The home range of control females was larger (mean  $\pm$  S.E.  $5.8 \pm 0.75$  km<sup>2</sup>) than that of trained females ( $0.54 \pm 0.58$  km<sup>2</sup>), and control and trained males

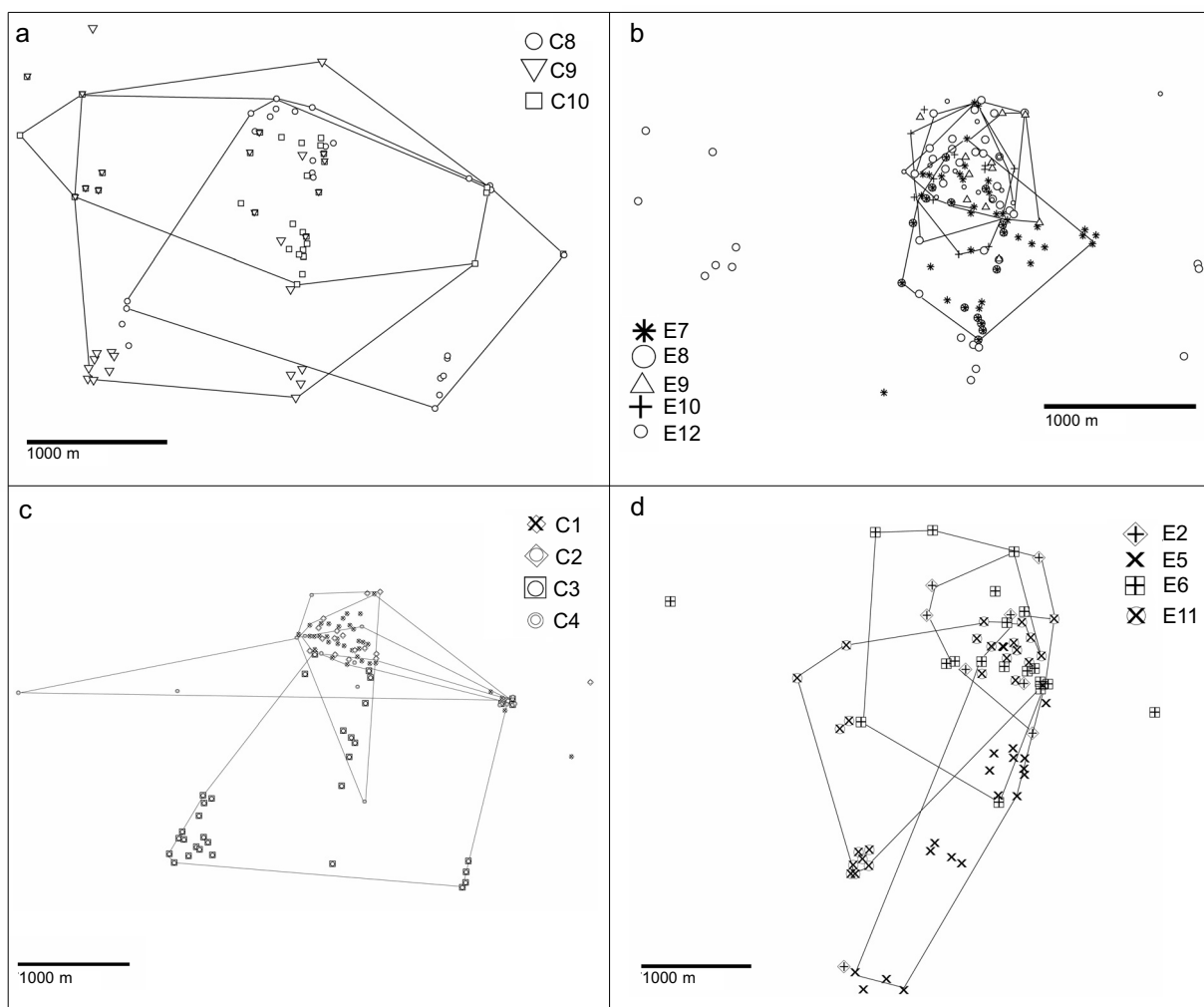


Fig. 1. Home range estimated by 95% Minimum Convex Polygon method, of Greater Rhea females (A — control, B — trained), and males (C — control, D — trained).

( $2.11 \pm 0.65 \text{ km}^2$  and  $2.96 \pm 0.65 \text{ km}^2$ , respectively) (Fig. 1 and Table 1).

The treatment did not influence the displacement distances (average of trained rheas:  $1.84 \pm 0.29 \text{ km}$  vs. control rheas:  $3.59 \pm 0.32 \text{ km}$ ;  $F_{1,12} = 2.63$ ,  $p = 0.13$ ). However, an effect of gender on this variable was observed, as all the males travelled farther than females ( $3.21 \pm 0.29 \text{ km}$  and  $2.21 \pm 0.32 \text{ km}$ , respectively;  $F_{1,12} = 9.48$ ,  $p = 0.001$ ; Table 1).

Average percentages of home range overlap were higher than 10%. In males, the treatment had no effect on this variable ( $F_{1,6} = 1.03$ ,  $p = 0.4803$ ). The individuals, disregarding their experimental group, showed similar percentages of inter and intrasexual home range overlap ( $F_{1,6} = 0.37$ ,  $p = 0.5640$ ). On the contrary, in females, the trained ones had greater overlap than the control ones ( $F_{1,6} = 50.04$ ,  $p = 0.0004$ ), where-

as if we consider all females as a whole, there were no intersexual differences in home range overlap ( $F_{1,6} = 4.30$ ,  $p = 0.0835$ ; Fig. 2).

The control and trained males used the habitats in a similar way. Both groups of males were more frequently located in crops (mean  $\pm$  S. E: control —  $64.03 \pm 9.86$ , and trained males —  $52.95 \pm 6.38\%$ ), and with no differences between open areas (control —  $21.13 \pm 3.31$ , trained —  $25.63 \pm 4.46$ ), and grasslands (control —  $13.95 \pm 7.62$  vs. trained —  $21.45 \pm 7.75$ ) ( $F_{4,20} = 47.31$ ,  $p > 0.05$ , trained  $F_{4,20} = 54.70$ ,  $p > 0.05$ ). There were no locations in shrublands and salty flats. The trained females were detected more frequently in crops ( $F_{4,25} = 72.86$ ,  $p < 0.0001$ ), and with no differences between open areas and grasslands. On the contrary, the control females showed equal frequencies of detection in the three areas ( $F_{5,18} = 39.97$ ,  $p > 0.05$ ) (Fig. 3).

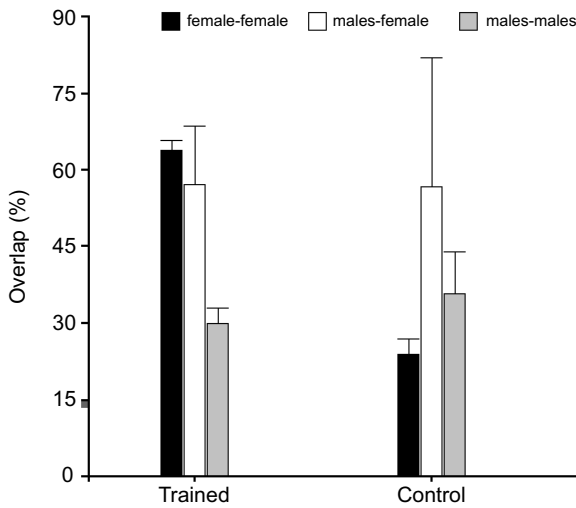


Fig. 2. Percentages (mean  $\pm$  S.E.) of the three possible types of Greater Rhea home range overlap, in relation to treatment.

## DISCUSSION

The displacement distances of the rheas and the size and overlap of their home ranges were influenced by the gender of the individuals of Greater Rhea and the antipredator training they had received. This conditioning, that modifies their abilities to recognize the predator (Azevedo & Young 2006a,b, Vera Cortez et al. 2015), affected somehow the dispersion of the rheas, and consequently their home range.

Only the trained females responded as we expected according to our hypothesis, possibly due to the fact that they reduced their movements and wandering. Thus, the trained females showed smaller home ranges with high degree of overlap, and would have used the space according to the variation in perceived risk, changing the size or location of their home range. Similarly, other species, such as the Elk *Cervus elaphus*, the Bison *Bison bison* (Laundré et al. 2001), and the Wild Boar *Sus scrofa* (Tolon et al. 2009), seem to evaluate the risk of the environment and modify their home range to minimize predation.

At the same time, although the conditioning applied in this study was not oriented to the recognition of risky habitats, the trained rheas avoided the grasslands, where visibility to possible attacks by predators is lower than in crops, which even include Alfalfa that is its preferred food (Martella et al. 1996). On the other hand, the control females would not make a distinction between low risk areas such as open areas (with low or none vegetation) from grasslands with high vegetation.

The way in which trained females used the habitats in the release area coincides with that observed in this species in the wild (Bazzano et al. 2002, Bellis et al. 2004a) and in other related species. The Puna Rhea *Rhea tarapacensis*, in its natural habitat of the Andean Puna, uses valleys and lowhill areas in relation to its nutritional

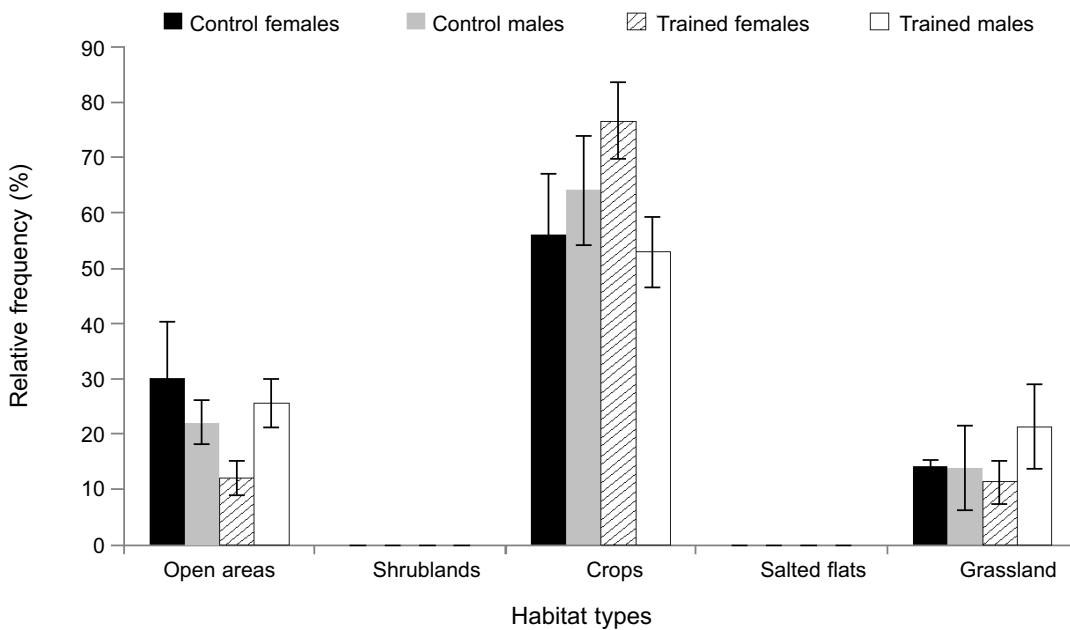


Fig. 3. Relative frequencies (mean  $\pm$  S.E.) of the localizations of Greater Rheas in the different habitat types.

requirements and predation risk, avoiding the topography that hinders vigilance and escape (Marinero et al. 2015). Also the Lesser Rhea, in the Argentine Patagonia, uses open habitats conditioned by the cost of predation (Bellis et al. 2006).

On the contrary, the males of Greater Rhea of both experimental groups (control and trained) had similar home range sizes, and their intra and intersexual overlap were higher than 10%. At the same time, these individuals used types of habitats in a similar way. This probably happens because the individuals share their territories to increase the encounter with receptive females, as they usually fight with other males and compete for access to females to form their own reproductive groups (Handford & Mares 1985). Therefore, the spatial behaviour of males is mostly influenced by the reproductive behaviour and the promiscuous mating system of this species (simultaneous and sequential polyandry combined with simultaneous polygyny; Martella et al. 2014) rather than by the training they have received.

Based on our results we can suggest that the home range of released individuals will be the result of a combination of several factors, such as the spatial distribution of resources (Mitchell & Powell 2012), the presence of conspecifics and possible competitors, the available habitat types, and the antipredator strategies of the species. In this sense, Laundré et al. (2001) have proposed that the animals perceive the landscape characteristics regarding predation risk and location of food, and then can structure a 'landscape of fear', and relocate to low risk areas.

In general, the sex variable is not taken into account in conservation programs, although it is a factor that exerts influence on stress, dispersion, reproduction and transportation (Lopes et al. 2017). As well as in this study, behavioral differences between sexes have been reported in the Greater Rhea either in captivity (Sales et al. 2000, Della Costa et al. 2013), as in the wild (Reboreda & Fernández 1997, Carro & Fernández 2009), but still a relationship could not be established among sex, the application of antipredator training, and survival (Vera Cortez et al. 2015). In Blue-fronted Amazon parrots *Amazona aestiva* that were subjected to an antipredator training previous to their release to the wild, behavioural differences were observed between males and females, and it was not possible to detect a relationship with the post-release survival (Lopes et al. 2017).

Our results show that the training applied would influence the home range size and overlap,

and the dispersal distances in a different way, according to sex. These factors have not been taken into account in previous studies about the home range in Greater Rhea (Bellis et al. 2004b, Bazzano et al. 2010), and consequently, this information was not considered when those reintroductions in this species were carried out. The pre-release conditioning would influence the animals to decrease their dispersal from optimal habitats and therefore shrink their home range, helping to achieve a better energetic balance. On the other hand, as we did not find differences in survival between trained and untrained rheas (Vera Cortez et al. 2015), nor between rheas that showed larger dispersion ranges than those with shorter ones, we have no evidences to conclusively affirm that the pre-release training such as the one we applied here has a measurable desirable effect in this parameter, and then recommend this conditioning for reintroducing individuals of this species. However, it should be stressed that in this experiment, the majority of the reintroduced rheas were killed by poachers or by dogs (either feral or brought by hunters), instead of Pumas (Vera Cortez et al. 2015).

On the other hand, the differences in the responses observed between trained and control animals could be conditioned on the personality of the individuals, linked to traits of shyness and boldness. Although in this work this characteristic was not analyzed, it should be considered that in general, after a release, bold animals would tend to be predated first; while the shy ones would naturally avoid great risks. In this sense, Azevedo & Young (2006c) have already shown in Greater Rhea under captive experimental conditions that personality influences antipredator training, so the animals react differently in each of the training sessions. Thus, these authors propose that the candidates to be released should be those with intermediate values of boldness, being able to explore the environment, find food or partner without taking fatal risks. Future reintroduction programs should include testing the link between antipredation training, personality, and survival, and should evaluate this hypothesis after release.

## CONCLUSION

Our work shows that gender composition should be a key aspect to be considered in future reintroductions of Greater Rheas. The different dispersal due to the distinct behaviour exhibited by both

sexes during the reproductive season is an important issue in this gregarious species with such a peculiar mating system. Taking into account that previous works suggest that reintroduction might be a useful conservation tool for this species (Navarro & Martella 2011, Bazzano et al. 2014), further research should be conducted to elucidate if a pre-release conditioning including the dog and the human as potential predators, will lead to a more appropriate spatial behavior and predator recognition, and a consequent higher survival in trained animals. Also, it will be important to verify if the spatial behavior of males would resemble that of females during the non-reproductive season, as there are no behavioural or glucocorticoids level differences between sexes in that part of the year (Lèche et al. 2015). We also suggest that releases should comprise more females than males, to reduce the fights that occur when the reproductive groups are conformed. This in turn will decrease the interference on the establishment of home ranges. Furthermore, the applied training will reduce the home range of the individuals, because those not subjected to it could be more prone to move farther, reaching less suitable surrounding habitats.

#### ACKNOWLEDGEMENTS

We thank the authorities of the Jardín Zoológico de Córdoba (Argentina) and their staff for allowing us to use their facilities, and the owners of Estancia Las Dos Hermanas Wildlife Refuge and the Rachel and Pamela Schiele Foundation for permitting us to conduct the research on their property. We are grateful to A. Lèche, N.S. Della Costa, A.M. and V. Delorenzi, N.V. Marinero, J.E. Minuzzi Fahn, D.J. Valdez, L.G. Vera Cortez and N.S. Zamboni, who collaborated in breeding and training the animals, and the field work. Thanks to K. Salle and G. Bazzano, for their help with Biotas, A. Steinmann for the home range overlap analysis, and G. Gonzalez for assisting us with statistical analyses. G. Giacone checked the English style. MVC is a postdoctoral fellow, and JLN and MBM are researchers of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). The proposal of this work was approved by the ethics committee of CONICET (Resolution No.1047 ANNEX II, 2005) before its implementation, as part of the author's doctoral fellowship project. This work was supported by the Secretaría de Ciencia y Tecnología

of the Universidad Nacional de Córdoba (SECyT - UNC) (grants N°162/12 and 124/13), and the Fondo para la Investigación Científica y Tecnológica de Argentina (FONCyT) (grant PICT2241).

#### REFERENCES

- Armstrong D. P., Seddon P. J. 2008. Directions in reintroduction biology. *Trends Ecol. Evol.* 23: 20–25.
- Armstrong D. P., Wittmer H. U. 2011. Incorporating Allee effects into reintroduction strategies. *Ecol. Res.* 26: 687–695.
- Azevedo C. S. De, Young R. J. 2006a. Behavioural responses of captive-born greater rheas *Rhea americana* Linnaeus (Rheiformes, Rheidae) submitted to antipredator training. *Revista Brasileira de Zoologia* 23: 186–193.
- Azevedo C. S. De, Young R. J. 2006b. Do captive-born greater Rheas *Rhea americana* Linnaeus (Rheiformes, Rheidae) remember antipredator training? *Revista Brasileira de Zoologia* 23: 194–201.
- Azevedo C. S. De, Young R. J. 2006c. Shyness and boldness in greater rheas *Rhea americana* Linnaeus (Rheiformes, Rheidae): effects of antipredator training on the personality of the birds. *Revista Brasileira de Zoologia* 23: 202–210.
- Bazzano G., Giordano P. F., Navarro J. L., Martella M. B. 2014. Linking landscape data with population viability analysis for evaluating translocations as a conservation strategy for Greater Rhea (*Rhea americana*) in central Argentina. *Ornitol. Neotrop.* 25: 25–35.
- Bazzano G., Martella M. B., Navarro J. L., Bruera N., Corbella C. 2002. [Habitat use of the Greater Rhea (*Rhea americana*) in a wildlife refuge: implications for the conservation and management of the species]. *Ornitol. Neotrop.* 13: 9–15.
- Bazzano G., Navarro J. L., Martella M. B. 2010. Behavioral responses of captive-born Greater Rheas reintroduced into the wild in central Argentina. In: Abstracts of the 25th International Ornithological Congress. Campos do Jordão, Brasil, p. 870.
- Beck B. B., Rapaport L. G., Price M. R., Wilson A. C. 1994. Reintroduction of captive born animals. In: Olney P. J. S., Mace G. M., Feistner A. T. C. (eds). *Creative conservation: interactive management of wild and captive animals*. Chapman & Hall, London, pp. 265–286.
- Bellis L. M., Martella M. B., Navarro J. L. 2004a. Habitat use by wild and captive-reared greater rheas *Rhea americana* in agricultural landscapes in Argentina. *Oryx* 38: 304–310.
- Bellis L. M., Martella M. B., Navarro J. L., Vignolo P. E. 2004b. Home range of greater and lesser rhea in Argentina: Relevance to conservation. *Biodiv. Conserv.* 13: 2589–2598.
- Bellis L. M., Navarro J. L., Vignolo P. E., Martella M. B. 2006. Habitat preferences of lesser rheas in Argentine Patagonia. *Biodiv. Conserv.* 15: 3065–3075.
- BirdLife International 2017. Species factsheet: *Rhea americana*. Downloaded from <http://www.birdlife.org> on 15/03/2018.
- Bonato F., Gomez D., Steinmann A., Priotto J. 2012. Mating strategies of Pampean mouse males. *Anim. Biol.* 62: 381–396.
- Börger L., Dalziel B. D., Fryxell J. M. 2008. Are there general mechanisms of animal home range behaviour? A review and prospects for future research. *Ecol. Lett.* 11: 637–650.
- Campioni L., Delgado M. del M., Lourenço R., Bastianelli G., Fernández N., Penteriani V. 2013. Individual and spatio-temporal variations in the home range behaviour of a long-lived, territorial species. *Oecologia* 172: 371–385.



- Cantero J. J., Nuñez C., Giayetto O., Cisneros J. M. 1994. [Floristic composition and dynamics of natural grasslands: proposed use]. Estancia Las Dos Hermanas, Arias, Córdoba, Argentina.
- Carro M. E., Fernández G. J. 2009. Scanning pattern of greater rheas, *Rhea americana*: collective vigilance would increase the probability of detecting a predator. *J. Ethol.* 27: 429–436.
- Del Hoyo J., Elliot A., Sargatal J. A. (eds) 1992. Handbook of the birds of the World. Vol. 1. Lynx Editions, Barcelona.
- Della Costa N. S., Lèche A., Guzmán D. A., Navarro J. L., Marin R. H., Martella M. B. 2013. Behavioral responses to short-term transport in male and female Greater Rheas (*Rhea americana*) reared in captivity. *Poult. Sci.* 92: 849–857.
- Di Rienzo J. A., Casanoves F., Balzarini M. G., Gonzalez L., Tablada M., Robledo C. W. 2012. InfoStat versión 2011. InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.
- Dickens M. J., Delehanty D. J., Romero L. M. 2010. Stress: An inevitable component of animal translocation. *Biol. Conserv.* 143: 1329–1341.
- Giordano P. F., Navarro J. L., Martella M. B. 2010. Building large-scale spatially explicit models to predict the distribution of suitable habitat patches for the Greater rhea (*Rhea americana*), a near-threatened species. *Biol. Conserv.* 143: 357–365.
- Handford P., Mares M. A. 1985. The mating systems of ratites and tinamous: an evolutionary perspective. *Biol. J. Linn. Soc.* 25: 77–104.
- Herrera L. P., Comparatore V. M., Lattera P. 2004. Habitat relations of *Rhea americana* in an agroecosystem of Buenos Aires Province, Argentina. *Biol. Conserv.* 119: 363–369.
- Islam M. Z. U., Ismail K., Boug A. 2008. Re-introduction of the Red-necked Ostrich, *Struthio camelus camelus*, in Mahazat as-Sayd Protected Area in central Saudi Arabia. *Zool. Middle East* 44: 31–40.
- IUCN 1998. Guidelines for Re-introductions. IUCN/SSC Re-introduction Specialist Group. Gland, Switzerland.
- Jahn P., Harper G. A., Gilchrist J. 2013. Home range sharing in family units of great spotted kiwi (*Apteryx haastii*) at Nelson Lakes National Park. *Notornis* 60: 201–209.
- Juan E., Bazzano G., Navarro J. L., Martella M. B. 2013. Space use by wild greater rhea (*Rhea americana*) in a relict grassland of central Argentina during the non-breeding season. *Hornero* 28: 1–6.
- Kesler B. D. C., Cox A. S., Albar G., Gouni A., Mejeur J., Plassé C. 2012. Translocation of Tuamotu kingfishers, post-release exploratory behavior and harvest effects on the donor population. *Pac. Sci.* 66: 467–480.
- Keye C., Roschak C., Ross J. 2011. Summer home range size and population density of great spotted kiwi (*Apteryx haastii*) in the North Branch of the Hurunui River, New Zealand. *Notornis* 58: 22–30.
- Kleiman D. G. 1989. Reintroduction of captive mammals for conservation. *BioScience* 39: 152–161.
- Laundré J. W., Hernandez L., Altendorf K. B. 2001. Wolves, elk, and bison: reestablishing the 'landscape of fear' in Yellowstone National Park, U.S.A. *Can. J. Zool.* 79: 1401–1409.
- Laver E. M., Kelly M. J. 2008. A critical review of home range studies. *J. Wildl. Manage.* 72: 290–298.
- Le Gouar P., Mihoub J. B., Sarrazin F. 2012. Dispersal and habitat selection: behavioural and spatial constraints for animal translocations. In: Ewen J. G., Armstrong D. P., Parker K. A., Seddon P. J. (eds). Reintroduction biology: integrating science and management. Wiley-Blackwell, Oxford, pp. 138–164.
- Lèche A. 2012. [Assessment of the adrenocortical stress response in captive reared and wild Greater Rheas (*Rhea americana*)]. Thesis, Universidad Nacional de Córdoba.
- Lèche A., Hansen C., Navarro J. L., Marin R., Martella M. B. 2015. Influence of breeding season on fecal glucocorticoid levels in captive Greater Rhea (*Rhea americana*). *Zoo Biol.* 34: 71–75.
- Lopes A. R. S., Rocha M. S., Junior M. G. J., Mesquita W. U., Silva G. G. R., Vilela D. A. R., Azevedo C. S. 2017. The influence of anti-predator training, personality and sex in the behavior, dispersion and survival rates of translocated captive-raised parrots. *Glob. Ecol. Conserv.* 11: 146–157.
- Marinero N. V., Navarro J. L., Martella M. B. 2015. [Factors that condition the habitat use of the Puna Rhea *Rhea tarapacensis* (synonym *Rhea pennata garleppi*) in the south of the Argentine Puna]. In: Abstracts of 'XVI Reunión Argentina de Ornitología'. Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Argentina, p. 117.
- Martella M. B., Navarro J. L., Gonnet J. M., Monge S. A. 1996. Diet of Greater Rheas in an agroecosystem of Central Argentina. *J. Wildl. Manage.* 60: 586–592.
- Martella M. B., Renny M., Chiappero M., Navarro J. L. 2014. A genetic approach to understanding the mating system of greater rheas. In: Abstracts of the 26<sup>th</sup> International Ornithological Congress, Tokio, p. 24.
- McPhee M. E., Silverman E. D. 2004. Increased behavioral variation and the calculation of release numbers for reintroduction programs. *Conserv. Biol.* 18: 705–715.
- Mitchell M. S., Powell R. A. 2012. Foraging optimally for home ranges. *J. Mammal.* 93: 917–928.
- Navarro J. L., Martella M. B. 2011. Ratite conservation: linking captive-release and welfare. In: Glatz P., Lunam C., Malecki I. (eds). The welfare of farmed ratites. Springer, Berlin, Heidelberg, pp. 237–258.
- Powell R. A., Mitchell M. S. 2012. What is a home range? *J. Mammal.* 93: 948–958.
- Rabin L. A. 2003. Maintaining behavioural diversity in captivity for conservation: Natural behaviour management. *Anim. Welfare* 12: 85–94.
- Reboreda J. C., Fernández G. J. 1997. Sexual, seasonal and group size differences in the allocation of time between vigilance and feeding in the Greater Rhea, *Rhea americana*. *Ethology* 103: 198–207.
- Sales J., Deeming D. C., van Deventer P. J. U., Martella M., Navarro J. 2000. Diurnal time activity budget of adult Greater Rheas (*Rhea americana*) in a farming environment. *Arch. Geflügelk.* 64: 207–210.
- Seddon P. J. 2010. From reintroduction to assisted colonization: moving along the conservation translocation spectrum. *Restoration Ecol.* 18: 796–802.
- Steinmann A. R., Bonatto M. F. 2015. Ecología comportamental. UniRío Editora, Río Cuarto, Córdoba, Argentina. Available at: <https://www.unrc.edu.ar/unrc/comunicacion/editorial/repositorio/978-987-688-141-8.pdf> (Accessed 1.05.2016).
- Steinmann A. R., Priotto J. W., Castillo E. A., Polop J. J. 2005. Size and overlap of home range in *Calomys musculinus* (Muridae: Sigmodontinae). *Acta Theriol.* 50: 197–206.
- Tolon V., Dray S., Loison A., Zeileis A., Fischer C., Baubet E. 2009. Responding to spatial and temporal variations in predation risk: space use of a game species in a changing landscape of fear. *Can. J. Zool.* 87: 1129–1137.
- Vera Cortez M., Valdez D. J., Navarro J. L., Martella M. B. 2015. Efficiency of antipredator training in captive-bred greater rheas reintroduced into the wild. *Acta Ethol.* 18: 187–195.
- Yoder J. M., Marschall E. A., Swanson D. A. 2004. The cost of dispersal: Predation as a function of movement and site familiarity in ruffed grouse. *Behav. Ecol.* 15: 469–476.

## STRESZCZENIE

**[Efekt treningu antydrapieżniczego na użytkowanie przestrzeni przez reintrodukowane samce i samice nandu szarego]**

Nandu szare to południowoamerykański nietlotny ptak, którego populacje zmniejszyły się z powodu kłusownictwa i fragmentacji odpowiednich siedlisk. Aby zapobiegać lokalnym i regionalnym zanikom populacji istnieje potrzeba wypracowania odpowiednich praktyk ochrony i zarządzania. Poprzednie badania wykazały, że działaniem, które zwiększa żywotność dzikich populacji tego gatunku jest reintrodukcja. Pomimo, że jest to często stosowana i skuteczna strategia ochrony czynnej, zwierzęta, które mają zostać wypuszczone na wolność, szczególnie, jeśli pochodzą z hodowli lub niewoli, powinny być przygotowane na różnorodne zagrożenia, takie jak drapieżnictwo, które mogą istotnie wpływać na powodzenie reintrodukcji.

W pracy oceniano efekt treningu mającego na celu rozpoznawanie zagrożenia związanego z drapieżnikami zastosowanego u hodowanych w niewoli osobników nandu szarego. Zakładano, że szkolenie to może wpływać na dyspersję oraz wielkość i nakładanie się arealów osobniczych u ptaków wypuszczonych na wolność. W szczególności przewidywano, że osobniki poddane szkoleniu będą unikać przemieszczania się na tereny, na których potencjalnie mogą występować drapieżniki, a to z kolei będzie prowadzić do zmniejszenia się ich areалу osobniczego. Ponadto, zbadano użytkowanie pięciu typów siedlisk (tereny otwarte, zakrzewienia, uprawy, solniska, tereny trawiaste) przez wypuszczone nandu.

Reintrodukowane osobniki (w wieku 2–3 lat) pochodziły z hodowli w ogrodzie zoologicznym. Zostały wypuszczone na wolność w miejscu, w którym istniała wcześniej populacja nandu, która, prawdopodobnie w wyniku kłusownictwa, zanikła w 2006 r. Jedenaście osobników poddanych treningowi (pokazywanie poruszającej się wypchanej pumy połączone z płoszeniem ptaków) oraz dziewięć zwierząt kontrolnych

(nieprzeszkolonych) zostało oznakowanych (obrączki a część osobników także obroże z nadajnikami – Tab. 1) i monitorowanych od dwóch do czterech razy dziennie w pierwszym tygodniu po wypuszczeniu oraz od 4 do 19 kolejnych dni w miesiącu przez dalsze cztery miesiące, w ciągu sezonu lęgowego i połęgowego. W analizach uwzględniono płeć wypuszczanych osobników. Prócz arealów osobniczych i ich nakładania się określono najdalszy dystans, na jaki przemieściły się ptaki od miejsca wypuszczenia.

Trening antydrapieżniczy wpływał istotnie na wielkość arealów osobniczych na wolności, ale wpływ ten zależny był od płci ptaków. Przeszkolone samice miały mniejsze areały (średnia  $\pm$  SE:  $0,54 \pm 0,58 \text{ km}^2$ ) niż pozostałe osobniki (samice kontrolne  $5,8 \pm 0,75 \text{ km}^2$ , samce kontrolne i poddane treningowi: odpowiednio  $2,11 \pm 0,65 \text{ km}^2$  i  $2,96 \pm 0,65 \text{ km}^2$ ). Natomiast nakładanie się arealów było znacznie większe u samic poddanych treningowi ( $63,83 \pm 16,95\%$ ) niż u samic kontrolnych ( $24,04 \pm 21,88\%$ ) (Fig. 1, 2). Samce z obu grup przemieszczały się na podobne odległości ( $3,21 \pm 0,29 \text{ km}$ ), dystans ten był znacznie większy niż w przypadku samic ( $2,21 \pm 0,32 \text{ km}$ ) (Tab. 1). Wielkość arealów osobniczych samców nie była związana z przebyciem treningu, także pokrywanie się arealów w obu grupach było podobne (ptaki kontrolne —  $29,96 \pm 18,95\%$ , przeszkolone —  $35,81 \pm 18,95\%$ , Fig. 1, 2). Obie grupy podobnie użytkowały przestrzeń w zależności od rodzaju siedliska preferując uprawy, i w mniejszym zakresie tereny otwarte i trawiaste (Fig. 3). Tylko samice poddane treningowi ograniczyły swoje przemieszczenia i użytkowały głównie tereny upraw lub obszary otwarte, unikając wysokiej roślinności, obecnej na terenach trawiastych, która może utrudniać wcześniejsze wykrycie drapieżnika. W przypadku samców wpływ sezonu lęgowego i złożonego systemu godowego wykazywanego przez ten gatunek przeważał nad potencjalnymi efektami treningu. Wyniki pracy pokazują nowe dane na temat użytkowania przestrzeni, które mogą być przydatne w reintrodukcji tego gatunku nandu.