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## **Bearing with noise: the effects of highway noise on behaviour and development in zebra finches**

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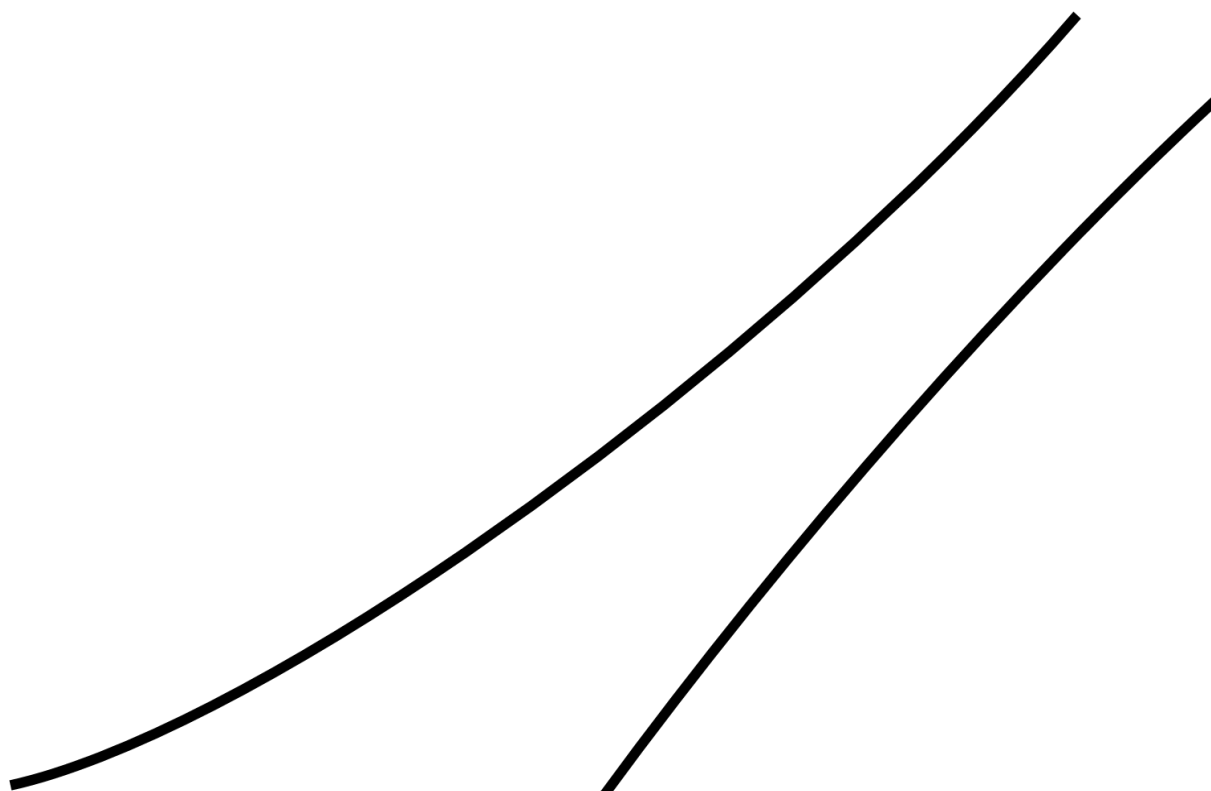
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# Chapter 3

## **The effects of chronic traffic noise exposure during breeding on zebra finch parental behaviour and breeding success**



Quanxiao Liu, Hans Slabbekoorn & Katharina Riebel



## **Abstract**

Traffic noise has been associated with decreased breeding outcomes in birds. However, whether and how noise-related behavioural changes in parental care may contribute to the suboptimal breeding outcomes is not well understood. To test this, we exposed zebra finch breeding pairs to continuous playbacks of two types of traffic noise that had previously been established as aversive and neutral in behavioural avoidance tests. Both noise stimuli were continuous 24h recordings of traffic noise taken at two different distances from a busy highway. The near- but not the far-distance highway recording had been actively avoided in spatial preference tests involving the same population of birds. The near-distance noise was thus chosen as experimental treatment and the far-distance as low-level non-aversive control. All pairs experienced each treatment once in a fully balanced design with cross-over. Noise playbacks did not affect breeding outcomes: latency to the first egg, clutch size, number of successfully hatched chicks, total weight of all hatched chicks and offspring mass at 11, 65 and 120 days of age did not differ between the two noise treatments. However, parents showed significantly higher nest attendance time when breeding under near distance high-level traffic noise conditions than under far distance low-level traffic noise. In the first breeding round, nest attendance time was correlated with feeding rate and the increase in feeding rate with increasing brood size was higher in the group exposed to near-distance noise. These results show that birds will breed in a no-choice situation at noise levels they had previously avoided in a free choice situation. Future work will have to investigate whether the altered parental behaviour is indicative of a compensatory strategy with hidden costs to the parents.

## Introduction

Roads, traffic and traffic noise are often associated with reduced wildlife diversity and abundance (Benítez-López et al., 2010; Newport et al., 2014; Bennett, 2017). This phenomenon is especially well documented in birds (Reijnen et al., 1996; Bayne et al., 2008; Arévalo & Newhard, 2011; Herrera-Montes & Aide, 2011). Noise has, for example, been found to mask mating signals (Swaddle & Page, 2007; Halfwerk et al., 2011) and affect intra-pair communication (Villain et al., 2016; Grabarczyk & Gill, 2020) and parent-offspring communication (Leonard & Horn, 2012; Leonard et al., 2015). Vigilance and anti-predator behaviour of breeding birds have also been found to be affected by noise (Meillère et al., 2015). Observational studies in the field comparing birds from noisy areas with conspecifics from more quiet areas have reported associations between higher noise levels and lower pairing success (Habib et al., 2007), lower hatching success (Kleist et al., 2018) and smaller brood sizes in some studies ( e.g. great tit *Parus major*, Halfwerk, et al., 2011; bluebirds *Sialia sialis*, Kight et al., 2012) but not in others (e.g. House sparrows *Passer domesticus*, Schroeder et al., 2012).

For the interpretations of these observational field studies, it is important to note that several studies found non-random distribution of birds in terms of breeding quality and territory quality within populations. Experimental noise exposure in the field has demonstrated that noise can deter high quality birds from noisy but otherwise suitable habitat but can also lead to selective settlement: in a population of North American tree swallows (*Tachycineta bicolor*) early birds settled in the more quiet nestboxes and nestboxes with higher levels of noise only became occupied by birds that arrived relatively late in the breeding area (Injaian et al., 2018). Similarly, when great tits could choose between nestboxes with or without noise playbacks, they preferred more quiet nest boxes (Halfwerk et al., 2016). Such noise avoidance behaviour may restrict birds of lower quality to the noisy areas. Therefore, instead of direct effects of noise on offspring, the quality of parents could explain lower breeding outcomes in noisy habitats. Consequently, the quality of the parents must be taken into consideration when investigating the effects of noise on breeding. One approach to do so, is using cross-fostering in the field (Schroeder et al., 2012) or randomly assigning family groups to noise/control treatments (Leonard et al., 2015). Such approaches can potentially help to reveal whether chicks are affected via their parents altered behaviour or whether they are directly affected by noise.

Traffic noise is associated with factors such as the presence of vehicles, pollution and changed vegetation that could also explain reduced breeding success near roads. Experimental noise exposure allows to separate the influence of noise from other factors. Such studies support that noise can directly affect parents and offspring. When exposed to noise, females may respond less to attractive male songs (Huet des Aunay et al., 2014), increase vigilance (Meillère et al., 2015) and delay egg laying (Injaian et al., 2018). Offspring raised in noisy conditions have been found to have altered reactivity of hypothalamic pituitary adrenal axis (Injaian et al., 2018; Kleist et al., 2018; Flores et al., 2020; Walthers & Barber, 2020; Zollinger et al., 2020), telomere attrition rate (Dorado-Correa et al., 2018; Injaian et al., 2019) and volumes of two brain nuclei of the song system (Potvin et al., 2016).

Previous studies have investigated either the effects of noise on parental care or the relationship between parental stress and offspring condition, taking quality of breeding pairs into account, but the combination of parental care and development of offspring have rarely, if at all, investigated at the same time. Reports on noise-dependent parental care include effects on nest visits (Naguib et al., 2013), provisioning rate (Schroeder et al., 2012; Lucass et al., 2016; Injaian et al., 2019) and begging calls from the chicks (Leonard et al., 2015). The communication among the partners within the breeding pairs may also change due to noise exposure (Villain et al., 2016). Zollinger et al. (2020) conducted a noise exposure study on captive zebra finches (*Taeniopygia guttata*), which were allowed to breed twice: once under quiet ambient conditions, and once with traffic noise playback until brood median age reached 17 days old. In that study, they found, for the noisy condition, that the parents were at lower baseline corticosterone levels and that their offspring were significantly lighter at 10 days old, but caught up later with the birds in the control group. The study demonstrates a noise impact on both parental stress levels (albeit in the opposite direction) and offspring body condition, raising the interesting follow-up question on the mechanisms underlying these effects. Since fledglings rely on their parents to feed them until nutritionally independent, noise-dependent changes in parental behaviour or direct effects of noise on chick physiology could explain noise-dependent offspring body condition.

Zebra finches are a suitable model species to study the effects of noise on parental care and breeding outcomes given the good knowledge on their behavioral development (Griffith & Buchanan, 2010) and well established lab populations (Griffith et al., 2017). Previous studies have successfully used this species to investigate the effects of a number of different environmental factors on offspring phenotypes (e.g. Brumm et al., 2009; Holveck & Riebel, 2010; Kriengwatana et al., 2013). A number of studies in zebra finches have used experimental noise exposures and have shown that noise exposure impacts intra-pair communication (Villain et al., 2016) and vigilance behaviour (Evans et al., 2018) of adult birds. Potvin

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& MacDougall-Shackleton (2015) exposed breeding birds to compiled traffic and urban noise and reported increased embryo mortality (assessed as unhatched fertilized eggs) in the treatment group exposed to noise in comparison to the control group but no differences in the total number of fledglings between both treatments. In an associated study (Potvin et al., 2016) on brain development involving 24 male offspring, there was an interaction between noise treatment and the volume of two brain nuclei of the song system (but not on overall telencephalon volume). The birds exposed to moderate and high levels of traffic noise had smaller volume of area X than the birds in the low level and control groups. The volume of HVC was, however, only reduced in the birds exposed to moderate level of traffic noise. In another experiment, zebra finches exposed to traffic noise during development were reported to have accelerated telomere loss as juveniles (Dorado-Correa et al., 2018). The various physiological effects in the nestlings are in line with both direct stress effects on the chicks or on the parents and either or both altering their behaviour in parent-offspring interactions. Both adult and juvenile zebra finches spatially avoid high level traffic noise in favor of more quiet space unless tested when previously housed in noise (Liu et al 2020 & chapter 4). Combined, these studies suggest developing zebra finches to be susceptible to traffic noise and to be a suitable model to investigate how traffic noise may affect parent-offspring behaviour and reproductive success.

In the current study, breeding zebra finches were exposed to traffic noise to explore the question as whether noise might impact parental care and breeding success. The study differed from previous studies by using original field recordings (highway noise recorded from near and far distances) and using two noise treatments to control the effects of non-aversive levels traffic noise versus too loud traffic noise (rather than comparing traffic noise versus silence). Biological meaningful playback levels for this experiment were experimentally established in behavioural tests prior to this study (causing no reaction or behavioural avoidance in spatial preference tests Liu et al., 2020, chapter 2). Building on these insights ensured that in the breeding experiments parents could be exposed to a previously avoided noise level and a control noise level that had not been actively avoided. To control for potential effects of differences in parental quality masking potential treatment effects, we used a repeated measures design with cross-over. Each pair participated in two breeding rounds, experiencing both types of traffic noise recordings once but in different sequences. Based on earlier reports on the negative impact on reproductive output and offspring physiology in zebra finches (Potvin & MacDougall-Shackleton, 2015; Dorado-Correa et al., 2018; Zollinger et al., 2020), we expected to find a negative effect of the near-traffic noise exposure on offspring development. If these changes are (partly) driven by changes in parental behaviour both parental care and offspring body mass should be affected. Adding observa-

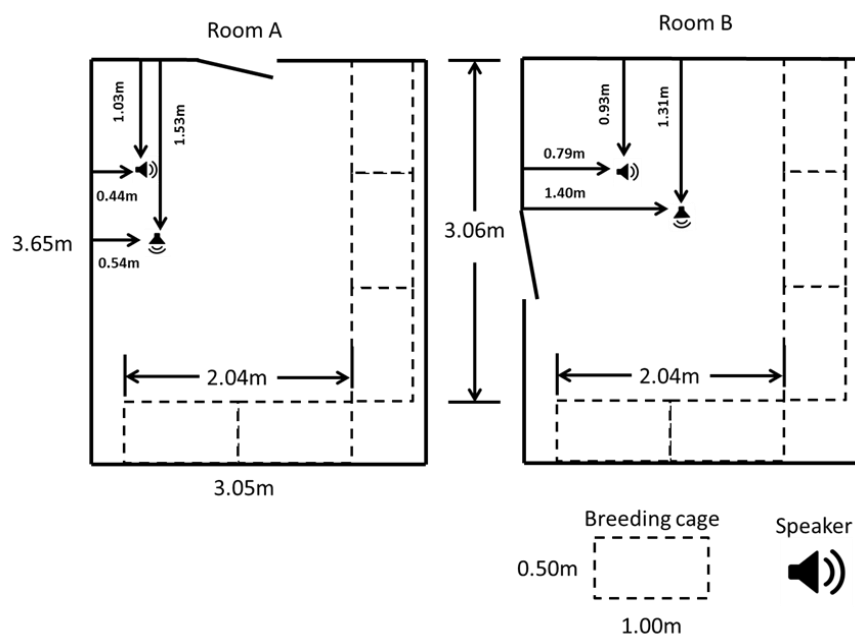


tions of parental behaviour to chicks' biometry markers of development, should help revealing associations between the two, thus helping to develop further regarding the underlying mechanism.

## Materials and Methods

### *Subjects and housing*

The zebra finches used in this study originated from the breeding colony at Leiden University. 30 males and 30 females (start of breeding experiment:  $900.9 \pm 60.3$  days of age) were drawn from a pool of birds that had participated in a behavioural noise avoidance test (Liu et al., 2020) 142 days earlier (at  $758 \pm 65$  days of age). They were caught from single-sex aviaries (L x W x H: 200 x 200 x 200 cm), randomly assigned into pairs of one male and one female and then moved to identical breeding cages (100 x 50 x 40 cm), placed into in one of two identical breeding rooms (365 x 305 x 300 cm, for details see Figure 1). In each breeding room, two blocks of 3 x 3 and 2 x 3 cages were stacked in an L-shape along two walls of the rooms. The cages in the lowest row were situated 60 cm above the ground. Every breeding cage had an opening (9 x 9 cm) to slide in a nestbox (11 x 9 x 9 cm) made of white plastic.



**Figure 1:** Top view of the two breeding rooms showing the location of the loudspeakers and breeding cages. In each room, 15 breeding cages (100 x 50 x 40 cm) were stacked into three layers onto supporting cabinets (100 x 50 x 60 cm), placed under the lowest layer. The dashed lines represent single columns of three breeding cages each.

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### *Sound stimuli*

For the noise playbacks, two 24 hour recordings of continuous highway noise were used (details in Liu et al. 2020, see chapter 2). These stimuli had been recorded with sound meters (Wildlife Acoustics, model SM1) at two distances from the A4 highway (the Netherlands, connecting Amsterdam and Rotterdam) between 10am July 27<sup>th</sup> and 10am July 28<sup>th</sup> 2017. One recording location was 15 meters from the highway (52.098504N, 4.439159E, ‘near distance’) the other 300 meters from the same highway (52.103469N, 4.441135E, ‘far distance’). At both locations the sound meters had been placed in open landscape at ca. 80 cm above the ground such that the microphone pointed across the open landscape towards the highway. The absolute sound pressure level (SPL) at the first three minutes was measured by a sound pressure meter (Pulsar Instruments Plc, Model 30, A weighted, reading LAT with an interval of 10 seconds). These SPL measurements were used later as reference to calculate the average sound levels per 30 seconds for the entire recordings (see details in Liu et al. 2020): near-distance:  $68.7 \pm 3.2$  dB(A), far-distance:  $52.8 \pm 5.2$  dB(A), ref = 20  $\mu$ Pa. A 30 min sequence of both recordings had been used as one of the stimuli sets in the noise avoidance test conducted with the parents. The parents had actively avoided the aviary with playback of the near- but not the far-distance recordings (Liu et al., 2020).

### *Traffic noise playbacks*

Two loudspeakers (Blaupunkt CB 4500) were positioned (details see Figure 1) in each breeding room opposite the rows of breeding cages and playback levels were adjusted such that the noise level during playback was roughly the same in every breeding cage (near-distance traffic noise: cages  $70.2 \pm 0.5$  dB(A), empty nestboxes  $68.5 \pm 0.9$  dB(A); far-distance traffic noise: cages  $51.5 \pm 0.4$  dB(A), empty nestboxes  $51.4 \pm 0.6$  dB(A)) and equivalent to the levels at the recording location when measured with the same sound pressure meter. Because it has been hypothesized that nesting material might act as an insulator to noise (Potvin, 2019), we also checked *post-hoc* whether noise levels were attenuated inside the nestboxes with zebra finch nests. To do so, the same type of nestboxes (n = 18) with completed zebra finch nests inside were obtained from our regular laboratory breeding colony after chicks had left the nests. These nestboxes were then placed into the exact same cages used in this study. When the noise stimuli were present, the sound levels inside these nestboxes were not different from what we had measured before with empty nestboxes (near-distance traffic noise:  $69.1 \pm 1.1$  dB(A), far-distance traffic noise:  $52.1 \pm 0.4$  dB(A)).

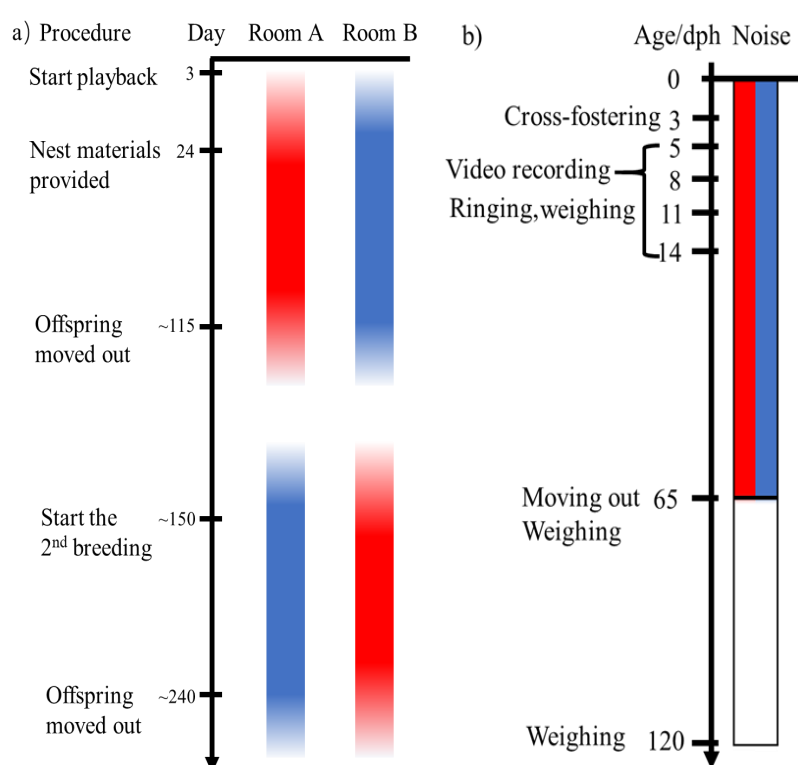
### *Experimental breeding*

All breeding pairs participated in two rounds of breeding, during which they were exposed to 24 hour continuous noise playback recorded at near or far distance from the highway (see

Figure 2). The pairs were introduced into the breeding cages 3 days before the onset of the noise playback. From day 3 onwards, the volume of the playback increased daily (by 5 dB (A) for near-distance noise and 4 dB(A) for far-distance noise) for 14 days until the noise level inside the breeding cages had reached the same level as at the original recording sites. After another week, all pairs were provided with a nestbox and hay and coconut fibre as nesting materials.

The nestboxes could be opened like drawers from outside the cage and were checked daily by one of the experimenters (QL, EG and KF) to track the dates of egg laying and hatching. When a chick hatched, it was weighed and marked uniquely within its brood by cutting its down feathers in one of several different patterns (head, back, leg or one wing, see Adam et al. (2014) for details). Chicks from different nests within the same breeding room were cross-fostered at age  $3.6 \pm 1.7$  days to spread siblings over as many broods as possible to break correlations between parental quality, brood size and offspring quality. The brood sizes before and after cross-fostering were not correlated (Spearman,  $r_s = 0.21$ ,  $N = 43$  broods,  $p = 0.18$ , pairs without offspring were excluded from this analysis). The age composition after the cross-fostering was ideally no more than a day between subsequent hatching dates, but because of asynchronous breeding this was up to two days in some nests resulting in a range of 0 – 2 days and mean of  $1.1 \pm 0.2$  days.

When the median age of a brood reached 11 days, chicks were banded with an orange plastic numbered ID-ring on their left leg. When individual chicks reached 65 days of age, they



**Figure 2:** Schematic view of the breeding procedure (a) and experimental procedure (b). The red and blue colour bars stand for near- and far-distance traffic noise. The strength of the colours indicates the relative intensity of the noise playback compared to the peak level. a) Y-axis shows the days since the start of the experiment. b) Y-axis shows offspring age in relation to reported procedures. The offspring were tested for noise avoidance at the same day when they were moved at 65 days old. The offspring were moved out to be weighed and to set up the parental generation for the second round of breeding.

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were moved from the breeding cages to participate in a noise avoidance test (see chapter 4) and then moved to single-sex aviaries (200 x 150 x 200 cm, 12 - 20 birds) in bird stock rooms without playbacks (ambient sound level without birds < 40 dB(A)). The parents remained in the breeding cages after all chicks had been moved from the room. Playback levels in both experimental rooms gradually faded out to ambient within the course of one week. The breeding pairs were then given a two-week period without any traffic noise playback before the noise playback procedure and a second breeding cycle as described above started again. For this second breeding round, pairs remained in the same cages but the type of noise playback (near- or far-distance traffic noise) was reversed between rooms.

#### *Breeding outcomes*

All breeding cages were checked daily from the day the nestboxes were provided to the day all chicks had fledged. We counted the latency to the first egg (in days, counting from the day the nestboxes were provided), the clutch size as total number of eggs laid without a break no longer than 3 days and the number of successfully hatched chicks per pair per round.

The offspring were weighed at age 1, 11, 65 and 120 days old. When the subjects were at 1 and 11 days old, the experimenter firstly prepared a dummy nest with hay and coconut fibres inside on a balance (Sartorius, BL600, +/- 0.1g). Then the experimenter inserted a partition between the two cage compartments when both parents were in the compartment without the nestbox. Then the experimenter opened the nestbox drawer, identified the individual chicks that needed to be weighed by the cutting pattern of their down feathers and transferred the chicks individually to the dummy nestbox on the balance to weight and quickly returned the chick to their nestbox. If the brood was also to be video recorded on the same day, video recording always preceded the weighing. When at 65 days old, individual birds were caught by a net from their cages and put in a bag to be weighed on a balance before being moved to the new aviary. At 120 days old, individual birds were also caught by a net from their aviary and put in a bag to be weighed on the same balance before participating a noise avoidance test (see chapter 4).

#### *Video recording of parental behaviour*

During the first breeding round, when the median age of a brood reached 5, 8, 11 or 14 days, a room camera (Panasonic HC-v500/JVC Everio HDD/JVC quad) was placed in the middle of the room to simultaneously film all focal cages from the front. These videos were later used to score nest attendance of all pairs. To validate whether nest attendance predicted feeding events, in-nest cameras (GoPro HERO 3+) that could be fixed under the roof of the nestbox were used to record inside the nest. These recordings were later used to count the

feeding events. To prevent a neophobia response to the in-nest cameras during filming, dummy in-nest cameras (a black cardboard dummy imitating the GoPro HERO 3+) had already been attached under the roofs of all nestboxes before introducing them to the breeding pairs. On the recording day, an experimenter (QL, EG or KF) slowly approached the cage and, by placing an opaque partition, separated the breeding pair from their nest. Then, the experimenter briefly withdrew the nestbox to replace the dummy with a real in-nest camera already switched on and immediately returned the nestbox and removed the partition. Then the experimenter - upon leaving the room - switched on the room camera to frontally film all focal cages. Fifty-five minutes later, the experimenter returned to the room, switched off the room camera and replaced the in-nest camera with the dummy. For the second breeding round, when nest attendance had been validated to predict feeding events (see below) the breeding pairs were only filmed by the room camera at the same time points as during the first breeding round. To keep the procedure identical to the first breeding round, the experimenter nonetheless repeated the movements of placing and removing the in-nest cameras at the beginning and at the end of the recording.

#### *Video scoring*

For all video recordings the first 5 min were excluded from analyses to make sure birds had enough time to resume normal activities after the disturbance. Recordings were then analysed from the 6<sup>th</sup> to the end of the 55<sup>th</sup> minute, to have exactly equally long sequences of 50 min for all recordings. Recordings were scored in BORIS video analysis software (Friard & Gamba, 2016, v.6.1.6). From the room camera recordings, we measured the duration of individual parent's nest visits by marking the time of all instances from when a bird had entered the nestbox (= both legs inside) to when the bird left the nestbox (= both legs outside). Male and female parent could easily be told apart by the species typical sex differences in plumage. The cumulative time spent in the nest box per parent per recording could then be obtained by adding up the duration of all the nest visits. This cumulative time was labelled as nest attendance.

From the recordings of the in-nest cameras used in the first breeding round, we scored individual feeding events in relation to nest visits. Each instance where a parent inserted its beak into a chick's mouth was counted as a feeding event. All videos were scored by one experimenter (EG) being blind with respect to the treatments. We had high inter- and intra-experimenter fidelity in scoring: two observers (QL and EG) separately scored the same five videos and their scores were highly similar (intraclass correlation coefficient = 0.99). EG also checked her own intra-observer repeatability by rescoring the first three videos after

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having finished all videos. Comparing the initial and later results of the first three videos also showed high consistency (intraclass correlation coefficient = 0.99).

#### *Statistical analyses*

##### Reproductive parameters

To test if noise exposure affected reproductive investment, we created several generalised linear mixed models in R (package *glmmTMB* in R 3.5.3) with the following response variables 1) latency to the first egg, 2) clutch size, 3) the number of hatched chicks, 4) total weight of all hatched chicks (measured as the sum of chicks' individual weights measured on each chick's first day) and 5) the number of unhatched eggs. The noise playback type (near/far), breeding round (1/2, categorical) and their interaction were fixed effects. Biological parent IDs were random intercepts.

To test if the weight of the offspring was affected by the noise, we created a linear mixed model. In this model, the weight of the offspring was the response variable. Type of noise playback, age of the offspring (11/65/120, categorical) and breeding round were fixed effects. Brood size (1-6) was a covariate. We also included the interactions among noise playback, age and brood size in the model. Bird IDs and social parent IDs were treated as random intercepts.

##### Parental behaviour

We checked if the sex of the parent affected the nest attendance using a linear mixed model. Nest attendance was the response variable, the sex of the parent (female/male), breeding round and the noise playback type were fixed effects, brood size and brood median age (5, 8, 11, 14) covariates. We also added the interactions between playback type and 1) sex, 2) brood size, 3) brood median age to the model. Social parent IDs were treated as random intercepts.

We tested whether noise exposure affected the combined nest attendance through a linear mixed model. Combined nest attendance per pair was the response variable, the noise playback type and breeding round fixed effects, brood size, brood median age covariates and social parent IDs random intercepts. The interactions between playback type and 1) brood size, 2) brood median age were also included in this model. We applied the same statistical method to test whether combined nest visits per pair was affected by noise playback.

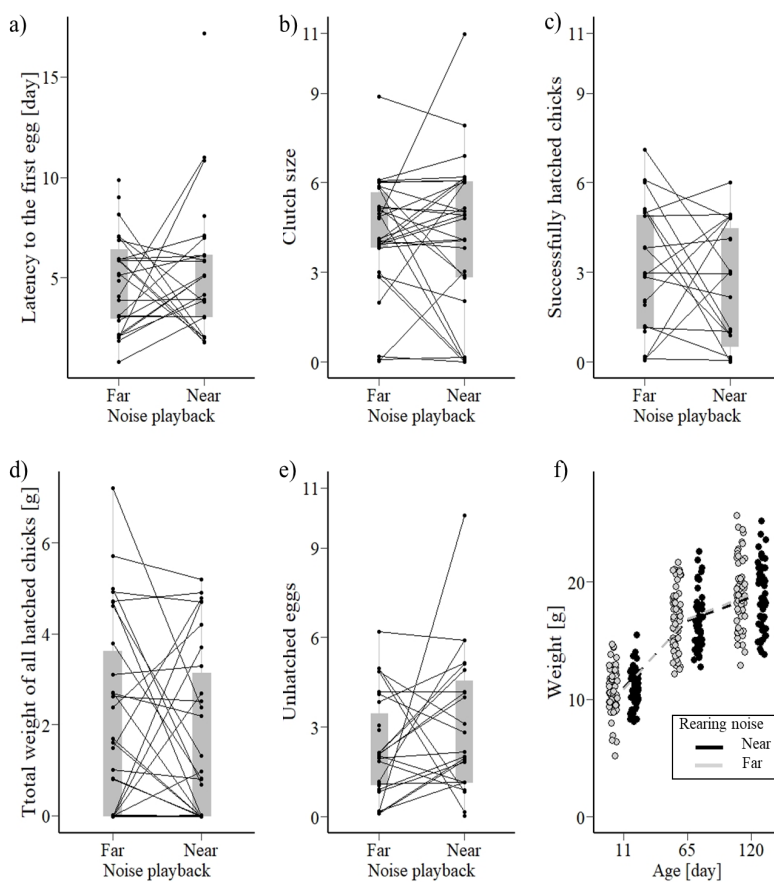
To test if combined nest attendance predicted feeding events, we created another generalised linear mixed model using data from the first breeding round. The total number of the feeding events was the response variable, the scaled (mean = 0, SD = 1 after scaling) combined nest attendance was the explanatory variable, the noise playback a fixed effect, brood size and

brood median age covariates and social parent IDs random intercepts. We also considered the interactions between playback type and 1) brood size and 2) brood median age in this model.

## Results

### Reproductive outcomes

The majority of pairs (23/30) bred in both breeding rounds. Four pairs did only breed during the far- and another two only during the near-distance traffic noise playback. Only one pair did not breed at all. The latency to the first egg, clutch size, the number of successfully hatched chicks, the total weight of all hatched chicks and the number of unhatched eggs did not differ between the two different noise treatments or breeding round (or their interactions, see Figure 3a-e, Table 1 & SEM 1).



**Figure 3:** Reproductive performance during breeding rounds with far- vs. near-distance traffic noise. a) Latency to the first egg, b) clutch size, c) successfully hatched chicks, d) total weight of all hatched chicks day 1, e) number of unhatched eggs of each pair in both breeding rounds. Each dot represents one measure from one pair (statistical details see Table 1). Two dots connected by lines originate from the same pair and show the measure for each of the two breeding rounds. Grey overlaid boxplots give the median, 1<sup>st</sup> and 3<sup>rd</sup> quartile. Overlapping data were jittered randomly for visualization. Details on individual pairs' breeding variables, see Table SEM 1. f) Offspring weight from both types of traffic noise exposure at 11, 65 and 120 days old. Each dot represents a single bird. The weight at different ages showed no significant differences between the treatment (see Table 2).

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**Table 1:** Results of (generalised) linear mixed model analyses of breeding outcomes: a) latency to the first egg, b) clutch size, c) successfully hatched chicks, d) total weight of all hatched chicks day 1 and e) number of unhatched eggs.

Effects	Level	Estimate	Std. Error	$\chi^2$	p-value
a) Latency to the first egg					
Noise				3.86	0.06
	<i>far</i>	-0.06	0.19		
Round				0.13	0.72
	<i>Second</i>	0.13	0.19		
Noise * round				0.01	0.92
b) Clutch size					
Noise				0.52	0.81
	<i>far</i>	-0.05	0.23		
Round				1.00	0.32
	<i>Second</i>	0.05	0.22		
Noise * round				0.16	0.69
c) Successfully hatched chicks					
Noise				1.25	0.26
	<i>far</i>	0.16	0.44		
Round				1.25	0.26
	<i>Second</i>	0.16	0.44		
Noise * round				00.01	0.92
d) Total weight of all hatched chicks					
Noise				0.94	0.33
	<i>far</i>	0.15	0.72		
Round				0.93	0.16
	<i>Second</i>	0.31	0.72		
Noise * round				0.16	0.69
e) Number of unhatched eggs					
Noise				0.62	0.43
	<i>far</i>	-0.18	0.33		
Round				0.08	0.78
	<i>Second</i>	0.00	0.32		
Noise * round				0.02	0.88

*Marginal and conditional R<sup>2</sup> for the models are a) 0.04 and 0.06, b) 0.01 and 0.35, c) 0.02 and 0.65, d) 0.03 and 0.41, e) 0.01 and 0.43. Biological parents' IDs were treated as random intercepts.*



The offspring weight at the age of 11, 65 and 120 days old was not affected by the noise exposure, nor the interaction between noise exposure, age and brood size (Figure 3f, Table 2). However, the weight at all ages was affected by brood size with chicks from larger broods being on average lighter than those from smaller broods.

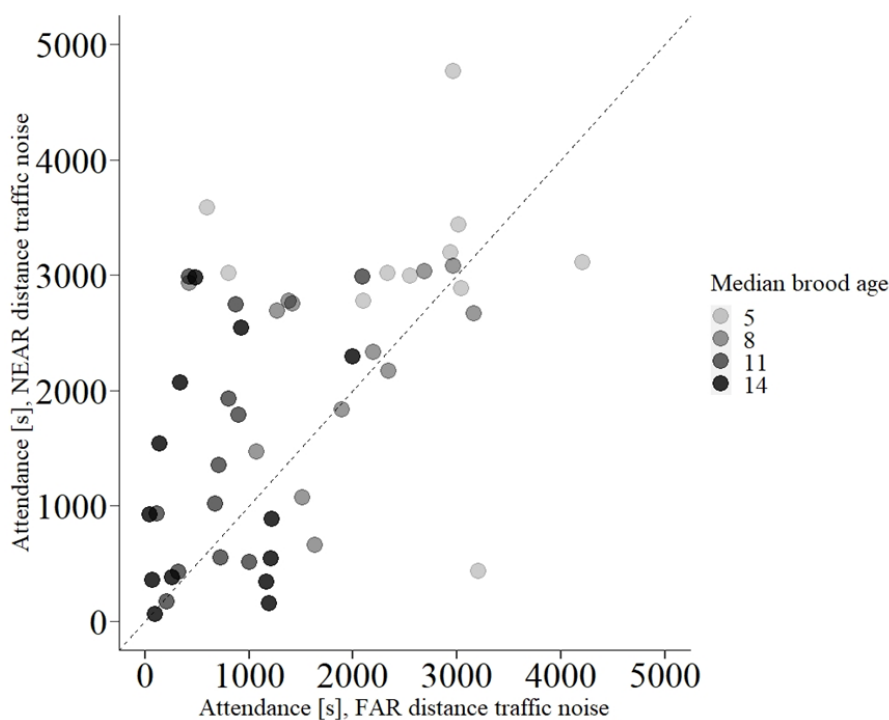
**Table 2:** Results of linear mixed model analyses of offspring weight measured at 11, 65 and 120 days old.

Effects	Level	Estimate	Std.Error	$\chi^2$	P value
Noise				0.41	0.52
	<i>Far</i>	-0.58	1.21		
<b>Age</b>				<b>1544.31</b>	<b>&lt;0.001</b>
	<i>65 days</i>	5.31	0.86		
	<i>120 days</i>	8.76	0.86		
Round				3.45	0.06
	<i>Second</i>	-0.68	0.37		
<b>Brood size</b>	<i>Covariate</i>	-0.60	0.23	<b>11.85</b>	<b>&lt; 0.001</b>
Noise * age				1.31	0.52
Noise * brood size				1.70	0.19
Noise * age * brood size				1.13	0.58

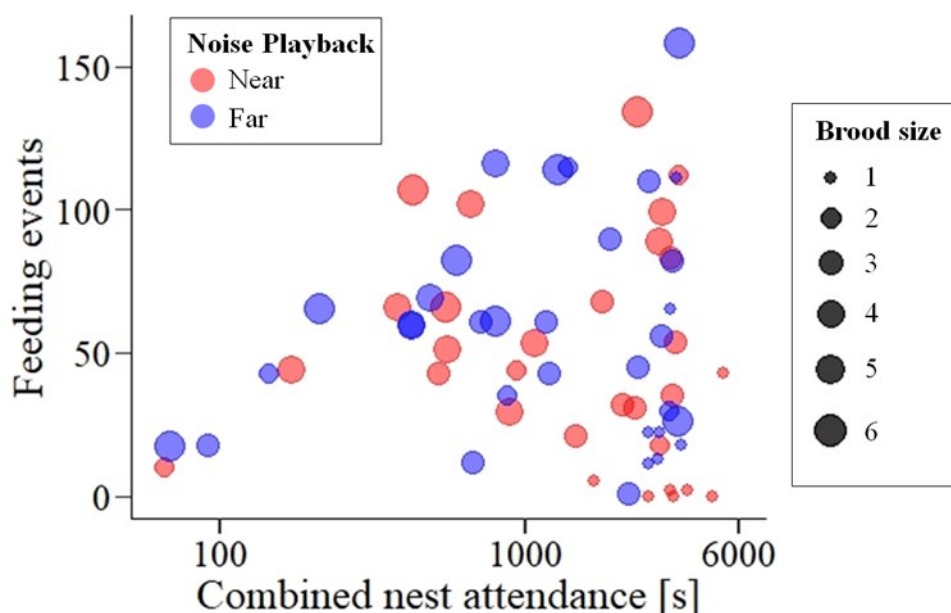
*Marginal and conditional R<sup>2</sup> for the model are 0.70 and 0.84. Social parents' IDs and bird ID were treated as random intercepts.*

In terms of parental behaviour, males spent overall less time attending nests than females (Table 3a). Comparing the birds exposed to the far- versus those exposed to the near-distance traffic noise showed that both males and females spent more time attending the nest when exposed to the high intensity, near-distance traffic. Nest attendance time was overall negatively associated with brood size and brood median age (Table 3a). Following analyses that combined data from both sexes of the same pair showed the same result in terms of effects of noise, brood size and brood median age (Table 3b, Figure 4). However, noise exposure did not affect the overall number of combined nest visits (Table 3c).

For the first breeding round, where parental behaviour had been filmed outside and inside the nest box, analyses of the in-nest video recordings showed a positive correlation between the combined nest attendance of both parents and total number of feeding events (Figure 5, Table 3d). Besides, the parents fed the chicks more often if the brood was younger. The significant interaction between brood size and noise shows that when exposed to near-distance traffic noise, the parents with large brood increased their feeding rate more than the parents exposed to far-distance noise (Table 3d and Figure 5).



**Figure 4:** Combined nest attendance of breeding pairs (in seconds). Each dot represents the same pair under two types of traffic noise exposure, with a brood at 5/8/11/14 days of median age. The dashed line gives the expectation for identical behaviour in both conditions ( $y = x$ ). Dots below above dashed line mean that pair spent more time attending the nest when exposed to traffic noise recorded at near distance.



**Figure 5:** Combined nest attendance is positively correlated with feeding events (data from the first breeding round only). X-axis is plotted on a logarithmic scale. The size of dots increases with brood size. Each dot represents the date from one recording. The increase of feeding rate with increasing brood size is bigger in the pairs exposed to near-distance traffic noise when compared to the pairs exposed to far-distance traffic noise.

**Table 3:** Results of generalised linear mixed model analyses of parental care. First three models explained if a) nest attendance per parent, b) combined nest attendance and c) combined nest visits were affected by the type of noise playback, breeding round, brood size and brood median age (5/8/11/14 days). Model d) explained if the feeding events were correlated with combined nest attendance time in the first breeding round.

Effects	Level	Estimate	Std.Error	$\chi^2$	P value
a) Nest attendance per parent					
<b>Sex</b>				<b>11.11</b>	<b>&lt; 0.001</b>
	<i>Male</i>	-0.10	0.04		
<b>Noise</b>				<b>11.00</b>	<b>&lt; 0.001</b>
	<i>Far</i>	-0.19	0.09		
Round				0.21	0.65
	<i>Second</i>	-0.01	0.02		
<b>Brood size</b>	<i>Covariate</i>	-0.07	0.01	<b>54.10</b>	<b>&lt; 0.001</b>
<b>Brood median age</b>	<i>Covariate</i>	-0.04	0.01	<b>83.29</b>	<b>&lt; 0.001</b>
Noise * sex				0.39	0.53
Noise * brood size				0.92	0.34
Noise * brood median age				0.40	0.53
b) Combined nest attendance					
<b>Noise</b>				<b>13.46</b>	<b>&lt; 0.001</b>
	<i>Far</i>	-0.16	0.07		
Round				0.15	0.70
	<i>Second</i>	-0.03	0.09		
<b>Brood size</b>	<i>Covariate</i>	-0.07	0.001	<b>55.48</b>	<b>&lt; 0.001</b>
<b>Brood median age</b>	<i>Covariate</i>	-0.03	0.00	<b>104.15</b>	<b>&lt; 0.001</b>
Noise * brood size				0.97	0.32
Noise * brood median age				0.50	0.48
c) Combined nest visits					
Noise				0.33	0.56
	<i>Far</i>	-0.03	0.32		
<b>Round</b>				<b>9.66</b>	<b>0.002</b>
	<i>Second</i>	-1.04	0.41		
Brood size	<i>Covariate</i>	-0.12	0.07	1.67	0.20
Brood median age	<i>Covariate</i>	0.00	0.02	0.02	0.88
Noise * brood size				0.06	0.80
Noise * brood median age				1.19	0.27
d) Feeding events (only measured in the first round)					
<b>Nest attendance</b>	<i>Covariate</i>	0.07	0.03	<b>4.56</b>	<b>0.03</b>
Noise				1.50	0.22
	<i>Far</i>	1.75	0.64		
Brood size		0.56	0.13	15.80	<0.001
<b>Brood median age</b>	<i>Covariate</i>	-0.08	0.01	<b>116.00</b>	<b>&lt; 0.001</b>
<b>Noise * brood size</b>				<b>6.02</b>	<b>0.01</b>
	<i>Far * brood size</i>	-0.41	0.16		
Noise * brood median age				0.00	0.99

Marginal and conditional  $R^2$  for the models are a) 0.36 and 0.36, b) 0.56 and 0.58, c) 0.09 and 0.11, d) 0.61 and 0.98. Social parents' IDs were treated as random intercepts.

## Discussion

To investigate the impact of continuous traffic noise exposure on parental care and breeding success, breeding zebra finches were experimentally exposed to two different types of highway noise: one that had been shown to be aversive (near-distance) and a control noise (far-distance) that was shown to be neutral in preference tests (Liu et al., 2020). Parental behaviour but not breeding outcome differed between the two noise treatments: using the feeding events measured in the first breeding round, we found feeding rate was increased in larger broods for both levels, but the effect was larger when the parents were exposed to near-distance noise compared to the parents exposed to far-distance traffic noise. In both breeding rounds, parents spent more time attending the nest when exposed to the near-traffic noise, which, in turn, was correlated to feeding rate in the measured subsample consisting of all breeding pairs during the first round. Overall nest visit rates by the zebra finch parents did not vary between noise treatments. While the parental behaviour seemed to be impacted by the noise, there were no significant effects of the noise treatment on any of the measurements of reproductive success (latency to the first egg, clutch size, successfully hatched chicks, the total weight of all hatched chicks at day 1 and the number of unhatched eggs). As reported previously for zebra finches, brood size affected chick mass (de Kogel, 1997; Naguib et al., 2004; Holveck & Riebel, 2010), but this effect was not reinforced by either of the noise treatments.

These results seem at first to contradict some of the results on effects of noise on offspring development in zebra finches. Potvin et al. (2015) reported, that captive zebra finch pairs exposed to traffic noise had more nesting attempts, delayed egg laying dates and a higher embryo mortality rate. However, the perhaps most salient measure of reproductive output namely the total number of chicks surviving to fledging in their - like in the present data set - was not affected. This means that despite different noise treatments, different populations and study design, the main results with respect to reproductive success are actually comparable. This seems further confirmed by a recent study that like the present study also used a paired design and likewise did not find any significant effects of noisy conditions on measurements of reproductive success (no effect on embryo mortality and offspring survival, Zollinger et al. 2020). The combined results from noise exposure studies on zebra finches suggest that: zebra finch parents might buffer some direct negative impacts of the noise. Such an interpretation would be in line with an absence of an effect on chick biometrical measures in our study or the observation that initial lower weight in noise exposed chicks disappeared later on (Potvin & MacDougall-Shackleton, 2015; Zollinger et al., 2020) but

those chicks' physiology is still impacted later in life as evidenced by altered HP-axis programming (Zollinger et al., 2020) and telomere attrition rate (Dorado-Correa et al., 2018). We did not measure physiological markers of increased stress levels in either parents or offspring, and we therefore cannot dismiss or exclude such impact that has been reported in quite a few studies (Injaian et al., 2018; Kleist et al., 2018; Flores et al., 2020; Walthers & Barber, 2020; Zollinger et al., 2020). Future work will have to address whether these effects result from the noise affecting chicks through altered behaviour of their parents (shown to be altered in our study) or direct effects of noise on chicks.

The absence of an effect on reproductive success in zebra finch laboratory studies is not necessarily in contrast with negative effects on breeding success (Schroeder et al., 2012; Injaian et al., 2018a) and parental behavioural (Naguib, 2013; Leonard et al., 2015; Lucass et al., 2016) reported by several observational field studies in other species. The general picture from these studies is that noisy conditions negatively affect both parental visit rates and offspring condition and that this is likely due to interference with parent-offspring or parent-parent communication. If parents do not hear begging calls well, or if chicks do not respond to parents arriving with food by begging (Leonard & Horn, 2012), feeding rates go down, which will cause a negative impact on chick growth. This will be much more pronounced in the field where parents have to work harder to find food and where additional impacts from roads (pollution) and increased predation risk in noise (McIntyre et al., 2014; Meillère et al., 2015) will add costs to parents' breeding efforts. A point to consider is that the majority of field studies concerned species breeding in natural environments. It may well be that masking effects on communication have a different, probably larger impact on birds in these real-world situations, but perhaps less so on zebra finches in the laboratory situation with housing in small cages, with close proximity between all birds. However, earlier experimental work in a comparable lab setting showed that wind noise exposure inside the nest did affect duet patterns within a zebra finch pair and increased time to vocally respond to the incubating partner's calls and these pairs (Villain et al., 2016). When comparing the field to the laboratory, one important substantial difference is that parents have unlimited easily accessible food resources under laboratory conditions, which might have made it easy for them to buffer any noise impacts on increased begging or metabolic changes in the chicks. We indeed found that zebra finch parents with a large brood size increased their feeding rates when exposed to near-distance traffic noise, which may potentially mitigate the effects of traffic noise on offspring mass. If this is the case, it is interesting to see that even though the pairs can mitigate the effects of noise, the brood size still affected offspring mass as commonly reported (de Kogel & Prijs, 1996; Naguib et al 2004; Tschirren et al., 2009).

The noise treatment significantly affected parental behaviour in two ways. First, breeding

### *Chapter 3*

pairs spent more time attending the nests when exposed to the near-distance traffic noise. Second, the near-distance traffic noise exposure was also associated with an increased feeding rate of the pairs with a large brood size. We could think of three possible explanations for these results. The first explanation concerns a noise impact counter strategy, as parents may flexibly adjust their parental care (Rehling et al., 2012; Vitousek et al., 2017). Parents visiting more may also have fed their chicks more. In the first breeding round, the feeding events positively correlated to the nest attendance, implying the parents may feed the chicks more when attending the nest for longer. An increased feeding rate may actually have been an indirect positive effect from noise on offspring, that may have compensated for a direct negative effect. Noise related feeding rates have been reported in tree swallows. Injaian et al. (2018) found that tree swallow parents fed their chicks more often after several days of noise exposure, although this was not sufficient to prevent a detrimental impact of noise on offspring body condition. The result from Leonard et al. (2015) showed a masking effect of noise of begging calls, but also showed that the parents exposed to noise seemed to feed their chicks more often in general. In wild breeding white-crowned sparrows, paternal nest attendance and feeding rate were found to be higher for nests closer to a road (Crino et al., 2011). While these studies all suggest that parents could adjust their parental behaviour with increasing noise levels, these behavioral changes in a noisy environment could also be due to noise induced differences in foraging efficiency, e.g. when there are insects easier to catch in noise, or when the quality of the parents varies with explorative birds breeding more often near the roads. This seems an unlikely explanation for our study which used a paired-design: the same pairs were observed to attend their broods in different ways when raising chicks in different noise conditions. Breeding pairs spent less time attending the nest when they were exposed to far-distance compared to near-distance traffic noise. However, while we had data on nest attendance for both rounds, we only measured feeding rate in the first round (where it was correlated with nest attendance) and used this information to assume that increased nest attendance in the second round also meant a higher feeding rate in the second round. We were also unable to measure the actual food intake of the chicks which in one study that did measure this was not fully predicted by feeding rates (Gilby et al., 2011). Thus, increased feeding rates can also arise from less actual food provisioning per feed. To test whether parents compensate the impacts of noise on developing birds by increasing the absolute amount of food delivered, future studies need to test if higher nest attendance does result in higher food intake in chicks.

A second explanation for the longer nest attendance may be that parents have been seeking acoustic shelter inside the nests. In an earlier experiment, the same adult zebra finches only avoided the near-distance traffic noise recordings in a spatial preference test using two con-

nected aviaries (Liu et al., 2020). However, in that experiment the birds could reduce experienced noise levels, as within the aviaries, birds could move along declining gradient of noise levels, while the noise levels in the nestboxes of the current experiment were not lower than other areas of the cage (post-hoc measurements, see Methods). This is in line with a study on noise attenuation in museum nest specimen from Australian passerine birds, which revealed that, apart from mud nests, most bird nests did not reduce noise levels significantly (Potvin, 2019). Consequently, although we cannot exclude that the zebra finches might seek acoustic shelter in other situations, in the experimental setup here, moving into the nest did not reduce noise exposure.

A third explanation for the longer nest attendance, may be that the communication between the zebra finch parents has been undermined by the noisy conditions. There is evidence that zebra finch pairs use duet-like vocal exchanges to arrange parental behaviour (Boucaud et al., 2016, 2017) and that the level of coordination predicts fitness (Mariette & Griffith, 2012, 2015). Noise from wind for example, has been shown to structurally change their duets (Villain et al., 2016). It is, therefore, theoretically possible that traffic noise exposure in our experiment had affected vocal communication and made the birds stay longer at the nest due to interrupted duty relief. Similarly, interrupted communication between parents and offspring (Leonard et al., 2015) may also have increased nest attendance time. However, as addressed above for the lack of impact on nest visits and masking may be less likely for the captive, close proximity conditions of our current experiment.

In conclusion, we found further evidence of noise-related effects on bird breeding behaviour namely that not all but higher intensity traffic noise, changed how zebra finch parents feed and attend their nestlings. In line with other studies in zebra finches we observed no negative impact on reproductive outcomes during the experiment. Whether this is due to the high flexibility of this nomadic and opportunistic breeder or whether benign conditions in captive breeding colonies buffer noise impact needs to be addressed in future studies. However, the observation of altered parental behaviour adds to the increasing insights that noisy conditions can have detrimental impact on birds and their reproductive behaviour in different ways, but that species and breeding conditions may affect to what extent this has fitness consequences and is measurable in the offspring.

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## Appendix

**Table SEM 1.** Breeding outcomes per pair in both rounds. Means and standard deviations are separately calculated from all pairs ('All pairs') and only pairs that laid eggs ('Bred pairs').

Pair ID	Latency to the first egg [days] <sup>1</sup>		Clutch size		Successfully hatched chicks		Total weight of all hatched chicks [g] <sup>2</sup>		Mean weight per hatched chick [g]		
	Near	Far	Near	Far	Near	Far	Near	Far	Near	Far	
1	7	6	4	4	0	0	-	-	-	-	
2	6	7	6	6	5	5	5.2	5.7	1.04	1.14	
3	4	4	8	9	3	7	2.4	7.2	0.8	1.03	
4	2	7	6	4	4	4	3.3	3.1	0.83	0.78	
5	2	6	5	6	1	4	0.8	3.8	0.8	0.95	
6	-	7	0	6	0	5	-	4.7	-	0.94	
7	8	-	0	0	0	0	-	-	-	-	
8	2	5	7	6	1	0	1.0	-	1	-	
9	-	5	0	3	0	1	0	0.8	-	0.80	
10	-	10	0	3	0	0	-	-	-	-	
11	5	3	4	4	0	2	-	1.6	-	0.80	
12	6	6	5	4	0	2	-	1.7	-	0.85	
13	7	3	6	6	6	5	4.9	4.7	0.82	0.94	
14	3	3	6	5	5	3	4.2	2.4	0.84	0.80	
15	3	3	4	5	1	1	0.8	1.0	0.80	1.00	
16	2	8	3	6	1	6	1.3	4.6	1.30	0.77	
17	17	-	3	0	3	0	2.7	-	0.90	-	
18	4	2	5	4	0	0	-	-	-	-	
19	11	2	6	4	0	3	-	2.7	-	0.90	
20	6	5	5	5	5	2	4.8	1.5	0.96	0.75	
21	5	2	2	3	0	1	-	0.8	-	0.80	
22	6	6	4	4	2	3	2.2	2.7	1.10	0.90	
23	-	9	0	5	0	0	-	-	-	-	
24	11	4	5	5	4	0	3.7	-	0.93	-	
25	-	-	0	0	0	0	-	-	-	-	
26	5	2	5	4	3	3	2.5	2.6	0.83	0.87	
27	4	2	6	2	5	0	4.7	-	0.94	-	
28	4	7	6	6	5	6	4.7	4.9	0.94	0.81	
29	2	6	11	5	1	5	0.7	5.0	0.70	1.00	
30	3	1	3	5	0	0	-	-	-	-	
All pairs	mean	-	-	4.2	4.3	1.8	2.2	-	-	-	-
	s.d.	-	-	2.4	2.0	2.1	2.3	-	-	-	-
Bred pairs	mean	5.4	4.9	5	4.7	2.2	2.5	2.0	2.3	0.91	0.89
	s.d.	3.5	2.4	2.1	1.4	2.1	2.3	1.9	2.1	0.14	0.10

<sup>1</sup> days were counted after providing nesting materials <sup>2</sup> chicks were weighted when founded hatched.