MASTERARBEIT / MASTER’S THESIS

Titel der Masterarbeit / Title of the Master‘s Thesis

„Effects of early social experiences on exploration and foraging in juvenile ravens (*Corvus corax*) “

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2023 / Vienna, 2023

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	UA 066 878
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Verhaltens-, Neuro- und Kognitionsbiologie
Betreut von / Supervisor:	Univ.-Prof. Mag. Dr. Thomas Bugnyar

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Acknowledgments

First and foremost, I want to thank my thesis supervisor Thomas Bugnyar for sparking my interest in animal behaviour during my bachelor studies, for subsequently providing me with countless opportunities to pursue my interests in science, for teaching me about the wonders of animal social life, for always being a kind and supportive mentor, and especially for introducing me to his study species, the raven. I also want to thank him for supplementing the data I collected myself with data from previous years, making this research endeavour possible.

As my thesis is only a small puzzle-piece in a large project, I want to especially thank Palmyre Boucherie, Mario Gallego-Abenza and Lisa-Claire Vanhooland, who were responsible for laying the theoretical groundwork and doing data collection even before I joined the project. They are responsible for teaching me most of what I know about ravens today, and I am truly grateful for their guidance. I also want to thank Aníta Rut for helping with both data collection in Grünau and subsequent video scoring.

I want to thank all my colleagues and friends that I met through my work with the ravens and at university, especially Anna, Anna, Awani, Marion, Aníta, Gloria and Vee, who were incredibly supportive throughout my studies.

Lastly, I want to thank my family for providing me with unconditional support and guidance through all of my struggles.

Zusammenfassung

Das soziale Umfeld in der Frühentwicklung hat starken Einfluss auf die Ontogenese des sozialen Verhaltens. Es ist jedoch nur wenig über den Einfluss auf andere Verhaltensweisen, wie Exploration und Nahrungssuche, bekannt. In dieser Studie habe ich 61 juvenile Raben (*Corvus corax*) nach Manipulation der Gelegegröße in einem „Novel-Environment Test“ (NE; eine etablierte Methode zur Messung individueller Reaktionen auf eine unbekannte Umgebung) beobachtet. Anhand explorativer Faktorenanalyse habe ich drei latente Verhaltensvariablen (*Exploration-Latenz*, *Exploration-Aktivität* und *Nahrungssuche*) im NE identifiziert. Ich wollte nun wissen, ob diese Verhaltensweisen durch die frühzeitige soziale Umgebung eines Individuums vorhergesagt werden können, insbesondere durch die *Gelegegröße* und das *Geschlechterverhältnis im Gelege*. Ich habe die Hypothese aufgestellt, dass erhöhter Stress in der Frühentwicklung zu einer erhöhten Exploration und Nahrungssuche im NE führt. Ich habe vorhergesagt, dass Küken aus großen Familien, sowie aus Familien mit einem höheren Anteil an Männchen, verstärkter Geschwisterkonkurrenz ausgesetzt sind und daher eine erhöhte Exploration und Nahrungssuche im NE vorweisen. Unsere Ergebnisse zeigen, dass die *Gelegegröße* keine Rückschlüsse auf das Verhalten im NE ermöglicht. Das *Geschlechterverhältnis im Gelege* konnte jedoch die *Exploration-Aktivität* vorhersagen, wobei es Individuen aus Gelegen mit mehr Weibchen waren, die am meisten erkundeten. Diese Beobachtung steht im Widerspruch zu meiner Vorhersage. Eine mögliche Erklärung dafür ist, dass Eltern mehr in männliche Küken investieren und daher Gelege mit mehr Weibchen verstärkter Ressourcenkonkurrenz und erhöhtem Stress in der Frühentwicklung ausgesetzt sind.

Abstract

Social experiences during early development strongly shape the ontogeny of social behaviour, but less is known about their impact on the development of other behavioural responses such as exploration and foraging. I subjected 61 juvenile ravens (*Corvus corax*) to a novel environment test (NE; an established method to measure individual responses to novel environment exposure) after brood sizes had been experimentally manipulated. Using exploratory factor analysis, I identified three latent behavioural variables (*Exploration-Latency*, *Exploration-Activity* and *Foraging*) in the NE. I asked if these behaviours can be predicted by an individual's early social environment, specifically *family size* and *sex ratio* in the brood. I hypothesized that increased stress, induced through competition, may lead to heightened exploration and foraging in the NE, as chicks might adapt their behaviour to cope with suboptimal resource availability in their early lives. I predicted that chicks from families with large broods would be subject to increased sibling competition, showing increased exploration and foraging in the NE. Since males are more resource-demanding than females, I similarly predicted that families with a higher percentage of males would show increased exploration and foraging in the NE. I found that *family size* was not a predictor of behaviour in the NE. *Sex ratio* in the brood significantly predicted *Exploration-Activity*, it were however individuals from female-biased broods that explored most. This observation runs contrary to my prediction. A possible explanation is that parents invest more heavily into male chicks, therefore subjecting female-biased broods to heightened resource competition and developmental stress.

Introduction

Background

To optimise behavioural responses to challenges posed throughout their life-history, individuals begin to shape their behavioural plasticity based on environmental conditions present in their early development (Gilbert, 2001; Metcalfe & Monaghan, 2001; Monaghan, 2008). In a predictable environment, this strategy can be highly beneficial and prepare individuals to cope with specific environmental challenges by shaping their phenotypic traits (Wells et al., 2007). This may help individuals to cope with challenges such as poor nutritional conditions, parasite exposure or high population density (Lindström, 1999; Metcalfe & Monaghan, 2001).

Social experiences during early development can have profound effects on the manifestation of behavioural phenotypes, especially concerning subsequent social behaviour (Gallego-Abenza et al., 2022; Sachser et al., 2011; Stamps & Groothuis, 2010). Notably, the absence of social partners during development can have significant modulating effects, particularly concerning social competence (i.e., the ability to process and respond to social information; Gallego-Abenza et al., 2022; Laviola & Terranova, 1998; Taborsky et al., 2012; Taborsky & Oliveira, 2012). For instance, it has been shown in mice that litter size and litter sex ratio affect social behaviour in later life stages (Laviola & Terranova, 1998) and experiments in ravens revealed that family size during upbringing affects how individuals value social information (Gallegao-Abenza et al., 2022). Similar patterns have been found across many taxa, including humans (Bick & Nelson, 2016; Tottenham, 2014).

Social experiences during early development can be more narrowly defined as the presence or absence of siblings, quantity and quality of parental care, and other rearing conditions, which directly influence sibling competition and subsequently individual stress (Gallego-Abenza, 2022). The developmental stress hypothesis proposes that stress during early development affects brain development of individuals on a structural level. It therefore provides a mechanistic explanation for the differential expression of behavioural phenotypes based on social factors during early development (Boogert et al., 2014; Buchanan, 2011). Many of the previous studies strongly manipulated access to social partners during early development, for instance by separating individuals from parents or siblings (Anderson & Mason, 1978; Arnold & Taborsky, 2010; Bick & Nelson, 2016). Less is therefore known about how natural variation in the social environment, such as differing family sizes and sex ratios affect stress and subsequently phenotypic behavioural expressions. In a recent study, Brandl et al. (2019) showed that zebra finch (*Taeniopygia castanotis*) nestlings experienced higher levels of stress in experimentally enlarged broods, due to increased sibling competition. On the other hand, Naguib et al. (2011) found that great tits (*Parus major*) showed increased stress responses in smaller broods compared to normal sized broods. Overall, it becomes clear that variation in nutritional and social conditions during early development can have lasting consequences on ontogenetic pathways, but there is a lack of experimental studies examining these relationships.

Early social conditions may not only shape behavioural responses towards social stimuli, but also towards factors of the physical environment, such as novelty. Animal personality is defined as between-individual differences in behaviour that are consistent through time and between contexts (Réale et al., 2007). An individual’s speed of exploration reflects the response to novelty, and is the focus of this experiment, as individuals were exposed to a novel environment test (also known as open-field test; Hall & Ballachey, 1932; Perals et al., 2017; Réale et al., 2007).

Groothius et al. (2005) found that great tit nestlings subjected to nutritional stress developed into bolder and faster exploring adults. In line with the developmental stress hypothesis, they propose that this shift in personality is a coping mechanism, allowing individuals to adjust to poor environmental conditions. In their study from 2011, Naguib et al. presented the first evidence that natural variation in social conditions in the nest could have lasting consequences on personality in great tits (Naguib et al., 2011). They found that while family size was not a predictor of exploration, the brood sex ratio was. However, there is a general lack in studies linking the early social conditions to non-social behaviour in later life stages. As Naguib (2011) proposed, heightened competition in the nest may lead to more aggressive and explorative phenotypes. As males are heavier and require more resources than females, a higher number of male chicks in the brood could lead to increased competition and stress, therefore leading to the same prediction (Ersoy et al., 2021). After being exposed to high levels of resource competition in the nest, juveniles might additionally be more likely to forage even in risk-prone situations such as novel environments. The individual tendency to forage in the novel environment could be interpreted as a measure of boldness, as chicks were familiar with the food items, but taking a risk by foraging in the unknown environment (Réale et al., 2007).

The common raven (*Corvus corax)* displays several characteristics that make it a fascinating model organism to address the questions of the current study. During early life stages, ravens are generally highly explorative and neophilic, before becoming highly neophobic as adults (Heinrich, 1995). However, little is known about individual differences in exploration during development and how they arise. Additionally, ravens display a highly complex social life: After fledging, individuals integrate into large non-breeder groups, where dynamics of social hierarchies require highly developed cognitive abilities in the social domain from a young age (Boucherie et al., 2019). Consequently, social conditions during early development might be especially influential predictors of life-history trajectories in this species.

Study Aims

The aim of the current study was to investigate the effects of several social factors during the early life stages of ravens on their behaviour in a subsequent novel environment test. Specifically, the influence of (1) *family size* and (2) sex distribution in the brood (*sex ratio*) were of interest. The *family size* was experimentally manipulated to create families ranging from one to four chicks while the natural variation in the *sex ratio* was used. To understand how these factors influence exploration and foraging behaviour, juvenile individuals were subjected to a novel environment test in the early non-breeder stage (approximately five months old). A wide range of behaviours in the novel environment was examined and latent variables for exploration and foraging extracted through means of factor analysis to be subjected to further regression analysis.

I hypothesized that an individuals' social experiences play an important role in shaping exploratory and foraging responses when exposed to a novel environment. I predicted that individuals from (1) larger families and (2) families with a higher percentage of male chicks experience heightened sibling competition (and consequently stress) during early life stages and therefore show heightened levels of exploration and foraging behaviour in the novel environment, to cope with suboptimal conditions during early development.

Methods

Study Subjects & Housing

The current study was conducted on 61 juvenile ravens (*Corvus corax*) from 27 families (not all families had distinct parents, see supplementary material Tab. S1) throughout four consecutive breeding seasons (2018-2021). The parents of the chicks were 10 captive breeding pairs, situated at the Haidlhof Research Station, Lower Austria (4 pairs), the Konrad Lorenz Research Center, Upper Austria (5 pairs) and Schönbrunn Zoo, Vienna (1 pair). Families were housed separately from each other in 80-120m^2 aviaries, and although some could hear each other, there was no physical interaction between chicks of different families during this stage (“family phase”). In each breeding season the broods of each family were experimentally manipulated to create families of varying size. This was done by removing eggs, resulting in four eggs per family. In half of the families, two of the four eggs were made infertile (by shaking), resulting in small (2 chicks) and large (4 chicks) families. In the first year of the study (2018), *family size* treatment was randomly allocated to the different families. Families with a large brood manipulation were treated with a small brood manipulation in the consecutive breeding season in a cross-design. In some cases, eggs did not hatch, or chicks died within the first days of the study, altering the *family size* (resulting in families with one or three chicks). Before fledging, chicks were marked with coloured foot rings, allowing individual identification of the chicks from this point onwards.

At approximately ten weeks post fledging (mid-July), the family phase ended, and juveniles were taken from the family aviaries and transported to the Konrad Lorenz Research Center (Grünau im Almtal, Upper Austria), where they were released into two large non-breeder aviaries (“group phase”). Each non-breeder group was comprised of five to ten individuals. Individuals were assigned to the groups based on their relationship: Families with three or four chicks were separated into the two nonbreeder-groups (so that two siblings stayed together) while families with two chicks were put into the same group. The non-breeder aviaries were approximately 60m^2 in size and contained numerous perching opportunities, covered areas to protect from weather, natural ground cover to allow for caching and digging and shallow pools for bathing. Neither visual nor acoustic contact was possible between the two non-breeder aviaries. Ravens were fed twice daily (vegetables, fruit and cereal in the mornings, meat in the afternoon) and had *ad libitum* access to water. After six weeks in the nonbreeder aviaries, the ravens were prepared to be released into the wild at the beginning of September. This preparation included catching the ravens and transporting them to the on-site laboratory where a wing tag and a GPS logger were attached to the birds and blood samples were taken for sexing. Since the individuals were already caught and separated, the opportunity was exploited to conduct the novel environment experiments. After processing, the birds were kept in a dark and quiet room for approximately 30 minutes, before being transferred to the novel environment aviaries.

Novel Environment Test

Setup

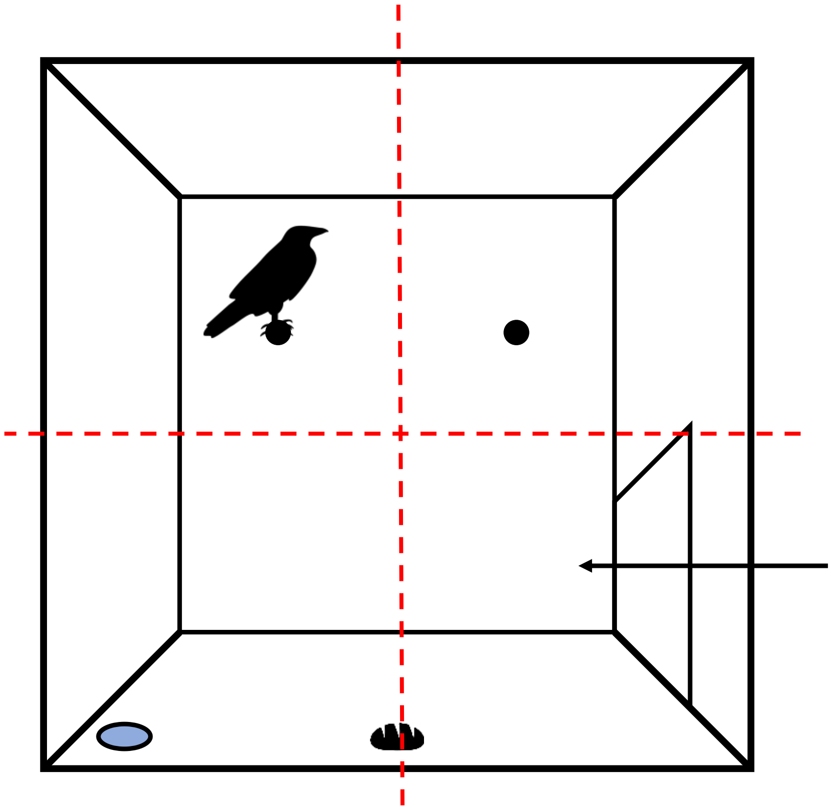

Fig. 1: Setup of the novel environment aviaries. There were two perches provided and food and water were freely available. At the bottom, a hatch could be opened to allow arriving ravens to enter the novel environment. Red lines represent the visual separation into quadrants that was used for video scoring.

The novel environment experiments were conducted approximately at the same time in each consecutive breeding season (last week of August or first week of September). The aviaries were 2m^3 in size and located in a sparsely forested area on the premises of the Cumberland Gamepark, Grünau im Almtal, Upper Austria. No other animal enclosures were located nearby, and park visitors and other disturbances were kept away while the experiments were conducted. The walls of the aviary were made of plastic mesh and therefore see-through. The aviaries contained two wooden perches, and ravens had *ad libitum* access to food and water during the experiment. In 2021, additional objects and tasks were added to the novel environment as part of a different study (two familiar and two novel objects, a string-pulling task and a bottle containing food). I did not expect this change to impact my question in a meaningful way, so data from 2021 was included in the study. As two individuals were tested simultaneously (in two separate novel environment aviaries spaced approximately two meters apart), one wall was covered with plastic tarp to block visual access to the second aviary. The tarp partially covered the top of the aviary, so that birds could take shelter. Birds could still hear each other during the experiment. The two birds that were simultaneously tested originated from the same non-breeder group but were not siblings. This was done to alleviate the stress of separation for the individuals being tested in the novel environment. In front of each aviary, a camera (either Panasonic HC-V770, Panasonic HC-V777, Panasonic HC-V880 or GoPro Hero 5) was mounted on a tripod to record for the entire duration of the experiment.

Procedure

The birds arrived at the site of the novel environment aviaries in animal carry boxes, covered with a towel to block visual access of the surroundings during approximately ten minutes of transportation. After the cameras were started, the ravens were placed at the entrance to the novel environment, uncovered, and the front of the carry box was opened to allow ravens to enter the novel environment of their free volition. If ravens were not willing to enter after three minutes, they were forced into the novel environment by tilting of the carry box. After the raven had entered the novel environment, the hatch was closed securely. Experimenters quickly left the site and returned after approximately thirty minutes to stop the recording. After the experiment was concluded, ravens were kept in the novel environment aviaries overnight (as part of a different study, see Gallego-Abenza et al., 2022) before being returned to their nonbreeder-group.

Video scoring

The video material was scored using Solomon Coder (Péter, 2019). First, the time to enter the novel environment was measured (*out of box latency*). After the raven had entered the novel environment, and experimenters had left the site, the coding process was started. To quantify movement of the ravens in the aviary, the aviary was visually separated into four quadrants (top and bottom left, top and bottom right, Fig. 1). Several behavioural latencies, durations and frequencies were transcribed (Tab. 1).

Tab. 1: Behavioural variables scored in the novel environment and their descriptions.

Variable Name	**Behaviour Description**
Out of box latency	Latency to enter the novel environment (measured from the opening of the carry box).
Latency to 2nd Quadrant	Latency to enter the second Quadrant (measured from the start of coding, i.e., after the experimenters left the site).
Latency to 3rd Quadrant	Latency to enter the third Quadrant (measured from the start of coding, i.e., after the experimenters left the site).
Latency to 4th Quadrant	Latency to enter the fourth Quadrant (measured from the start of coding, i.e., after the experimenters left the site).
Total Quadrants Visited	Number of quadrants visited in the duration of the experiment (ranges from 1 to 4).
No. Quadrant Changes	Number of quadrant changes in the duration of the experiment.
No. Wall Jumps	Number of wall jumps (jumps from a perch to the wall and back) in the duration of the experiment.
Foraging frequency	Number of ground-foraging pecks (pecking the ground) in the duration of the experiment.
Duration Eating	Time spent eating in the duration of the experiment.
Duration Inactive	Time spent inactive (no movement for at least three seconds) in the duration of the experiment.
Duration on Ground	Time spent on the ground in the duration of the experiment.

For further analysis, *latency to 3rd quadrant* and *latency to 4th quadrant* were excluded, as not all individuals reached more than two quadrants in the duration of the experiment. Videos were scored by two coders (Al. R. and An. R.). Both coders scored 7 of the 61 videos (11.5%) from different years of the study. The interclass correlation coefficient was calculated for each behaviour to ensure interrater reliability. ICCs were excellent across all behaviours scored, with the lowest ICC for *duration on ground* (ICC = 0.987) and the mean across all behaviours at ICC = 0.997. Calculations were done in R (R Core Team, 2022) using the “IRR” package (Gamer et al., 2019).

Statistical Analysis

All analyses were performed in R version 4.2.2 (R Core Team, 2022).

Data Processing

Since recording times were not consistently 30 minutes for each individual (recording times ranged from 16.4 to 33.8 minutes, mean = 28.0), behavioural variables were adjusted by dividing through the total time of the respective experiment (in minutes).

Factor Analysis

Exploratory factor analysis was performed on the time adjusted behavioural variables (N = 61) using the package “psych” (Revelle, 2022). As the goal was to subject the factors to interpretation based on underlying factor loadings, factor analysis was deemed more appropriate than principal component analysis in this case (Budaev, 2010). First, the *KMO* function was used to measure sampling adequacy of the correlation matrix. Variables with MSA < 0.5 were excluded from the correlation matrix, resulting in the exclusion of *duration inactive* from further analysis (Kaiser, 1974). The new correlation matrix (see supplementary material, Fig. S2) possessed an overall sampling adequacy of MSA = 0.63, which is described as “mediocre” by Kaiser (1974) but nonetheless implies that the correlation matrix is factorable. Next, the adequate number of factors to be extracted was determined to be three, using parallel analysis (function *fa.parallel*). The factor analysis was performed on the correlation matrix using the *fa* function. A maximum likelihood estimation procedure was chosen and varimax rotation applied to the resulting factors, as this is suggested to be most appropriate for small sample sizes (Budaev, 2010).

The factor scores were extracted using the bartlett algorithm, as this is less likely to produce biased factor scores than the standard regression method (Skrondal & Laake, 2001). After examining and interpreting the factor loadings, factor scores were used as response variables in regression analysis to test the predictions of the study.

Regression Analysis

Regression analysis was performed using the package "glmmTMB" (Brooks et al., 2017). Three separate mixed effects models were fitted using the factor scores isolated in the factor analysis as response variables (*Exploration-Latency, Exploration-Activity* and *Foraging*). For each model, *family size* (discrete; 1 to 4 chicks) and *sex ratio* (continuous; % male chicks in the nest) were included as fixed effects to test the hypothesis of the study. Additionally, *sex* (categorical; male or female) was added as a fixed effect to control for a potential main effect of sex. Similarly, *rain* (categorical; yes or no) was added as a last fixed effect to account for the effect of rain during the experiments (rain was set to yes when there was rainfall during the majority of the video recording). Neither *sex* nor *rain* were intentionally manipulated as part of the study but could not be added as random effects due to the low number of levels in each categorical variable.

Both *parent identity* (categorical; refers to the parents of the chick) and *nonbreeder group* (categorical; 2 groups in each year of the study) were added as crossed random effects, to control for genetic and rearing effects provided by the parents as well as possible differences between social groups and years. Full models were fitted using different "family" parameters to achieve satisfactory model fit and residual diagnostics for each response variable. The *Exploration-Latency* model was fitted with a gaussian error distribution. The models *Exploration-Activity* and *Foraging* were fitted using a negative binomial error distribution and checked for overdispersion. Response variables (factor scores) for the *Exploration-Activity* and *Foraging* models were transformed to allow model fitting (factor scores were made positive by adding the minimum score to all scores, then multiplied by 100 and rounded to obtain discrete values).

In the next step, fixed effects were reduced in a stepwise fashion for each full model using the *dredge* function in the package “MuMIn” (Bartoń, 2022). Optimal models were determined in each case by inspection of the model selection table (based on AIC; Akaike, 1974). Residual diagnostics and checks for overdispersion were performed on the reduced models using the package “DHARMa” (Hartig, 2022) and deemed appropriate for all models. Chi-squared and p-values were calculated using the *Anova* function in the package “car” (Fox & Weisberg, 2019). Lastly, R-Squared values were calculated using the function *r.squaredGLMM* in the package “MuMIn” (Bartoń, 2022). Model tables were created using the “sjPlot” package (Lüdecke, 2022) and graphs were created using a combination of “sjPlot” and “ggplot2” (Wickham, 2016).

Results

Factor Analysis

Exploratory factor analysis reduced the eight behavioural variables to three factors, that could be labelled according to the variable factor loadings (Tab. 2). The first factor, *F1*, explained 22% of the variance in the data and was strongly influenced by *duration on ground* and *duration eating*, as well as *foraging frequency*. This first factor was labelled *Foraging*. The second factor, *F2*, explained 19% of the variance and was negatively correlated with *total quadrants visited* and positively with *latency to 2nd quadrant* and *latency to enter arena*. This factor was labelled *Exploration-Latency*. The last factor, *F3*, explained 18% of the variance and was strongly loaded by both *no. quadrant changes* and *no. wall jumps*. Factor *F3* was labelled *Exploration-Activity*.

Tab. 2: Table of factor loadings. F1, F2 and F3 represent the three factors extracted in the factor analysis. Factor loadings <0.5 are not displayed for visual clarity. Communality of each variable is given to the right of the factor loadings. Sample size for factor analysis is N = 61.

	F1	F2	F3	communality
Duration on Ground	0.97			1
Duration Eating	0.72			0.55
Foraging Frequency	0.52			0.27
Total Quadrants Visited		-0.75		0.61
Latency to 2nd Quadrant		0.69		0.5
Latency to enter Arena		0.57		0.35
No. Quadrant Changes			0.85	0.84
No. Wall Jumps			0.8	0.67
Variance explained	22%	19%	18%	

Regression Analysis

Foraging

The best model for the response variable *Foraging* was the null model, containing no fixed effects (Tab. 3).

Tab. 3: Model table for the response variable *Foraging*.

	Foraging		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	4.88	4.65 – 5.11	**<0.001**
Random Effects			
$N_{\text{parent identity}}$	10		
$N_{\text{nonbreeder group}}$	8		
Observations	61		
Marginal R^2 / Conditional R^2	0.000 / 0.055		

Exploration-Latency

The model reduction procedure revealed that the best model to explain *Exploration-Latency* only contained the random effects (Tab. 4). The variation of *parent identity* explained 33.6% of the total variation (Fig. 2).

Tab. 4: Model table for the response variable *Exploration-Latency*.

	Exploration-Latency		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-0.09	-0.55 – 0.38	0.719
Random Effects			
N $_{\text{parent identity}}$	10		
N $_{\text{nonbreeder group}}$	8		
Observations	61		
Marginal R^2 / Conditional R^2	0.000 / 0.336		

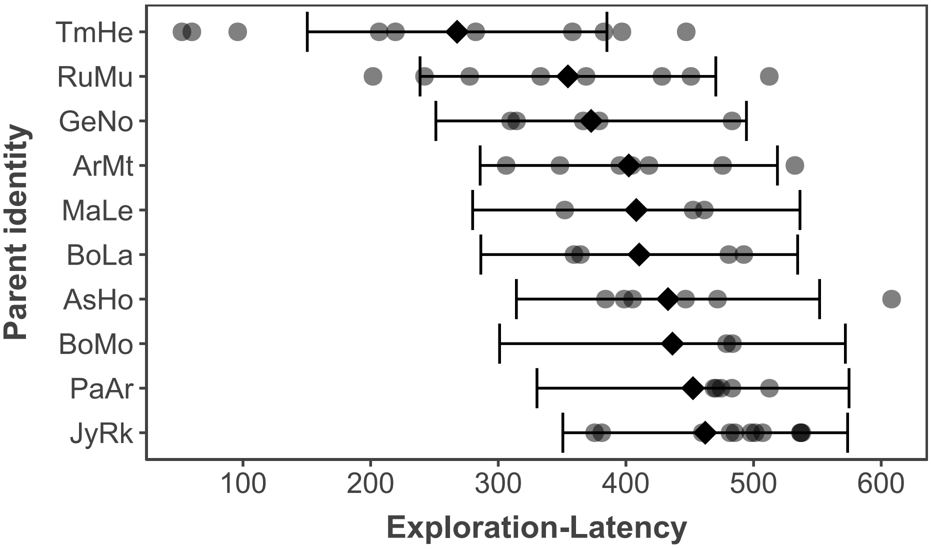

Fig. 2: Random effects plot of *parent identity* with *Exploration-Latency* (factor-score, not a measurement of time. A larger score reflects shorter behavioural latencies) as the response variable. Exploration-Latency is plotted on the x-axis, the different families are listed on the y-axis. Model estimates (black diamonds) are plotted along with 95% confidence intervals. Data are plotted as grey dots.

Exploration-Activity

For the response variable *Exploration-Activity,* the optimal model contained the variables *sex ratio* and *rain* as fixed effects (Tab. 5). All fixed variables had significant effects on the response variable *Exploration-Activity*. With an increasing percentage of male chicks in the brood, *Exploration-Activity* decreased significantly ($\chi^2 = 6.93$, $p = 0.008$; Fig. 3; For a more detailed breakup of *Exploration-Activity* as a function of brood sex combinations, see Fig. 4).

Additionally, birds showed significantly less *Exploration-Activity* in rain ($\chi^2 = 8.38$, $p = 0.004$, supplementary material Fig. S3).

Tab. 5: Model table for the response variable *Exploration-Activity*.

		Exploration-Activity		
Predictors		*Estimates*	*CI*	*p*
(Intercept)		5.327	4.931 – 5.723	**<0.001**
Sex Ratio		-0.009	-0.015 – -0.002	**0.008**
Rain [yes]		-0.662	-1.118 – -0.206	**0.004**
Random Effects				
$N_{\text{parent identity}}$	10			
$N_{\text{nonbreeder group}}$	8			
Observations	61			
Marginal R^2 / Conditional R^2	0.17 / 0.17			

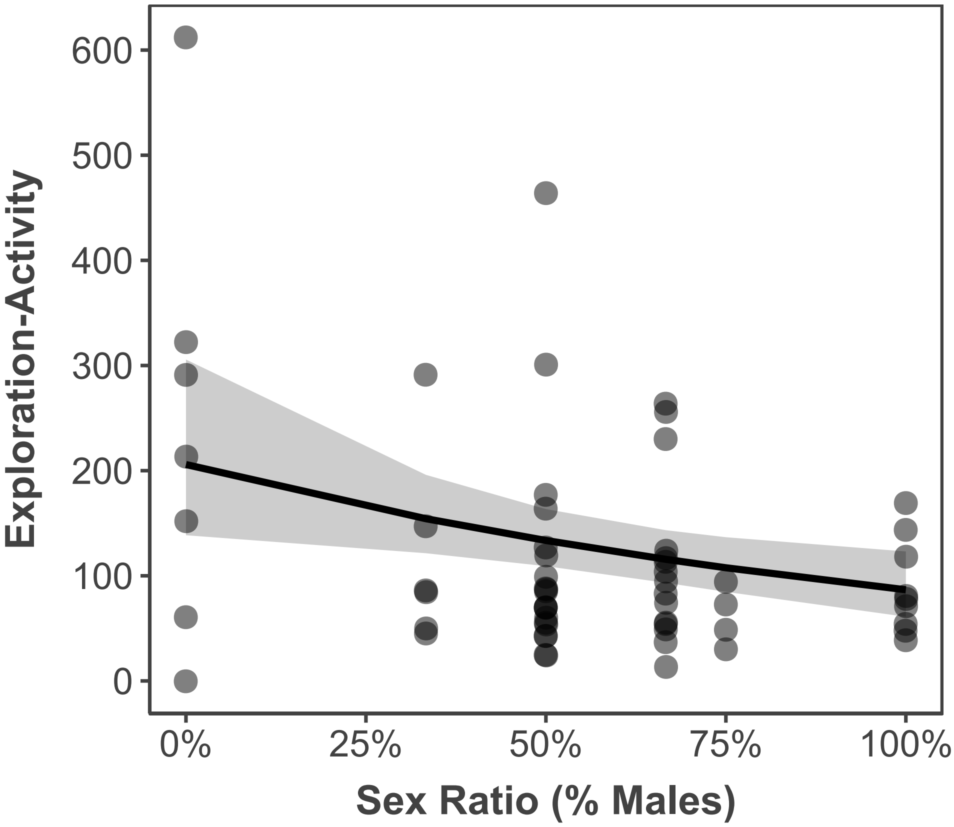

Fig. 3: The *Exploration-Activity* score decreased in response to % males in the clutch (χ^2 = 6.93, p = 0.008). The graph shows the regression line in black, with a 95% confidence ribbon. Data are plotted as grey dots.

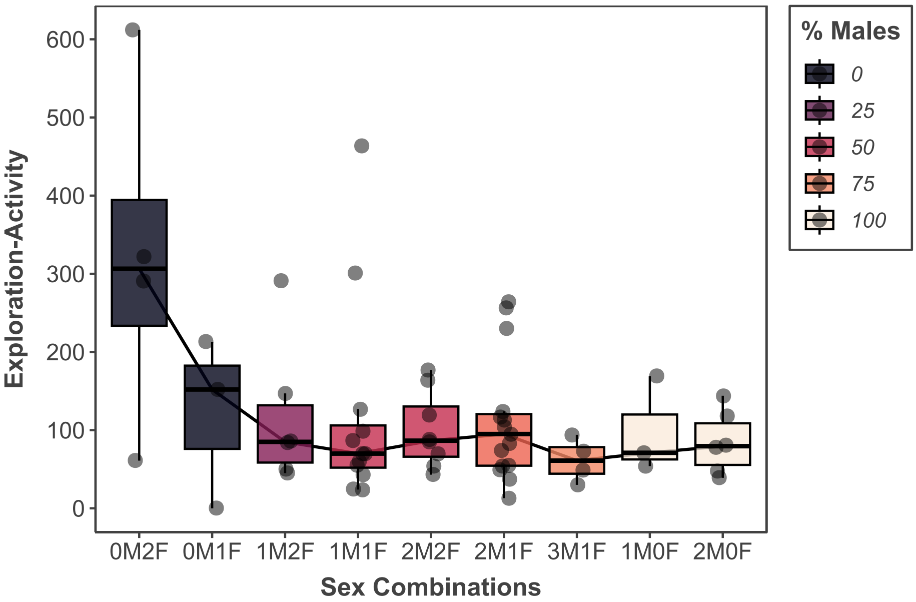

Fig. 4: Descriptive boxplots of the *Exploration-Activity* as a function of different sibling sex combinations. Sex combinations are given as the number of masles (M) and number of females (F) in the brood. The % of males in the brood corresponds to the shading of the boxplots, see figure legend.

Discussion

The aim of the current study was to test the effects of early social conditions on the exploration and foraging behaviour of juvenile ravens in a novel environment test. Contrary to my expectations, *family size* could not explain subsequent behaviour in the novel environment. While *sex ratio* in the nest was a significant predictor of the latent variable *Exploration-Activity,* the observed effect ran contrary to my prediction: chicks from female-biased families explored the most.

It is well established that nutritional restriction during early development is a substantial stressor, which can have lasting consequences on individual behavioural plasticity during later life stages (Groothuis et al., 2005; Krause et al., 2009; Oers et al., 2015; Stamps & Groothuis, 2010). Brood size directly affects parental food provisioning, with individual intake decreasing with increasing brood size (Emms & Verbeek, 1991; Ersoy et al., 2021). In fact, in a recent publication investigating the post-fledging parental investment in ravens, individuals from smaller families (1-2 chicks) received significantly more food than chicks from larger broods (2-4 chicks; Ersoy et al., 2021). Furthermore, in zebra finches, individuals from experimentally enlarged broods showed higher stress responses than chicks from smaller broods (Brandl et al., 2019). Considering this, it seems surprising that no effect of *family size* was found in this study. Stress during development might not increase exploration and foraging as predicted. This seems unlikely, as previous evidence from great tits supports increased exploration as a coping mechanism for poor nutritional conditions during early development (Groothius et al., 2005). Groothius et al. (2005) tested the effects of early food provisioning on exploration. They found that food-rationed chicks showed increased begging behaviour in the nest and subsequently became significantly faster explorers than untreated birds. Possibly, the manipulation of *family size* did not create a sufficiently stressful environment to have lasting effects on exploratory tendencies in the juvenile ravens. Broods of wild ravens in Grünau im Almtal, Upper Austria, range from one to four chicks (mean = 2.84; unpublished data), closely resembling the manipulated range of *family size* in the current study. However, because ravens in this study had unlimited access to food, providing for large broods of four chicks might not have been particularly challenging to the parents. Additionally, chicks from smaller families could be experiencing increased stress not due to a lack of food, but other aspects of the social environment. Naguib et al. (2011) showed that in great tits, chicks from small families showed higher stress responses than those from average sized families. They suggest that this could be the consequence of increased thermoregulatory costs or differential parental behaviour (Naguib et al., 2011). Another detrimental factor might be a lack of social interaction between chicks in smaller families (Taborsky et al., 2012; Taborsky & Oliveira, 2012). After dispersal, ravens integrate into large social groups of non-breeding individuals before they reach sexual maturity and become territorial (Heinrich, 2014). Non-breeder groups are characterised by a hierarchical dominance system and fission-fusion dynamics (Boucherie et al., 2022, Loretto et al., 2017). These conditions likely promote complex social cognition and the ability to process social information (social competence) is partly shaped by social experiences in the early development (Boucherie et al., 2019; Gallego-Abenza et al., 2022). A lack of social partners in this sensitive stage might be especially detrimental in ravens, therefore also affecting the development of behavioural phenotypes. Taken together, the connection of *family size* and subsequent development of behavioural traits may be more complex than anticipated and mediated by several factors, including both nutritional and social factors of the early environment.

The effect of brood *sex ratio* on the behaviour in the novel environment ran contrary to my predictions. Male chicks are heavier and require more resources (Ersoy et al., 2021). Therefore, I predicted male-biased broods to be a more stressful environment, leading to increased exploration and foraging. However, the smaller the percentage of males in the brood was, the larger were *Exploration-Activity* scores, indicating the opposite effect. Female-biased broods showed higher exploration scores in the novel environment which was unexpected in the light of the developmental stress hypothesis, although it is not an unprecedented finding. Naguib et al. (2011) found the same pattern in their experiment with great tits, where female-biased families were likewise the ones to explore more. In great tits, parental food provisioning is not dependant on brood sex ratio, leading Naguib et al. (2011) to conclude that their results were most likely not mediated by parental behaviour (Lessells et al., 1998). However, there is evidence of increased nest defence in male biased broods, showing that sex ratio might nonetheless be a predictor of parental investment in great tits (Leech et al., 2006; Radford & Blakey, 2000). In ravens, mothers feed all chicks equally, while fathers selectively feed heavy males (Ersoy et al., 2021). It seems plausible that increased parental investment into male chicks might mediate nutritional stress in male-biased broods. Consequently, female-biased broods might be the ones exposed to higher levels of nutritional stress and therefore show higher *Exploration-Activity* scores in the novel environment.

In addition to brood *sex ratio*, *rain* was also a significant predictor of the *Exploration-Activity* score. Chicks were slower and less active in exploring the novel environment during bad weather conditions, which might be due to individuals preferring to take cover instead of exploring the environment.

The variation of *Exploration-Latency* could not be explained by fixed effects in this study. Interestingly, *Exploration-Latency* showed substantial random effect variation between parent identities. It is likely that other family differences, stemming from genetic predisposition or rearing conditions, are more suitable to explain the development of *Exploration-Latency* (Stamps & Groothuis, 2010).

Foraging behaviour in the novel environment could not be explained through *family size* or *sex ratio* and in contrast to *Exploration-Latency*, there was no large random-effect variation either. This indicates that foraging behaviour in the novel environment could not be explained by any of the variables of this study, and it is likely that the observed variation was in response to individual hunger levels of the chicks. Although chicks were always fed at the same time in the social groups, food access might have varied between individuals due to social dynamics in the non-breeder group (Boucherie et al., 2022).

One important drawback of the current study is that individual responses to novelty were only measured once for each individual, i.e., in a novel environment test. As per the largely agreed upon definition of animal personality (Réale et al., 2007), repeated measures are an essential constituent to determine the consistency of behavioural differences between individuals. When taking only one measure of behaviour, the observed variation might not be consistent between individuals, but reflect within-individual variation. Although only one session of behavioural measures was taken, it was taken over the course of 30 minutes, summarising individual tendencies in at least that timeframe. Factor analysis allowed the grouping of related behaviours based on previously established personality axis (Groothuis & Carere, 2005; Réale et al., 2007).

In this study, I separated the observed behavioural measures of exploration into two latent variables, *Exploration-Latency* (i.e., behaviours related to latencies of movement in the novel environment) and *Exploration-Activity* (i.e., behaviours related to frequencies of movement in the novel environment). *Exploration-Activity* in this case is interpreted as a measure of exploration, not activity, since the behaviours were observed in an unfamiliar environment (Réale et al., 2007). However, it is nonetheless important to consider that variables related to *Exploration-Activity* were not correlated with those of *Exploration-Latency* and that the influence of the early social environment differed strongly between the two. In a recent review of novel environment experiments, Perals et al. (2017) concluded that more care needs to be taken when measuring behaviour in the novel environment: it might be problematic to simply assign the construct of exploration to all behaviours. To provide construct validity for measures of exploration, secondary experiments should be conducted to determine both convergent and discriminant validity (Perals et al., 2017). This was unfortunately not in the scope of the current study, so the results regarding especially *Exploration-Activity* should be interpreted carefully. It is, for instance, possible that the observed behaviours are related to individual stress or fear responses rather than exploration (Cockrem, 2007).

In future studies, the repeatability of individual behaviour should be more firmly established and behavioural measures in the novel environment should be validated through secondary experiments. I nonetheless propose that the current findings contribute to better understanding the developmental influences on behavioural plasticity in a highly social species, the raven.

Conclusions

I found no effect of *family size* on exploration or foraging scores in the novel environment, which was surprising as the developmental stress hypothesis predicts a link between sibling competition and subsequent behaviour. Likely, the manipulation of *family size* did not affect individual stress in the predicted way. *Exploration-Activity* increased in response to the percentage of female chicks in the brood, which was contradictory to my prediction. I propose that in ravens, female biased broods might be subjected to higher levels of developmental stress, as fathers selectively invest in sons (Ersoy et al., 2021). Finally, I conclude that my results reconfirm previous findings that natural variation in the brood *sex ratio* during early development play an important role in shaping subsequent exploratory behaviour (Naguib et al., 2011). To better understand the effects of developmental stress, future studies should include either behavioural or physiological measures of stress in that period. Additionally, the presence of exploration as a personality trait needs to be better established in the study subjects.

Ethical Statement

The novel environment experiment on individually separated ravens was performed under the license for animal experimentation of the Austrian government (BMBWF-66.006/0015-V/3b/2018). All ravens were kept according to the guidelines and permissions of the federal states Lower and Upper Austria.

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Supplementary Material

Tab. S1: Overview of all raven families and individuals contained within across the four breeding seasons 2018-2021.

Parent identity	Year	Family members	Family size	Sex combination	Sex ratio (% males)
AsHo	2018	Bacchus, Diana, Minerva	4	2M1F	66.7
BoLa	2018	Coal, Draggy, Ila, Talisker	4	3M1F	75
JyRk	2018	Jolly, Twinkle	2	1M1F	50
RuMu	2018	Gusgus, Mufasa	2	1M1F	50
TmHe	2018	Apollo, Arthemis	2	1M1F	50
ArMt	2019	Summer, Winter	2	0M2F	0
AsHo	2019	Sansa	1	0M1F	0
Geno	2019	Hazel, Hickory	2	0M2F	0
JyRk	2019	Hamlet, Juliet, Othello	3	2M1F	66.7
MaLe	2019	Ganymed, Io, Kallisto	3	1M2F	33.3
RuMu	2019	Toma	1	1M0F	100
TmHe	2019	Cleo, Genghis, Xerxes	3	2M1F	66.7
ArMt	2020	Cirrus, Cumulus, Nimbus	3	2M1F	66.7
AsHo	2020	Nyx	1	0M1F	0
Geno	2020	Oak	1	1M0F	100
JyRk	2020	Ebony, Inky	2	1M1F	50
PaAr	2020	Eragon, Saphira	2	2M0F	100
RuMu	2020	Arakusa, Shiba, Uenu	3	2M1F	66.7
TmHe	2020	Tango	1	0M1F	0
ArMt	2021	Merry, Pippin	2	1M1F	50
AsHo	2021	Cheddar	1	1M0F	100
BoMo	2021	Cobain, Hendrix	2	2M0F	100
Geno	2021	Gnocci, Tortellini	2	2M0F	100
JyRk	2021	Gamay, Merlot, Pinot, Riesling	4	2M2F	50
PaAr	2021	Earth, Fire, Water	3	1M2F	33.3
RuMu	2021	Auguste, Hippolyte	2	1M1F	50
TmHe	2021	Foxtrot, Rumba, Salsa, Samba	4	2M2F	50

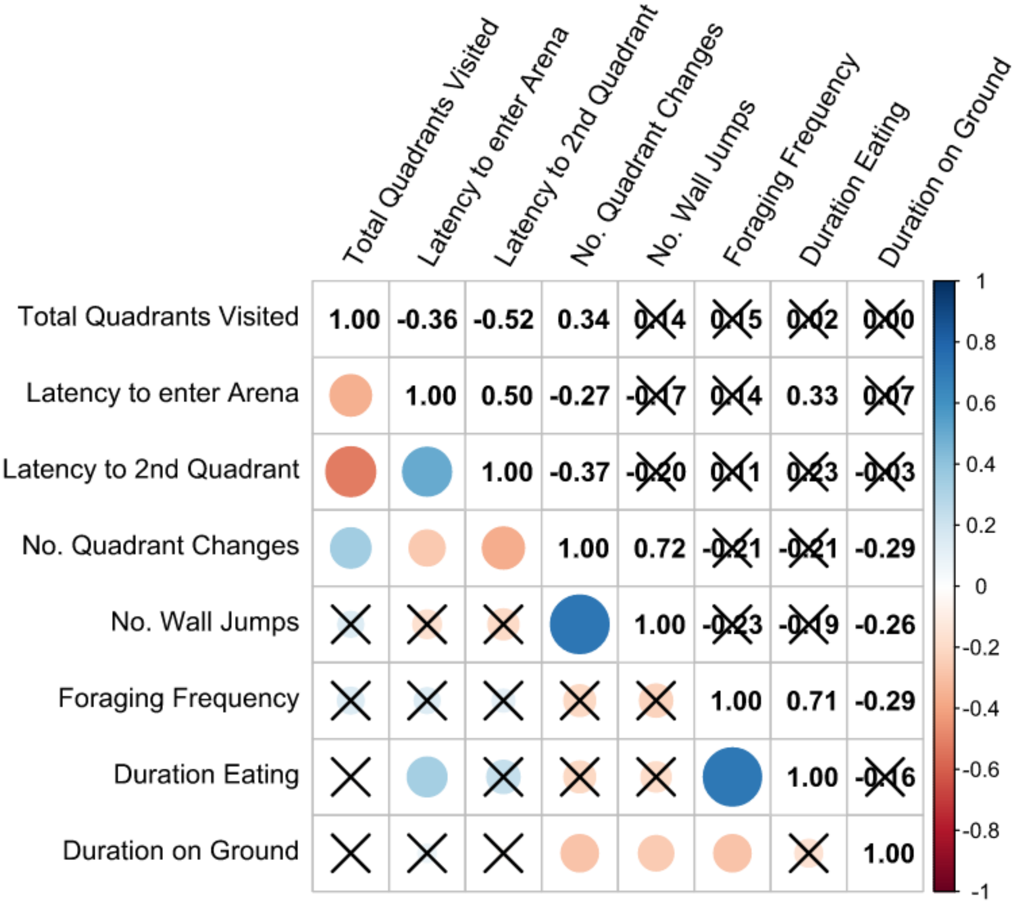

Fig. S2: Correlation matrix used in the factor analysis. Pearson correlation coefficients are plotted in the top right of the graph. In the bottom left of the graph, correlation coefficients are represented as circles: Lager circles imply a stronger correlation, blue circles imply a positive correlation. Non-significant (p > 0.05) correlations are crossed out.

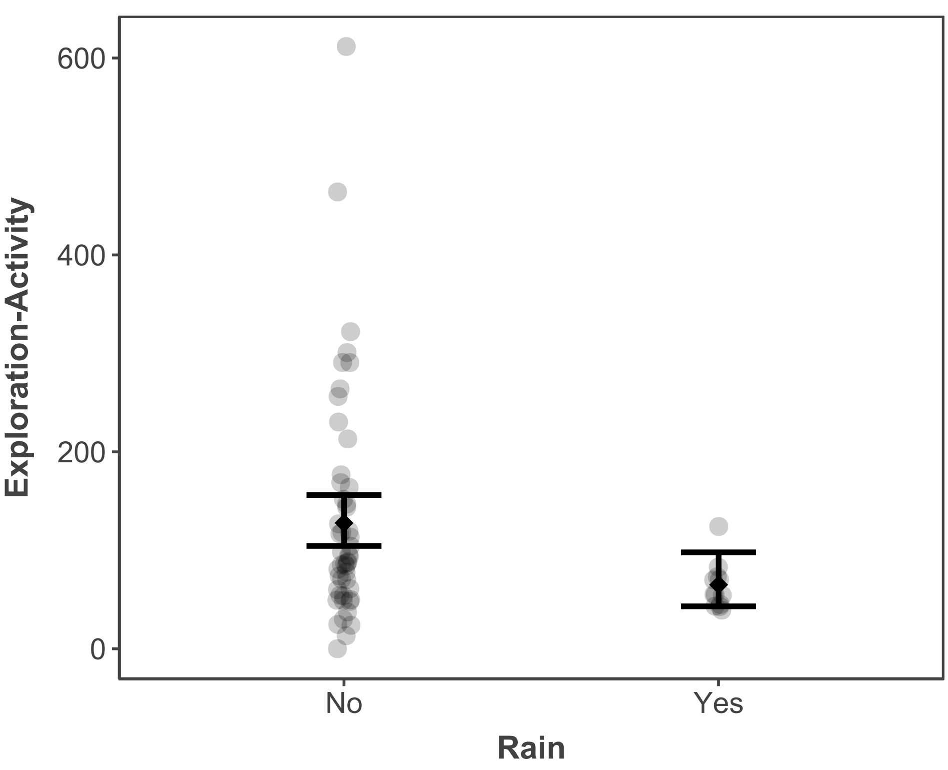

Fig. S3: The *Exploration-Activity* score decreased in response to rain (χ^2 = 8.38, p = 0.004). The graph shows the model estimates, with 95% confidence intevals. Data are plotted as grey dots.