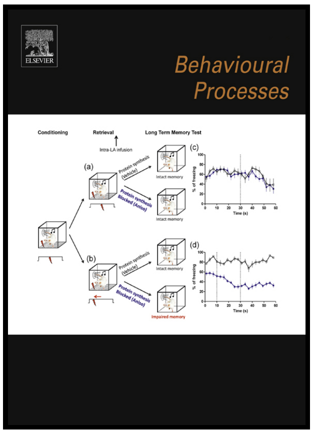


The effect of early experiences in Barn Owl (*Tyto alba*) behaviour. Acquisition-expression time of neophobia and filial imprinting. Implications for management and conservation

L Lezana, R Hernández-Soto, M Díez-León, M Ríos, E Baquero, D Galicia

PII: S0376-6357(21)00253-9

DOI: https://doi.org/10.1016/j.beproc.2021.104569

Reference: BEPROC104569

To appear in: *Behavioural Processes*

Received date: 16 December 2020
Revised date: 10 December 2021
Accepted date: 19 December 2021

Please cite this article as: L Lezana, R Hernández-Soto, M Díez-León, M Ríos, E Baquero and D Galicia, The effect of early experiences in Barn Owl (*Tyto alba*) behaviour. Acquisition-expression time of neophobia and filial imprinting. Implications for management and conservation, *Behavioural Processes,* (2020) doi:https://doi.org/10.1016/j.beproc.2021.104569

The effect of early experiences in Barn Owl (*Tyto alba*) behaviour. Acquisition-expression time of neophobia and filial imprinting. Implications for management and conservation

Lezana, L. *[1,2], Hernández-Soto, R.[1], Díez-León, M.[3], Ríos, M.[2], Baquero, E.[1], Galicia, D.[1]

[1]Department of Environmental Biology, University of Navarra, Spain

[2]Tierra Rapaz, Calahorra, Spain

[3]Deparment of Pathobiology and Population Sciences, Royal Veterinary College, University of London, UK

*Corresponding author: info@tierrarapaz.com

Abstract

In birds, early experiences determine the later behavioural phenotype of individuals and their way of adapting to the challenges they encounter in their environment. We investigated how the degree of exposure of barn owl chicks to humans and their biological parents influenced their behavioural response to humans and different environments. Only the treatment groups raised by human beings, or those that remained for less time with their biological parents (15 days posthatching), learned to fly towards their trainer. However, the two groups of chicks that were raised the longest by their biological parents (20 and 25 days) never flew towards their trainer. In these last groups, the filial imprint was shown not to be able to be reversed. Neophobia was estimated to emerge between 17 and 19 days of age, as barn owls were able to recognize the environment in which they were habituated, showing fear of a new environment.

Birds were able to recognize the person who raised them and objects with which they had been raised. The results obtained in this work can help to establish breeding protocols in this and other species of birds of prey, which improve their adaptability to the environment where they will live, whether in captivity or in the wild.

Keywords: rearing method, barn owls, imprinting, neophobia, conservation.

1. Introduction

Bird reintroduction projects as a method of recovering endangered, threatened or vulnerable populations have been greatly developed in recent years (Carter and Newbery, 2004; Morandini and Ferrer, 2017). However, avian reintroductions using captive-bred individuals are frequently less successful than the avian reintroductions using wild-caught animals (Griffith et al., 1989; Scott and Carpenter, 1987). One species for which several reintroduction initiatives are ongoing is the barn owl (*Tyto alba* (Scopoli, 1769)), a strigiform bird from the Tytonidae family. Barn owls are currently bred in captivity for reintroduction projects and conservation purposes (Fajardo et al., 2000; Meek et al., 2003), for two main reasons: i) their potential as a biological pest controller (Meyrom et al., 2009; Motro, 2011); and ii) the threat and decline of local populations due to abandonment of traditional farming buildings, road collisions, poisonings, etc. (Martí and Del Moral, 2003; Vallée, 2004). Reintroductions of captive-bred barn owls in particular have been unsuccessful as a result of poor preparation of birds for life in the wild (Taylor, 2017).

Early experiences have a profound impact on behavioural phenotypes, affecting the ability of individuals to adapt to the environment (Drickamer et al., 1992; Fox and Millam, 2004; Watters and Meehan, 2007). In birds, social preferences – towards a particular living being, an object or class of object – are acquired early in life through the process of imprinting (Bolhuis, 1991). Imprinting occurs on a short time scale, especially in precocial species (Bateson and Jaeckel, 1974; Lickliter et al., 1993; Manning and Stamp, 1998) and in several altricial species (Bischof and Rollenhagen, 1999; Immelmann and Suomi, 1981). For example, individuals bred by humans showed at a later time less fear towards humans compared to individuals who had no contact with humans (Zulkifli et al., 2002; Fox and Millam, 2004; Feenders et al., 2011). While less fear of humans is likely beneficial for captive individuals, as typically linked to good welfare (Bonato et al., 2013; Jones and Waddington, 1993), less fear of humans is considered detrimental for individuals who will be released into the wild (e.g., puppet rearing is recommended over hand rearing, as it preserves fearful responses towards predators (Valutis and Marzluff, 1999)). Nonetheless, hand rearing might be required when parents do not brood eggs that are otherwise fertile and might be genetically valuable from a conservation point of view (Glasier, 1978).

Neophobia can be defined as the fear of new resources (whether food or objects in general) that birds find in the environment (Greenberg, 2003). Birds exposed to a higher level of novelty in early life show shorter latencies to feed in the presence of a novel object months later (Amazon winged parrots (Fox and Millam, 2004)). Generalized neophobic behaviour in reintroduced birds could lead to a failure to adapt to the environment by rejecting new potentially beneficial resources found at the reintroduction site and/or leaving the release area too soon towards other more hostile areas. Therefore, understanding how early experiences affect adult neophobic behaviour to design best breeding, rearing and management practices is essential for conservation breeding programmes, yet accurate information is often lacking for species of interest, particularly in birds (Feenders et al., 2011).

Barn owl is a common species in zoological parks, breeding or rehabilitation centres. Extensive scientific knowledge of its ecological characteristics (Mikkola, 1983; Roulin, 2020) and the facility for its breeding in captivity (Parry-Jones, 2001) allow us to suggest this species as ideal for this study. There is also no information about when the development processes of social preferences or fear of novelty take place in this species: a critical point if the goal of artificial breeding is oriented to reintroduction or conservation.

Here, we tested how the degree of exposure to human beings, biological parents or individual siblings during the upbringing period influences the behavioural response of barn owls to perform a task learned at the age of emancipation in environments with different degrees of novelty. This study will also allow us to approximate the timing of filial imprinting and neophobic behaviour acquisition and to determine if these processes can be modified later through training and/or accustoming to a stimulus. We predicted that the response latencies in a learned task—flying up to a trainer's glove when called, as routine training in falconry—will increase in all experimental groups as the degree of novelty in the environment increases, but the response latency will be relatively greater for birds raised with their parents for a longer period compared to birds raised by humans.

In addition, in birds of prey, the implantation in the release area of certain elements with which the birds were raised (pens, cages, perches, etc.) can help to better control the release conditions and promote greater confidence of birds in the release area (Cade, 2000; Csermely, 2000; Wallace, 1994). In contrast, training captive-bred birds during their early stages to avoid specific objects that are harmful to them in their natural habitats can help the birds to avoid these objects after they are released and contribute to their survival (Wallace, 1997). In this work, we also investigate whether translocation of birds to an unknown environment, but with a known object (a bird perch) during their early stages, can improve their confidence level, allowing them to perform learned tasks more easily.

Knowledge gained from this study would help characterize the most appropriate early rearing method for each individual, according to the environment in which they will live as adults, increasing the chances of success in the reintroduction of individuals to the natural environment and improving the welfare of birds that are part of conservation breeding centres and/or zoos.

2. Materials and Methods

2.1. Birds, housing, and treatment groups

The study was carried out during the breeding seasons of 2011 to 2013 in the Birds of Prey Educational Centre and Zoological Park 'Tierra Rapaz' (Calahorra, La Rioja, Spain). We used a total of 70 barn owls. These experimental individuals were born from 13 unrelated breeding pairs that had been reared by their biological parents. The offspring of these breeding pairs were part of a reintroduction project approved by the Environment Ministry of the Spanish Government as well as educational projects in environmental education centres, zoos and other institutions. Each breeding pair was housed in an aviary measuring 3.5 m×2.3 m×2.8 m high, above minimum recommended housing for barn owls (Parry-Jones, 2001; Platt et al., 2007).

Chicks from these breeding pairs were assigned to five different treatment groups: isolation-reared by humans (IH n=10), group-reared by humans (GH, n=25), and group-reared by parents (GP). In addition, the chicks of the GP group were subdivided into three further groups (GP15, n = 6; GP20, n = 11 and GP25, n = 8) according to how many days after hatching (posthatching days, PHD) were spent with their biological parents. In this way, the GP15 group is made up of chicks that had been with their biological parents during the first 15 days and had been extracted from their biological parents on the 15th day to be fed from that moment by humans.

The distribution of the chicks among the experimental groups was such that not all the siblings of the same breeding pair were assigned to the same experimental group. For each experimental group, a maximum of 3 chicks from the same breeding pair were included, thus avoiding a bias in the results linked to a possible dominance of a certain behavioural phenotype, motivated by the same genetic origin of the specimens. The high number of breeding pairs (n = 13) as well as the number of chicks (n = 70), which this work has counted, has allowed us to have a large sample of individuals of each clutch size and of each individual position according to hatching order. Barn owl chicks commence to open their eyes after PHD 12 (Bunn et al., 2010), and their eyes remain completely open during their second week. According to the literature, we chose an experimental group that included this opening process (GP15), and the following group distanced itself by 5 days to verify possible differences after fully opening the eyes and visually identifying their surroundings (GP20). The day when the eyelids began to open and the day they were fully open were observed, since visual recognition of the environment would be a critical point in their neophobic behaviour and in the acquisition of their social preferences (Bischof et al., 2002; Bolhuis and Honey, 1998; Immelmann, 1959). Previous anecdotal observations at this breeding centre showed that specimens removed from their biological parents at the beginning of their fifth week (PHD 30) had an irreversible aversion to humans and could not be trained for educational flights. Therefore, for our GP group, we included birds that did not exceed their fourth week of life (PHD 25 in our study), since beyond seems to be a development time point by which their social preferences are already established.

Human breeding

To create the IH group, each egg was taken to the incubator 24 hours after it was laid. Egg fertility was checked with a cold-light egg scope and with an egg electrocardiogram system (Digital egg monitor-Buddy- of Avitronics, Threemilestone, Cornwall, UK) at 10 days of incubation. Infertile eggs were discarded. In this group, incubators, brooders and nest boxes were always used with only one egg or chick. These chicks were fed and trained individually throughout their development, without the possibility of observing other chicks. In the GH group, the procedure was the same as for IH, without biological parents, but neither the eggs nor the newborn chicks were isolated and always were in the presence of other eggs or chicks.

After artificial hatching, chicks in the IH and GH groups were transferred to their respective isolated (IH) or group (GH) brooders on PHD 2. Each brooder consisted of a 60 cm×40 cm×45 cm wooden bin with glass along one of the sides so that chicks could partially monitor the environment and see the trainer when he came to feed them. The brooder had an electric heating mat under a slope of riverbed stones that created a thermal gradient on the brooder floor so that the birds could choose their preferred thermal environment.

At PHD, 38 chicks from all groups were moved to a wooden enclosure or “nursery” (1.5 m×1.5 m×0.8 m). For the IH group, this enclosure was divided into individualized 0.75 cm×0.75 cm compartments that impeded visual contact among chicks. The rest of the experimental groups were placed in the nursery without physical separation between them in groups of up to 4 individuals. The front of the enclosure was covered with wire mesh so chicks could monitor the environment, including the first flying zone “known zone” that would be used in the flight trials.

This flying zone was an open gardened zone that included a wooden perch from which birds would make their experimental flights. In addition to this zone, the experiments were carried out in two other zones not known to the birds until the time of experimentation. The perch was 1.80 m tall, T-shaped, and covered with artificial grass on top. Between PHDs 38 and 65, twice a day, the trainer called each bird with a sound that imitated the sound that female barn owls make when they feed their chicks (Chandler, 2011; König et al., 2008) to encourage them to follow him and would feed them once the bird climbed onto the glove. The birds were also fed on the T-shaped perch so that they would habituate to this element. While a particular individual was being trained in this way, the rest of the birds could observe this procedure through the front of the wooden enclosure, except for birds in the IH group. Between PHD 65 and 85, birds were trained to fly with standard falconry techniques (Glasier, 1978) in two daily sessions (morning and evening). Birds were called by the falconer with meat bait so that they flew to his hand from the perch, situated 5 m away from the trainer. Flight trials were carried out between PHD 85 and 90 (see below).

Parental Breeding

For the GP group, eggs were totally incubated by their biological parents. Chicks remained in the nest box with their parents until PHD 15, 20 and 25, depending on the group. At that moment, the chicks were moved from their nests to a brooder, where they were cared for following the same protocol as groups IH and GH.

2.2. Feeding procedure

Barn owl chicks were fed day-old chicken and laboratory mouse meat supplemented with Nekton-S vitamin complex; all chicks, regardless of treatment group, received the same diet. For groups that were totally or partially raised by humans, the food was offered by the trainer with dissection forceps (up until PHD 40) or directly by hand (from PHD 40 onwards). For groups IH and GH, the feeding and contact time of each barn owl chick with the trainer was limited to 15 minutes per day (distributed in 5 sessions of 3 minutes each) until the chicks reached PHD 15. As the chicks developed, the number of feeding and contact sessions decreased because the chicks were able to eat more food per session. Three daily sessions were necessary between PHD 15-38 (9:00, 13:00, 19:00) and two between PHD 38-65 (9:00, 19:00). However, from PHD 15 on, the time per session was increased to 15 minutes until PHD 65, as birds had better thermoregulatory ability. Therefore, to facilitate imprinting, each time that a morsel of food was provided, the trainer imitated the vocalizations emitted by barn owl females when feeding their offspring and touched the bristles around the beak of the birds. These sensory feathers are used by owl chicks that have not yet opened their eyes to locate meat strips hanging from their mother's beak (Bunn and Warburton, 1977; Cunningham et al., 2011). When birds began to fly from PHD 65 onwards, food was administered in two daily flying sessions during training (PHD 65-85) and flight trials (PHD 85-90).

During the experiments, chicks did not suffer at any time from food shortages, which could have caused patterns of aggressive behaviours previously described in the wild (Roulin and Dreiss, 2012). For the experimental groups where the trainer fed the birds, the chicks received the maximum amount of food they could ingest and were satiated after each feeding session. For those chicks that were being fed by their parents in the experimental nests, the parents were fed "ad libitum", thus allowing them to feed the chicks until they were satisfied. Therefore, the differences found in more or less friendly reactions, elusive or aggressive, collected in these experiments do not present a bias related to food scarcity.

2.3. Opportunistic observations

Breeding and training the different treatment groups provided additional data (opportunistic observations) on the behaviour of the different individuals, which we now describe.

2.3.1. Response to human beings (filial imprinting tests in PG groups)

Data from 31 chicks belonging to the GP groups were recorded during their transfer from the nest box of their biological parents to the artificial brooder. The trainer came into the aviary and opened the door of the nest box. The trainer introduced his arm with dissection forceps to touch the sensorial feathers before taking birds with the hands. Chick reactions were gathered into three main groups and classified as normal-friendly or fear-aggressive according to the literature (Table 1). To avoid any influence of this protocol on the rest of the experimental procedures, the method of extraction of all the chicks raised by their parents that were part of the experiments was carried out according to these same guidelines.

Table 1. Classification of reactions of GP chicks to trainer manipulation when moving to artificial brooder. NF: normal-friendly; FA: fear-aggressive.

Group	Reaction	Type	Description
Postural	No change	NF	Chick stays in the same position as before the introduction of trainer arm.
	Rejection or fear	FA	Chick suddenly bends down to the floor. Sometimes it goes back to the farthest part of the box trying to escape (Bischof and Lassek, 1985)
Vocalizations	Communication snoring	NF	Chick plays the typical strident snoring of chicks and females during feeding (Chandler, 2011; König et al., 2008)
	Alarm whistle	FA	Chick plays high sounds and alarm squeals, tongue cracks or typical defensive whistles against potential predators (Bunn et al., 2010)
Physical contact	Forceps pecking	NF	Chick pecks the dissection forceps in the same way that they feed from the beak of their parents.
	Defence/Attack	FA	Chick stays face up trying to show/throw their claws to the trainer

2.3.2. Evolution of feeding behaviour after separation from parents

Data from 25 chicks belonging to the parental groups were recorded during the 10 days after moving to the artificial brooder. Following the same procedure as with the IH and GH groups, the chicks were fed inside the brooder during three daily sessions (morning 9:00, afternoon 13:00 and evening 19:00). The number of pieces of meat eaten in each session was recorded. As the chicks grew, the sizes of the pieces of food became larger, with an average weight per piece of food of 1.5-2 g, 2-2.5 g and 3-3.5 g for chicks of PHD15, PHD20 and PHD25, respectively.

In turn, several behavioural aspects related to acclimatization to their trainer were also recorded: feeding velocity, attitude when feeding and a general assessment about reaction to trainer (Table 2).

Table 2. Behavioural aspects recorded during chick feeding. See Table 1 for a complete description of NF and FA reactions.

Group	Reaction	Description
General	Normal-friendly	In general, chick shows NF reactions during feeding.
	Fear-aggressive	Chick performs one or several of the FA reactions.
Feeding velocity	Apathetic (slow)	Chick shows an apparent lack of appetite, the insistence of the trainer is required to feed the animal.
	Normal	Chick feeds normally, at the rhythm that meat is offered.
	Ravenous (fast)	Chick eats the meat rapidly and actively seeks more pieces from the trainer.
Attitude	Request	Chick pecks the dissection forceps or moves forwards requesting more food.
	Avoidance	Chick moves backwards avoiding the contact with the dissection forceps or the meal.
	Defence/Attack	Chick shows/throws the claws defensively against the coach.
	Still	Chick stays relaxed in its position.
	Stiff	Chick erects and adopts a stiff posture usually against a wall of the box.
	Escape	Chick runs away to a secure zone of the box.

2.3.3. Fear response to novelty during the 13-23 PHD period

Monitoring the behaviour of 14 chicks of the human reared group (GH) during feeding allowed us to design a small post hoc experiment to more precisely describe the changes in their fear of the novelty response during the 10 days of the development of the chick (PHD 13-23), a period of time that includes the process of opening the eyes. These birds were not used in any other experiment.

Each chick was extracted from the brooder, a known environment in which it stayed all the day and where they had been regularly fed until that moment (known environment) and moved to a different spot for feeding (always with a sibling next to them). This new environment was placed on a table in an adjacent room where they had never been before. This environment was therefore initially unknown to the birds (unknown environment). There, food was offered as usual, and after 3 minutes, the chick was returned to the brooder. Regardless of whether the chick had fed or not, they were given the opportunity to feed again inside the brooder. The number of eaten pieces in each environment (known or unknown) and fear reactions (defined as in Table 1) were registered for each feeding session.

2.4. Flying trials

Flight trials were carried out between PHD 85 and 90. In the wild, this marks the time when parents expel their offspring from their breeding territories and the young disperse and therefore a critical period for survival (Chandler, 2011; König et al., 2008). At that point in their lives, owls face new situations and environments, and the highest mortality rates occur due to lack of adaptability to the environment (Petty and Thirgood, 1989; Sunde, 2005). This period is also the period most used for the release of these birds in reintroduction projects (Green and Ramsden, 2001; Karapan, 2012). This time is also of great interest for studying the response of the birds to stimuli of novelty and social preferences. Two days before flight trials began, each individual was weighed to keep track of their natural training weight. This weight served as a reference to avoid excessive weight loss during flight trials and to ensure that all birds flew between 80-90%

of their natural training weight, as routine practice when training birds of prey, using hunger as a motivation to fly in a way similar to the way flight would occur in the wild (Fox, 1995), but without excessive hunger and avoiding different motivational states among individuals.

Of the 70 barn owl chicks raised, only 47 were used in this experiment: IH (n = 10), GH (n = 11), GP15 (n = 8), GP20 (n = 10) and GP25 (n = 8). All the barn owls in the first three groups (IH, GH and GP15) completed their training and flew to the trainer in all experimental settings. With a single exception (a bird from Group G20), no birds from Groups G20 and G25 flew towards the trainer.

Three zones were used for flight trials. Each of these areas presented 250 m^2, with low-height vegetation (grass) and small gravel surfaces, where birds could fly freely and without obstacles. Each zone varied in addition to their own location in the distribution of the grass and the areas covered with gravel. Flying zones had open ceilings but were peripherally closed by 2.3 m tall concrete walls to prevent birds from seeing beyond the experimental zone, minimizing external influences. Flight trials were performed under 6 different scenarios (see Table 3) grouped in two blocks, according to the degree of novelty introduced, either in the environment (block 1) or the trainer (block 2). In the first block, scenarios varied in the degree of novelty gradually introduced (A to D, from least to most).

Table 3. Description of the different scenarios for flight trials. The four elements that varied among the scenarios (type of perch, perch location, flying zone in block 1 and trainer in block 2) were classified as known (K) or unknown (U) according to the rearing conditions, conforming to 6 different scenarios. K*: the same trainer dressed in a different way.

Block	Scenario	Description	Perch	Location	Zone	Trainer
1- Environment	A	Birds fly in the same zone where they were trained (zone 1), perching on the same perch as used in training, and called	K	K	K	K

		by their usual trainer.				
	B	Birds fly in the same zone where they were trained (zone 1), perching on the same perch which is now located in a different place within their known zone of training, with the same trainer.	K	U	K	K
	C	Birds fly in an unknown zone (zone 2), but perching on the same perch used in training, with the same trainer.	K	U	U	K
	D	Birds fly in an unknown zone (zone 3), but perching on a different perch, with the same trainer.	U	U	U	K
2- Trainer	E	Birds fly in the same zone where they were trained (zone 1), perching on the same perch as used in training, but called by an unknown trainer using the same clothes as the known trainer.	K	K	K	U
	F	Birds fly in the same zone where they were trained (zone	K	K	K	K*

	1), perching on the same perch as used in training, and called by the same trainer using different clothing.

Every flying scenario involved three flying sessions (replicates) per bird. Each flying session consisted of a series of continuous flights from a perch to the trainer's hand (approximately 12 flights per bird). Bird flight latencies were recorded when called, as latencies to perform behaviour have been shown to reflect motivational state and to increase when fearful birds are exposed to novel situations (Boogert et al., 2006). Thus, we recorded the number of seconds elapsed since the trainer called the bird and raised his hand with the bait (both gestures occurred simultaneously) until the bird took off from the perch and flew towards the trainer. Once the bird perched on the hand and ate a morsel of meat, the bird was placed back on the perch to make the next flight. A flight was considered a failure when the bird did not fly to the trainer's hand within 3 minutes or if it flew in another direction. When a failed flight occurred, the flying session ended, and the individual did not fly until the next flying session. Trials were performed sequentially, from A to F, with an additional replica of the A scenario (A*) after D to test possible behavioural changes derived from an improvement in the response to the call acquired in the previous flight sessions. Flying sessions were video-recorded, and videos were analysed by a researcher blind to the hypotheses under the test and rearing groups.

2.5. Statistical analyses

All statistical analyses were performed with the statistical software R version 3.3.2 (R Development Core Team, 2016). Sex and family were randomized among groups and not considered explanatory variables in the models. Additional packages *nlme* (Pinheiro et al., 2017) and *multcomp* (Hothorn et al., 2008) were used for mixed models and post hoc comparisons, respectively. Significance level was set at p<0.05. All tests performed were two-tailed, as there were no clear *a priori* directional predictions.

2.5.1. Opportunistic observations

For data related to initial trainer manipulation, the number of barn owls that showed the described reactions (Table 1) was used in the contingency tests of independence. During the 10-day monitoring period (Table 2), attitude to feeding was quantified in a similar way, counting how many birds of each treatment group showed every reaction in any of the feeding sessions. For general and feeding velocity reactions, the daily lectures of each bird were summarized in only one of each group's categories, giving priority to nonnormal reactions when a bird performed two different types. Only on two occasions did two birds show all the feeding velocity categories on the same day, leading to discarding those days from analyses. Counts of reactions were used in statistical analyses. Separate analyses were performed for the different main groups of reactions to check for the independence between the type of PR group and the type of reaction. Standardized residuals were calculated to explore the relative weight of each cell, and absolute values above 2 were considered significant deviations of the expected values (Agresti, 2002).

Additionally, in the study of the evolution of feeding behaviour, two variables were calculated for the statistical analyses: the total number of pieces eaten (Amount) and the ratio between the variance and mean of pieces eaten during the three feeding sessions per day (Dispersion). The former provides information about the quantity of food that each chick consumes every day, while the second provides information about the uniformity of feeding each day. Dispersion should be close to or below 1 when feeding is randomly or uniformly spread along the day and above 1 when feeding is mainly centred (clumped) in one of the sessions (Krebs, 1999). The amount of pieces eaten each day was statistically analysed by Kruskal-Wallis tests using Wilcoxon signed rank tests for *a posteriori* comparisons. The trend across days in Amount and Dispersion variables was checked by linear regressions.

In the section related to the response of fear to novelty during the 13-23 PDH period, for each feeding session, the difference between meat pieces eaten in the brood and the initially unknown environment was calculated. The three values obtained per day were averaged to describe the bird's use of the two environments: for each day, a positive value indicates that the bird fed mostly in the brooder, while a negative value shows that feeding was mainly in the initially unknown environment. We compared the mean values between consecutive days by means of a Wilcoxon signed rank test, looking for a significant change in feeding pattern during the experiment.

Behavioural reactions during feeding were coded in a binary variable (Fear) that took a value of 1 if the chick showed any FA reaction (Table 1) during any of the feeding sessions of a day and 0 otherwise. A change in the type of reactions during feeding was expected to occur around the period of eye opening. Thus, to characterize this binary variable in time, a logistic regression was adjusted to find a relationship between the eye-opening period and the probability of showing a fear response when feeding.

2.5.2 Flying trials

To eliminate individual motivational differences as flight trials progressed, only the first 8 flights of every session were used for analysis after verifying that the probability of a flight failure increased notably from the 10th flight onwardss. Thus, the latency records of all the sessions (flights and replicates) were averaged per bird and experimental situation.

Latency was log-transformed to meet normality criteria. The two experimental blocks (environment and trainer) were analysed separately by means of a generalized linear mixed model (GLMM) with birds as repeated measures and scenarios, treatment groups and their interaction as fixed factors. In the case of a significant interaction between main factors, separate models were performed for each scenario (ANOVAs for testing differences between treatment groups) and for each treatment group (GLMMs for testing differences between scenarios, bird as repeated measure) with Tukey tests for post hoc tests. Model fit was explored by analysing residual normality and the correlation between observed and predicted values.

3. **Results**

3.1 Opportunistic observations

3.1.1. Response to the human being (filial imprinting tests)

GP 15 responses to the handler were significantly different from the responses of GP20 and GP25 (Table 4). Within GP15, all chicks but one did not appear fearful when the trainer approached them with his arm carrying the feeding forceps. Over half of the chicks (57.1%) vocalized to solicit food, and some of them even pecked forceps in a seeming food solicitation attitude (35.7%). In contrast, all of the GP20 chicks showed fear-like behaviour when handled (77.8% of them emitted alarm calls, and 33% defended themselves by showing/throwing their claws). Chicks on GP25 showed a reaction to the trainer similar to chicks on GP20, with performing alarm calls (62.5%) and showing/throwing their claws to the trainer (25%).

Table 4. Contingency tests of reactions to trainer manipulation of chicks (GP) in group reared by parents when moving to artificial brooder. The number of birds and standardized residuals for each cell are shown in parentheses.

Group	Reaction	GP15 (n=14)	GP20 (n=9)	GP25 (n=8)	Chi2	df	Fisher's exact p value
Postural	No change	13 (2.94)	0 (-1.94)	0 (-1.83)	27.19	2	<0.001
	Rejection or fear	1 (-2.5)	9 (1.65)	8 (1.56)			
Vocalizations	Snoring	8 (2.31)	0 (-1.52)	0 (-1.44)	17.72	4	<0.001
	Alarm whistle	1	7	5			

		(-2.01)	(1.66)	(0.90)			
	None	5	2	3			
		(0.23)	(-0.53)	(0.26)			
Physical contact	Forceps pecking	5	0	0	10.46	4	0.021
		(1.82)	(-1.20)	(-1.14)			
	Attack or defence	0	3	2			
		(-1.50)	(1.29)	(0.62)			
	None	9	6	6			
		(-0.16)	(-0.04)	(0.25)			

During the following ten days after separation from their parents, the GP groups showed different responses to human feeding (Table 5). Less than 5% of the registered reactions in the GP15 group were considered fearful or aggressive, while in the GP20 and GP25 groups, these reactions accounted for more than 15% of the reactions (18.6% and 17.5%, respectively). The feeding velocity of owlets of the GP15 was frequently classified as fast (30.8%), and it is the only group in which a requesting attitude was recorded (in all birds). In none of the GP20 birds and only on one occasion in the GP25 group was a fast feeding velocity recorded. Contrary to GP15, no animal requested food, and reactions of avoidance, defence or escape were frequently detected. No data could be registered in two individuals belonging to the GP25 group, since, as soon as they were removed from their parents, they rejected the food coming from the human being for two consecutive days and were returned with their biological parents to complete their breeding.

Table 5. Contingency tests of reactions during the first 10 days of artificial feeding of chicks (GP) groups reared by parents. Counts are the number of reactions recorded for each group during the experimental period (general and feeding velocity groups) or the number of birds (attitude group). Standardized residuals for each cell are shown in parentheses. * asymptotic probability (exact value not available).

Group	Reaction	GP15 (n=12)	GP20 (n=9)	GP25 (n=4)	Chi2	df	Fisher's exact p value
General	Normal-friendly	113 (5.82)	16 (-4.61)	7 (-3.24)	148.40	2	<0.001
	Fear-aggressive	5 (-6.54)	70 (5.18)	33 (3.64)			
Feeding velocity	Apathetic	6 (-4.87)	56 (6.05)	10 (-0.54)	106.75	4	<0.001*
	Normal	75 (1.30)	30 (-2.53)	29 (1.48)			
	Ravenous	36 (4.31)	0 (-3.62)	1 (-2.06)			
Attitude	Request	12 (3.39)	0 (-2.12)	0 (-1.69)	38.08	10	<0.001
	Avoidance	5 (-0.76)	9 (0.88)	4 (-0.13)			
	Defence	0 (-1.65)	5 (1.48)	2 (0.26)			
	Still	6 (-0.10)	7 (0.42)	3 (-0.41)			
	Stiff	0 (-0.88)	0 (-0.86)	2 (2.21)			

Escape	0	1	3
	(-1.25)	(-0.40)	(2.11)

Similarly, there were also clear differences between GP15, GP20 and GP25 in the evolution of food consumption after transfer to the brooder. While chicks on GP15 ate a regular quantity of food from the beginning (Figure 1A), GP20 and GP25 chicks took several days to reach the same levels, barely eating for the first few days and gradually increasing the number of pieces consumed per day. This trend is statistically supported by the significant and positive slopes of GP20 and GP25 regressions (11.84, t=4.09, p=0.003; 17.43, t=3.111, p=0.014, respectively) and the absence of a significant slope in GP15 (-3.56, t=-1.1445, p=0.186). The dispersion parameter (Figure 1B) was also nearly constant for the GP15 group (0.30, t=0.317, p=0.759), while slopes in GP20 and GP25 were negative, although only significant in the former (-6.34, -3.222, p=0.012; -3.28, t=-0.934, p=0.378, respectively). The analysis of the number of pieces eaten showed significant differences between groups only until Day 6.

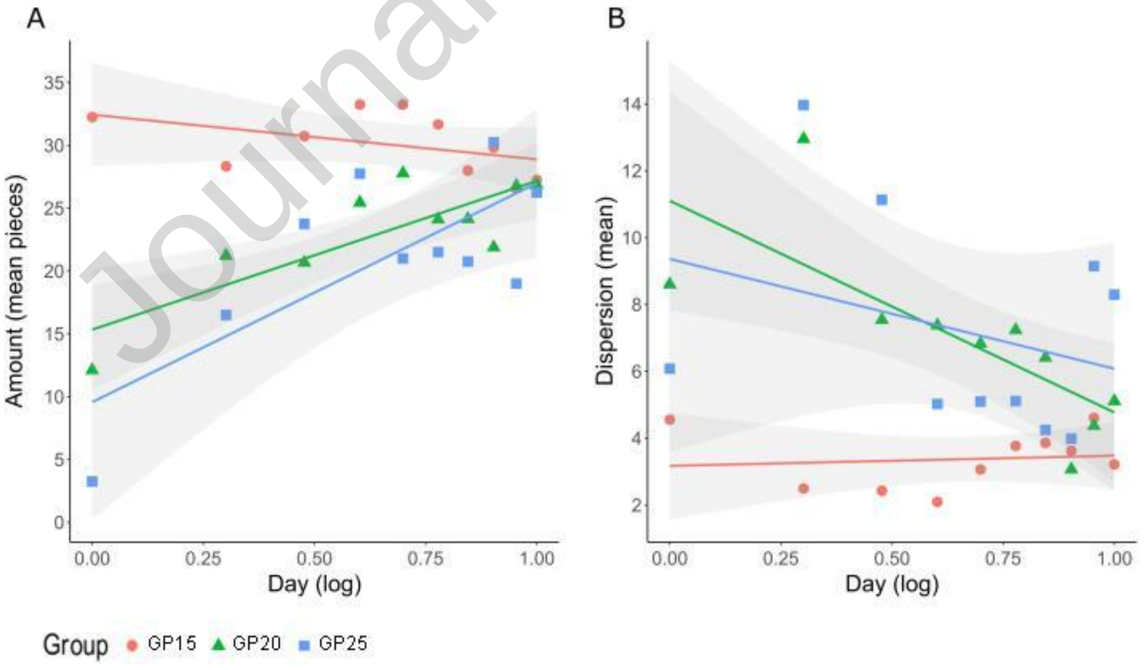


Figure 1. Feeding evolution during the ten days after separation from parents. Day variable has been $\log_{10}$ transformed. A) Number of pieces (mean of the total of pieces eaten per day); B)

Dispersion (mean of the dispersion index of each bird). Linear regressions (solid lines) are shown (shaded areas indicate the 95% CI of the regressions).

3.1.2. Fear response to novelty during the 13-23 PHD period (neophobia) in the GH group

No FA reaction was observed in the brooder (known environment), and all were registered when owlets were in the unknown environment, especially from PHD 17 onwards. The probability of expressing an FA reaction was statistically related to the age of the chicks, as the logistic regression shows (Figure 2, Deviance=3.817, df=9, *p*<0.001). The model predicts a drastic change in probability between PHD 17 and 19. Wilcoxon sign tests found the same critical period, providing significant results only in comparisons between PHD 17 and 18 (z=-2.827, *p*=0.003) and PHD 18 and 19 (z=-2.731, *p*=0.004). Out of this range, feeding took place mainly in the unknown environment (PHD 13 to 16, negative values) or in the brooder (PHD 20 to 23, positive values).

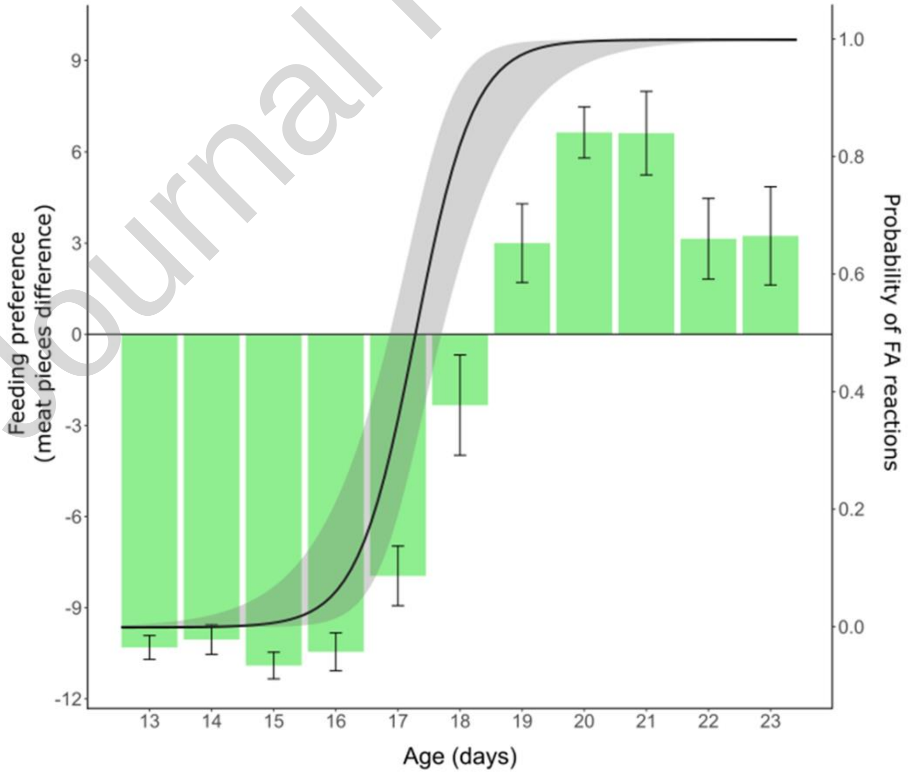


Figure 2. Change in feeding preference during the 13-23 PHD period in the GH group. Green bars (left axis) show the average and standard error of the difference between the number of

meat pieces eaten in the known (brooder) and unknown environments. Logistic regression (right axis) models the probability of a chick showing an FA reaction as it grows (shaded area indicates the CI 95% of the regression).

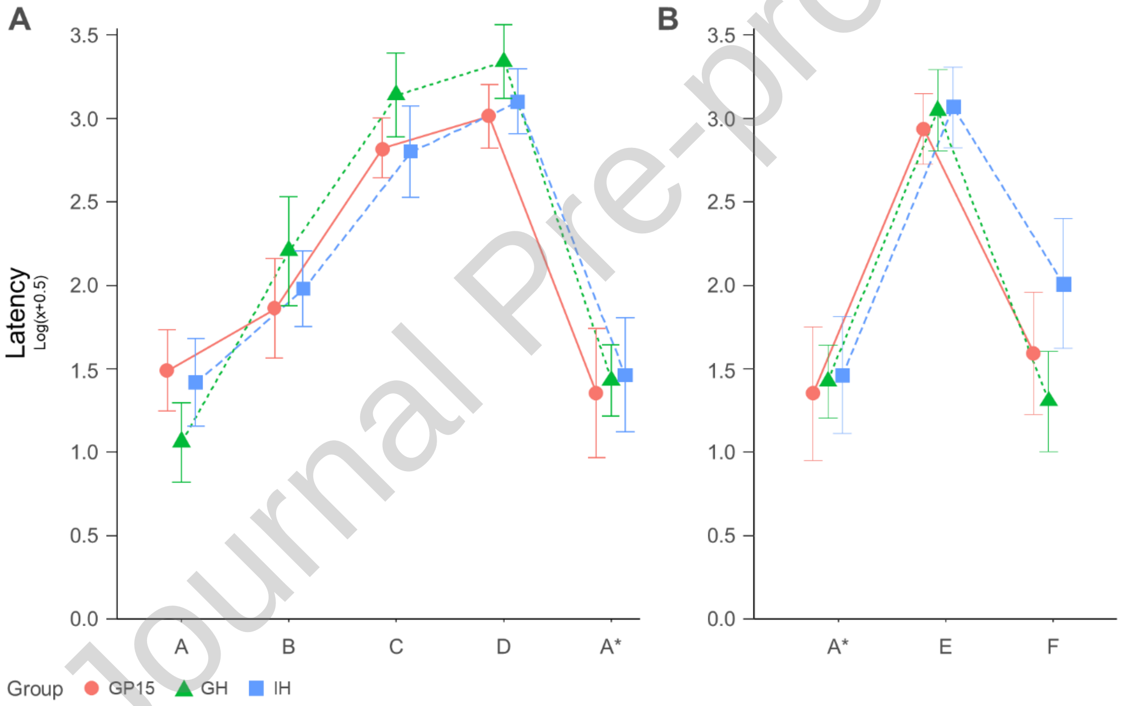
Therefore, from Day 17, birds had a fearful and elusive attitude when they were placed in an unknown environment, cowering or leaning back and trying to hide and sometimes emitting their classic sound of alarm. However, immediately after refusing to feed in an unknown place, their attitude changed radically when they were introduced to the brooder (known area). Then, they approached the trainer, emitting classic sounds of food request (Chandler 2011) and eating normally.

3.2. Flight trials

All the barn owls of the groups IH, GH and GP15 could be flown by the trainer in the experiments. However, the birds of groups GP20 and GP25 did not fly to the trainer´s call (except one of the GP20 groups, which only flew on stage A). Flight failures had a very low incidence in the rest of the experimental groups (approximately 1.5% of all flights considered) and were not statistically analysed (see Supplementary Material for a summary of test failures for each group and scenario). Regarding the latency variable, statistical analyses showed a significant effect of the scenarios presented both in relation to the environment (F=95.67, ndf=4, ddf=99, p<0.001) and trainer (F=60.672, ndf=2, ddf=48, p<0.001), but no differences among treatment groups were detected (F=0.020, ndf=2, ddf=26 p=0.980; F=0.112, ndf=2, ddf=26, p=0.895, respectively). Both models provided normally distributed residuals (p>0.05) and a good linear fit between predicted and observed values (p<0.001, adjusted R square=0.857 and 0.804, respectively). However, a significant interaction (F=2.277, ndf=8, ddf=99, p=0.028) was observed between the treatment group and the Scenario in the Environment situation.

To delve into the interaction, the five scenarios (A to D and A*) were analysed separately (see Supplementary Material to see all ANOVAs performed and post hoc results). None of the five scenarios analysed provided significant results, which indicates that within each scenario, all experimental groups behaved similarly. By dividing the dataset by experimental treatment, we found a significant effect of scenario in all the cases (GH: F=15.8, df=4, p<0.001; IH: F=10.03, df=4, p<0.001; GP15: F=10.3, df=4, p<0.001), although post hoc comparisons provided slightly different trends depending on the group considered.

Figure 3. Latency during flight trials (mean and SE of log transformed values). A) Effect of changes in the environment (see Table 3). A*: repetition of situation A after the rest of the scenarios. B) Effect of changes in trainer: A repetition was introduced as a reference for scenario F (same trainer with different clothes, see Table 3).



The shortest latencies are associated with the scenarios in which all the conditions are known (trainer, zone, perch and spatial location of perch); Scenarios A and A*. As the novelties in the different scenarios increased (from A to D), all three groups took longer to fly when they were called, being statistically not different in the IH and GP15 groups. These differences in flight latencies to the same trainer were less significant when the introduced environmental innovations were of lesser importance, such as changes in the position of the flight perch (from A to B) and greater when the flight zones were changed (from B to C). In summary, although small differences can be detected between experimental groups, the statistical relationship between scenarios can be expressed as (A = A* = B) < (C = D).

Regarding the trainer and clothing effect, post hoc tests confirmed that there were significant differences in all treatment groups when the birds had to fly to an unknown trainer (scenario E, $p<0.001$) but not to a known trainer in different clothing (scenario F, p=0.938).

3.3 Eye-opening period

In our study, owl chicks began to open their eyes (slanted eyes) at the age of 11 days (11.33 ± 0.83; n = 67). The most premature chick in this process began to open at the age of 10 days, and the latest began to open at 13. From then on in an intermittent opening sequence, the chicks managed to fully open their eyes at the age of 13 days (13, 10 ± 0.83; n = 67). The most premature chick in this process was 12 days old, and the latest was 15 days old.

4. Discussion

In general, our study found that between Days 15 and 20 of life, barn owls expressed their social preferences and fear of novelty, with social preferences being stable over time and neophobic behaviour being more plastic and able to revert under habituation to environmental novelties.

4.1. Critical periods during growth

4.1.1. Acquisition-expression time of Filial Imprinting

When the handler touched PHD 15 chicks in their parents' nests, they remained calm as normal, and some of them even stretched their necks towards the forceps by pecking at them and making the typical food request sound. No individual backed down in defensive attitude or crouched in fearful attitude. In contrast, the chicks of the other GP groups threw back in defensive attitude, emitting a sound of threat, even though some of them stayed on their backs attacking with their claws. This attitude occurs in nature when a predator or intruder enters the nest of these birds (König et al., 2008), showing aversion to the human figure. Our data, therefore, suggest that owl chicks begin to express their social preferences and rejections between PHD 15 and PHD 20.

4.1.2. Acquisition-expression time of Neophobia.

The GH birds that were followed from PHD 13 to PHD 23 showed a drastic change in their feeding behaviour during this period (Figure 2). Before PHD 17, they fed normally in the unknown environment. However, from this age, birds refused to feed in this unknown place, showing an attitude of fear and elusiveness towards a known person (trainer) who was trying to feed them. However, immediately after refusing to feed in an unknown place, their attitude changed radically when they were introduced to the brooder (known environment). Then, they approached the trainer, emitting sounds of food request and eating normally. Therefore, between 17 and 19 days of age, owls seem to be able to recognize the environment they are in and were scared when they suddenly moved to a different one, thus emerging in them the neophobia or fear of novelty. In nature, the rejection of novelty can be an advantage for the survival of individuals, allowing them, for example, to seek refuge in familiar environments, avoiding new ones where they can easily be preyed upon (Tsurim et al., 2008).

Several studies have linked the development of visual capacity in chicks and their subsequent identification of the elements of the environment with the expression of fear towards unknown stimuli (Bischof et al., 2002; Bolhuis and Honey, 1998; Immelmann, 1959). The development of the neural circuit in vertebrates, which allows visual identification of the environment, is stimulated only by visual experiences at an early age (Antonini and Stryker, 1993; Wiesel and Hubel, 1965). Visual identification of the environment in turn seems to stimulate the neural circuits needed to develop the behaviour of the acquired preferences throughout the life of the individual (Bischof, 1994; Knudsen, 2004).

In this study, birds opened their eyes at PHD 13. The acquisition phase of the fear behaviour of novelty could therefore begin from this day until PHD 18, when basically all of the birds started to show fear of environmental change. Other published work on bird imprinting has suggested that it takes several days from the opening of the eyelids until the complete perception and identification of the stimuli by the visual system (Bischof, 1994). It is therefore likely that, as in filial imprinting on other birds where the phases of acquisition and development of behaviour occur almost simultaneously (Bischof, 1979), in barn owl chicks, the acquisition phase of fear of novelty begins days after the opening of the eyes, in the days or even hours just before the exact moment they expressed this behaviour (PHD 17 and PHD 18). Knowing with more precision the exact time in which the acquisition of this behaviour takes place would require more study and would allow us to establish management programs optimized for each bird depending on the destination of each individual. For birds destined for reintroduction projects, exposing them through positive association with the reintroduction area during this period of acquisition and/or emergence of neophobia or at least to a set of objects with which the bird can become familiar with that area could generate greater confidence in this habitat and permanence in the trained birds. This fact would favour their survival, since the reintroduction area has been chosen previously against other areas due to its suitability for the species. However, the exposure of birds destined for captivity projects, to a variability of the environments in this period, could reduce their neophobic behaviour, something already suggested for other species (Fox and Milan, 2004; Feenders and Bateson, 2013), and allow them to reduce the development of stress with the continuous changes that humans cause in their environment.

Exposure to the unknown environment during the opening of the eyes and in subsequent days, at the times marked by our experimental procedure, did not prevent the fear reaction to this environment from being different from their usual brooder. In all birds, the fear reaction towards a new environment appeared days after they opened their eyes. This phenomenon has already been observed in other bird species (Bischof and Lassek, 1985). It would be interesting to know if a longer exposure time to the new environment or to a combination of new environments in the days prior to the expression of fear would have mitigated or even canceled the appearance of neophobia in the birds or at least the reduction or loss of fear of a specific initially unknown environment.

4.2. Adaptability to new conditions and behavioural plasticity

Deep changes in rearing conditions, such as the transition from being cared for by their biological parents to being cared for by a human, resulted in very different behavioural responses in the GP groups. From the first day of exposure to the trainer, reactions of GP15 birds differed from the reactions of GP20 and GP25 birds, showing less fear or aggression towards the trainer (Table 5). It took approximately a week for the feeding behaviour of these last two groups to resemble the feeding behaviour of the GP15 birds, both in the number of pieces consumed and the regularity of intake in each session (Figure 1). However, the feeding velocity of owlets of the GP15 group was frequently classified as fast (30.8%), and it is the only group in which request behaviours were recorded (in all birds). In none of the GP20 birds and only on one occasion in the GP25 group was a fast feeding velocity recorded. Contrary to GP15, no animal requested food, and reactions of avoidance, defence or escape were frequently detected among individuals.

In addition, two birds of the GP25 group did not accept food from humans for two days after being transferred to the brooder and had to be returned to their biological parents to avoid endangering their lives. Another relevant fact that confirmed this behavioural difference was that while all the owls of GP15 could be flown by the trainer in the experiments, practically all the specimens of groups GP20 and GP25 did not fly. These results, along with anecdotal evidence from falconers reporting inability to train chicks removed from their parents with 5 weeks of life, allow us to suggest that from 20 days posthatching onwards, filial imprinting shows low plasticity and is hardly reversible.

Unlike this type of imprinting, the fear of new environments or objects showed greater plasticity in our experiments. Although it is logical to think that the fear of novelty will always remain throughout the life of the bird, as a guarantee of its survival, a prolonged exposure time to a new environment caused the habituation of the birds to an initially unknown environment. This phenomenon could be detected in the three groups of birds that performed the flying trials (IH, GH and GP15). They showed significantly shorter latencies in a flight area known during training (the environment of their nest cage) compared to other areas not known to them (see below). The fact that birds were able to habituate to the zone around their wooden enclosure or “nursery”, even though they were introduced there at PHD 38, more than two weeks after the fear of novelty arises in them according to our previous observations, comes to demonstrate their ability to get used to an initially unknown environment. Logically, if this were not the case, birds would not be able to adapt to any other environment except the environment of birth or the nest itself, something that does not occur in wild birds.

4.3. Response of birds to different types of novelties in the environment and trainer recognition

Fear of a novel environment therefore existed in all the birds that flew in the study (IH, GH, GP15). On average, when birds flew in an unknown zone from a known perch (scenario C), they tended to show shorter latencies than when they flew in an unknown zone from an unknown perch (scenario D), although the differences were only clearly significant in the GH group. This response suggests that the birds might be able to recognize the perch from which they had flown during their training. Frequently, in the release of birds of prey into the wild, the chicks inside their nest cages or portable nests (in which the chicks have been raised) are placed at the release point without opening their enclosures for a time until they gain trust and are released later (Csermely, 2000; Monti et al., 2012; Wallace and Temple, 1987). The results obtained in this work allow us to suggest that for barn owls, the conditions of the release areas could be improved by including known objects in their early stages of development. If birds are more confident in the reintroduction area, they will stay there for longer and be able to better control their adaptation, resulting in greater success of the reintroduction project (Meek et al., 2003).

Within the known zone, birds experienced an increase in their latencies when the typical position of their known perch was changed (scenario B). This fact suggests that barn owls also recognize the spatial situation of objects in their environment. In birds, spatial memory is a widely studied feature in different species (Balda et al., 1987) and in owls (Knudsen, 2002). The implications that managing the spatial distribution of these previously known elements by birds in the reintroduction areas should be part of future studies. For example, in a release area to which birds are adapting, any changes in the distribution or incorporation of different elements during the process, instead of having been placed prior to the release of the birds, could put their adaptation at risk.

The results of this work also suggest the importance of a study that provides information on how the type of breeding in barn owls affects their ability to hunt and, therefore, to adapt to the natural environment where they are introduced. In the same way, it would be of great interest to know if early experiences with live prey and/or with specific prey that are already present in the wild will allow them a better adaptation to the new environment.

In relation to the trainer's recognition, in the known flight zone, the three groups of barn owls showed longer latencies to fly when the person calling them was not their regular trainer (scenario E, Figure 3). The birds thus were able to show their capacity for individual recognition of the person who raised them, with whom they had socialized and had greater confidence. However, barn owls came to feed with the trainer regardless of the clothes he was wearing (Scenario F). The manner in which birds identify the person who raises them may be related to their physical appearance, the way they moved (Gray and Howard, 1957), the sound of their call (Kent, 1987), or probably a combination of all these characteristics.

5. Conclusions

This study allows us to suggest that fear of novelty in the environment appears in barn owl chicks around PHD 17, and it has been shown to be a reversible behaviour that allows a certain plasticity through a period of adaptation or acclimatization to the novel environment. However, the identification of the filial figure has been shown to be less plastic. Between PHD 15 and 20, behavioural changes took place in barn owl chicks that could determine the recognition of their parents and their environment. At this point, it could be of great interest to know if these changes are the result of certain physiological changes at the brain level, which would be susceptible to further study.

Data obtained here could allow the future development of individuals with specific behavioural traits by means of specific rearing models, depending on the breeding objectives. For example, individuals bred to be released into the wild should stay with their natural parents at least until PHD 20 so that they can socialize with their own species in the wild and stay away from humans. However, these same individuals could be more prone to suffering stress in zoological parks or research centres because they are continuously exposed to humans. In this case, it might be considered that the birds best-adapted to these places would be those kept isolated and fed exclusively by humans, as they would be stress-free in their presence. However, these birds, confident with humans, could have serious problems recognizing other individuals of their same species, which would biologically limit them, preventing any type of intraspecific relationship. The search for double imprinting, where birds are not fearful of human beings but also recognize their conspecifics, would be of great interest for keeping birds in captivity.

This study also reflects the importance of the presence of certain objects known to birds through prior training in their early stages in a potential reintroduction area. This method could increase the confidence of the released individuals and their stay in the area without drastic situations of evasion and stress. In the same way, birds could be trained to negatively associate elements such as vehicles or power lines, which kill large numbers of barn owls each year, thereby contributing to lower mortality of introduced birds.

References

Agresti, A. (2002). *Categorical Data Analysis*. *John Wiley & Sons, Inc* (2nd ed.). Hoboken, New Jersey: John Wiley & Sons, Inc.

Antonini, A., & Stryker, M. P. (1993). Development of individual geniculocortical arbors in cat striate cortex and effects of binocular impulse blockade. *Journal of Neuroscience*, *13*(8), 3549–3573. https://doi.org/10.1523/JNEUROSCI.13-08-03549.1993

Balda, J. C., Hadingham, M. F., Fairbairn, R. E., Harley, R. G., & Eitelberg, E. (1987). Measurement of synchronous machine parameters by a modified frequency response method - Part I: Theory. *IEEE Transactions on Energy Conversion*, *2*(4), 646–651.

Bateson, P. P. G., & Jaeckel, J. B. (1974). Imprinting: Correlations between activities of chicks during training and testing. *Animal Behaviour*, *22*(4), 899–906. https://doi.org/10.1016/0003-3472(74)90013-X

Bischof, H J. (1979). A model of imprinting evolved from neurophysiological concepts. *Journal of Comparative Ethology*, *51*(2), 126–139. https://doi.org/10.1111/j.1439-0310.1979.tb00677.x

Bischof, H J. (1994). Sexual imprinting as a two-stage process. In J. A. Hogan & J. J. Bolhuis (Eds.), *Causal mechanisms of behavioural development* (pp. 82–97). Cambridge University Press. https://doi.org/10.1006/anbe.1995.0254

Bischof, H J, & Lassek, R. (1985). The gaping reaction and the development of fear in young zebra finches (Taeniopygia guttata castanotis). *Zeitschrift Für Tierpsychologie*, *69*(1), 55–65. https://doi.org/10.1111/j.1439-0310.1985.tb00756.x

Bischof, H J, & Rollenhagen, A. (1999). Behavioural and neurophysiological aspects of sexual imprinting in zebra finches. *Behavioural Brain Research*, *98*(2), 267–276. https://doi.org/10.1016/S0166-4328(98)00093-X

Bischof, H.J., Geißler, E., & Rollenhagen, A. (2002). Limitations of the sensitive period for sexual imprinting: neuroanatomical and behavioral experiments in the zebra finch (Taeniopygia guttata). *Behavioural Brain Research*, *133*(2), 317–322. https://doi.org/10.1016/S0166-4328(02)00016-5

Bolhuis, J. J. (1991). Mechanisms of avian imprinting: a review. *Biological Reviews of the Cambridge Philosophical Society*, *66*(4), 303–345. https://doi.org/10.1111/j.1469-185X.1991.tb01145.x

Bolhuis, J. J., & Honey, R. C. (1998). Imprinting, learning and development: From behaviour to brain and back. *Trends in Neurosciences*, *21*(7), 306–311. https://doi.org/10.1016/S0166-2236(98)01258-2

Bonato, M., Malecki, I. A., Wang, M. D., & Cloete, S. W. P. (2013). Extensive human presence at an early age of ostriches improves the docility of birds at a later stage of life. *Applied Animal Behaviour Science*, *148*(3–4), 232–239. https://doi.org/10.1016/j.applanim.2013.08.003

Boogert, N. J., Reader, S. M., & Laland, K. N. (2006). The relation between social rank, neophobia and individual learning in starlings. *Animal Behaviour*, *72*(6), 1229–1239. https://doi.org/10.1016/j.anbehav.2006.02.021

Bunn, D. S., & Warburton, A. B. (1977). Observations on breeding Barn Owls. *British Birds*, *70*(March 1968), 246–256.

Bunn, D. S., Warburton, A. B., & Wilson, R. D. S. (2010). *The Barn Owl*. London: T & AD Poyser Ltd.

Cade, T. J. (2000). Progress in translocation of diurnal raptors. In R. D. Chancellor & B. D. Meyburg (Eds.), *Raptors at risk* (pp. 343–372). Surrey, British Columbia, Canada: Hancock House Pub Ltd.

Carter, I. & Newbery, P.(2004). Reintroduction as a tool for population recovery of farmland birds. *Ibis* 146: 221-229.

Chandler, D. (2011). *Barn Owl*. (E. Rose, Ed.) (1st ed.). London: New Holland Publishers Ltd.

Csermely, D. (2000). Behaviour of hand-reared orphaned long-eared owls and tawny owls after release in the wild. *Italian Journal of Zoology*, *67*(1), 57–62. https://doi.org/10.1080/11250000009356295

Cunningham, S. J., Alley, M. R., & Castro, I. (2011). Facial bristle feather histology and morphology in New Zealand birds: Implications for function. *Journal of Morphology*, *272*(1), 118–128. https://doi.org/10.1002/jmor.10908

Drickamer, L. C., Vessey, S. H., & Meikle, D. (1992). *Animal behavior: Mechanisms, ecology, and evolution*. (K. Kane, Ed.) (3rd ed.). Wm. C. Brown Publishers.

Epple, W., & Rogl, M. (1992). *Barn Owls*. Carolrhoda.

Fajardo, I., Babiloni, G., & Miranda, Y. (2000). Rehabilitated and wild Barn Owls (Tyto alba): dispersal, life expectancy and mortality in Spain. *Biological Conservation*, *94*(3), 287–295. https://doi.org/10.1016/S0006-3207(00)00003-3

Feenders, G., Klaus, K., & Bateson, M. (2011). Fear and exploration in European starlings (Sturnus vulgaris): a comparison of hand-reared and wild-caught birds. *PloS One*, *6*(4), e19074. https://doi.org/10.1371/journal.pone.0019074

Feenders,G. & Bateson, M. (2013). Hand rearing affects emotional responses but not basic cognitive performance in European starlings. *Animal Behaviour* 86: 127-138.

Fox, N. (1995). *Understanding the bird of prey*. Hancock House Pub Ltd.

Fox, R., & Millam, J. R. (2004). The effect of early environment on neophobia in orange-winged Amazon parrots (Amazona amazonica). *Applied Animal Behaviour Science*, *89*(1–2), 117–129. https://doi.org/10.1016/j.applanim.2004.05.002

Glasier, P. (1978). *Falconry and hawking*. HarperCollins Distribution Services.

Gray, P. H., & Howard, K. I. (1957). Specific recognition of humans in imprinted chicks. *Perceptual and Motor Skills*, *7*, 301–304. https://doi.org/10.2466/PMS.7.7.301-304

Green, M., & Ramsden, D. (2001). *Barn Owl Trust second reintroduction report*. Ashburton, United Kingdom.

Greenberg, R.S.(2003). The role of neophobia and neophilia in the development of innovative behaviour of birds. *Animal Innovation*, chapter 8, 1-22.

Griffith, B., Scott, J. M., Carpenter, J. W., & Reed, C. (1989). Translocation as a Species Conservation Tool : Status and Strategy. *Science*, *245*(4917), 477–480. https://doi.org/10.1126/science.245.4917.477

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Immelmann, K. (1959). Experimentelle Untersuchungen uber die biologische Bedeutung artspezifischer Merkmale beim Zebrafinken (Taeniopygia castanotis Gould). *Zoologische Jahrbuecher Systematik*, 83, 437–592. https://doi.org/10.1111/j.1439-0310.1977.tb02118.x

Immelmann, K., & Suomi, S. J. (1981). Sensitive phases in development. In K. Immelmann, G. W. Barlow, L. Petrinovich, & M. Main (Eds.), *Behavioral development. The bielefeld interdisciplinary project* (pp. 395–431). Cambridge: Cambridge University Press.

Jones, R. B, & Waddington, D. (1993). Attenuation of the domestic chick’s fear of human beings via regular handling: in search of a sensitive period. *Applied Animal Behaviour Science*, *36*(2–3), 185–195. https://doi.org/10.1016/0168-1591(93)90009-E

Karapan, S. (2012). *Survival and foraging area of the released Barn Owl fledglings (Tyto alba) in oil palm plantation under different traing techniques* (Vol. 66). Retrieved from https://www.semanticscholar.org/paper/Survival-and-foraging-area-of-the-released-barn-owl-Karapan/9609515288632f4d7e84aa5fd473dde529fc6697

Kent, J. P. (1987). Experiments on the relationship between the hen and chick (Gallus gallus): the role of the auditory mode in recognition and the effects of maternal separation. *Behaviour, 102*(1), 14.

Knudsen, E. (2004). Sensitive periods in the development of the brain and behavior. *Journal of Cognitive Neuroscience*, *16*(8), 1412–1425. https://doi.org/10.1162/0898929042304796

Knudsen, E. I. (2002). Instructed learning in the auditory localization pathway of the Barn Owl. *Nature*, *417*(6886), 322–328. https://doi.org/10.1038/417322a

König, C., Weick, F., & Becking, J-H. (2008). *Owls of the World*. Second edition. Cristopher Helm, London.

Krebs, C. J. (1999). *Ecological methodology*. Addison-Wesley Educational Publishers, Inc.

Lickliter, R., Dyer, A. B., & Mcbride, T. (1993). Perceptual consequences of early social experience in precocial birds. *Behavioural Processes*, *30*(3), 185–200. https://doi.org/10.1016/0376-6357(93)90132-B

Manning, A., & Stamp, M. (1998). *An Introduction to Animal Behaviour*. Cambridge University Press.

Martí, R., & Del Moral, J. C. (2003). *Atlas de las aves reproductoras de España*. Madrid: Dirección General de Conservación de la Naturaleza-Sociedad Española de Ornitología.

Meek, W. R., Burman, P. J., Nowakowski, M., Sparks, T. H., & Burman, N. J. (2003). Barn Owl release in lowland southern England - a twenty-one year study. *Biological Conservation*, *109*(2), 271–282.

Meyrom, K., Motro, Y., Leshem, Y., Aviel, S., Izhaki, I., Argyle, F., & Charter, M. (2009). Nest-Box use by the Barn Owl Tyto alba in a Biological Pest Control Program in the Beit She’an Valley, Israel. *Ardea*, *97*(4), 463–467. https://doi.org/10.5253/078.097.0410

Mikkola, H. (1983). *Owls of Europe*. T & AD Poyser Ltd.

Monti ,F., Sforzi ,A. & Dominic, J. M. (2012). Post-fledging dependence period of ospreys Pa*ndion Haliaetus* released in central italy: home ranges, space use and aggregation *Ardeola* 59 (1): 17-30

Morandini, V. & Ferrer M. (2017). How to plan reintroductions of long-lived birds. PLoS One. 2017; 12(4): e0174186.

Motro, Y. (2011). Economic evaluation of biological rodent control using Barn Owls Tyto alba in alfalfa. *8th European vertebrate pest management conference*. Berlin.

Parry-Jones, J. (2001). *Training birds of prey* (1st ed.). Davis & Charles.

Petty, S. J., & Thirgood, S. J. (1989). A radio tracking study of post-fledging mortality and movements of tawny owls in argyll. *Ringing and Migration*, *10*(2), 75–82. https://doi.org/10.1080/03078698.1989.9673943

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & Core Team, R. (2017). nlme: Linear and Nonlinear Mixed Effects Models.

Platt, J. B., Bird, D. M., & Bardo, L. (2007). Raptor research and management techniches. In *Raptor research and management techniches* (1st ed., pp. 383–400). Hancock House Publishers.

R Development Core Team. (2016). *R: a Language and Environment for Statistical Computing*. *http://www.R-project.org/* (Vol. 2).

Roulin A., Kölliker M.and Richner H.(2000). Barn Owl (Tyto alba) siblings vocally negotiate resources. *Proc. R. Soc. Lond. B.* (Vol 267, pp. 459-463).

Roulin, A. (2020). *Barn Owls. Evolution and Ecology.* Cambridge University Press. 291 pp.

Ruppli, C.A., Almasi, B., Dreiss, A.N., Battesti, M., Jeni,L.& Roulin, A. (2012) Corticosterone Promotes Scramble Competition Over Sibling Negotiation in Barn Owl Nestlings (Tyto alba). *Evol. Biol.* 39:348-358.

Scott, J. M., & Carpenter, J. W. (1987). Release of captive-reared or translocated endangered birds: What do we need to know? *The Auk*, *104*(3), 544–545. https://doi.org/10.2307/4087562

Sunde, P. (2005). Predators control post-fledging mortality in tawny owls, Strix aluco. *Oikos*, *110*(3), 461–472. https://doi.org/10.1111/j.0030-1299.2005.14069.x

Taylor, M. (2017). *Owls*. Bloomsbury Natural History. 128 pp.

Tsurim, I., Abramsky, Z., & Kotler, B. P. (2008). Foraging behavior of urban birds: are human commensals less sensitive to predation risk than their nonurban counterparts? *The Condor*, *110*(4), 772–776. https://doi.org/10.1525/cond.2008.8572

Vallée, J. L. (2004). *La lechuza*. Ediciones Omega S.A.

Valutis, L. L., & Marzluff, J. M. (1999). The appropriateness of puppet-rearing birds for reintroduction. *Conservation Biology*, *13*(3), 584–591. https://doi.org/10.1046/j.1523-1739.1999.97443.x

Wallace, M.P. (1997). Carcasses, people, and power lines. *Zoo View* 31:12–17.

Wallace, M. P. (1994). Control of behavioral development in the context of reintroduction programs for birds. *Zoo Biology*, 13(5), 491–499. https://doi.org/10.1002/zoo.1430130511

Wallace, M.P. & Temple, S.A. (1987) Releasing captive- reared Andean condors to the wild. *Journal of Wildlife Management* 51 541-550,

Watters, J. V., & Meehan, C. L. (2007). Different strokes: Can managing behavioral types increase post-release success? *Applied Animal Behaviour Science*, *102*(3–4), 364–379. https://doi.org/10.1016/j.applanim.2006.05.036

Wiesel, T. N., & Hubel, D. H. (1965). Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. *Journal of Neurophysiology*, *28*(6), 1029–1040. https://doi.org/10.1152/jn.1965.28.6.1029

Zulkifli, I., Gilbert, J., Liew, P. K., & Ginsos, J. (2002). The effects of regular visual contact with human beings on fear, stress, antibody and growth responses in broiler chickens. *Applied Animal Behaviour Science*, *79*(2), 103–112. https://doi.org/10.1016/S0168-1591(02)00135-1

AUTHORSHIP STATEMENT

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Behavioural Processes

Highlights

- The way barn owls are imprinted determines their adaptability to the environment where they will live, whether in captivity or in the wild.
- Filial imprint and neophobia in barn owls determine their survival success in wilderness.
- The presence of certain objects known to birds in their habitat with which they were previously trained in their early life stages, can determine attraction or elusive responses to them.
- The physiological changes that occur in the third week of life in barn owls largely determine the recognition of their parents (filial imprinting) and environment (neophobia).