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

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The effects of highway noise on behaviour and development in zebra finches

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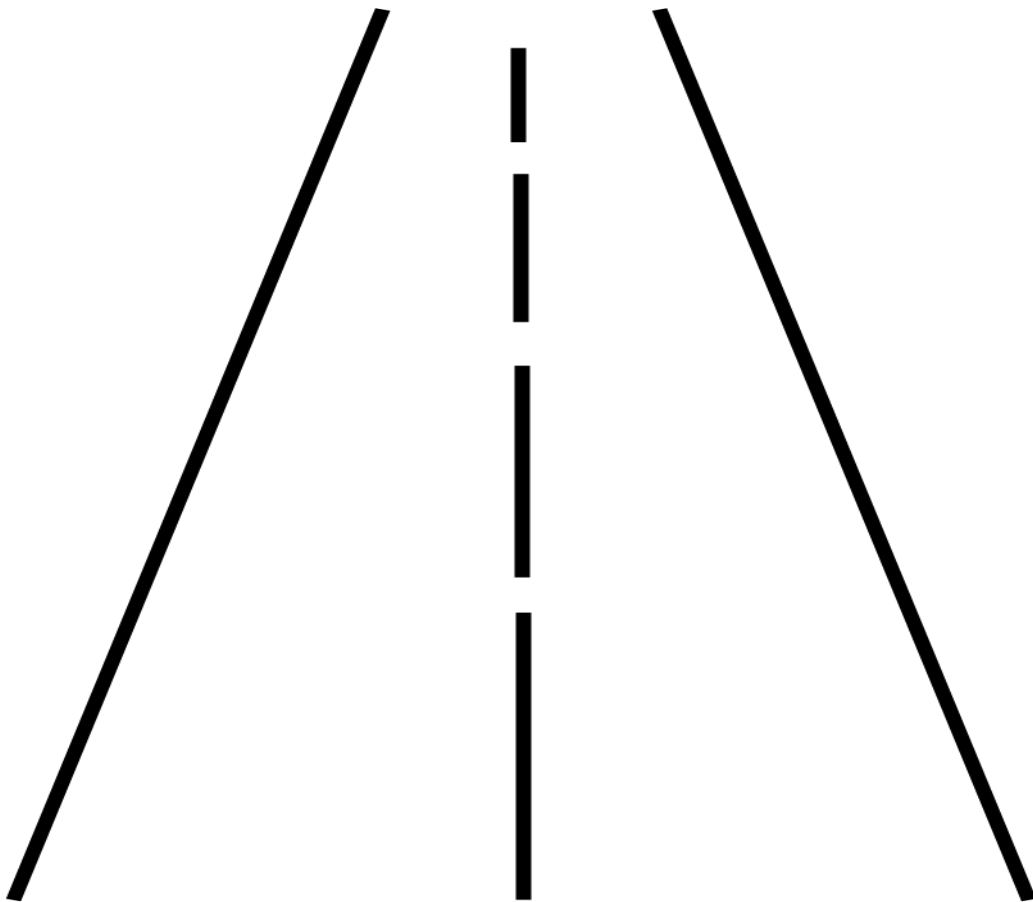
Table of contents

Chapter 1	General introduction	1
Chapter 2	Zebra finches show spatial avoidance of near but not far distance traffic noise	11
Chapter 3	The effects of chronic traffic noise exposure during breeding on zebra finch parental behaviour and breeding success	35
Chapter 4	The effects of rearing noise condition on spatial avoidance of traffic noise in juvenile and adult zebra finches	57
Chapter 5	The effects of rearing noise conditions on singing effort and exploration tendency in adult zebra finches	76
Chapter 6	Thesis summary & General discussion	97
	Nederlandse samenvatting	107
	Chinese summary [中文概述]	112
	Bibliography	115
	Curriculum Vitae	140



Chapter 1

General Introduction



Introduction

The past decades have seen a continuous rise in anthropogenic noise pollution. Worldwide, anthropogenic noise is affecting millions of people day and night (EEA, 2015; Wu et al., 2019) who suffer not only from short-term annoyance and sleep disturbance but also from long-term hypertension and cardiac disease (reviewed by Clark & Stansfeld, 2007). Traffic, especially road traffic, is a dominant source of anthropogenic noise (Barber et al., 2010; Mennitt et al., 2015) that reaches many animals: not only those living in urban areas, but also those in remote and protected areas, due to the ever increasing network of roads and highways (Barber et al., 2011; Buxton et al., 2017). This has raised concerns that animals are also being negatively affected by anthropogenic noise pollution (Kull & McGarrity, 2003; Wright et al., 2007; Slabbekoorn et al., 2010; Kunc & Schmidt, 2019). Birds are a taxonomic group in which noise pollution problems are especially well documented, perhaps because they are well visible and relatively easy to monitor, but they may also be particularly sensitive to anthropogenic noise (Dooling & Popper, 2007; Hutton & McGraw, 2016; Gilbert et al., 2017; Kleist et al., 2018).

Traffic noise can have a wide variety of effects on birds. Habitats near roads often have lower breeding bird densities (Reijnen et al., 1996; Bayne et al., 2008; Benítez-López et al., 2010) and different community structure compared to more quiet areas (Francis et al., 2009). Birds in noisy areas are also reported to be of lower body condition than conspecifics in more quiet areas (Phillips et al., 2018). Birds breeding in territories near noisy generators or along noisy highways are less likely to attract a mate (Habib et al., 2007), have a smaller clutch size (Halfwerk et al., 2011), and produce fewer fledglings (Kight et al., 2012) compared to conspecifics in relatively more quiet territories.

Next to differences in reproductive success, birds occupying noisy habitats also behave differently in comparison to conspecifics in more quiet areas. Birds near airports across the world have a clock-shifted dawn chorus beginning earlier than in control areas (Gil et al., 2015; Dominoni et al., 2016). Additional examples of noise-related changes in behaviour near airports are for example, European blackbirds (*Turdus merula*) singing less twitters in their songs (Sierro et al., 2017) and chiffchaffs (*Phylloscopus collybita*) reacting more aggressive to audio playbacks (Wolfenden et al., 2019). Similarly, great tits (*Parus major*) in urban areas start their dawn chorus earlier, but sing less later in the afternoon (Bermúdez-Cuamatzin et al., 2020). House finches (*Haemorrhous mexicanus*) in noisy urban areas are less avoidant of novel noise stimuli than rural populations (Weaver et al., 2019). Birds typi-

cally sing louder (Brumm & Todt, 2002) and at a higher pitch (Slabbekoorn & Peet, 2003) when noise levels rise, a feature they share with many other animal species (Brumm & Zollinger, 2011). Many species also sing at higher sound frequencies when exposed to noise playbacks, as for example great tits (Slabbekoorn & Halfwerk, 2009), silveryeyes (*Zosterops lateralis*, Potvin & Mulder, 2013) or house finches (*Carpodacus mexicanus*, Bermúdez-Cuamatzin et al., 2011). Black-capped chickadees (*Poecile atricapillus*) shifted their songs upwards in frequency only when they were familiar with noisy conditions (LaZerte et al., 2016). Urban but not rural white-crowned sparrows (*Zonotrichia leucophrys nuttalli*) similarly showed upward frequency shifts in their songs in response to noise playbacks (Gentry et al., 2017). While these examples show differences between birds in noisy versus quiet habitats, for many of the examples it is unclear whether differences in behaviour are examples of selection for particular genotypes or of short- or long-term behavioural plasticity and flexibility. The behavioural patterns could be a reaction to acute or chronic noise exposure or an ontogenetic result of a noisy rearing environment.

To better understand and address how noise affects animals, several studies have started to address the mechanisms underlying the behavioural and physiological impact of noise and how it relates to specific noise properties. Pioneering experimental studies testing how birds adjust timing of their singing to avoid fluctuating short-term increases of noise did not find a consistent pattern across species. Eurasian wrens (*Troglodytes troglodytes*) did not temporally adjust their singing pattern to fluctuations in traffic noise, nor did they avoid singing during playbacks of broadband white-noise (Yang & Slabbekoorn, 2014), while chiffchaffs along train tracks interrupted their sequence of songs for a passing train (Slabbekoorn et al. unpublished data). The mechanism of physiological effects of long-term noise exposure on neuroendocrine system, metabolism, and development of the auditory system (reviewed by Kight & Swaddle, 2011) has also been studied. In house sparrows (*Passer domesticus*), feather corticosterone levels of juveniles before moulting (reflecting long-term corticosterone levels) were positively correlated with urbanization levels (Beaugeard et al., 2019), indicating that noisy urban environment may be a stressor to developing birds. Behaviourally, animals have been reported to respond to noise through changes in their spatial distribution, the use and structure of vocalizations, and in foraging patterns related to predation or anti-predatory behaviour (reviewed by Mellinger, 2016). Any of these effects, could play a role in generating associations found between traffic noise and reductions in bird density and diversity (reviewed by Newport et al., 2014).

The behavioural and physiological differences between bird populations in noisy and quiet areas could come about by a number of different and mutually non-exclusive processes acting at different life stages and at different time scales (Figure 1). Noise could elicit an imme-

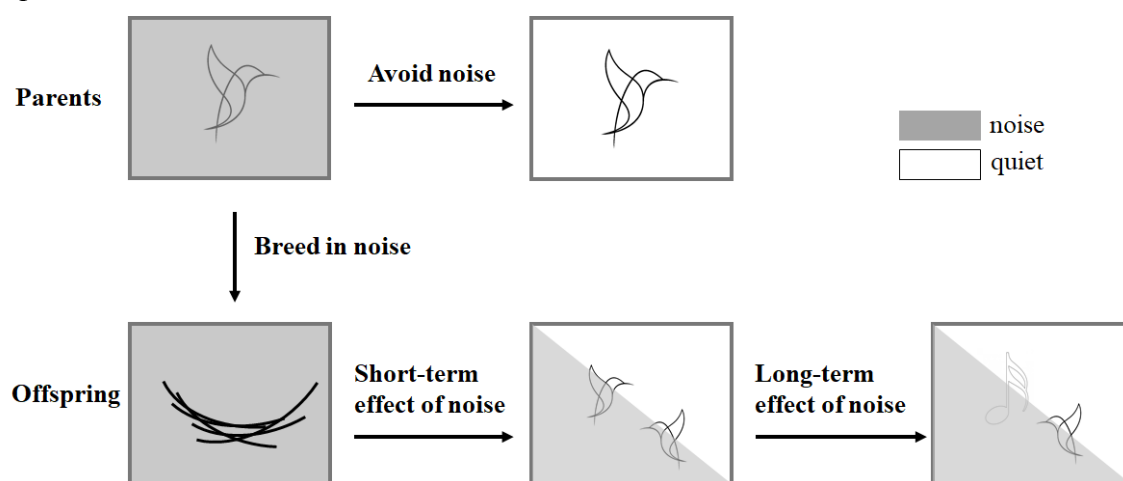


Figure 1: Different life stages and time scales at which traffic noise may affect bird behaviour. When exposed to traffic noise, birds may directly avoid noisy habitats. If birds do not avoid the noise and stay on to breed in high level traffic noise, direct or indirect effects on the parents could affect their behaviour towards their offspring. In addition, noise can also have direct short- and long-term effects on offspring phenotypes and behaviour.

mediate response (e.g. avoidance) but also cause long-term effects (e.g. singing less complex song). The background noise of the rearing environment of birds may affect their responses to noise later in life. Investigating the mechanisms and processes underlying the possible explanations for noise-related patterns and identifying whether they are due to (self) selection or phenotypic plasticity can facilitate the understanding and possibly the mitigation of the effects of traffic noise on birds and their reduced numbers near roads. An important question the field has to address now experimentally is whether decreasing numbers near roads arise just from avoiding moving cars, chemical pollution, or poorer habitats or whether birds are also repelled by noise *per se*. Two studies tested this question in the field: Blickley et al. (2012) played back drilling and road noise at leks of greater sage-grouse (*Centrocercus urophasianus*) and found that there were fewer males attending the noisy leks compared to the control leks. McClure et al. (2013) used a speaker array playing back traffic noise to imitate a noisy road ('phantom highway') at an avian migratory stop-over site. On days with playback they observed fewer visiting individuals from multiple bird species. These results are in line with both deterring or masking effects of noise. In both these field tests, birds were tested in a context where they would seek out and be attracted by the presence of vocally active conspecifics. If the noise masked the vocalizations normally attracting the birds (Mönkkönen & Forsman, 2002; Schepers & Proppe, 2017) to mating and stop-over sites then they might have failed to locate the other birds, rather than having been deterred by noise. For now, these studies thus show that noise alters birds' spatial distribution but do not address the underlying mechanism.

Likewise, observations of reduced breeding outcomes near roads are not conclusive with respect to the underlying processes leading to these outcomes. Both observational and experimental approaches in the laboratory and the field have aimed to gain an understanding from different perspectives. Several studies have provided experimental evidence for noise interfering with acoustic communication within breeding pairs (zebra finches, Villain et al., 2016) and between parents and offspring (tree swallows *Tachycineta bicolor*, McIntyre et al., 2014; Leonard et al., 2015; blue tits *Cyanistes caeruleus*, Lucass et al., 2016). Noise playbacks can also directly affect the behaviour of the breeding parents. Female house sparrows fed their young with less food in noisier areas (Schroeder et al., 2012) and when experimentally exposed to noise during breeding, females had longer flush distance (Meillère et al., 2015). However, these impacts on parental behaviour seem to depend on various factors including the traits of the parents (sex and personality traits, Naguib et al., 2013) and even in the same species, the impacts of noise on parental behaviour are not always consistent (feeding rate, Injaian et al., 2018b, 2018a). Complementary study is required to see if traffic noise causes the changes in parental behaviour.

Changes in parental behaviour could potentially explain the observed differences in reproductive outcomes, but noise can also directly affect offspring if the chronic noise constitutes a stressor. Offspring reared in noisy conditions were affected morphologically (lighter mass, Potvin & MacDougall-Shackleton, 2015; Zollinger et al., 2020), and physiologically (lower metabolic rate, Brischoux et al., 2017 and faster telomere attrition, Salmón et al., 2016; Dorado-Correa et al., 2018; Injaian et al., 2019; Grunst et al., 2020). A number of studies investigated whether chronic noise exposure during development would affect the HPA-axis programming and physiological stress responses in several bird species (white-crowned sparrows, Crino et al., 2013; house sparrows, Angelier et al., 2016; tree swallows, Injaian et al., 2019; Japanese quails *Coturnix japonica*, Flores et al., 2020; zebra finches *Taeniopygia guttata*, Zollinger et al., 2020) but these studies have not shown a clear consistent pattern yet and also varied considerably in methodology. Perinatal exposure of repeated bouts of 5 minute traffic noise was associated with increased baseline corticosterone in tree swallows (Injaian et al., 2019) but in another study, white-crowned sparrow chicks continuously exposed to short recordings of passing traffic for five days after hatching, had decreased baseline corticosterone (Crino et al., 2013). House sparrow nestlings exposed to repeated short highway recordings for six hours per day (Angelier et al., 2016) had no altered baseline corticosterone levels at the age of nine days. There was likewise no effect of daily, eight hours playbacks of sound clips of downtown, highway and subway noise to Japanese quail chicks during the first eight days posthatching (Flores et al., 2020).

Chapter 1

From the studies above, the interactions between type and duration of noise exposure and physiology cannot be fully characterised yet, and future studies will have to disentangle the effects from different noise treatments and species differences. It is worth noting though, that most of the studies to date either used artificial noise stimuli or repeated, short clips of traffic noise, that do not fully capture the dynamics of a busy road. Studies with realistic traffic noise stimuli can help validate the aforementioned results. For now several of these studies demonstrated an effect on physiological parameters known to covary with behaviour: developmental plasticity of the HPA axis reactivity is associated with behavioural differences in many birds (Schoech et al., 2011), but the long-term effects of experimental noise exposure to date have mostly focussed on physiology rather than behaviour (Injaian et al., 2020).

Little is known on how growing up in noisy conditions affects adult behaviour (other than song), but there are now a number of studies that suggest long-term effects in several songbird species. Chronic exposure of simulated traffic noise in captive adult Carolina chickadees (*Poecile carolinensis*) and tufted titmice (*Baeolophus bicolor*) made both bird species stay closer to conspecifics and have longer latencies to approach a novel food container (Owens et al., 2012). Moseley et al. (2018) presented white-crowned sparrow nestlings with tutor songs categorized as being of relatively high or low frequency, determined by how much the song was masked by low frequency city-like noises. When song was simultaneously played back with noise, the tutees showed a bias in copying the high frequency songs, which were less masked by noise. However, a similar study in great tits, exposing nestlings to broad bandwidth noise, masking either high (3 - 6 kHz) or low (0 - 3 kHz) frequencies of great tit songs, did not find any effect of traffic noise on song learning (Zollinger et al., 2017). These studies indicate that more studies are required on whether and how adult phenotypes are affected by noisy rearing conditions.

By far the largest number of developmental studies on effects of noise on adult phenotypes have been conducted in the zebra finch. This is probably due to widely accessible laboratory populations of zebra finches and because the zebra finch is a very suitable model to study behavioural development. Zebra finches breed easily in captivity and mature in about 100 days only, much faster than most songbirds. In combination with the extensive knowledge on their behaviour and development (Zann, 1996; Griffith & Buchanan, 2010), this species is suitable to study the short- and long-term behavioural effects of being exposed to noisy conditions during development. From the experiments to date (Table 1), we know from work on hearing physiology that song development in zebra finches can be delayed by overexposure with extremely loud noise, but actual hearing damage occurs only at extreme high amplitudes (120 dB SPL, 2-6 kHz, Ryals et al., 1999). Using much lower levels of compiled urban noise, Potvin et al. (2016) found that zebra finches, raised in noise levels similar to condi-

Table 1: Recent experimental noise exposure studies in zebra finches.

Research focus	Noise type	Noise level	Exposure period ¹	Measurement ²	reference
Single playback studies					
Foraging	Broadband noise + colony vocalisation	70 dBA	2 h	Vigilance* and foraging*.	Evans et al. (2018)
Communication	Repeated short wind noise	59 – 64 dBA	Overnight (~ 16 hours)	Structure of duets*, calls*, pair spatial proximity during the duets*	Villain et al. (2018)
Pair preference	White noise	90, 75 and 45 dBC	40 min	Time spent near mate*	Swaddle & Page (2007)
Continuous playback studies					
Nestling growth and physiology	Repeated short traffic noise	Day: 65 – 85 dBA; Night: 45 – 75 dBA	Perinatal - 16 dph	Baseline corticosterone levels in parents*, granulocyte/lymphocyte ratio, embryo mortality, offspring survival at 120 days, offspring mass*, Telomere attrition rate*	Zollinger et al. (2020)
Telomere attrition	Same as Zollinger et al. (2020)		Perinatal - 18 dph or 18 – 120 dph		Dorado-Correa et al. (2018)
Reproduction, parental corticosterone	Urban noise edited with vehicle sound	40 – 80 dBA, avg 65 dBA	0 – 100 dph	# offspring, embryo mortality*, offspring mass*, baseline corticosterone levels in parents.	Potvin et al. (2015a)
Adult song	The fathers in Potvin et al. (2015a)			Baseline corticosterone, song similarity, frequency*, duration and notes per song	Potvin et al. (2015b)
Offspring physiology, song and brain development	The offspring in Potvin et al. (2015a)			Song similarity*, song structure*, song similarity to tutor*, baseline corticosterone, telencephalon, RA, hvc* and area X*	Potvin et al. (2018)
Song learning	Pink noise	75 dBA	0 - 200 dph	Min, max and peak* frequency, song similarity	
Song learning	White noise	110 dBA	35 – 129 dph	Reproduction of tutor syllables*, song temporal pattern* and syllable sequences*	Funabiki & Konishi (2003)
Song learning	White noise	110 dBA	35 – 137 dph	Song learning after sensitive phase*	Funabiki & Funabiki (2009)

¹ I period is expressed in days post hatching (dph): 0 dph = hatching date. ² statistical significance is indicated by *

Chapter 1

tions close to a busy urban road, still learned their tutor songs, but with lowered song sequential similarity to their tutors (of sample size ≤ 6). Several studies looked at other physiological effects and found accelerated telomere attrition rate and decreased mass in noise exposed juveniles showing that noise can contribute to differences in condition (Dorado-Correa et al., 2018; Zollinger et al., 2020). To fully understand how traffic affects birds, it seems paramount to also investigate how noise affects their behavioural phenotypes.

It is clear from the reviewed observational and experimental studies that traffic noise has many effects on bird behaviour and physiology. However, most experimental noise exposure studies either tested birds of unknown rearing background (in the wild) or, when exposing them to noise in the laboratory, birds that had grown up at low ambient noise levels. There is thus still a substantial gap between the observations that bird numbers are dwindling along roads and potential behavioural drivers of this phenomenon. Are birds disappearing because they show aversion to noise, noise related habitat quality change, or because noise induced short- and/or long-term behavioural effects reduce their fitness? How important are rearing noise levels in shaping reactions of adult birds to noise? There is increasing documentation that growing up in noise can affect growth and physiology in birds, but are there long-term behavioural consequences of such physiological changes in young birds that manifest only in adult birds?

In this introduction, I mostly used traffic noise as a generic term. However, noise effects depend on dosage and type of noise (Dooling, 2011). Stimuli to date have been diverse and only few studies have used external validation of experimented stimuli. Moreover, almost all experimental noise exposure studies used high levels of exposure as treatment and no-playback as a control condition. Without comparison of different types of noise, effects of a traffic noise playback might not be specific to traffic noise but to any noisy or even any novel sound. Identifying aversive and non-aversive levels of sounds and comparing the birds treated with aversive to non-aversive playback might avoid confounding effects from the mere presence of any type of anthropogenic sound (rather than its intensity). In this thesis, I addressed some of these open questions in a series of experiments with zebra finches.

Outline of the Thesis

To test what levels of highway traffic noise triggered noise avoidance in adult zebra finches, I developed a method to test spatial preferences in relation to noise levels in a two-way

choice test (**Chapter 2**). Experimental exposure studies in birds either used edited sound stimuli (high stimulus control) or original recordings from highways (high external validity) but rarely, if ever first established at which levels sounds would be aversive. I therefore developed a behavioural test to assess deterrence effects of different intensities of traffic noise. I first made 24-hour field recordings of two Dutch highways from near and far distances. Based on these recordings, ‘far’ and ‘near’ distance noise profiles were characterised and parts of the recording were used for behavioural testing. Birds were tested in flocks of four, and could freely choose between two aviaries (offering choices between near- and far-distance traffic noise or quiet, i.e. no playback). If traffic noise *per se* is sufficient to deter birds, the tested birds should always prefer the quiet aviary over the one with noise playback. If the properties of the noise affect the avoidance behaviour, the birds should show different levels of avoidance of the two types of traffic noise.

Based on the insights of the noise avoidance test, a breeding experiment was designed to test whether breeding in behaviourally avoided sound would affect reproduction (**Chapter 3**). The noise avoidance test in Chapter 2 demonstrated that birds’ avoidance behaviour was affected differently by different types of highway noise. Breeding pairs were given two opportunities to breed: once being continuously exposed to near-distance traffic noise (avoided) and once being exposed to far-distance traffic noise (not avoided). This cross-over design using two types of sound avoids interpretation problems that can arise when using a ‘no-playback’ control where differences between groups might be caused by the difference between sound and no sound rather than being specific to the properties of traffic noise. If the behavioural avoidance in the spatial preference test is a good measure to assess which noise levels are aversive to the birds, then parental behaviour and reproduction should differ in breeding events with actively avoided noise versus noise that had not been actively avoided.

Rearing birds with different noise exposure also allowed testing whether rearing levels of traffic noise would affect birds’ tolerance to noise (**Chapter 4**). Habituation or sensory adaptation are discussed widely as possible coping mechanisms during sustained noise exposure. To date, studies testing birds’ behavioural responses to noise have either tested young birds in the nest or adult birds with unknown rearing background, but not whether rearing in noisy conditions leads to higher noise tolerance levels. Using the same experimental set-up used for testing the parents (**Chapter 2**), the offspring generation was tested for noise avoidance tendency at 65 days (with an additional same-age control group raised without any noise) and at 120 days post hatching. If being reared/housed in noise prior to testing affects noise

Chapter 1

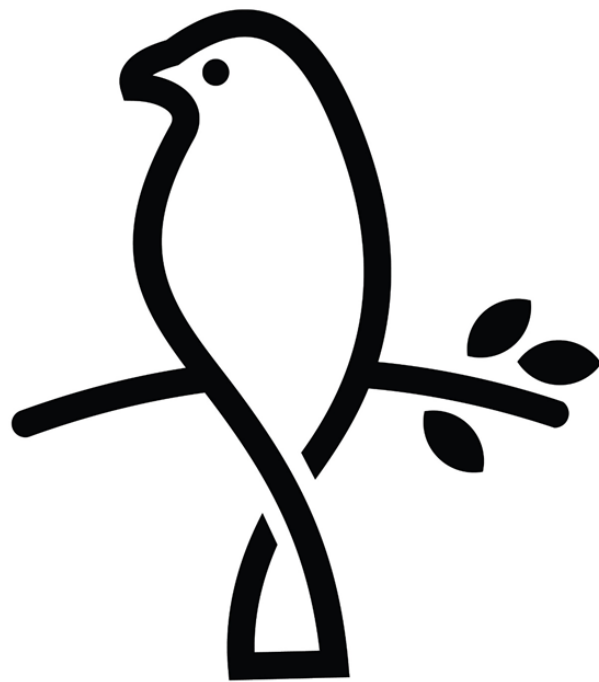
tolerance, the offspring should have behaved differently from the control group as subadults and should have behaved differently from their (not noise reared) parents as adults.

Once the offspring were adults, I also investigated whether there were long-term behavioural effects of traffic noise exposure during development (**Chapter 5**). Observational data in several bird species show individuals residing in noisy/urban areas to be bolder and to sing differently than individuals from quiet/rural areas. This has been hypothesised to be a phenotypic effect of chronic high noise levels on stress physiology and acoustic signals. While effects of traffic noise on song learning have now been tested in several species, effects on singing effort remain unclear. I therefore tested if rearing noise conditions affect singing effort and whether singing effort is correlated to explorative behaviour. If singing effort and exploration are affected by rearing noise conditions, the birds from different rearing noise backgrounds should have been different in these behaviours.

In the final **Chapter 6**, I briefly summarised the results of Chapter 2-5. I discussed the main conclusions and insights then on how to proceed with investigating the effects of traffic noise on animals.

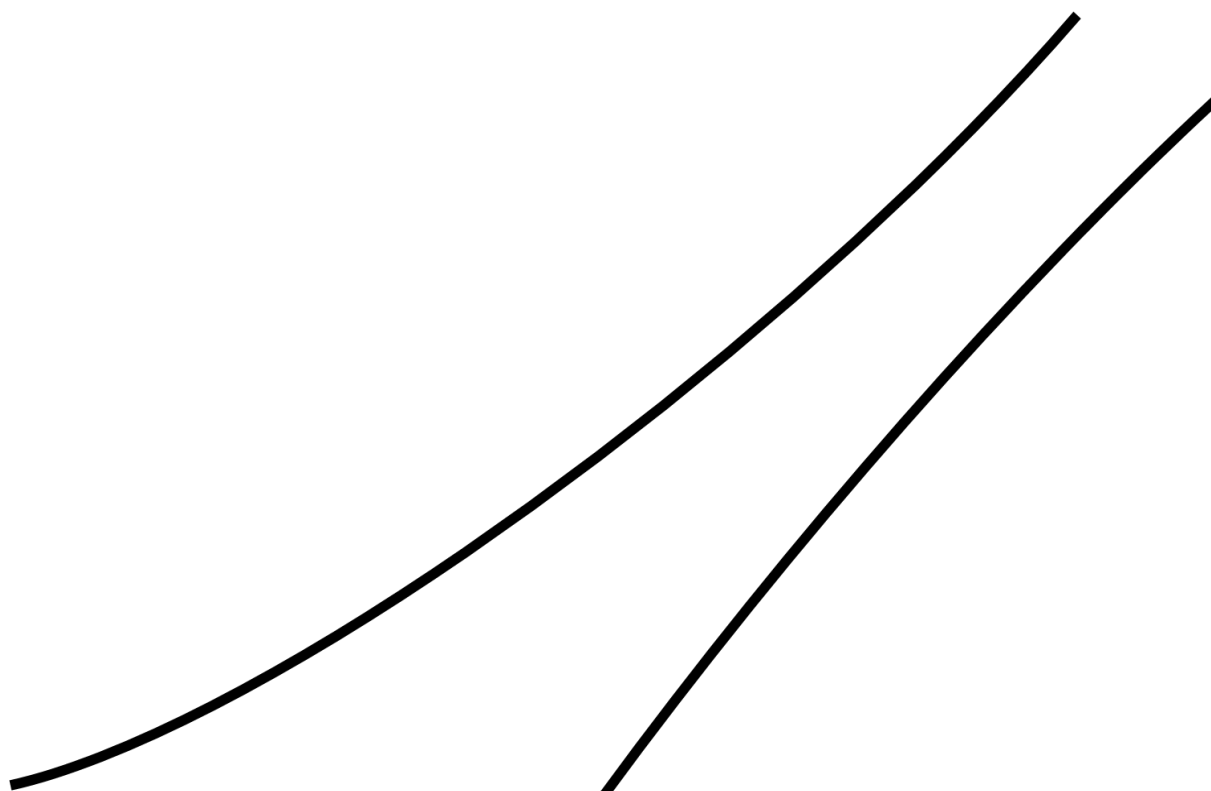
Chapter 2

Zebra finches show spatial avoidance of near but not far distance traffic noise



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Abstract

Traffic noise is on the rise worldwide. Birds have been reported to decrease in number and diversity near highways. This could be indirectly caused by traffic if birds avoid overall poorer habitat quality near highways or directly if birds actively avoid noisy conditions. To test whether traffic noise directly affects birds' spatial preferences, we designed a preference test where zebra finches (*Taeniopygia guttata*) could move freely between noisy and quiet aviaries that only differed in the type of noise playbacks. During playback of high amplitude traffic noise recorded near from highways (5–15 m), birds spent significantly more time in the quieter aviary. Such spatial preferences were not observed during playbacks of moderate amplitude traffic noise recorded further away (200–400 m). Our result provides experimental support for the growing notion that traffic noise itself rather than the presence of vehicles may suffice to deter birds from busy roads.

Introduction

Anthropogenic noise continues to increase worldwide and traffic noise has now become the most prominent source of noise pollution (Barber et al., 2010; Mennitt et al., 2015). This is of great concern as chronic exposure to traffic noise has been found to be associated with physiological stress, sleep disturbance, hearing deficits and suboptimal cognitive performance in humans (Basner et al., 2014; WHO, 2017). In the EU alone, 28 million citizens are thought to suffer health effects from chronic noise exposure (Nugent et al., 2014). Traffic noise is affecting more and more rural areas, including national parks and conservation zones, and there is increasing concern that this may negatively affect wildlife (Barber et al., 2011; Iglesias Merchan et al., 2014). An increasing number of field studies have now reported declining diversity and abundance of animals near roads (reviewed by Newport et al., 2014) a phenomenon especially well documented in birds (Reijnen et al., 1996; Bayne et al., 2008; Francis et al., 2009; Parris & Schneider, 2009; Benítez-López et al., 2010; Arévalo & Newhard, 2011; Goodwin & Shriver, 2011; Herrera-Montes & Aide, 2011; Proppe et al., 2013).

Declines in animal populations near roads could come about by several different mechanisms directly or indirectly affecting fitness. High noise levels can trigger physiological and behavioural stress responses (Wright et al., 2007; Kight & Swaddle, 2011; Shannon et al., 2016; Injaian et al., 2018a, b, 2019; Kleist et al., 2018). Traffic noise can mask acoustic signals and undermine their reception, as demonstrated in avian (Grade & Sieving, 2016), anuran (Tennesen et al., 2014), fish (Vasconcelos et al., 2007) and invertebrate (Bent et al., 2018) species. Impaired communication could affect attraction to a particular site, mate attraction and/or survival. Although some species have been observed to partly compensate the effects of low-frequency masking noise by singing or calling at higher frequency or amplitude, or adjusting when to produce sounds (Brumm & Slabbekoorn, 2005; Parks et al., 2011; Derryberry et al., 2016; Luther et al., 2016), these changes can compromise signal quality and functionality (Halfwerk et al., 2011a; Huet des Aunay et al., 2014). Increased noise levels have also been found to be associated with reduced foraging performance and predator avoidance in birds (Ware et al., 2015; Grade & Sieving, 2016), but also in other taxa, for example, in bats (Siemers & Schaub, 2011), fish (Voellmy et al., 2014) and crustaceans (Wale et al., 2013; Hubert et al., 2018). Any of these reported effects of noise exposure on acoustic signals could potentially affect individual fitness and reproductive success. In line with this, within-population comparisons have revealed that birds breeding in relatively noisy territo-

Zebra finches show spatial avoidance of near but not far distance traffic noise

ries can have reduced pairing success (Habib et al., 2007), smaller clutch sizes (Halfwerk et al., 2011b), reduced parental care (Naguib, 2013) and smaller and lighter fledglings (Kight et al., 2012; Kleist et al., 2018) compared to birds in more quiet territories.

However, observations of noise-related patterns of reduced breeding performances do not necessarily reveal the underlying processes. Reduced breeding performance near roads could also arise if these territories are of lower quality because of chemical pollution, the presence and movement of cars and different vegetation (Reijnen et al., 1996; Summers et al., 2011; Jack et al., 2015), and/or if these territories are more likely to be occupied by lower quality individuals (e.g. first time breeders) unable to secure better quality territories further away from roads (Injaian et al., 2018c). Moreover, noise does not always reduce bird abundance: Francis et al. (2009) investigated noise impact on community composition at gas well exploitation sites and found that noisy sites had fewer predator species than quiet sites, but that the total number of birds did not differ between sites. Accordingly, some species were more abundant, presumably because of reduced predation pressure. These observations suggest species differences in sensitivity to noise, but also that next to direct effects of noise, additional indirect effects can arise through species interactions. Such ecological complexity can make it difficult to identify which mechanisms underlie noise-associated distributions. Experimental studies are required to test whether the noise itself or other factors associated with the actual noise sources (e.g. chemical pollution, presence of moving machinery or vehicles) deter birds from noisy territories (Francis et al., 2009; Slabbekoorn & Halfwerk, 2009; Halfwerk et al., 2011b; Hubert et al., 2018).

Experimental approaches, for example using noise playbacks in the field, allow excluding traffic associated factors other than noise. Using such an approach, Blickley et al. (2012) found that playbacks of sound recordings of oil drilling activity and heavy vehicle traffic reduced lek attendance of greater sage-grouse (*Centrocercus urophasianus*) at playback versus control sites. McClure et al. (2013) constructed a ‘phantom road’ — a line of loudspeakers placed in trees playing back road noise at a known migratory stop-over site in a nature reserve. In alternating blocks of four days with and without road noise playbacks, the total number of individuals using the stopover site was significantly lower during days with road noise playbacks. This experiment convincingly demonstrated an impact of road noise (rather than the road itself) on the usage of a migratory stop-over site. However, for both of the aforementioned field playback studies different mechanisms could have been underlying the reductions in birds attending the sites: the noise itself could have kept the birds away and/or the noise masked the vocalizations of the birds already on site and thereby prevented the phonotactic aggregation of more birds (Mönkkönen & Forsman, 2002; Winger et al., 2019), a phenomenon that has been demonstrated experimentally (Schepers & Proppe, 2017).

Chapter 2

To test whether noise becomes so aversive that it affects spatial preferences requires testing paradigms that allow individuals to choose among identical locations only differing in noise characteristics as well as suitable model species. Domesticated zebra finches and other related estrildid finches have already been successfully studied in experiments investigating the effect of masking noise on their vocal communication (Cynx et al., 1998; Tumer & Brainard, 2007; Potvin & MacDougall-Shackleton, 2015; Villain et al., 2016; Tachibana et al., 2017). These studies, however, passively exposed birds to noise without offering quiet(er) alternative spaces. To the best of our knowledge, only one bird study offered birds to move between different noise levels (Evans et al., 2018), while testing the masking effect of noise on foraging behaviour. In this study, high level noise affected individuals' vigilance during foraging, but not their spatial choices: birds were not observed to move from louder to more quiet foraging chambers after having entered either one of them. In this set-up, entering a chamber enabled the individually tested subjects of this social species not only to feed but also to come physically closer to sounds of conspecifics. Perhaps the birds stayed on because of these factors that were not tested separately but only in conjunction with the noise. Several other studies in other taxa also tested for noise avoidance, but generally did not provide choices between otherwise identical locations (see Table 4). However, we could identify one study that offered animals identical spaces only differing in the amount of noise. Schaub et al. (2008), tested mouse-eared bats (*Myotis myotis*) foraging forays from a central space into two identical chambers differing in the level of noise playback and found that the bats preferentially and more successfully foraged in the quieter space. Birds have not yet been tested in controlled experimental exposure studies that explicitly compared avoidance of different types of traffic noise in favour of quieter locations.

In the study presented here, we opted for a two-choice set-up offering two options that were identical but for the type or absence of noise. This with the aim to test if birds would always avoid highway noise in favour of quieter locations or whether such behaviour was dependent on the type of noise (in this case highway noise recorded at different distances). In our set-up, small groups of adult zebra finches could move freely between two interconnected aviaries: one with the relatively quiet ambient laboratory sound level and one with highway noise playbacks recorded at either near or far distances. If (highway) noise *per se* is aversive to birds, we expect birds to show active avoidance of noise by moving to and spending more time in quiet than noisy compartments during highway noise playbacks.

Material and Methods

Subjects and housing

Test subjects were adult domesticated zebra finches (32 males, 778 ± 58 days old; 32 females, 739 ± 76 days old) from an outbred breeding colony at Leiden University. Prior to testing, birds had either been housed in aviaries or cages of different sizes (width \times depth \times height ranging from $80 \times 40 \times 40$ cm to $170 \times 80 \times 200$ cm). All birds were moved into the same type of aviaries ($170 \times 80 \times 200$ cm) at least one week prior to experiments. Each of these holding aviaries housed only males or only females (group size 4–16 individuals). All housing and testing rooms had a light regime of 14:10 h light: dark with 15 min of light fading in and out at the start and end of the light period. The temperature was kept between 20–22°C and humidity between 35 and 50%. Birds had *ad libitum* access to water, mixed seeds (Deli Nature, Beyers, Schoten, Belgium), grit and cuttlefish bone. This daily diet was complemented twice a week with egg food, fresh fruit and vegetables and once a week with germinated seeds. All birds were marked with a numbered orange colour ring (Hayes, UK) on their right leg.

Experimental set-up: two-choice aviaries

In the testing room, two identical aviaries of equal size ($200 \times 200 \times 200$ cm) were interconnected by a wire mesh tunnel ($100 \times 50 \times 50$ cm, see Figure 1). Each aviary contained one loudspeaker (CB4500, Blaupunkt, Hildesheim, Germany) placed at 1 m height in the corner the furthest away from the tunnel and a webcam (HD Pro C920, Logitech, Lausanne, Switzerland) at the top of the corner facing the tunnel. In each aviary there were four parallel perches perpendicular to the tunnel and a fifth parallel perch in the tunnel (see Figure 1). All side walls with the exception of the tunnel were covered with 3 cm thick sound attenuating material. Food and water dispensers were suspended underneath the two outermost perches, providing *ad libitum* access to food and water in both aviaries. The connecting tunnel had an entrance (50×50 cm) at each end that could be opened and closed remotely by operating a wire mesh trap door. A wire mesh separator (50×50 cm) in the middle of the tunnel could be passed only via a smaller opening of 30×30 cm surrounded by a black metal antenna (ANTSER300, Dorset, Aalten, The Netherlands) which was connected to a PC outside the room and registered the ID of the tags of passing birds.

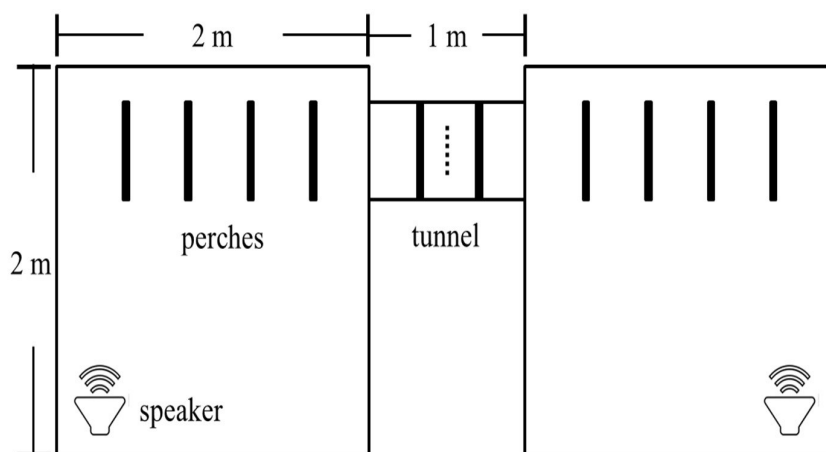


Figure 1: Top view of the experimental set-up. Two aviaries were interconnected by a tunnel with a central wire mesh partition with a small opening (30×30 cm) surrounded by a rectangular shaped antenna of the same size (dashed line) that automatically read the ID's of each passing bird's electronic tag. Thick black lines indicate perches (50 cm long, 12 mm in diameter). Each aviary contained a loudspeaker (speaker symbol) on a 1-m tripod.

Highway noise recordings and playback design

Continuous 24-h recordings (44100 Hz sampling rate, 32 bits, waveform audio file format) of highway traffic noise were made between 5 July 2017 and 10 August 2018 using two song meters (Model SM1, Wildlife Acoustics, Maynard, MA, USA). Recordings from four different sites in the Netherlands were used for this experiment: (1) Vlietland $52^{\circ}06'12.5''N$ $4^{\circ}26'28.1''E$, (2) Polderpark Cronesteyn $52^{\circ}08'12.9''N$ $4^{\circ}30'07.5''E$, (3) Oegstgeest $52^{\circ}10'34.2''N$ $4^{\circ}27'33.4''E$ and (4) Park Landskroon, $52^{\circ}10'22.7''N$ $4^{\circ}27'07.6''E$. At each site, two song meters were fixed to trees or shrubs in such a way that the microphone pointed in an uninterrupted line across open pasture to the highway. One sound meter was always placed at a far (200–400 m) and one at near (5–15 m) distance to the highway to then record near and far highway noise for a continuous period of 24 h. This resulted in four sets of matched recordings where each set had been recorded simultaneously at the same site but at two different distances from the same highway. Before starting each recording (between 1030 and 1200 h), a sound pressure meter (Model 30, Pulsar Instruments, Filey, UK; mode: A-weighted, LAT reading at 30 s intervals, re: $20 \mu\text{Pa}$) was positioned 2 cm above the sound meter microphone and pointed in the same direction. Absolute sound pressure levels were then measured at 30-s intervals for at least 2 min as the A-weighted equivalent continuous sound level (L_{Aeq}). With these measures the L_{Aeq} of the remainder of the entire 24-h recordings were calculated using 30-s bins. The medians of these bins (in dB(A)) for locations 1–4 for the near-distance recordings were 68.7, 68.7, 69.9, 71.6 and for the far-distance recordings were 53.2, 52.6 55.3, 55.3.

Zebra finches show spatial avoidance of near but not far distance traffic noise

From each of these eight 24-h recordings, the hour from 11 to 12 am (to avoid rush hours) was selected for further processing and stimulus preparation. Each of these one-hour recordings was split into 0.02-s bins. For these bins, the decibels relative to full scale were obtained by assigning the level of 0 dB FS to the maximum digital level in the recording and then scaling the remainder of the recording to this maximum using the ‘to intensity’ function in Praat (v. 6.04.40, Boersma & Weenink, 2019) that squares and convolves the sound values with a Gaussian analysis window of 0.02 s. The bin values were transferred to the statistical software R (v. 3.4.1, R Core Team, 2019) to search and flag all time stamps with extremely high amplitudes (>2 standard deviations, i.e., louder than 97.5% of all 0.02 bins). Using the Audacity(R) (v. 2.1.2; Audacity Team, 2019) acoustic software (v. 2.1.2) the flagged passages were inspected acoustically and visually (by QL). This revealed that these extreme amplitudes always occurred when, in addition to the highway noise, other sudden onset sounds had been picked up by the microphone such as car honking or dogs barking or the noise of low overflying aircraft. Sudden sounds easily startle birds (and make them fly away); to prevent this we edited out these extreme amplitude sounds so that they could not confound or exacerbate highway noise avoidance. The editing consisted of deleting the bin associated with the event using the ‘delete’ function in Audacity. Overall, this procedure removed only 90 s per 3600-s-long sound file. After this step, the recordings were checked in their entirety again while listening to the recordings via headphones (by QL) which confirmed that the above procedure had removed all sudden onset sounds. Next, 30 continuous minutes from each of the 8 recordings were selected by using a random number generator to pick a number between 0 and 28 and then using this number as the minute to start the extraction of the subsequent 30 min as stimuli. As final editing step, the first and last two minutes of each 30-min stimulus were faded in and out in Audacity. Generally, the near-distance highway noise recordings were much louder than those recorded at far distances and because of frequency-dependent degradation, far-distance recording differed in aspects of spectral composition from the near-distance recordings (see Figure 2). Next to these overall differences, we need to point out that all recordings were real time non-stop field recordings and both recording categories had transient biotic and abiotic sound sources that added stochastic variation akin to what a wild animal would experience in a 30-min period next to roads.

For playbacks, stimulus amplitude at the 4th perch (the most distant from the tunnel) was set to match the sound level at the original recording locations using the same sound pressure level meter as described above. After the adjustment, we also took sound pressure level measures during the noise playbacks at all other perches. Peak noise levels within the aviary decreased from the 1st perch (furthest from the speaker and closest to the tunnel) to the 4th perch (nearest to the speaker) and ranged from 45–55 dB(A) during playbacks of the far-

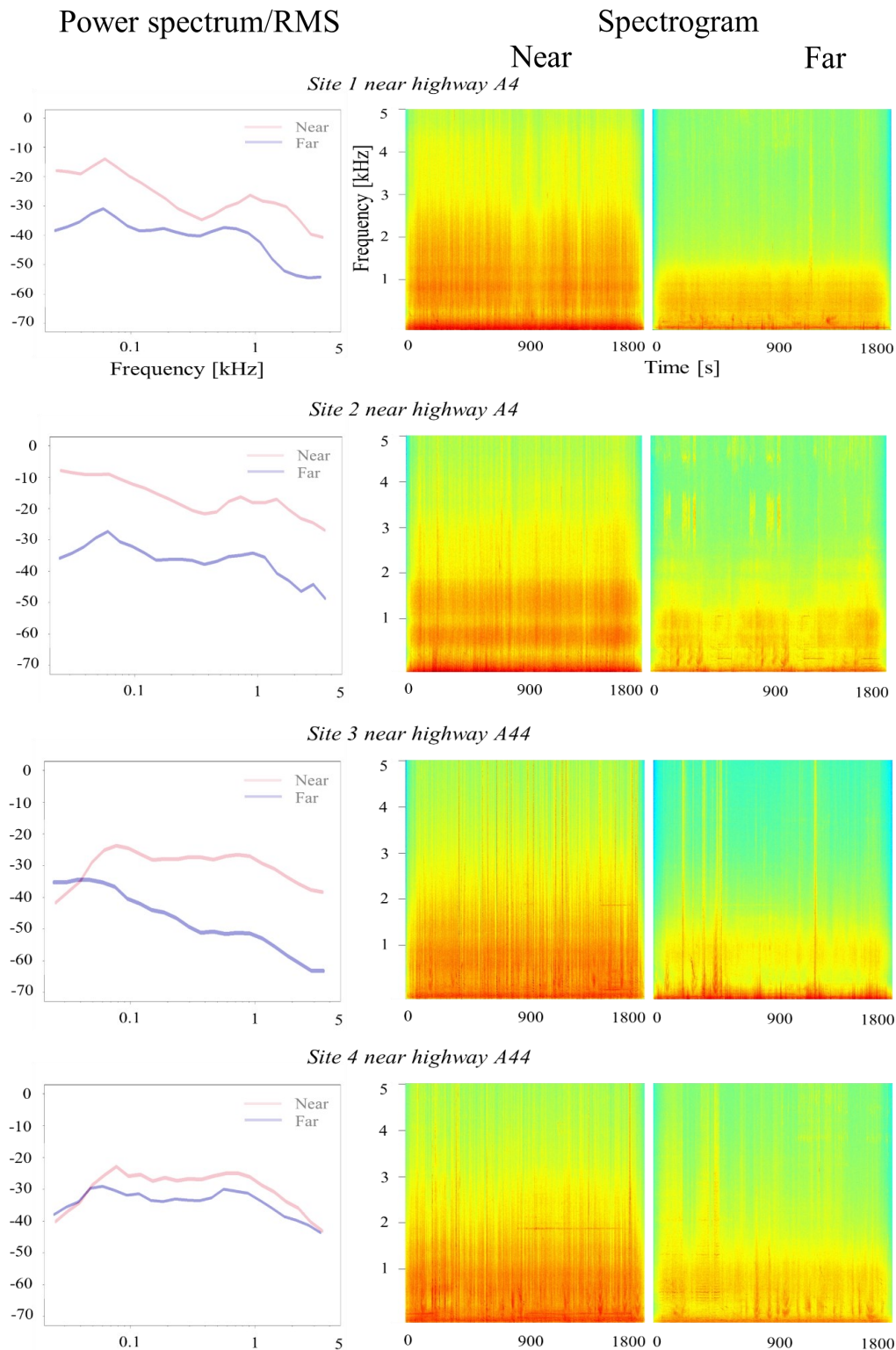


Figure 2: Power spectra (left column) and spectrograms (centre and right columns) of all near- and far- distance highway noise stimuli. Each pair of stimuli was recorded from two different distances at one of four different locations (GPS details see Table A1 in the Appendix). The power spectra show the decibels relative to full scale (dB FS) at 0–5 kHz. The spectrograms show the power spectral density (PSD). Relative sound pressure levels are indicated by the colour gradient. All plots were calculated using the entire 30 min of each stimulus (R package PAMGuide, Hamming window, 1s resolution, 0–5 kHz, 50% window overlap; data averaging: Welch method set as 5, following the recommendations by Merchant et al., 2015).

Zebra finches show spatial avoidance of near but not far distance traffic noise

distance highway recordings to 55–75 dB(A) during playbacks of the near-distance highway recordings. With either playback on in one aviary, noise levels in the quiet aviary without playback ranged from 35–40 dB(A). To put these measures into perspective: zebra finches generally experience sound levels of 46–68 dB(A) in our breeding rooms and background noise in natural habitats is estimated to range from 35–75 dB(Z) on windy days (Villain et al., 2016). Note in this context that decibel is measured on a log scale, and doubles in intensity every 6 dB, so the differences between ambient and either of the two highway noises were in the order of one to several magnitudes but the absolute maximum levels of 75dB(A) were far from the level (approx. 100 dB(A)) where hearing and auditory feedback are blocked and way below the thresholds for hearing damage in zebra finches (Funabiki & Konishi, 2003; Zevin et al., 2004). The playback levels however fall into the range of background noise that has been found to affect parameters of acoustic communication in zebra finches (Cynx et al., 1998; Villain et al., 2016).

Playback trials

Zebra finches are highly social (Zann, 1996) and explore new environments faster in groups than when alone (Coleman & Mellgren, 1994; Schuett & Dall, 2009). Because zebra finches prefer being in groups and birds had to be moved into a new room for testing, we aimed to reduce neophobia by testing birds in small groups (consisting either of four males or four females). Birds were gently caught from their home aviaries the day before a playback test (19 ± 2 h before the first playback) and each bird was fitted with a small plastic leg ring with a micro transponder tag (ID100A, Dorset). Birds were then released into the central tunnel connecting the two experimental aviaries. At this stage, the trap doors at either end were closed and were not opened until after a short delay of about 5 min. This was the time it took the experimenter to leave the experimental room to enter the adjacent observation chamber and to remotely open both trap doors from there. From the moment the trap doors at either end of the tunnel were opened, birds could freely move and explore both aviaries. The next morning, at around 10:00h (± 90 min) the data log of the antenna readings was checked and in all 16 tested groups, birds had crossed the tunnel between the aviaries multiple times which was the criterion to start the first experimental playback.

There were three playback trials for each experimental group. The first two playbacks were fully balanced with respect to the order (near or far tested first against quiet) and whether aviary 1 or 2 was used for the first playback (for a schematic overview of the playback procedure see Figure 3c). Testing started by switching on both loudspeakers without playing back sound to register birds' baseline behaviour (pre-playback) for 15 min. Then, the first 30-min playback started in one of the aviaries while the other remained quiet. After 30 min the

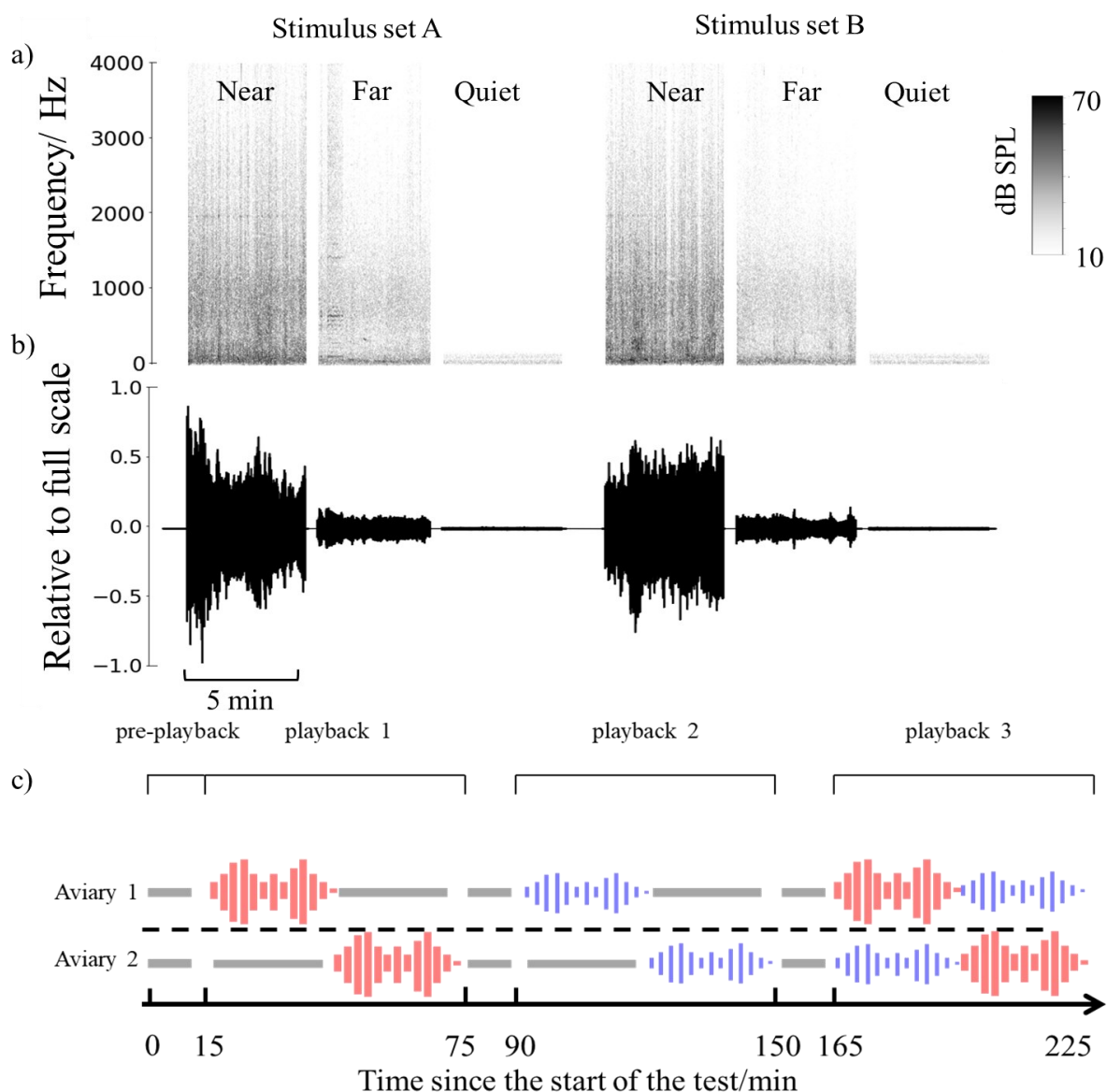


Figure 3: Highway noise stimuli and playback design: (a) Frequency spectrograms of 5 min of recordings in the experimental aviaries during the near- and far-distance highway noise and no playback ('quiet') condition. The spectrograms were computed by Fast Fourier Transformations (using scipy library in python 3.7, window length 4096 points, overlap 512 points, time step 0.08 s, frequency step 11 Hz). (b) Waveforms of the same stimulus sets (y-axis: SPL to full scale). (c) Timeline: 15 min pre-playback (grey bars: no playback, but loudspeakers already switched on) were followed by a 30 min playback of traffic noise (red, near-; blue, far-distance highway noise) in one aviary and no playback in the other (quiet) aviary, followed by another 30 min after stimulus reversal. After a 15-min silent break, this procedure was repeated with the other noise category. After another 15-min break, playback 3 exposed the birds to simultaneous playback of the near- and far-distance highway noise for 30 min. For illustration, the timeline shows only one presentation order (red, near-distance highway noise first), but presentation order was fully balanced and half the trials started with far-distance noise first).

Zebra finches show spatial avoidance of near but not far distance traffic noise

playback stopped and then started in the other aviary for another 30 min. Afterwards, there was a 15 min break without any playback which was followed by the second 2×30 min playback that now was either near- or far-distance highway noise (whichever sound had not yet been played back during the first trial). After the second playback another 15-min break followed before the third playback trial started, where near- and far-distance noise playback each started simultaneously in one of the two aviaries and then lasted for 30 min. We fully balanced the aviary \times stimulus type combinations and stimulus sets. However, due to a playback file error during playback 3, four groups had to be excluded from the analyses of playback 3.

To measure the time the birds spent in each aviary, all video recordings were analysed by the same observer (QL) using the BORIS video analysis software (v. 6.1.6, Friard & Gamba, 2016) playing back the recordings at $3 \times$ normal speed. All videos were scored with the sound switched off so that the observer did not know which playback treatment the birds on the screen were experiencing. Videos were analysed by scoring ‘the number of birds in aviary 1’ as a single state event as follows: whenever a bird passed from one aviary to the other via the small opening with the antenna in the middle of the tunnel, the video was paused and the state event was updated with the new number of birds in aviary 1. If a bird stayed in the tunnel without passing through the antenna, the time spent in the tunnel counted toward the aviary on the same side. Sometimes, individual birds were sitting on the antenna for a while. In such cases, 50% of the duration of this event was added as ‘time spent’ to aviary 1 and the other 50% to aviary 2. For each playback, the cumulative time spent by all birds in aviary 1 (from now on referred to as ‘total time in aviary 1’) was then calculated (multiplying the duration of each state event with the corresponding number of birds during this event). The maximum total time all four birds could spend in either aviary thus was 120 min (= 4 birds \times 30 min playback) and once aviary 1 was scored, time spent in aviary 2 could be calculated (120 min total time all birds – total time in aviary 1 = total time aviary 2). Note that this means that for analyses, only one value per group was used, as the movements of the four birds in a group cannot be assumed to be independent of each other. Total time spent per aviary was then transformed to the relative proportion of time spent in either aviary; this parameter could range from 0 (no bird visited an aviary during playback) to 1 (all birds stayed in the same aviary for the whole duration of the playback).

Ethical note

The experiments described here were reviewed and approved by the committee for animal experimentation at Leiden University and the Centrale Commissie voor Dierproeven (CCD)

Chapter 2

of the Netherlands (permit AVD1060020171409), and monitored by the Animal Welfare Body of Leiden University, in accordance with national and European legislation.

Analyses

Prior to statistical analyses, the proportion of time spent in either aviary was calculated (time in aviary 1 or 2/total time). Because proportional data tend to centre around the mean, all proportional data were arcsine square root transformed (Sokal & Rohlf, 1995) prior to statistical analyses (but were back transformed for illustrations and reporting in the text).

To verify whether birds preferred one of the two aviaries independently of sound treatment, we first tested whether the proportion of time spent in the left aviary during the baseline observations deviated from chance level using a one-sample *t*-test.

For playbacks 1 and 2 (where a one-sample *t*-test would result in pseudo-replication of groups and stimuli), we used a mixed model approach (using lme4 in R) to first test whether the time spent in the quiet aviary deviated from chance level by subtracting 0.5 from the observed proportions and then testing whether the intercept of a mixed linear model with only random effects ('stimulus ID' and 'group ID') deviated significantly from 0 (Tables 1 and 2, Model A). Next, we asked whether a preference for the quiet aviary differed between noise treatments, by adding noise type as factor to the model (Tables 1 and 2, Model B). To explore whether there was a difference in response to noise playback between sexes, sex was added to Model B as fixed factor to create Model C (Tables 1 and 2, Model C).

While all previous analyses tested the groups' behaviour as a whole, we also ran an additional analysis checking whether the playback treatment affected whether individual birds were more likely to split from the group. To this end, we compared all events (and their duration) where a single individual was observed in an aviary with respectively near- or far-distance highway noise during playbacks 1 and 2 with a generalised mixed linear model, using a Poisson distribution, with the cumulative time individual birds were staying alone in a particular aviary as response variable, playback in that aviary as fixed (quiet/far/near) and the group ID as random effect (Table 3, Model D).

For playback 3, where near- and far-distance highway noise playbacks were presented simultaneously (resulting in one data point 'relative time spent in the relatively more quiet aviary' per tested group), we tested whether the proportion of time spent in the aviary with far-distance highway noise playback differed from chance level with a one-sample *t*-test.

Results

The different playback treatments affected how birds allocated their visiting time to the different aviaries (see Figure 4 & 5). In the absence of playback (during the ‘pre-playback’ phase) there was no difference in the amount of time birds spent in the left (or right) aviary (one-sample t -test: testing ‘proportion of time spent in aviary 1’ for deviation from a 0.5 chance level $t_{15} = -0.34$, $p = 0.74$).

During playbacks 1 and 2, which offered the choice between a quiet (no playback) and a noisy aviary (playback of either near- or far-distance highway noise), birds spent more time in the quiet aviary, as evidenced by the significant deviation of the intercept from zero (Table 1, Model A). This effect was stronger for the near-distance noise; playback noise type (near or far) significantly affected the amount of time birds spent in the quiet aviary (Table 1, Model B). Comparing the proportion of time spent in the quiet aviary versus chance level for the two noise types separately, revealed a significant preference for the quiet aviary over the aviary with near-distance highway noise (one-sample t -test: $t_{15} = 5.31$, $p < 0.001$), but not over the far-distance highway noise (one-sample t -test: $t_{15} = 1.33$, $p = 0.20$).

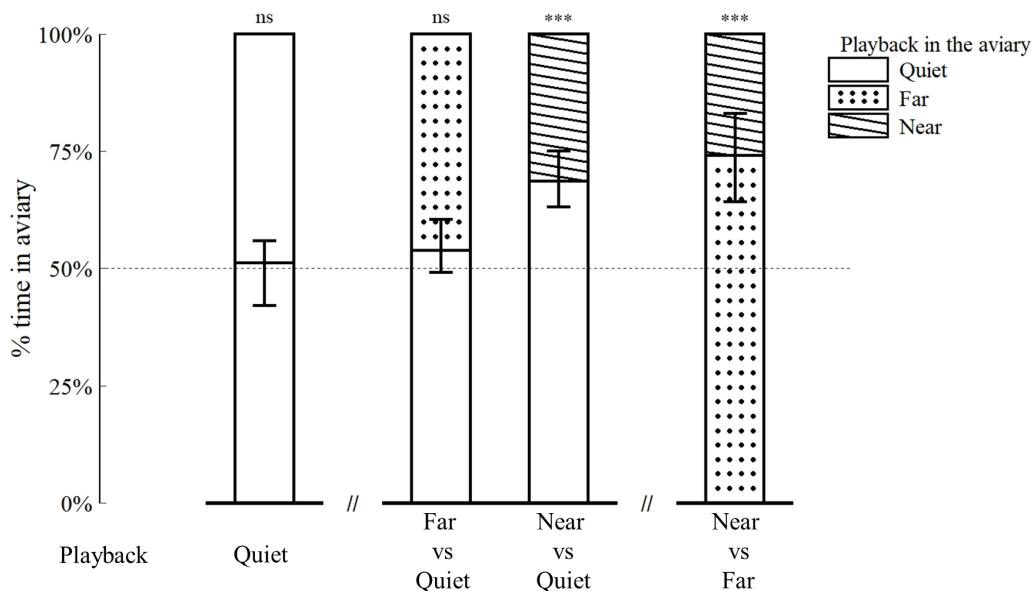


Figure 4: Time spent in the different aviaries during the different playback conditions. Bars show the percentage of time (mean \pm 95% CI) groups spent in each of the two aviaries during the different playback conditions. Note that the values and their CIs are reciprocals within a playback condition. ***Significant, ns: not significant, see Tables 1 and 3.

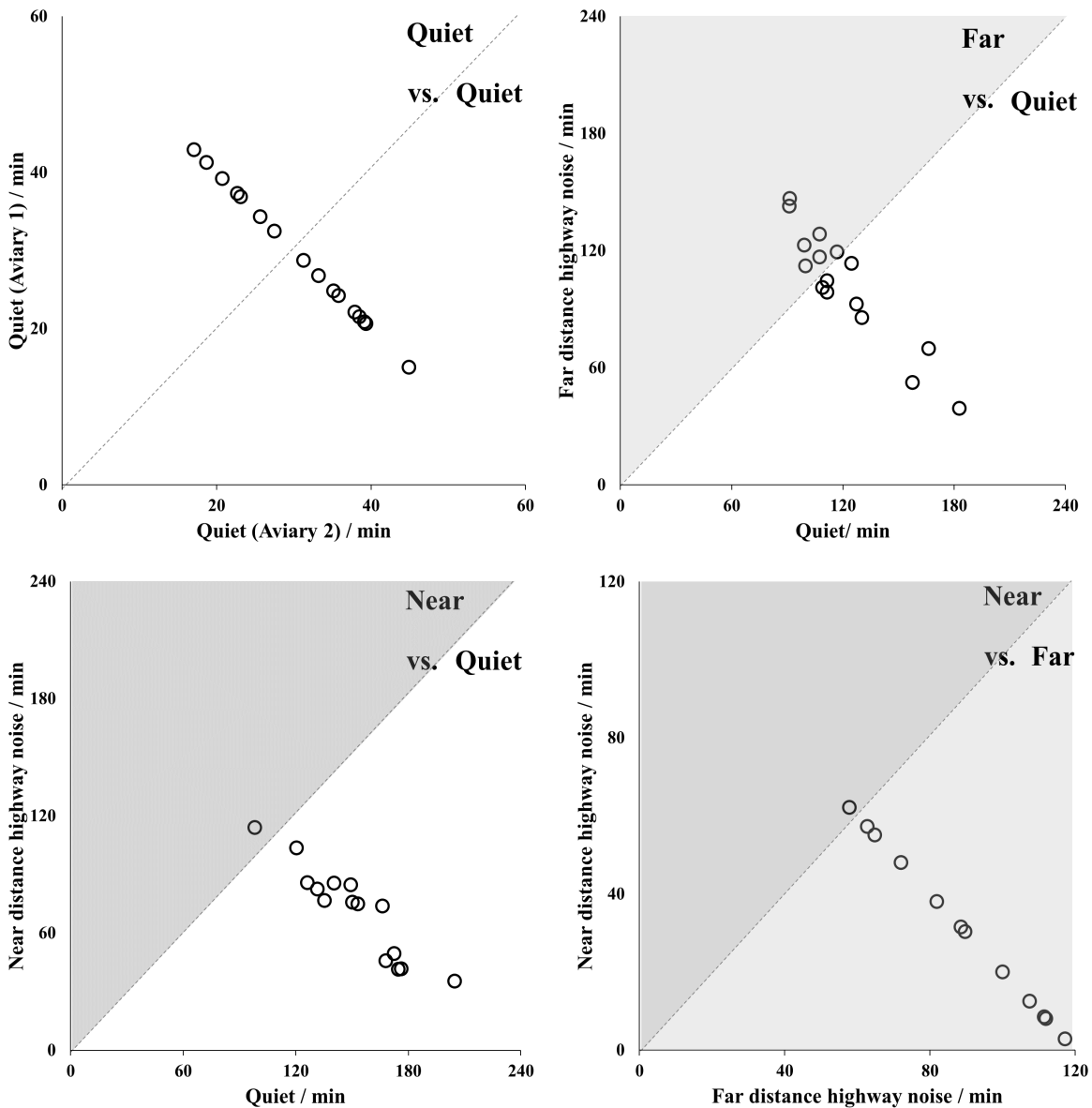


Figure 5: Total time each group of birds spent in either of the two aviaries during different playback treatments. a) Quiet vs. quiet during pre-playback ($n = 16$ groups, maximal time spent = 60 min), b) far vs. quiet ($n = 16$ groups, maximal time spent = 240 min) c) near vs. quiet ($n = 16$ groups, maximal time spent = 240 min) and d) near vs. far during simultaneous playback 3 ($n = 12$ groups, maximal time spent = 120 min). Each dot is one tested group. Dots in b) and c) are jittered for visualization. The intensity of the greyness indicates the intensity of the noise playback. Each plot is separated by the $y=x$ line (all dots would be on this line if birds showed an equal preference).

Zebra finches show spatial avoidance of near but not far distance traffic noise

Table 1: Mixed linear models testing whether total time spent in the quiet aviary (per group) deviates from chance level. Model A: null model, Model B: playback added as fixed factor and Model C: playback + sex as fixed factors.

	estimate	Std. Error	t value	p value
Model A¹				
Intercept	0.12	0.04	-2.73	= 0.01
Model B²: Playback				
Intercept	0.20	0.05	-4.27	< 0.001
Playback: Far	-0.16	0.03	4.94	< 0.001
Model C: Playback + sex				
Intercept	0.18	0.06	-2.80	= 0.009
Playback: Far	-0.16	0.03	4.94	< 0.001
Sex: Male	0.05	0.06	-0.73	= 0.47

¹ group ID and stimulus ID are included in all models as random effect. ² marginal and conditional r square are 0.25 and 0.68 for Model B.

Table 2: Parameters of Models A–C

Models:	AICc	ΔAICc	Weight
Model B	-23.5	0.0	0.94
Model C	-17.1	6.4	0.04
Model A	-15.9	7.6	0.02

Males and females showed no difference in how they behaved in the tests (adding sex as additional factor did not improve the model see Table 1, Model C).

In addition, we also checked whether the birds' grouping behaviour was affected by the type of playback in a particular aviary by summing up all events where a single bird was alone in one of the aviaries. This revealed that the proportion of time a single bird was observed was slightly, but significantly higher in an aviary with near-distance noise than aviaries with either no or far-distance noise playbacks, but was overall very low during all conditions (median time single bird was observed: near = 972 s, far = 733 s and no playback = 67 s out of a total of 14440s; see Table 3, Model D)

Table 3: Mixed linear model testing whether birds spent more time alone in the aviary with playback (Model D).

Model D: Playback	estimate	Std. Error	z value	p value
Intercept	6.66	0.20	33.47	< 0.001
Playback: Far	-0.12	0.01	- 10.47	< 0.001
Playback: Quiet	-0.56	0.01	-41.63	< 0.001

¹Marginal and conditional r^2 are 0.08 and 0.99 for Model D

Chapter 2

During playback 3, with near- and far-distance highway noise presented simultaneously, birds also spent less time in the aviary with the near-distance highway noise than expected by chance (deviation from 0.5 tested by a one-sample t -test: $t_{11} = 4.39$, $p = 0.001$).

Discussion

The aim of our experiments was to test whether zebra finches would prefer quiet over noisy locations in an unconstrained choice situation. During the playback tests, birds could freely choose to move between two identical locations that only differed in their simulated soundscape (near- vs. far-distance highway noise). Birds did not avoid all noise playbacks but showed stimulus-dependent spatial preferences: they clearly avoided the near- but not the far-distance highway noise in favour of the quiet aviary.

Birds likewise avoided the near-distance highway noise during the simultaneous playbacks of near- and far-distance traffic noise. The combined observations from the sequential and simultaneous playbacks showed that the birds were showing stimulus-dependent avoidance (near-distance highway noise was avoided) rather than showing general avoidance or neophobic behaviour to a location with playback of (for them unknown) highway noise (far-distance highway noise was not avoided)

Noise avoidance in the laboratory and in the field

Our study adds to experimental work in other vertebrate taxa testing active noise avoidance (see Table 4). Only one of these studies — by testing bats allowed to hunt for prey in two identical free flight chambers (Schaub et al., 2008) — gave subjects a choice between two identical chambers only differing in noise levels. Bats hunted more in the relatively quieter chamber, but note that this bat species orientates on the sounds produced by their prey to locate them. This means that their behaviour can be both interpreted as attraction to the sounds of their prey (which were audible in the low level noise chamber but masked in the noisy chamber) or as an avoidance of the noise in the other chamber. Zebra finches are seed eaters and do not need to hear their prey. By using a comparable two-way choice design for the first time in a songbird allowed testing of whether noise avoidance *per se* guided birds' spatial behaviour. In the experiment, the tested birds avoided noisy conditions to move into a quieter space. This behaviour would confer an advantage outside the laboratory as complementary research in zebra finches has already shown that noise negatively impacts zebra finches: it increases vigilance reducing food uptake during foraging (Evans et al., 2018), af-

Zebra finches show spatial avoidance of near but not far distance traffic noise

Table 4: Studies using choice set-ups to test noise avoidance behaviour

	Species	Choice set-up (m) ¹	Stimuli ⁴	Noise avoided	Study
Mammals	Greater mouse-eared bat (<i>Myotis myotis</i>)	Moving from quiet start area into noisy vs. quiet identical foraging rooms (2.5 x 3 x 2)	Silence, broadband or traffic noise ~80 dB, moving vegetation 68 dB	Yes	Schaub et al., 2008
	Holstein Friesian cattle (<i>Bos Taurus</i>)	T maze, one end noise playback	Milk factory noise 85 dB	Yes	Arnold et al., 2008
	Harbor porpoise (<i>Phocoena phocoena</i>)	Noisy outdoor (12 x 8 x 2) vs. quiet indoor pool (8 x 7 x 2)	Intermittent and continuous brown noise between 100 and 130 dB re 1µPa	Yes	Kok et al., 2018
	Pig (<i>Sus scrofa</i>)	Operant control of noise-off switch	Transportation vibration and noise between 80-90 dB	Yes	Stephens et al., 1985
Reptiles	Rat (<i>Rattus norvegicus domesticus</i>)	Noisy dark box vs. quiet open enclosure	60 or 90 dB noise at 2-20, 2-8 and 16-20 kHz	Yes	Manohar et al., 2017
	Eastern blue tongued lizard (<i>Tiliqua scincoides</i>)	Row of three chambers from noisy to quiet ²	Highpass > 500 Hz vs. lowpass < 500 Hz truck noise 82.2-90 dB(A)	No	Mancera et al., 2014
	Eastern blue tongued lizard	Row of three chambers from noisy to quiet ³	74 vs. ~63 dB(A) noise with high- > 2kHz or low-pass < 2 kHz mining machinery noise	Yes	Mancera et al., 2017
Fish	Zebra fish (<i>Danio rerio</i>)	Two connected tanks (0.7 x 0.5 x 0.5)	White noise 0.1-1kHz from 120 – 80 dB re 1µPa vs no playback	No	Shafiei Sabet et al., 2016a
	Zebra fish (<i>Danio rerio</i>) and Lake Victoria cichlid (<i>Haplochromis piceatus</i>)	Tank with noisy vs. quiet zone (2 x 0.35 x 0.45)	Intermittent and continuous white noise 122 dB re 1µPa	No	Shafiei Sabet et al., 2016b
Birds	Zebra finch (<i>Taeniopygia guttata</i>)	Foraging choices during simultaneous playback of noise and conspecific sounds	broadband noise 70 dB(A)	No. but increase vigilance	Evans et al., 2018
	Zebra finch (<i>Taeniopygia guttata</i>)	Choice noisy vs. quiet aviary (2 x 2 x 2)	Highway noise 55 or 70 dB(A) vs. no playback	Yes	Present research

¹ Length x width x height/depth

² 1st and 3rd chamber: 0.2 x 0.1 x 0.2, 2nd chamber: 0.8 x 0.4 x 0.2

³ 1st chamber: 0.57 x 0.12 x 0.2, 2nd chamber: 0.81 x 0.42 x 0.2 and 3rd chamber: 0.5 x 0.12 x 0.2.

⁴ the reference level of sound pressure is 20 µPa if not mentioned otherwise.

The studies were selected by screening the search results in Web of Science, Google scholar, Mendeley data and ResearchGate obtained when using the key words: 'anthropogenic noise'/'noise' + 'avoidance'/'spatial distribution' +/- 'animal' +/- 'experiment'.

Chapter 2

fects intra-pair communication (Villain et al., 2016), offspring initial growth rates (Potvin & MacDougall-Shackleton, 2015), reduces telomere length (Dorado-Correa et al., 2018) and affects song learning (Funabiki & Funabiki, 2009; Potvin et al., 2016).

To date, only one other study also offered zebra finches a choice between quiet and noisy spaces: however, this was concurrent with access to food and playback of conspecific vocalisations (Evans et al. 2018). Birds showed increased vigilance in noise but little spatial sampling (most birds stayed in the first chamber they entered) and no preferences for quiet versus noisy cage compartments. This is different from the pronounced spatial choices observed in the birds in our study. However, the set-up used by Evans et al. (2018) was different in several aspects from our two-aviary study: a much smaller testing apparatus, individual rather than group testing of birds, manipulation of food availability, and simultaneous playbacks of noise and conspecifics. Any of these differences in design could be the reason why most birds in the set-up by Evans et al. (2018) only entered one compartment and stayed there. These differences stress the importance of potential context-dependency of active noise avoidance. Because zebra finches are highly social and engage in almost continuous acoustic exchange (Elie et al., 2011; L. Gill et al., 2015) when exploring the environment, behavioural decisions of the birds were likely not only driven by trying to avoid noise but also by their motivation to find food and/or join other birds after 2 h without food and company. In our set-up, the zebra finches were tested in groups of four and had ad libitum access to food, meaning that spatial preferences did not have to be weighted against food or company. To test whether social context indeed affects noise avoidance behaviour, our or comparable set-ups can be used in the future to investigate whether and how social companions and noisy conditions interact in affecting exploration and/or avoidance tendencies (Coleman & Mellgren, 1994; Schuett & Dall, 2009; Templeton et al., 2014).

Environmental noise can mask avian communication signals (Brumm & Slabbekoorn, 2005; Halfwerk & Slabbekoorn, 2015; Derryberry et al., 2016). In a highly social species like the zebra finch, this could affect group cohesion. Birds could become less explorative as not to lose visual contact if the exchange of information through vocal signals is impaired through masking (e.g. Villain et al., 2016). In the tests reported here, group cohesion was generally strong. In all three playback conditions birds spent around 93% of their time in the group, suggesting that masking of contact calls is not likely to have influenced group cohesion in the experiments described here. These percentages may or may not look different for larger groups of birds or when housed in noisy conditions for longer than during the short tests presented here. Effects of noise on social behaviour and group structure could be investigated in the future with our aviary design by specifically monitoring calling behaviour and/or reinforcing it by playbacks (Schepers & Proppe, 2017).

Zebra finches show spatial avoidance of near but not far distance traffic noise

An aviary study in the laboratory cannot simulate how effects of noise exposure affect spatial preferences and noise-dependent distribution patterns in the field. Conversely, direct causality between ambient noise and the target species' behaviour is difficult to establish in the field where experimentally elevated sound levels could affect individuals directly, but inadvertently also affect other organisms in the ecosystem. This may yield indirect effects of noise on the target species via interactions with other species (Francis et al., 2009; Slabekoor & Halfwerk, 2009; Hubert et al., 2018). Furthermore, factors like the reproductive stage or migratory status might affect responsiveness in the field and thus make it difficult to infer direct effects of noise (Ware et al., 2015). Laboratory studies provide a less natural environment but can keep potential confounds to a minimum, and can thus demonstrate a deterrent effect of traffic noise playback *per se*. Experiments, both in the field and in the laboratory, thus contribute to our understanding about the causal factors that explain the reduced species diversity and abundance in noisy versus quiet areas (Blickley et al., 2012; McClure et al., 2013).

Recording distance dependent response pattern

In the experiment presented here, only the playback of the near- (but not the far-) distance highway noise affected how often and how long birds spent time in the different aviaries. These two types of highway noise differed substantially in amplitudes (approx. 15 dB(A)) and this seems the most likely reason why birds only avoided the aviary with playbacks of near-distance highway noise but not the playback of far-distance highway noise. It is important to note that the near-distance noise levels at around 70 dB(A) are several orders of magnitude (dB is a log scale) below the threshold where hearing cell damage occurs in zebra finches (>110 dB(A); Funabiki & Konishi 2003; Zevin et al., 2004) and well within the range that wild birds in urban and rural populations may experience (Wickham & Riitters, 2003; Barber et al., 2011; Halfwerk et al., 2011b; Gil et al., 2015). This raises the question as to how easily birds can habituate to noisy conditions and whether birds habituated to high noise levels would have reacted differently, and/or whether birds found near busy roads have habituated to the traffic noise, or have no alternative habitat to choose.

The near- and far-distance highway noise recordings used in this study did not only differ in amplitude but also in their spectral characteristics. Overall, the far-distance highway noise has less energy at 1–4 kHz than the near-distance noise. This is the range where zebra finches have the lowest hearing threshold. Therefore, moving from near-distance to far-distance noise, might perceptually release the birds from masking or noxious effects more than just the absolute reduction in amplitude. It is important to note that this difference in spectral characteristics is not an artefact of the method, but also applies to near- and far-distance lo-

Chapter 2

cations in the vicinity of real highways. In the field, locations near and further from a highway also differ both in amplitude and spectral composition.

What our results unambiguously show is that the birds did not move away from the noisy conditions because they were unfamiliar with the sounds on the traffic noise recordings or were startled by it: although the far-distance noise was less loud than the near-distance recording, it was still around 55 dB(A) which is above the level the WHO considers safe for human chronic exposure (see WHO Environmental Noise Guidelines, 2019). However, playbacks with these levels did not make the birds move to the quiet aviary. This makes neophobia or startle reactions unlikely explanations for our results but suggests that the louder near-distance noise (rather than any audible and unfamiliar sound) was being actively avoided. This avoidance response could prevent the negative impact of noisy conditions on acoustic communication, social decision making and foraging which have been reported from other experimental studies exposing zebra finches to noise playbacks (Swaddle et al., 2006; Swaddle & Page, 2007; Villain et al., 2016; Evans et al., 2018).

Without being able to pinpoint down the lowest threshold of negative impact yet, the combined evidence from our and other studies suggests that for sound levels from 70 dB(A) and higher (<15 m to the highway), traffic noise may affect communication, foraging, physiology and reproduction (Cynx et al., 1998; Potvin & MacDougall-Shackleton, 2015; Potvin et al., 2016; Villain et al., 2016; Dorado-Correa et al., 2018). Wild birds of other species that were chronically exposed to anthropogenic noise were found to have altered glucocorticoid levels (Kleist et al., 2018; but see Crino et al., 2013), which in turn will raise energy demands (Jimeno et al., 2018). In combination with raised vigilance levels in noisy conditions (Quinn et al., 2006; Chan et al., 2010), this may further impact fitness prospects in noisy areas. Hence, avoiding noisy areas (given a choice) might well be beneficial for the birds and is in line with observed distribution patterns in the field in different songbird species (Parris & Schneider, 2009; Arévalo & Newhard, 2011; Goodwin & Shriver, 2011; Herrera-Montes & Aide, 2011; Proppe et al., 2013) and with avoidance patterns of (experimentally induced) noisy nest boxes (Kleist et al., 2017; Injaian et al., 2018a) or stopover sites near an experimental ‘phantom road’ (McClure et al., 2013).

The approach used with birds in this study could also be used in other taxa and independent of taxon specific modes of locomotion, which suggests it could be a suitable method for comparative work. Table 4 lists a number of species from other taxa that also suggest active preferences for quiet over noisy space. However, given the methodological differences and types of stimuli used, absence of such preferences in some of the examples is more difficult to interpret, for example in the fish studies, where active noise avoidance was not observed

Zebra finches show spatial avoidance of near but not far distance traffic noise

but where the complexity of sound directivity and propagation underwater, especially in small tanks makes it difficult to create linear noise gradients (Parvulescu, 1967; Akamatsu et al., 2002; Slabbekoorn, 2016). This adds to growing realisation that species, type, and level of anthropogenic noise exposure (and their fluctuations) need better characterisation (S. Gill et al., 2015).

Conclusions

The birds in our test avoided space with near-distance, high amplitude traffic noise when given a choice at no cost of reduced access to food or increased risk of predation. This shows that, although additional (in)-direct factors and interactions will contribute to patterns in the field, active behavioural avoidance of anthropogenic noise (above a certain threshold) will likely contribute to the distribution of birds along roads. We here tested only relatively short-term exposure (hours) and short-term reactions, but the birds clearly showed that given a choice, they preferred quiet over noisy, when above a particular threshold. Future work will have to test how habituation may or may not reduce such avoidance behaviour. Traffic noise is affecting more and more areas on our planet. The chronic noise exposure for animals living in such areas may detrimentally affect them, but may also make animals avoid settling in such areas in the first place. It is thus important to keep in mind that sound can reduce the value of an area of otherwise suitable animal habitat immediately up to quite some distance away from a given noise source. Assessing the distances and thresholds at which different species are affected might be an important aspect to consider for better understanding traffic noise impact and appropriate policy making.

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Appendix

SEM Table 1: Recording locations of the highway noise stimuli

File	Location	coordinates
Near-Site1 A4	Vlietland	52°06'12.5"N 4°26'28.1"E
Far-Site1 A4	Vlietland	52°06'12.5"N 4°26'28.1"E
Near -Site2 A4	Polderpark Cronesteyn	52°08'12.9"N 4°30'07.5"E
Far-Site2 A4	Polderpark Cronesteyn	52°08'12.9"N 4°30'07.5"E
Near -Site3 A44	Oegstgeest	52°10'34.2"N 4°27'33.4"E
Far-Site3 A44	Oegstgeest	52°10'34.2"N 4°27'33.4"E
Near-Site4 A44	Park Landskroon	52°10'22.7"N 4°27'07.6"E
Far-Site4 A44	Park Landskroon	52°10'22.7"N 4°27'07.6"E

Formula used to calculate dB FS

dB to full scale (dB FS) conversion formula used in editing stimuli and making figure 2 and 3.

Step 1: Relative amplitude to full scale (0, 1] = Positive Sample / maximal positive value

Negative sample / minimal negative value

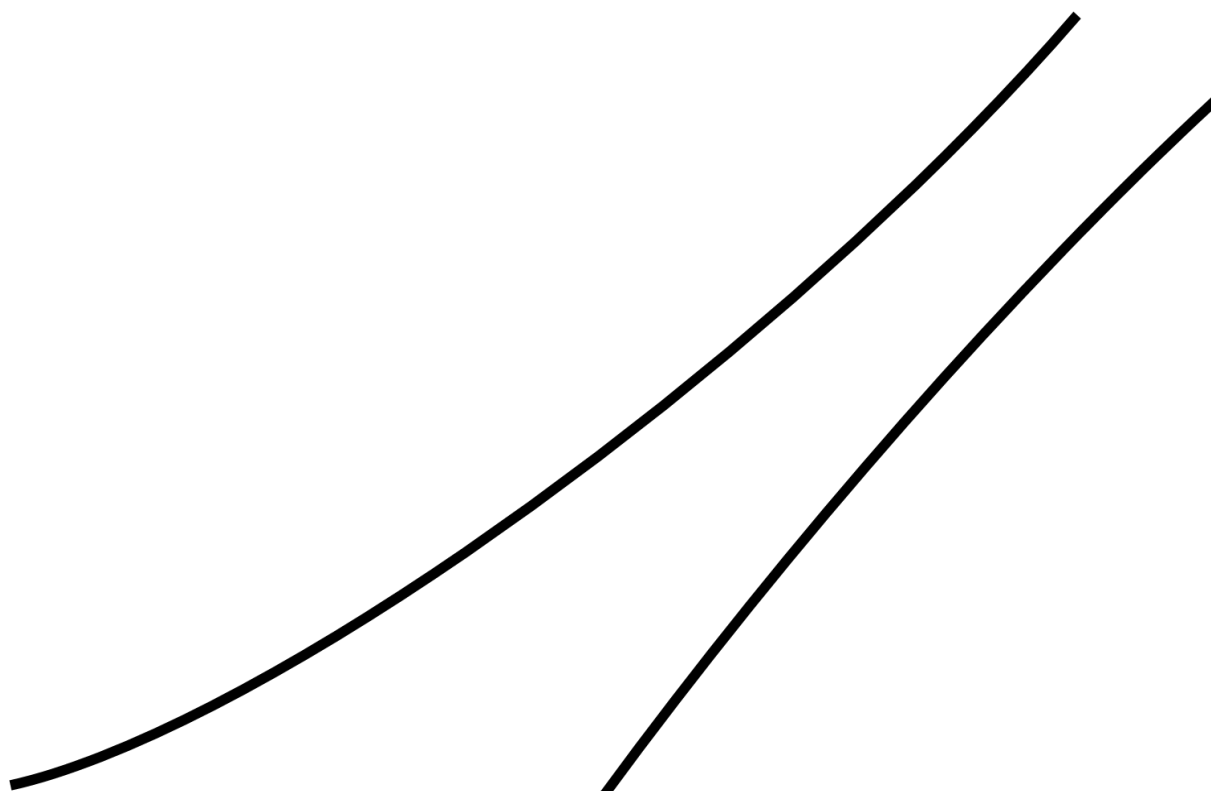
Step 2: Relative dB to full scale $(-\infty, 0] = 20 * \log_{10}^{\text{(Relative amplitude to full scale)}}$

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

Chapter 3

& 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 ± 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 ± 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.

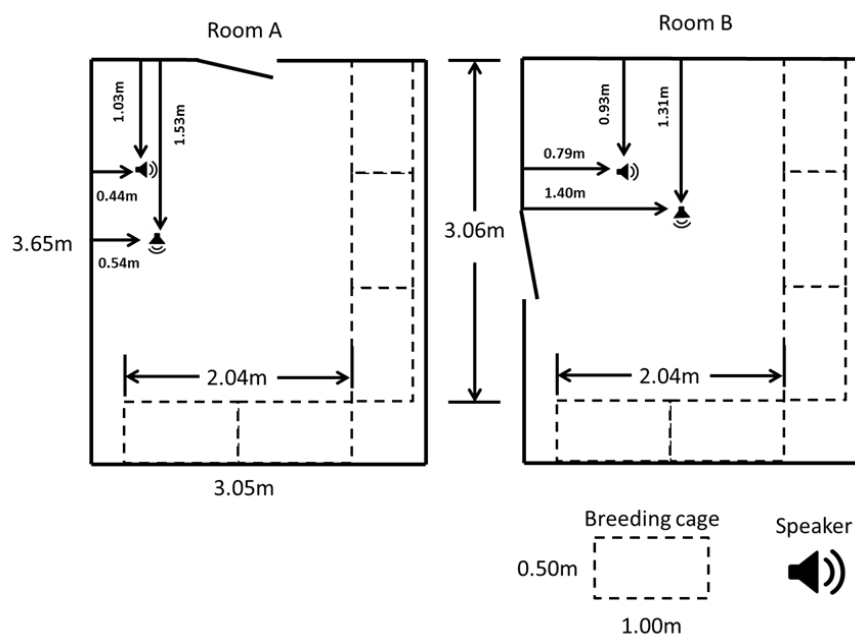


Figure 1: Top view of the two breeding rooms showing the location of the loudspeakers and breeding cages. In each room, 15 breeding cages (100 x 50 x 40 cm) were stacked into three layers onto supporting cabinets (100 x 50 x 60 cm), placed under the lowest layer. The dashed lines represent single columns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

Chapter 3

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 27th and 10am July 28th 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 ± 3.2 dB(A), far-distance: 52.8 ± 5.2 dB(A), ref = 20 μ 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 ± 0.5 dB(A), empty nestboxes 68.5 ± 0.9 dB(A); far-distance traffic noise: cages 51.5 ± 0.4 dB(A), empty nestboxes 51.4 ± 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 ± 1.1 dB(A), far-distance traffic noise: 52.1 ± 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 ± 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 ± 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

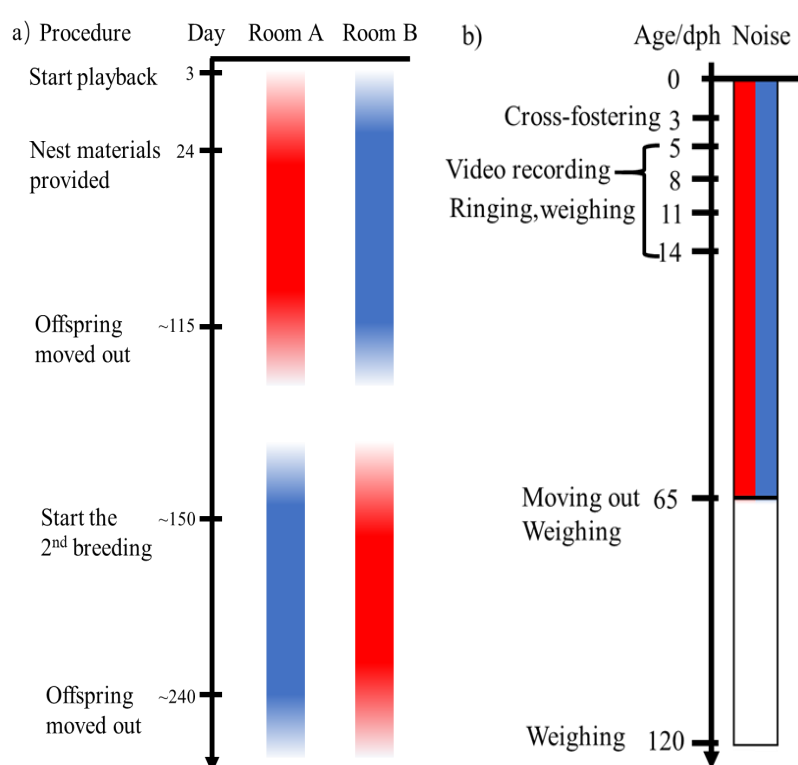


Figure 2: Schematic view of the breeding procedure (a) and experimental procedure (b). The red and blue colour bars stand for near- and far-distance traffic noise. The strength of the colours indicates the relative intensity of the noise playback compared to the peak level. a) Y-axis shows the days since 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.

Chapter 3

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 6th to the end of the 55th 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

Chapter 3

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).

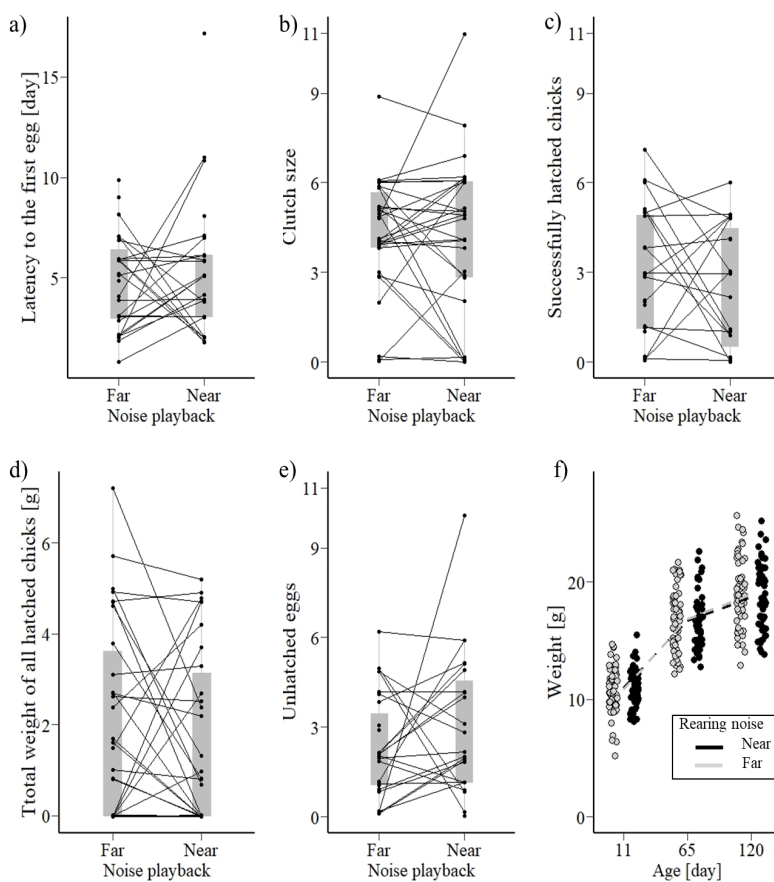


Figure 3: Reproductive performance during breeding rounds with far- vs. near-distance traffic noise. a) Latency to the first egg, b) clutch size, c) successfully hatched chicks, d) total weight of all hatched chicks day 1, e) number of unhatched eggs of each pair in both breeding rounds. Each dot represents 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, 1st and 3rd 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).

Chapter 3

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	χ^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 R² 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	χ^2	P value
Noise				0.41	0.52
	<i>Far</i>	-0.58	1.21		
Age				1544.31	<0.001
	<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		
Brood size	<i>Covariate</i>	-0.60	0.23	11.85	< 0.001
Noise * age				1.31	0.52
Noise * brood size				1.70	0.19
Noise * age * brood size				1.13	0.58

Marginal and conditional R² 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).

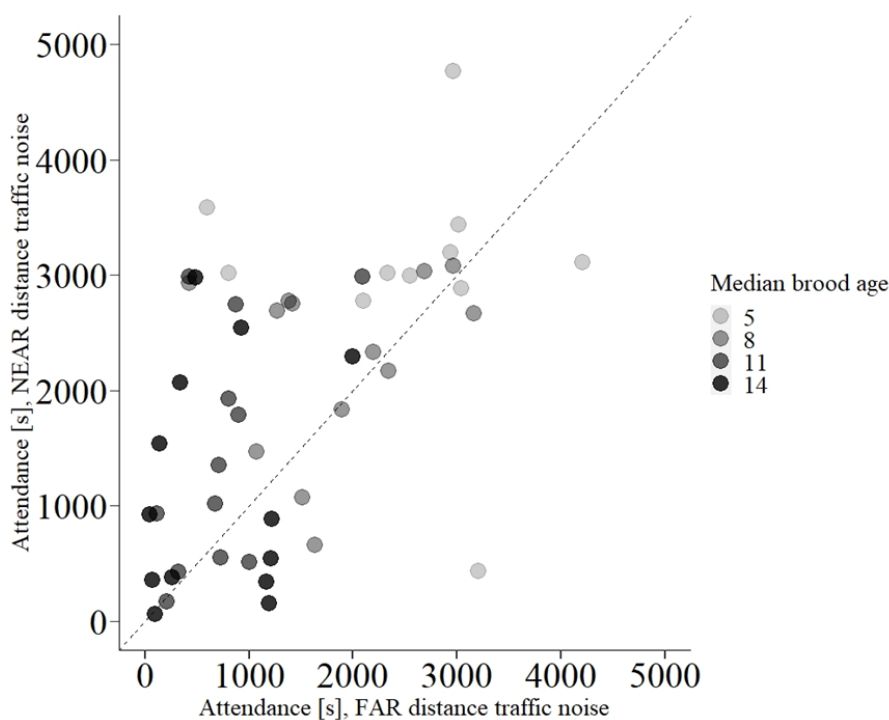


Figure 4: Combined nest attendance of breeding pairs (in seconds). Each dot represents the same pair under two types of traffic noise exposure, with a brood at 5/8/11/14 days of median age. The dashed line gives the expectation for identical behaviour in both conditions ($y = x$). Dots below above dashed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

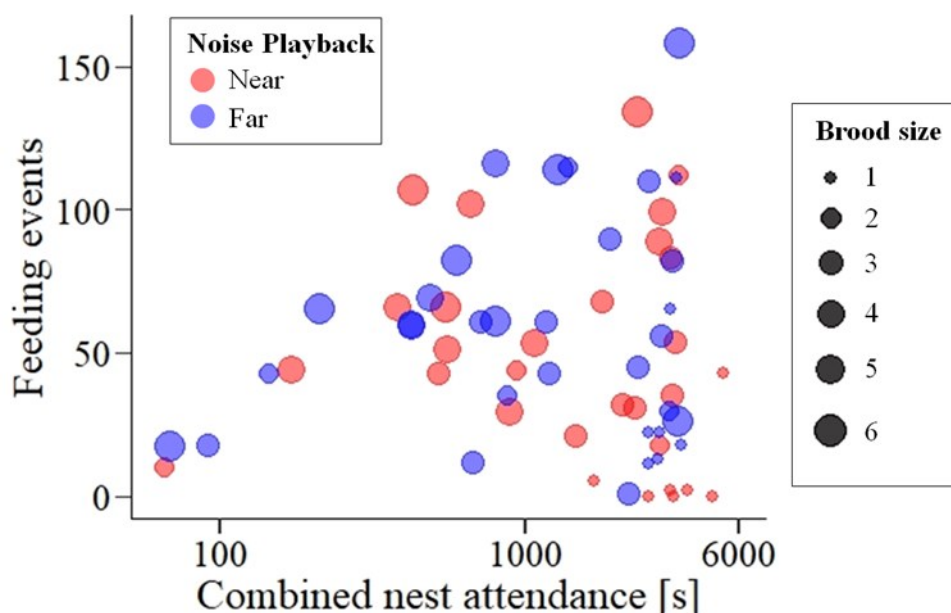


Figure 5: Combined nest attendance is positively correlated with feeding events (data from the first breeding round only). X-axis is plotted on a logarithmic scale. The size of dots increases with brood size. Each dot represents the date from one recording. The increase of feeding rate with increasing 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	χ^2	P value
a) Nest attendance per parent					
Sex				11.11	< 0.001
	<i>Male</i>	-0.10	0.04		
Noise				11.00	< 0.001
	<i>Far</i>	-0.19	0.09		
Round				0.21	0.65
	<i>Second</i>	-0.01	0.02		
Brood size	<i>Covariate</i>	-0.07	0.01	54.10	< 0.001
Brood median age	<i>Covariate</i>	-0.04	0.01	83.29	< 0.001
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					
Noise				13.46	< 0.001
	<i>Far</i>	-0.16	0.07		
Round				0.15	0.70
	<i>Second</i>	-0.03	0.09		
Brood size	<i>Covariate</i>	-0.07	0.001	55.48	< 0.001
Brood median age	<i>Covariate</i>	-0.03	0.00	104.15	< 0.001
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		
Round				9.66	0.002
	<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)					
Nest attendance	<i>Covariate</i>	0.07	0.03	4.56	0.03
Noise				1.50	0.22
	<i>Far</i>	1.75	0.64		
Brood size		0.56	0.13	15.80	<0.001
Brood median age	<i>Covariate</i>	-0.08	0.01	116.00	< 0.001
Noise * brood size				6.02	0.01
	<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; Walther & 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.

Acknowledgements

We would like to thank Esther Gelok and Kiki Fontein for checking breeding birds and scoring video recordings. QL was funded by the Chinese Scholarship Council.

Appendix

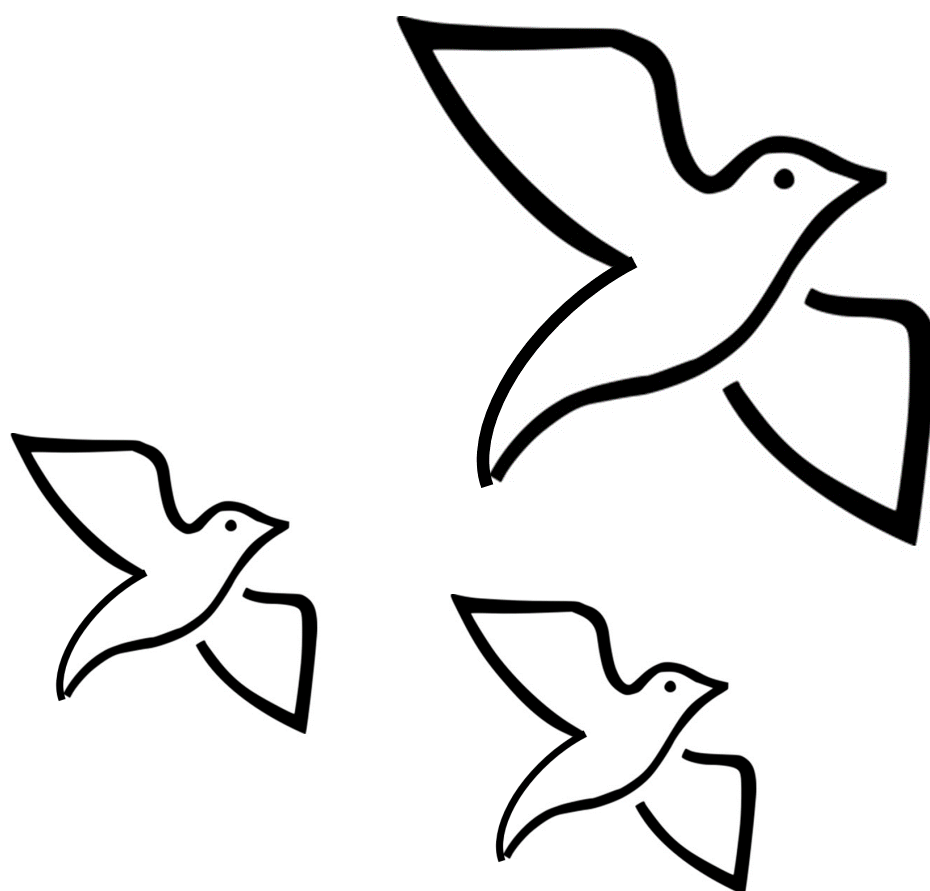
Table SEM 1. Breeding outcomes per pair in both rounds. Means and standard deviations are separately calculated from all pairs ('All pairs') and only pairs that laid eggs ('Bred pairs').

Pair ID	Latency to the first egg [days] ¹		Clutch size		Successfully hatched chicks		Total weight of all hatched chicks [g] ²		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

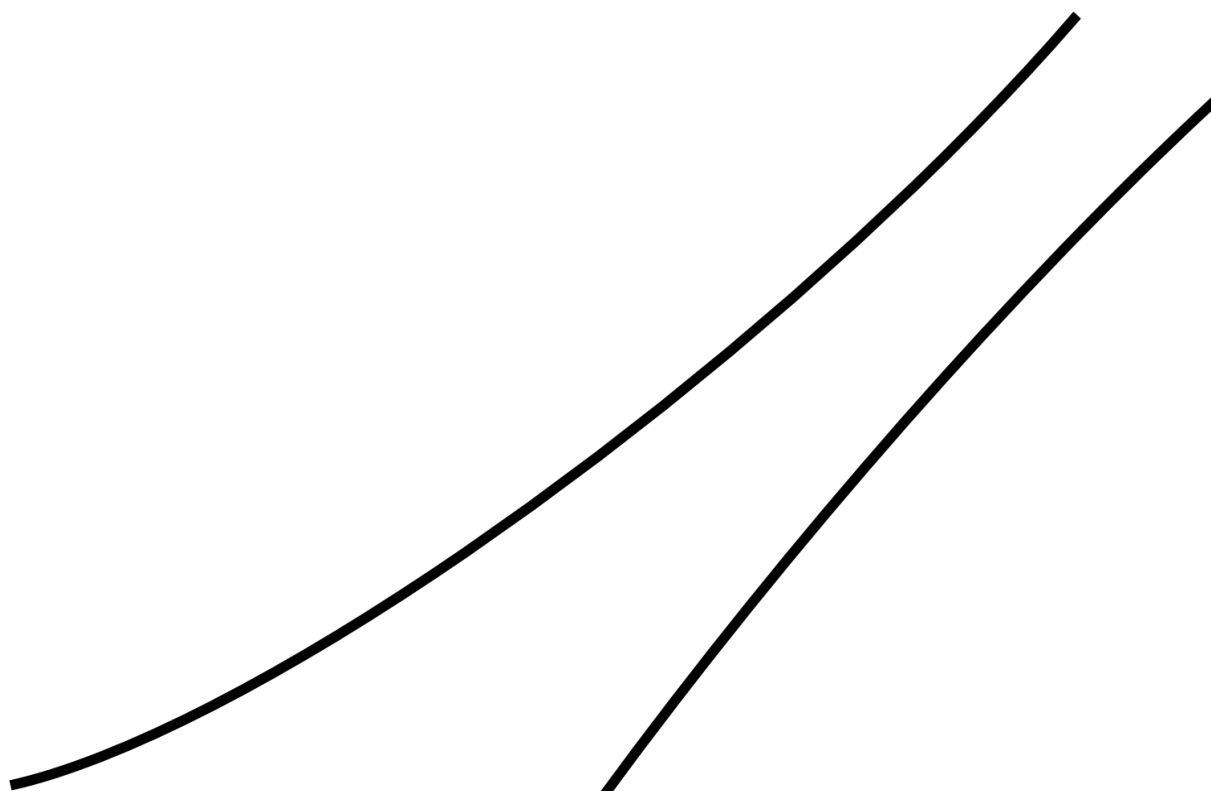
¹ days were counted after providing nesting materials ² chicks were weighted when founded hatched.

Chapter 4

The effects of rearing noise condition on spatial avoidance of traffic noise in juvenile and adult zebra finches



Quanxiao Liu, Hans Slabbekoorn & Katharina Riebel



Abstract

Traffic noise is increasingly recognized to affect the distribution of wildlife and this is especially well documented in birds. Globally, the diversity and the abundance of birds are declining along roads. Noise avoidance has been suggested to be one of the factors contributing to this decline. However, the causality between noise and spatial avoidance is not well established as studies are either observational or, if experimental, only tested adult birds known to be naïve to noise or of unknown origin. To investigate whether early experience with noise would affect noise avoidance behaviour, we here tested experimentally whether chronic exposure to different types of traffic noise during development affected noise avoidance behaviour in zebra finches (*Taeniopygia guttata*). At the age of 65 days, all offspring were tested for noise avoidance in a two-choice set-up allowing free movement between two interconnected aviaries offering different noise conditions. After this first test, experimental birds were housed without noise playbacks and were re-tested two months later when the birds had matured into adults. When the offspring were juveniles, birds from both noise treatment groups did not avoid either near- or far-distance traffic noise but both types of noise conditions were avoided by same-age juveniles raised without noise playbacks (controls). In the second test, all offspring from both noise treatments now avoided both near- and far-distance traffic noise. The results demonstrate behavioural flexibility in noise avoidance, but provide no evidence for early noise exposure permanently increasing tolerance to noise. We interpret the increased tolerance in the noise-reared (but not the control) juveniles as a transient effect of noise experience because the birds reared in noise did not develop into noise tolerant adult phenotypes. The results suggest that noise tolerance is a behavioural trait of behavioural flexibility. Such flexibility might be an adaptation to cope with short-term fluctuations in ambient noise levels across time and space. However, rearing in chronic noise does not necessarily lead to noise tolerant adult phenotypes.

Introduction

Traffic noise is increasingly associated with negative effects on wildlife (Barber et al., 2011; Iglesias Merchan et al., 2014). A number of reports have now revealed noise-related declines in diversity and abundance, particularly for birds (Reijnen et al., 1996; Bayne et al., 2008; Francis et al., 2009; Parris & Schneider, 2009; Arévalo & Newhard, 2011; Goodwin & Shriver, 2011; Herrera-Montes & Aide, 2011; Proppe et al., 2013). Birds may fare less well in noisy conditions because their acoustic signals are masked (Brumm & Slabbekoorn, 2005; Grade & Sieving, 2016; Villain et al., 2016), or because noise makes them forage less efficiently due to distraction or increased vigilance (Quinn et al., 2006; Ware et al., 2015). The above have been hypothesized to contribute to less successful breeding observed at noisy compared to quiet breeding sites (Halfwerk et al., 2011; Kight & Swaddle, 2011; Schroeder et al., 2012). Bird numbers may also decline because birds simply move away to avoid noisy but otherwise suitable habitat (Brown, 1990; Blickley et al., 2012; McClure et al., 2013; Liu et al., 2020). There is as yet little knowledge on transgenerational versus ontogenetic effects of noise on birds, and whether behavioural phenotypes of birds in different habitats arise from selective or from ontogenetic effects from being raised in quiet versus noisy conditions (Dooling & Popper, 2007; Potvin, 2017).

Noise exposure could affect animals' development resulting in phenotypic differences among individuals and populations, but the degree of impact will likely be influenced by the level, frequency, duration and context of exposure. The effects of these noise characteristics are difficult to systematically assess in the field. In controlled experimental laboratory studies, high level sounds near hearing damage levels can cause permanent or temporary hearing threshold shifts (Dooling & Saunders, 1974; Ryals et al., 1999; Valero et al., 2017). Rats (*Rattus norvegicus domestica*) normally avoid low- and high-frequency noise, but experimental long-term exposure to loud high-frequency sound, resulted in permanently shifted hearing thresholds: the rats stopped avoiding high-frequency noise, but started showing stronger avoidances of low-frequency noise (Manohar et al., 2017). Moderate level sound exposure during development could change sensory processing. For example, grey squirrels (*Sciurus carolinensis*) raised in noisy urban area responded more often to visual than acoustic warning signals from a robot squirrel than their rural counterparts (Partan et al., 2010).

Differences such as the one reported between urban and rural squirrel populations (Partan et al., 2010) could be the result of selection for particular pheno- or genotypes that are more

easily settling or surviving in either habitat, but they could also be the result of a chronic noise induced developmental effect. In rats, for example, continuous exposure to moderate level of white noise during development made the primary auditory cortex develop more slowly (Chang & Merzenich, 2003). In guppies (*Poecilia reticulata*), noisy rearing conditions can hamper the development of multisensory integration (Xu et al., 2014). However, some of these shifts might also be a case of adaptive developmental plasticity and sensory switching to other modalities; a way to optimally adapt to environmental conditions, as has been reported for dark-reared guppies switching from vision to olfaction to forage (Chapman et al., 2010; Kimbell et al., 2019). Noise can also affect cognition: juvenile lizard fish (*Synodus dermatogenys*) and moonwrasses (*Thalassoma lunare*) exposed to boat noise during training failed to learn predator odour and survived less well after being released to the sea, indicating the impact of noise on learning about predation risk (Ferrari et al., 2018).

We do not know the underlying mechanisms for most of the above reported changes in the sensory systems and processing. However, at least for birds, there is some evidence that noise exposure during development can affect the pituitary-hippocampus-adrenal axis (Kleist et al., 2018; Beauguard et al., 2019, but also see Angelier et al., 2016), which can affect many aspects of an individuals' behavioural phenotype (Spencer, 2017). However, any of these physiological changes could arise from the noise exposure directly, or be an indirect effect of the noise on the parents and the quality of parental care they provide. Several studies have reported noise-level dependent variation in parental behaviour (Schroeder et al., 2012; Naguib et al., 2013; McIntyre et al., 2014; Leonard et al., 2015; Meillère et al., 2015; Injaian et al., 2018a), highlighting the complexity of these issues and the difficulties to understand the effect of long-term noise exposure on birds directly and on their distribution patterns along roads.

An area that has seen quite some experimental work into the effect of long-term noise exposure during development on adult behaviour concerns vocal development. For example, when male zebra finches were exposed to very loud sound masking part or all of their song during vocal development, the timing and results of song development and crystallization were severely affected (Marler et al., 1973; Funabiki & Konishi, 2003; Zevin et al., 2004; Funabiki & Funabiki, 2009). Even less severe masking and less intense sound were reported to affect vocal learning. Developing white-crowned sparrows (*Zonotrichia leucophrys*) learned preferentially high-frequency songs when exposed to city-like low-frequency noise (Moseley et al., 2018). This result could be explained by selective masking of low-frequency songs leading the birds to only copy the songs they could hear better. This phenomenon may not apply to all species. In great tits (*Parus major*), chicks exposed to noise, masking either

Chapter 4

high (3 - 6 kHz) or low frequencies (0 – 3 kHz), did not show any masking dependent learning of song types (Zollinger et al., 2017).

Although noise exposure during development has been shown to cause adjustments in physiology and song, we know little to nothing about the avoidance behaviour of birds raised in noisy conditions. Observational studies in the wild documenting settlement tendencies to vary across areas with different ambient noise levels (Halfwerk et al., 2011; Kight et al., 2012; Schroeder et al., 2012) cannot rule out confounding effects of other factors varying among territories. Moreover, these studies have no information of the rearing noise levels the settling individuals may have experienced. It is important to stress that while sound level dependent settlement patterns in adult birds can be a direct effect of some birds avoiding settling under aversive noise levels, differential settlement can also arise from condition-dependent success in getting a high-quality territory (Reijnen & Foppen, 1991). Playback experiments in the field (Blickley et al., 2012; McClure et al., 2013) and the laboratory (Evans et al., 2018; Liu et al., 2020) are useful to gain insight into noise avoidance tendencies, but such studies have typically used adult birds that were naïve to noise or were of unknown rearing background. Furthermore, birds may show experience-dependent changes in their noise avoidance tendencies in the course of their development, while most exposure experiments only tested birds once at a specific stage in life. Therefore, to examine if chronic exposure to traffic noise affects noise avoidance behaviour in birds, a controlled experiment with tests at different stages during development is required.

Zebra finches are an excellent model to test how sound exposure during development affects noise avoidance. Zebra finches have a relatively short generation time and breed easily in captivity, allowing to breed the same pairs under different acoustic conditions in brief succession. The hearing curve and thresholds for auditory damage are also known for this species (Okanoya & Dooling, 1987; Ryals et al., 1999; Noirot et al., 2011), which allows working with sound levels of potential behavioural consequences, but that do not accidentally lead to physiological hearing impairment. Zebra finches have been shown to be affected by sound levels elevated above regular ambient noise with respect to song learning, communication and foraging (Cynx et al., 1998; Tumer & Brainard, 2007; Villain et al., 2016; Tachibana et al., 2017; Evans et al., 2018). Furthermore, in an earlier experiment, we have shown with a two-choice paradigm that domesticated adult zebra finches naïve to noise, avoided traffic noise recorded at near but not at far distances from highways (Liu et al., 2020). This setup thus provides an excellent approach to test noise reared offspring, of the previously (naïve to noise) tested adults, to investigate whether noisy rearing conditions affect noise avoidance behaviour as well as whether this behaviour changes over time.

In the current study, we tested whether birds, that grew up under constant exposure to two types of traffic noise, showed noise avoidance behaviour that differed from their parents, and whether it was dependent on the type of rearing noise. To this end, one group was raised at a level that had been avoided by their parents (high intensity traffic noise, recorded near a Dutch highway), while another group was raised with a noise level that was shown not to be aversive to their parents (recorded far from the same highway, Liu et al. 2020). The birds were tested twice, once as juveniles and once as adults. If early noise exposure has an effect on noise avoidance, the offspring should behave differently than control (no noise playback) birds of the same age when tested as juveniles. If there is a long-term effect, the offspring raised in noisy conditions should also behave differently from their parents when tested as adults.

Material and Methods

Subjects and housing

All subjects originated from the breeding colony at Leiden University. To set up breeding pairs, 30 males and females were selected from the experimental subjects that had been tested by Liu et al. (2020) for noise avoidance (details see below). Preceding the experimental breeding, the birds (age: 900.9 ± 60.3 days old) had been housed in one all-male and one all-female aviary of identical make and size (L x W x H: 200 x 200 x 200 cm). To start breeding, birds were randomly paired and each pair was assigned to a breeding cage (100 x 50 x 40 cm) in one of two identical breeding rooms (365 x 305 x 300 cm, see Figure 1 in Chapter 3). In each room, 15 identical breeding cages were stacked in 5 (3+2) columns along two adjacent walls with 3 cage layers, so that they formed an L-shape (with the lowest cages 60 cm above the floor). For each cage, a nest box (11 x 9 x 9 cm) could be inserted via a small trap door at the top right corner of each cage.

Playback stimuli for continuous noise exposure

Traffic noise of a Dutch highway was recorded for 24 hours, from 10 am - 10 am on 27 - 28 July 2017, using two song meters (Wildlife Acoustics, model SM1), at two locations, near (52.098504N, 4.439159E, 50 meters from highway) and far (52.103469N, 4.441135E, 300 meters from highway) from the highway A4 (main route for cars between Amsterdam and Rotterdam/The Hague) near Park Vlietland (details in Liu et al. 2020). During the first three minutes of each recording, a sound pressure meter (Model 30, Pulsar Instruments Plc, Filey,

Chapter 4

UK, settings: A-weighted, reading LAT at a 10 s interval) was positioned at the microphone of the song meters to measure absolute sound pressure levels at the recording locations. The sound pressure levels (reference level throughout this study: SPL re 20 μ Pa) were 68.7 ± 3.2 and 52.8 ± 5.2 dB(A), for near- and far-distance traffic noise respectively. These levels were used as reference for later playback levels.

Experimental procedure for breeding with chronic noise exposure

Two loudspeakers (Blaupunkt CB 4500) were positioned in each room so that during playback of the highway recordings, noise levels were almost the same in all cages (near-distance traffic noise treatment: cages: 70.2 ± 0.5 dB(A), nest boxes: 68.5 ± 0.9 dB(A), far-distance traffic noise treatment: cages 51.5 ± 0.4 dB(A), nest boxes 51.4 ± 0.6 dB(A), measured with a sound pressure level meter (Model 30, Pulsar Instruments Plc, Filey, UK, settings: A-weighted, reading LAT at a 10 s interval).

Each pair participated in two rounds of breeding: once with continuous playback of the near-distance noise and once with continuous playback of the far-distance noise. In the first round, Room A was assigned to the near-distance traffic noise exposure and Room B to the far-distance traffic noise exposure treatment. The experiment started with introducing the breeding pairs to the breeding cages and leaving them for 3 days to acclimate before the noise playbacks started. For the next 14 days, the volume of the playbacks was gradually increased daily, starting from zero, by 5 dB(A) for near-distance traffic noise, and by 4 dB(A) for far-distance traffic, until the sound level inside the breeding cages reached the amplitude levels that had been measured at the recording site. Once the final levels had been reached the breeding pairs were given another week to acclimate before we introduced nest boxes (11 x 9 x 9 cm, attached to the top right front corner of each cage) and nesting material (hay and coconut fibre).

From then on, all nest boxes were checked daily for new eggs and hatched chicks. Chicks were individually marked on the day of hatching, or the day after, by cutting down feathers at one or two places of six specific body parts (head, back, left or right leg, or left or right wing, see Adam et al. 2014). Chicks (within a treatment) were cross-fostered around 3.6 ± 1.7 dph to break any correlation between parental quality and brood size, and to ensure also having a number of larger brood sizes, as we expected potential interactions between noise treatment and brood size dependent body condition of the chicks (see chapter 3). Full sibling groups were split up whenever possible and the age composition was aimed to emulate those found in natural broods. Final brood sizes ranged from 1 to 6. At 11 days post hatching (dph), just before the down feather cuts were no longer recognisable, chicks were marked by attaching an orange plastic ring with a unique identity number to their left leg.

The effects of rearing noise condition on spatial avoidance of traffic noise in juvenile and adult zebra finches

At 65 ± 4 dph, chicks were caught and weighed and they received an additional ring on the right leg with a micro transponder tag (ID100A, Dorset, Aalten, The Netherlands). After tagging, they were moved to the noise avoidance test aviaries (see below). After all chicks from both breeding rooms had been moved out, the traffic noise playbacks were gradually faded out in the course of one week until levels were back to base line ambient levels (10 dB(A)/day for near-distance traffic noise and 8 dB(A)/day for far-distance traffic noise). Breeding pairs were given a two week break with these ambient levels, before the second breeding round started. Pairs remained in their cages. The noise treatment was now reversed between rooms but all other procedures were an exact replicate of the first breeding round. Figure 1 gives a schematic overview of the procedures and the timeline. Most pairs bred in both conditions (details see chapter 3) and all offspring (near-distance: 37 females and 22 males; far-distance: 45 females and 18 males) were subsequently tested in the noise avoidance test. In addition, another 28 control subjects (14 females and 14 males) were recruited from the la-

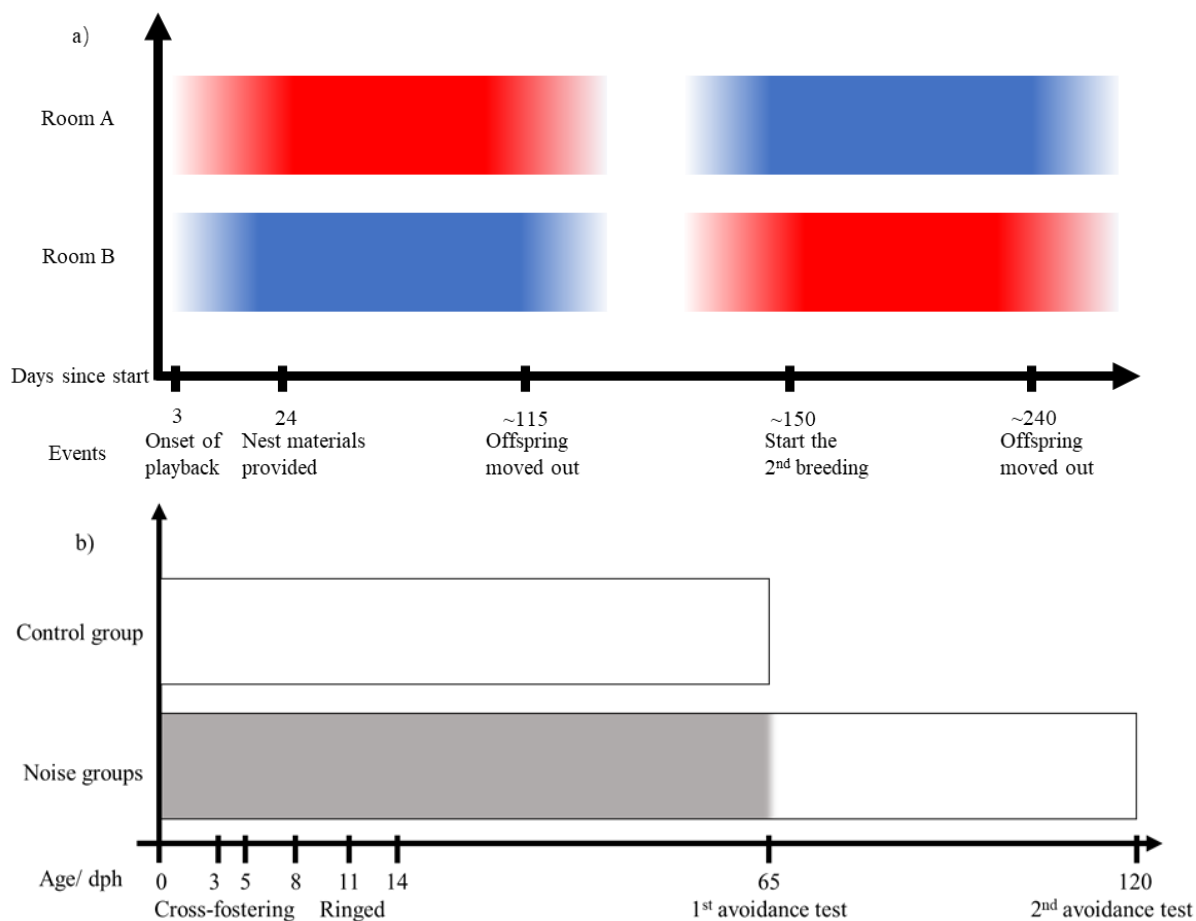


Figure 1: a) Overview of the treatment schedule for the breeding pairs in the two experimental rooms. Red and blue colours indicate exposure to noise recorded at a near or far distance from the highway. The intensity of the colours stands for the relative noise levels to their peak level. b) Experimental procedure for the offspring. The grey bar symbolises the noise exposure (either level), the white bar the absence of playback (= “lab ambient”).

Chapter 4

boratory breeding stock after the second breeding round. These young birds had been raised without any noise playback (ambient levels in their breeding room had always been lower than 40 dB(A) when the birds were quiet).

Behavioural testing: noise avoidance test

Experimental subjects were tested twice: as juveniles (65 ± 4 dph) and as young adults (115 ± 17 dph). Control birds were only tested as juveniles, but not again as adults as testing all the experimental offspring at a comparable age was given priority and because a large number of naïve adult birds (the parents of the subjects) had already been tested in the setup previously (Liu et al. 2020). Birds were tested in groups of four, and with subject originating from at least 2 different nests (number of groups, 1st round = 26, 2nd round = 19) with the exception of a total of eight groups that were tested in smaller groups because no same age peers were available (seven groups of three: 1st round = 2, 2nd round = 5; and one group of two birds in the 1st round). Juveniles were tested as they reached 65 days, and groups could be all-male or all-female or mixed, but adults were tested in all-male or all-female groups only to avoid males and females engaging in courtship. To start a test, a test group was moved into the experimental setup to acclimate 18 hours before the test started. During this phase, there was continuous playback of the test group's rearing background noise.

The two-choice set-up consisted of aviaries (200 x 200 x 200 cm) that were connected by a wire mesh tunnel (100 x 50 x 50 cm). All side walls of the aviaries and the tunnel were covered by 3 cm thick sound attenuating foam. Inside each aviary, we placed one loudspeaker (CB4500, Blaupunkt, Hildesheim, Germany) at 1 m height in the corner furthest away from the tunnel. A webcam (HD Pro C920, Logitech, Lausanne, Switzerland) was installed at the top of the corner facing the tunnel. Four parallel perches, perpendicular to the tunnel, were hung from the top of the aviary and a fifth perch was placed in the tunnel. Food and water dispensers were suspended from the two outermost perches, granting birds *ad libitum* access to food and water. The connecting tunnel had an opening (50 x 50 cm) at each end that could be opened and closed remotely. The middle of the tunnel could be passed only through a 30 x 30 cm black metal antenna (ANTSER300, Dorset, Aalten, The Netherlands) connected to a PC outside the room to register the IDs of the tags of passing birds.

On test days, a noise avoidance test always started between 9 am and 10 am. After a pre-playback phase of 15-minute rearing noise, the groups were exposed to playback 1 and playback 2, which included presentation (in a fully balanced design) of the following playback stimuli: 1) quiet vs far-distance traffic noise (~55 dB(A)) or 2) quiet vs near-distance traffic noise (~70 dB(A)), with a 15-minute break in between. For each of these first two playback trials, the loudspeaker in one aviary started to play a 30-minute-long noise stimulus with a 2

min fade-in and 2 min fade-out time while the loudspeaker in the other aviary was turned on but not playing back any sound. Once the first 30 min playback was finished, the previously silent speaker started to play the same stimulus (for another 30 min) and the previously noisy speaker became silent. This playback was followed by a 15 min break, then birds were exposed to playback 3. The procedure of playback 3 was the same as for the two previous playbacks, but now the loudspeakers in the two aviaries played near- and far-distance traffic noise simultaneously for 30 minutes, and then after stimulus reversal for another 30 minutes. All playbacks were balanced with respect to which type of noise was played back in which aviary first. This test procedure was identical to the procedure used by Liu et al (2020), except for two minor modifications: 1) during the acclimation and pre-playback phase, the speakers in both aviaries were playing back birds' rearing noise and 2) the simultaneous playback phase in Liu et al (2020) consisted of one 30-minute simultaneous playback, now this phase was extended for another 30 minutes to also have a stimulus reversal during playback 3.

Each group that had completed their first preference test was then moved and from then onwards housed with other groups in single-sex aviaries (100 x 200 x 200 cm, quiet laboratory ambient conditions (< 40 dB(A))). Two months later, as adults, these birds were tested for a second time, but now always in male or female only groups to avoid interest in potential partners affecting spatial behaviour (group size four birds: n = 20 groups; three birds: n = 5). The protocol of the second test was identical to the first, except that birds now received no noise playback during the acclimation and pre-playback phases, because the acclimation phase was set to match housing noise levels (which were without noise playbacks at this stage). After completing the second test, subjects were returned to their home aviaries.

All tests were filmed with the webcam in the aviary and behaviours were scored using BORIS video analysis software (v. 6.1.6; Friard & Gamba, 2016). One of us (QL) conducted all video analyses blindly with respect to the noise playback (sound was turned off during scoring) and subjects' rearing conditions (videos were coded by random numbers). The main response variable for the tests was the cumulative time birds spent in either aviary during each phase of the experiment (pre-playback, playback 1, 2 and 3). The cumulative score for an aviary could range from 0 min (not a single bird visited) to 240 minutes (4*60 minutes, 4 all birds stayed for the whole time).

Analyses

Prior to statistical analyses, the proportion of time spent in the relatively more quiet aviary was calculated for all groups and all playbacks (for pre-playback: quiet vs. quiet, we used the time spent in aviary A). These values were arcsine square root transformed for normality.

Chapter 4

All statistical analyses were performed in R 3.5.2 (R Core Team, 2019). To test whether proportion of time spent in the quiet room was deviating from a 0.5 chance level, we subtracted the (arcsine square root transformed) chance level of 0.5 from each of these values and then tested for a significant deviation from zero.

We then analysed if juvenile birds from all rearing conditions used both aviaries equally during the pre-playback phase using one-way ANOVA, with rearing noise condition (near/far/control) as the factor. To test whether rearing noise levels affected time spent in the aviary during playbacks 1 and 2, we used a mixed linear model with ‘time spent in the quiet aviary’ (without noise playback) as response variable, ‘group ID’ as random intercept, rearing condition (near/far/control), noise playback type (near/far) and their interaction as fixed factors (Table 1, Model A, lmer function from the ‘lme4’ package in R). To further analyse the differences among treatments, the least-squares means (EMMs) for three rearing conditions were computed and compared with the ‘emmeans’ function in the R package ‘emmeans’ (Table 1, Model A, EMMs).

For Playback 3, a linear regression model was created with time spent in the aviary with far-distance traffic noise as response variable and rearing condition (near/far/control) as the fixed factor (Table 1, Model B, ‘lm’ function from package ‘stats’ in R). Similar to playback 1 & 2, EMMs were computed to analyse the differences among all three rearing conditions (Table 1, Model B, EMMs). We applied the same statistical methods to the data from the second test round with the adults, but then with two, instead of three, rearing conditions (near/far).

Results

As juveniles, birds from all rearing conditions spent equal amounts of time in either aviary during the pre-playback phase (one-way ANOVA, $F_{2,46} = 0.16$, $p = 0.85$, Figure 2a). During playback 1 & 2, zebra finches from both noise treatments did not avoid either type of traffic noise while the control birds, raised in quiet ambient conditions, always avoided noise (Figure 2b-c). In addition, the avoidance was stronger towards near- than far-distance traffic noise (EMM contrast test: playback near vs. far, $p = 0.006$). During playback 3, when exposed to simultaneous playback of near- and far-distance traffic noise, control birds and birds raised in far-distance traffic noise avoided near-distance traffic noise. In contrast, the

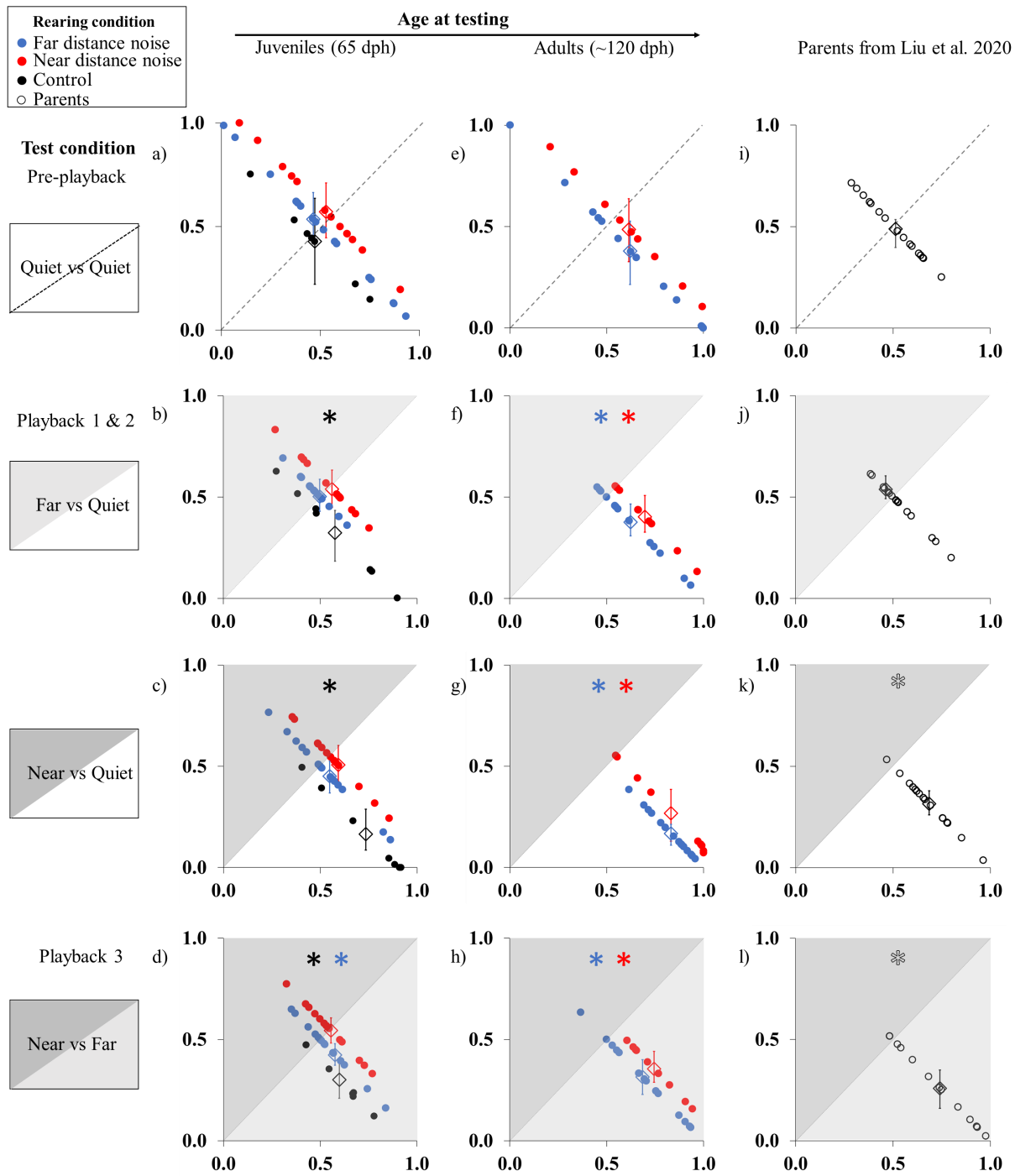


Figure 2: Percentage of time spent by each group in either aviary during the noise avoidance tests. Each dot represents one tested bird group (blue/red/black indicate near-distance noise/far-distance noise/control rearing conditions). Open diamonds with error bars represent mean \pm se per treatment. The data of the parental generation (from Liu et al., 2020) are provided for reference. To avoid dots overlapping, dots were slightly shifted diagonally away from the ‘ $y = 1 - x$ ’ line by adding 0.05. Significances are indicated by * in the colours corresponding to the rearing conditions.

Table 1: Models and least-squares means (EMMs) comparisons of time spent in the more quiet aviary of the tested juvenile zebra finch groups from the three different rearing conditions in playback 1, 2 & 3.

Rearing conditions	estimate	Std. Error	p value	lower limit	upper limit
<i>Model A: Far/Near vs Quiet: Rearing condition (R)* + Playback type (P)* + R:P¹, as juveniles</i>					
<i>Model A – EMMs1, Playback type = Near</i>					
Control birds	0.35	0.07		0.21	0.48
Far	0.03	0.05		-0.06	0.12
Near	0.03	0.05		-0.08	0.12
Control birds – Far	0.32	0.08	0.0005		
Control birds – Near	0.33	0.09	0.0004		
Near – Far	-0.01	0.07	0.99		
<i>Model A – EMMs2, Playback type = Far</i>					
Control birds	0.15	0.07		0.01	0.29
Far	-0.01	0.05		-0.06	0.14
Near	0.04	0.05		-0.10	0.08
Control birds – Far	0.16	0.09	0.42		
Control birds – Near	0.11	0.08	0.13		
Near - Far	0.05	0.07	0.73		
<i>Model B: Far vs Near: Rearing condition*, as juveniles</i>					
<i>Model B – EMMs1, Playback type = Near vs Far</i>					
Control birds	0.16	0.05		0.06	0.26
Far	0.04	0.03		0.03	0.13
Near	0.00	0.04		-0.06	0.07
Control birds – Far	0.11	0.06	0.37		
Control birds – Near	0.15	0.06	0.04		
Near - Far	-0.04	0.05	0.18		

¹ R:P stands for the interaction between rearing conditions and playback types; * the factor is significant in the model, $p < 0.05$

Table 2: Models and least-squares means (EMMs) comparisons of time spent in the more quiet aviary of the adult zebra finch groups from two rearing conditions in playback 1, 2 & 3.

Rearing conditions	estimate	Std. Error	p value	lower limit	upper limit
<i>Model C: Near/Far vs Quiet: Rearing condition (R) + Playback type (P)* + R:P¹, as adults</i>					
<i>EMMs, Playback type = Near</i>					
Far	0.38	0.05		0.28	0.48
Near	0.35	0.06		0.22	0.47
Near - Far	-0.03	0.08	0.67		
<i>EMMs, Playback type = Far</i>					
Far	0.14	0.05		0.04	0.23
Near	0.16	0.06		0.03	0.29
Near - Far	0.02	0.08	0.76		
<i>Model D: Far vs Near: Rearing condition, as adults</i>					
Far	0.21	0.05		0.11	0.31
Near	0.21	0.06		0.08	0.34
Near - Far	0.00	0.09	0.98		

¹ R:P stands for the interaction between rearing conditions and playback types; * the factor is significant in the model, $p < 0.05$

birds raised in near-distance traffic noise did not avoid the near-distance noise and spent similar amounts of time in both aviaries (Figure 2d).

In the second test round, which took place two months after the first test (the juvenile birds had now matured into adults), we only tested the birds from the two noise rearing groups. They used both aviaries equally during the pre-playback phase (one-way ANOVA, $F_{1,22} = 0.21$, $p = 0.65$, Figure 2e). During playbacks 1 & 2, birds from both treatments avoided either noise playback and more strongly so the louder, near-distance traffic noise playbacks (Table 2, Figure 2f-g). During playback 3, birds from both treatments also avoided near-distance traffic noise (Figure 2h).

Discussion

We tested whether chronic exposure to traffic noise during development affected noise avoidance behaviour in zebra finches. To this end, breeding pairs were given the opportunity to breed in two different noise conditions. Noise avoidance behaviour in their offspring was tested at both the juvenile and adult stage. Additionally, a control group of zebra finches was tested as juveniles. Experimental rearing noise affected the noise avoidance behaviour in juveniles. Juveniles from both chronic noise exposure treatments did not avoid noise recorded at near or far distance from a highway. In contrast, same-age juveniles from the control group, reared in quiet conditions, avoided both the near- and far-distance traffic noise and preferred the quiet space. When simultaneously presented with both near- and far-distance traffic noise, juveniles raised in near-distance traffic noise behaved indifferently and showed no preference or avoidance of either type of noise. In contrast, both the controls and the birds raised in far-distance traffic noise significantly avoided the near-distance traffic noise and preferentially stayed in the aviary with the playback of the far-distance traffic noise. In the second test round, as adults, birds from both treatment groups now avoided both near- and far-distance traffic noise (see Figure 2f and 2g). Birds from both noise treatments equally strongly avoided near-distance traffic noise and moved into the aviary with far-distance traffic noise during the simultaneous playbacks (see Figure 2h).

The experimental birds that were reared with chronic highway noise exposure, when tested as juveniles, did not avoid traffic noise in favour of quiet and thus behaved strikingly different from controls that did move away from noise in favour of quiet. The treatments seemed to make juvenile birds more tolerant to the noise, a phenomenon that could have been in-

Chapter 4

duced by changes in hearing physiology, such as hearing damage or temporary threshold shift. Hearing damage is however an unlikely cause of the behaviour of the birds in this experiment. The birds were exposed to noises around 55 and 70 dB(A) and these two levels are considerably below the estimated threshold of around 93 dB(A), identified as the level to cause temporary threshold shifts in zebra finches (Dooling, 2011). Zebra finches exposed to continuous noise at 110 dB(A) from 35 days old for more than hundred days (Funabiki & Konishi, 2003), were reported to have some hair cell damage, but the hearing of these birds started to recover within two days after the exposure and was fully recovered after four days. Since 110 dB(A) contains around 10000 times more energy than the 70 dB(A) we used, and earlier work has already demonstrated that exposure up to 93dB(A) does not cause hearing damage, hearing damage seems no likely explanation for the behaviour during the preference test. It is also unlikely that the lack of avoidance was caused by the lack of familiarity to the set-up in the first test because the control groups were also naïve to the set-up, but showed clear avoidance of the noise.

Although hearing damage is thus most unlikely, the lack of avoidance in our juvenile zebra finches might still originate from other effects of the chronic noise on the development of receiving and processing sounds. Noise can induce delayed song maturation (Funabiki & Konishi, 2003). Exposure to loud noise after 35 days of age caused juvenile males to crystallize their songs much later than the normal age. The delay was associated with a lack of structural and physiological changes in song nuclei during the noise exposure, probably caused by the absence of auditory feedback. Among these subjects, birds exposed to noise for more than 80 days had lower resemblance to their tutor in terms of temporal order of syllables. Similarly, noise exposure during early development may have delayed some aspects of auditory processing involved in the noise avoidance response. This could have changed during the subsequent two months without noise and this might have led to recovery or catch up of a noise avoidance response which the birds did exhibit at the later test as adults.

We lack sufficient insight into the potential impact of noise exposure on brain development as an explanation for our results. Nevertheless, exposure to traffic noise has been shown to impact developing birds in several ways including body condition (Schroeder et al., 2012; Injaian et al., 2018b), growth rate, oxidative stress (Injaian et al., 2018a), telomere degradation (Dorado-Correa et al., 2018; Injaian et al., 2019), immune system (Crino et al., 2013) and corticosterone levels (Kight & Swaddle, 2011; Kleist et al., 2018; Beaugeard et al., 2019, but see Angelier et al., 2016). Only one study to date has measured two brain areas related to song learning (Area X and HVC, Potvin et al., 2016) in birds exposed to recorded traffic urban noise during development. The authors report effects of noise on brain development, but the results are difficult to interpret, as only the traffic noise of moderate level but

not the other (including the high levels) had an effect on the structure volume of HVC. Even less is known regarding the effects of noise exposure on the processing of non-song learning related sounds. Therefore, physiological and neurological studies are required to confirm whether noise exposure delays the development of the auditory system and/or the processing of sounds.

Another explanation for our results may be a compensatory sensory switch. Birds have been observed to temporarily switch from using auditory to visual cues in noisy areas while foraging. Zebra finches and chaffinches (*Fringilla coelebs*) have been shown to tilt their heads up more often for scanning, when foraging in noisier conditions (Quinn et al., 2006; Evans et al., 2018). Similarly, the juveniles in our study, raised in noisy conditions, may also have been relying more on visual signals and less on acoustic signals to orientate in their environment, therefore showing no avoidance to the noise. Such sensory switches have been documented in several species when one of multiple sensory modalities is compromised by the environment (reviewed by Partan, 2017). In guppies, a sensory switch has been linked to rearing environment. Guppies raised in low-light conditions tended to respond more to chemical instead of visual cues (Chapman et al., 2010) and turbid water rearing conditions may also make guppies to develop opsins that are more sensitive to long wave lengths that could help motion detection in turbid waters (Ehlman et al., 2015). This particular type of compensatory plasticity normally restricts the animals to the developed phenotype, which was clearly not the case in our birds. However, a less restricted type of sensory plasticity may still explain our results.

Habituation, which can act on a much shorter time scale, and for a much shorter period than sensory plasticity, could be another mechanism (Rankin et al., 2009) that can explain the behaviour of the birds in our test. In experimental tests, exposing previously naïve birds to high levels of noise, depending on the species and the characteristics of the ambient noise, adult animals were observed to habituate to noisy environments from within a day to tens of days (Conomy et al., 1998; Brumm & Slabbekoorn, 2005; Masini et al., 2008; Linley et al., 2018; Neo et al., 2018). In the experiment described here, the juveniles are likely to have habituated to the noise after having been continuously exposed to the same noise stimuli for around 65 days. It is also possible that not the upbringing for the whole period in the noise was important, but that only the exposure in the days just before the tests mattered. The second test took place two months after the first test and after two months housing in the more quiet conditions, any habituation that might have been present would have been lost and that would explain why the adult noise reared birds now started avoiding the noise. That the experimental birds were habituated to the noise when tested as juveniles, also seems a more likely explanation than that the experimental birds lacked the skills to manoeuvre the large

Chapter 4

aviaries because they had been reared in cages. This is unlikely, because the control birds, like the experimental birds, had also been raised in cages and not experienced the aviaries before the test but showed clear noise avoidance and moved into the more quiet aviaries. Since the aim of this study was to test how noise exposure during development would affect birds rather than how long it takes for juveniles to habituate to noise, our experimental design did not include sufficient experimental noise rearing groups to vary the duration of the noise exposure which would have been necessary to titrate how long it would take the birds to habituate and to dishabituate. We therefore cannot draw any further conclusions now as to whether habituation or a temporary sensory threshold shift was most likely responsible for the difference between experimental and control birds.

It is also possible that the birds from the experimental treatments did not avoid the noise, because they had not yet experienced that they could actively move away from a noise source because the noise in their rearing environment was ubiquitous and showed no gradients. The position of the loudspeakers had specifically been arranged such that the noise levels were constant in the rearing cages. The birds had thus never experienced that they could actively fly away from noise before they participated in the noise avoidance tests in the aviaries. This may explain why the birds did not avoid the noise during their first test, as exposure to non-avoidable negative stimuli has been shown to affect the acquisition of appropriate performance in an avoidance task (Seligman & Beagley, 1975; Peterson, 2010; Swanson et al., 2012). This could also explain why as adults, the same birds that now had experienced quiet environments, avoided the noisy aviary.

Variation in physiological state arising from developmental stress has been shown to affect information processing in zebra finches in the contexts of mate choice, social learning and exploration (e.g. Holveck & Riebel, 2009; Krause & Naguib, 2011; Riebel et al., 2012). It is of interest to establish whether chronic noise exposure during development would have similar effects (for discussion, see Potvin, 2017). As yet, little is known about effects of noise exposure on animal decision making and cognition although it might have far reaching fitness consequences. For example, juvenile lizard fish and moonwrasses, failed to associate an odour with a predator when the odour was presented simultaneously with boat noise during training sessions in the laboratory (Ferrari et al., 2018). In the following experiment, a new batch of fishes underwent the same procedure. Fishes exposed to noise during training survived less well after being released to the sea than the control groups experienced no noise during training. This result indicates the potential impact of noise on learning about predation risk and suggests that it might also affect learning in other contexts.

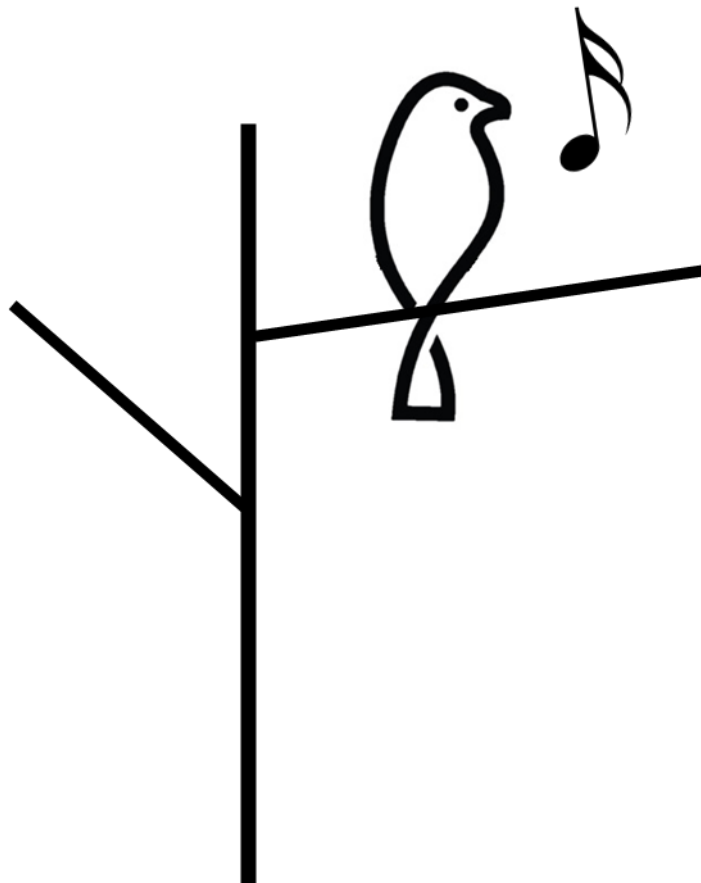
To summarise, from our results, it does not seem likely that noisy rearing conditions could

potentially create a more noise resistant next generation. While it remains to be further investigated which mechanisms underlie the differences in behaviour between the control and the experimental groups at day 65, we can infer for now that the effects of the noise treatment had different consequences in the short and in the long term. The traffic noise exposure until 65 days affected the birds' behaviour in the first (juveniles) but not in the second test (adults). This suggests that the rearing noise did not induce adult noise tolerance. Instead, the noise tolerance of our test birds decreased after the birds had also experienced quiet conditions. If rearing noise does not induce more noise tolerant phenotypes, noise avoidance behaviour will be likely to continue to contribute to diminishing bird numbers in noisy habitats. Our results are not only in line with but also providing a possible explanation for field observations showing that noisy but otherwise suitable habitats often have lower bird numbers than comparable quiet control habitats, although the presence of the noise source has spanned several generations and despite ongoing breeding activities in the noisy areas. Noise avoidance of otherwise suitable habitat may not be unique to birds. Mammals, reptiles, fish and insects also show reduced abundances in noisy versus quiet habitats (Shannon et al., 2016; Bunkley et al., 2017). Future studies of the developmental effects of noise exposure are required to fully understand the mechanism(s) underlying transient, but apparently reversible effects of experience-dependent noise tolerance as found in the current experiments. What can be concluded for now is that the increased noise tolerance in the juveniles is not long lasting: rearing in noise did not lead to a noise resistant phenotype. Our observations may have implications for further investigations into declines in bird diversity and breeding densities along noisy roads.

Acknowledgments

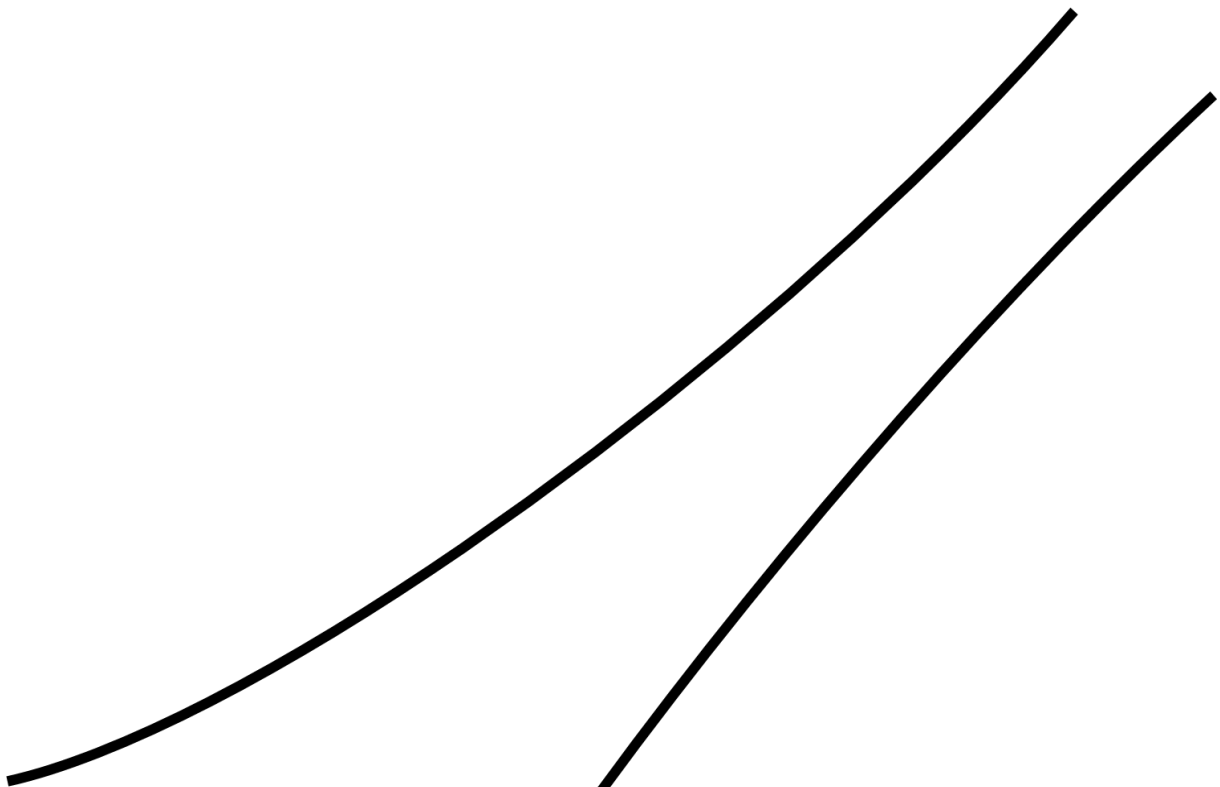
We would like to thank Susanne Campo, Esther Gelok and Kiki Fontein for helping with the experiments. QL was funded by the Chinese Scholarship Council. This research was supported by the Dr. J.L Dobberke Foundation for Comparative Psychology (UPS/BP/3927 2015-19).

The effect of rearing noise conditions on singing effort and exploration tendency in adult zebra finches



Chapter 5

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Abstract

Birds are increasingly exposed to anthropogenic noise worldwide. Traffic noise is particularly pervasive and birds in populations in noisy areas show behavioural differences in contexts such as foraging, breeding, singing and responsiveness to threats when compared to those in more quiet areas. However, it is as yet unclear whether these behavioural changes arise from noise and if so whether it is current, recent or chronic exposure to noise. A particular vulnerable period could be juvenile development but there is little experimental work on the question as to which extent noisy rearing conditions shape adult behavioural phenotypes. Relatively high ambient noise levels could affect development directly or indirectly (via parental effects) and result in phenotypic differences in adult behaviour. We here investigated if rearing noise levels affect singing effort (number of song bouts, song bout duration and total singing duration) and exploration tendency, two well studied behaviours known to show phenotypic plasticity in songbirds. Zebra finch (*Taeniopygia guttata*) siblings raised under different types of traffic noise exposure were compared in their singing effort (males) and their explorative tendencies (both sexes). Noise treatment had no significant effect on the singing efforts of males nor on the variation in performance in the exploration test. We discuss these results in view of findings in the literature and discuss future avenues to explore potentially long-term effects of chronic noise rearing conditions on a broad range of adult behaviours.

Introduction

Bird populations in urban areas are often reported to behave differently from birds in rural areas (Evans et al., 2010; Lowry et al., 2013; Papp et al., 2015). The difference in anthropogenic noise levels between the noisy urban areas and the more quiet rural areas may potentially be a causal factor explaining or contributing to the changes in behaviour (Slabbekoorn, 2013). Birds living in high level noise habitats have been found to be more aggressive towards same-sex territorial competitors (Phillips & Derryberry, 2018; Wolfenden et al., 2019), sing with different acoustic structures (Ríos-Chelén et al., 2012; Guo et al., 2016; Luther et al., 2016; Hill et al., 2018), or at different times (Dominoni et al., 2016; Bermúdez-Cuamatzin et al., 2020) than individuals from the same species living in more quiet habitats. Such population differences could be the outcome of selection for particular genotypes in noisy environments. However, if chronic noise exposure is also a form of development stress that can induce phenotypic behavioural differences in birds (Buchanan, 2011; Farrell et al., 2015), population differences may also arise from being exposed to chronic noise during development.

Individuals could also differ in their behaviour because of shorter-term behavioural adjustments. A number of studies using a variety of methods have investigated whether short-term noise exposure ranging from minutes to hours affects birds' behaviour. Using audio playbacks of traffic noise, birds have been reported to avoid traffic noise in the field (McClure et al., 2013) and in the laboratory (Liu et al., 2020). Noise playback has also been shown to increase vigilance (Owens et al., 2012) and, if played back during foraging, to reduce food intake in birds (Quinn et al., 2006; Evans et al., 2018). Playbacks of noise can advance the dawn chorus (Arroyo-Solís et al., 2013), cause an immediate increase in song amplitude (Cynx et al., 1998; Brumm & Todt, 2002; Templeton et al., 2016), or lead to change in song structure (Bermúdez-Cuamatzin et al., 2011; Potvin & Mulder, 2013). During breeding, artificially elevated noise levels in the nest can hamper the detection of a returning feeding parent (Leonard & Horn, 2012; Lucass et al., 2016) and make the breeding birds more vigilant (Meillère et al., 2015). These studies focused primarily on behavioural changes in birds' responses to experimental, temporary noise exposure which was higher than they normally experience in their environment. It is unclear whether birds that have been familiar with such noise levels for long periods, or even grew up under such noisy conditions will show the same type of behavioural reaction to the experimental noise exposure.

Chapter 5

While still relatively little is known about long-term effects of noise on behaviour, several studies have investigated the long-term effects of rearing noise conditions on stress physiology. The baseline corticosterone of nestlings in three songbird species was negatively correlated with noise levels near their nestboxes. However, the level of acute corticosterone response among the same birds was positively correlated with their rearing noise levels (Kleist et al., 2018). When exposed to traffic noise playbacks until fledging, tree swallow (*Tachycineta bicolor*) chicks had higher baseline corticosterone levels and stronger stress-induced response levels (Injaian et al., 2018, 2019). Changes in baseline corticosterone levels can indicate developmental stress, which may lead to consistent phenotypic differences later in life (Buchanan, 2011; Boogert et al., 2014; Farrell et al., 2015; Brandl et al., 2019), including song development (Spencer et al., 2003). As there is increasing evidence that chronic noise is a potential stressor, we can expect long-term behavioural effects of chronic noise exposure during development (Potvin, 2017).

Several studies have touched upon the long-term effects of developmental noise exposure on singing behaviour in birds. Extremely loud noise can mask the auditory feedback needed for song learning in birds (Marler et al., 1973; Funabiki & Konishi, 2003; Funabiki & Funabiki, 2009). Zebra finches and white-crowned sparrows (*Zonotrichia leucophrys*) chicks exposed to anthropogenic noise at more ecologically relevant levels during song development were found to change the spectral components of their songs (Potvin et al., 2016; Moseley et al., 2018) but a comparable study (Zollinger et al., 2017) in great tits (*Parus major*) did not find any effect on song spectral parameters. These experimental studies focused on structural aspects of bird songs but other, more performance related aspects of singing can also be affected. For example, blackbirds (*Turdus merula*) near Madrid Airport sung more and earlier than birds at a nearby control site (Sierro et al., 2017) and great tits at urban versus rural sites differed in the timing and amount of singing (Bermúdez-Cuamatzin et al., 2020).

Variation in singing effort has not been investigated experimentally in the context of rearing noise levels in songbirds, but singing effort has been found to be associated with other phenotypic traits known to contribute to avian behavioural syndromes like exploration (Naguib et al., 2010, 2016). Interestingly, exploration is often tested as a part of the shy-bold continuum in birds and may differ systematically between populations of the same species occupying noisy and quiet habitats (Lowry et al., 2013; Tryjanowski et al., 2016; Thompson et al., 2018; Biondi et al., 2020). Consistent habitat-dependent differences in both singing behaviour (Slabbekoorn & den Boer-Visser, 2006; Slabbekoorn & Ripmeester, 2008) and exploration could result from developmental plasticity or selection for different song and behavioural types (Slabbekoorn & Smith, 2002; Patricelli & Blickley, 2006; Slabbekoorn, 2013). Experimental studies specifically testing whether chronic noise exposure during develop-

ment is a causal factor contributing to behavioural differences in adult phenotypes could help understand how such population differences could arise.

Zebra finches are a suitable model system to test for long-term behavioural effects of noise exposure on singing and exploration. Zebra finches have been tested experimentally for impact of short-term noise exposure on intra-pair communication, foraging and spatial preferences (Villain et al., 2016; Evans et al., 2018; Liu et al., 2020). How early developmental stress affects singing behaviour of this species has been studied extensively (e.g. Spencer et al., 2003; Buchanan et al., 2004; Gil et al., 2006; Brumm et al., 2009, Holveck et al. 2008). Zebra finches show interindividual differences in explorative behaviour that can be easily measured using already validated exploration tests (Schuett & Dall, 2009; David et al., 2011). In a previous study, we have shown that birds raised in different levels of traffic noise may behave differently in terms of noise avoidance (Chapter 4). This suggests noisy rearing conditions potentially have other behavioural effects in zebra finches. Good candidate behaviours known to show phenotypic plasticity, namely singing and explorative behaviour, seem good starting points to investigate this further.

In the current study, we studied the effect of high level noise exposure during rearing conditions on adult behaviour focusing on singing effort and explorative behaviour. We first examined the repeatability of our exploration test in our laboratory population and then used these birds as parental generation to raise offspring under two noise exposure treatments: continuous highway playback recorded at either near (15 m) or far (200 m) distance from a highway. In the offspring generation, we tested the explorative behaviour of both sexes in a novel environment test and measured the singing effort of males when moved to a recording chamber away from the colony.

Material and Methods

Subjects and housing

All subjects from this study originated from the breeding colony at Leiden University (The Netherlands). The individuals from the parental generation were the birds reported in Liu et al. (2020) and their offspring (see chapters 2 & 3). Figure 1 gives a schematic overview of all procedures: subjects of the parental generation (N = 48; 24 males and 24 females) were tested for behavioural syndromes among several traits (Liu, Amin et al. in prep) and noise avoidance (Liu et al., 2020). Birds had been raised in family groups and from 65 days old

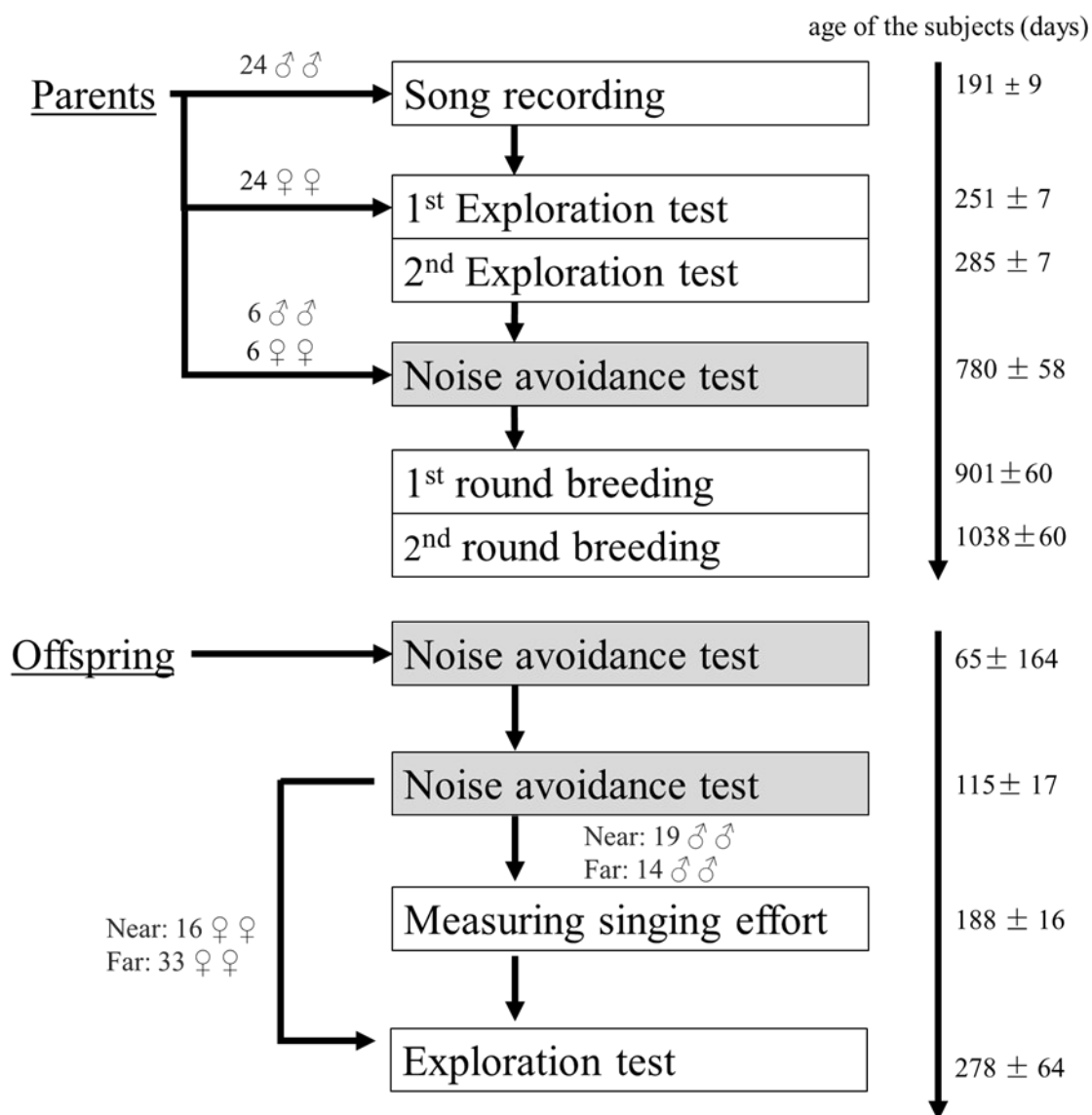


Figure 1: Timeline and procedures of the study. In the parental generation, males were recorded for their songs first. Males and females were then tested in a novel environment for explorative behaviour. Subsequently, all tested birds plus an additional 12 new birds (6 males, 6 females) were randomly paired to breed while exposed to one of two types of traffic noise. All male offspring’s singing effort was recorded at about 4 months of age, and at around 10 months all offspring were tested in an exploration test.

onwards subjects were housed in same-sex aviaries (L x W x H: 180 x 80 x 200 cm, 10 - 20 birds). Before testing, birds were moved from the aviaries to smaller group cages (80 x 40 x 40 cm) holding four individuals each. From these cages, individual males were taken to sound proof chambers for song recordings (see below). At this stage males were on average 191 ± 9 days old. One month later, all birds had been recorded and four weeks after the recording of the last bird, the exploration test with all males and females started (the average of subjects of both sexes was now 251 ± 7 days). The exploration tests were all conducted within the course of two weeks. The subjects were tested a second time about a month later (at 285 ± 7 days old). Between and after the tests, birds continued to be housed in their group cage. All subjects, plus an additional 6 males and 6 females, were tested at 901 ± 60 days old to establish preference between two types of traffic noise recordings in an unconstrained two-way choice test (Liu et al., 2020). Using the insights from this experiment, three months later, birds were then allowed to breed with continuous exposure to 24 hour traffic noise recordings, one level of which had been avoided in the aforementioned experiment and another level that had not been avoided (details in Liu et al 2020, see chapter 2). Using these types of noise stimuli for the breeding experiment, would allow us later to compare explorative behaviour of birds raised in two different noise conditions: one that was aversive to their parents (noise experimental) and another one that was not (noise control).

To initiate breeding, males and females were randomly paired and moved to individual breeding cages (100 x 50 x 40 cm). Each cage had a nestbox that could be attached or removed any time during the experiment via a removable door of metal bars. All breeding cages were placed in one of two breeding rooms of the same size (365 x 305 x 300 cm) and made from the same materials. The breeding experiment is described in detail by Liu et al. (see Chapter 3). Briefly, in each breeding room, 15 cages were stacked along two of the walls forming an L-shape of three and two cages wide and three cages high. In the corner of the room, opposite the L-shaped cage wall, two loudspeakers (Blaupunkt CB 4500) were positioned to continuously play back traffic noise. In one room this was the near-distance highway noise, in the other the far-distance highway noise. The loudspeakers were positioned and calibrated such that during playback sound pressure levels were almost the same in all the cages and within the nestboxes (reference level: SPL re 20 μ Pa, far-distance: cages 51.5 ± 0.4 dB(A), empty nestbox 51.4 ± 0.6 dB(A), nestbox with nest: 52.1 ± 0.4 dB(A); near-distance: cages 70.2 ± 0.5 dB(A), empty nestboxes: 68.5 ± 0.9 dB(A), nestbox with nest: 69.1 ± 1.1 dB(A)). The levels in the room and cages were equivalent to the levels at the original recording sites in the field (for more information on stimuli, see Liu et al. 2020). Before starting the noise exposure playback, pairs were given 14 days to acclimate to the room at ambient sound levels. Playbacks were then started and were linearly increased over the next

Chapter 5

two weeks until the full levels of the original recordings were reached. At this stage nest boxes were introduced. Pairs were then allowed to breed and the noise continued until chicks in both rooms were moved out at 65 dph. Nestboxes and nesting material were also removed now and the amplitude of the playbacks was gradually faded out over the next seven days returning the rooms to their baseline level of ambient sound (< 40 dB(A) when birds were quiet). After a break of 14 days without playback (and a total of 146 days after the start of the first breeding), playback treatments were resumed but reversed between rooms. For the next 14 days playback levels were thus gradually raised again to the respective full treatment levels when birds were given nestboxes for a second breeding round. After the second breeding round, each pair had thus bred once with continuous playback of near- and once with far-distance traffic noise. Because parental quality and brood size are both factors known to affect nestling development and song learning, chicks were cross-fostered with non-siblings whenever possible keeping brood sizes between 1-6 chicks, the range observed in our colony (for all details see Chapter 3). Chicks stayed with their parents until 65 dph. Then they were tested for noise avoidance behaviour for 2 days (for details see Chapter 4), after which the birds were moved into aviaries (100 x 200 x 200 cm), with birds of the same sex. At 188 ± 16 dph, male singing effort was recorded. At 278 ± 64 dph, offspring of both sexes could voluntarily participate in an exploration test (see below).

Throughout the experiments, all birds were kept at a temperature of 20 - 22 degree Celsius with humidity levels ranging from 45 - 63%. The light:dark regime at all rooms was 14:10 with a daily 30 minute transition period between light and dark where the lights were gradually in- or decreased until on or off. Birds had *ad libitum* access to water and food throughout. This was supplemented with egg food, fruits and vegetables, every other day, and germinated seeds twice per week.

Song recordings and singing effort measurements

To record songs, males were caught from their home cage or aviary between 0800 and 1200h and moved individually to a recording cage (100 x 60 x 30 cm), placed into one of two identical sound attenuated recording chambers (300 x 125 x 240 cm). The cage had a solid floor but the sidewalls and ceiling were made from mesh wire and thereby acoustically transparent. An omnidirectional microphone (Sennheiser MKH50-P48) hung above the cage (50 cm above the perches in the cage) and recorded directly onto the hard disk of a computer placed outside the chamber (sound card: TASCAM UH-7000, Ishmael software: version 1.0.2.0 with sampling frequency of 44.1 kHz, accuracy of 16 bits, file format: .wav). As soon as a bird had been placed into the setup, the experimenter started the software that automati-

cally recorded sounds in the cage. The next day, after 24 hours of recordings, the experimenter stopped the recording software and returned the bird to its home aviary.

To measure the singing effort, all audio recordings from the 24 hours after the software started were subsequently screened for song utterances with a self-written script in R that used validated amplitude interval thresholds to automatically find and then save songs as .wav files (for script, see <https://github.com/TempestMars/Songdection>). The extracted songs were inspected (by QL) using spectrogram displays (Audacity 2.2.0, window size: 1024, window type: Hanning, zero padding factor: 1, gain (dB): 20, range (dB): 80, frequency Gain (dB/dec): 0, Audacity Team, 2019) with concurrent audio playbacks to remove the false positives (this was very rare, and these were mostly long series of calls). From these files, the number of song bouts, song bout duration and the total singing duration within the recorded 24 hours were obtained as aspects of singing effort.

Exploration tests: parental generation

In the parental generation, 24 males and 24 females were tested twice (1st: 251 ± 7 days of age, 2nd: 304 ± 7 days of age) for explorative behaviour in one of two novel aviaries (200 x 150 x 200 cm) with an identical interior but mirrored in the arrangement of features. Each aviary had two artificial trees (2 m high wooden poles) placed at ca. 73 cm distance on the diagonal of the aviary. Each tree had two wooden rods (100 cm long, 18 mm diameter) pushed through a hole in the pole at 80 or 100 cm creating two perpendicular branches. In sum, the novel aviary had two trees and eight branches and one water and one food dispenser placed on the ground. To start a test, a subject was carefully caught in the home cage, put in a transportation box (20 x 30 x 20 cm) and placed within this box onto the floor in the novel aviary by the experimenter (BA) who would immediately leave the room afterwards. The door of the box could be opened remotely from outside of the aviary by pulling on a string that opened the bolt of the door which marked the start of the trial. The door was always opened within a minute after the bird in the transport box had been moved into the aviary. All trials lasted one hour and were video recorded (webcam Logitech Pro Webcam C920). After one hour, the trial ended and the experimenter entered the aviary, caught the bird and returned it to its home cage. All trials took place between 0800 and 1600 h. Each bird's starting time during the first trial was also used again as starting time when the bird was tested for the second time 34 days later.

Exploration tests: offspring generation

All offspring were tested for participation in a voluntary exploration test at 278 ± 64 dph. Birds of the same sex from the same breeding round were housed in one large aviary (200 x 200 x 200 cm), connected to a second 'novel' aviary of the same size through a mesh wire

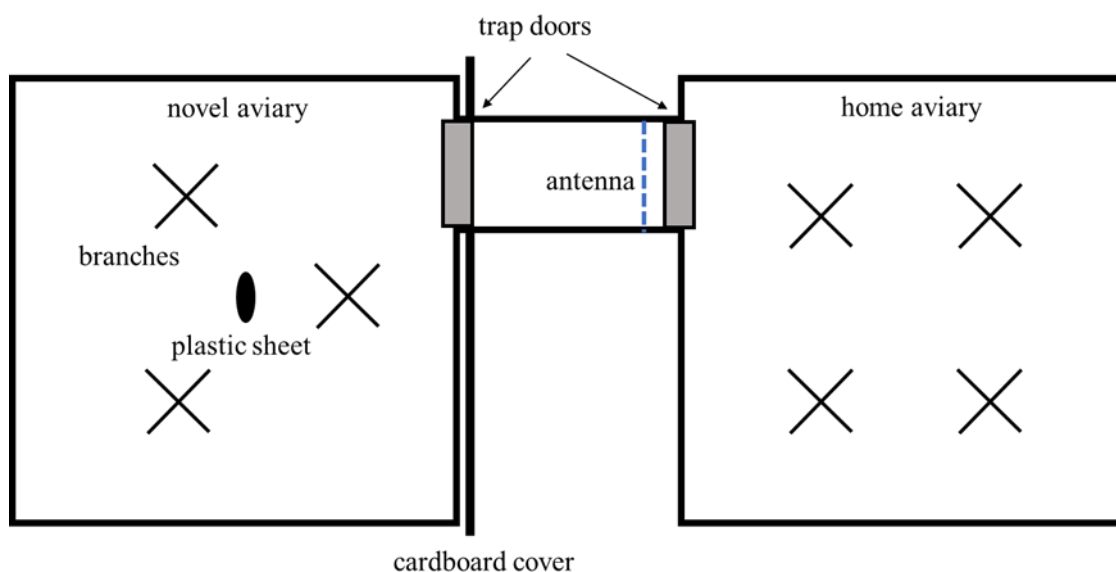


Figure 2: Top view of the voluntary exploration test setup. The novel aviary was connected to birds' home aviary through a tunnel with a trap door at either end which could be remotely opened and closed. An antenna near the entrance to the novel aviary identified passing birds ID tags and send a signal with this information on identification(s) to a PC placed outside the room.

tunnel (see Figure 2, 100 x 50 x 50 cm). At both ends of the tunnel, there was a trap door that could be opened or closed from outside the room via a simple string-pulling mechanism. The entrance to the tunnel and the novel aviary were filmed via webcams (webcam Logitech Pro Webcam C920) and also connected to a PC and monitor outside the room. An antenna was placed right behind the trap door at the end to the home aviary for automatic, real-time identification of passing birds through a leg-mounted micro transponder tag (antenna ANT-SER300, tags ID100A, Dorset, Aalten, The Netherlands) allowing immediate identification of a passing bird so that the experimenter outside the room could decide to allow or deny the bird to proceed to the novel aviary.

The outer walls of the novel aviary facing the subjects' home aviary were fully covered by cardboard pieces so that birds could not see this aviary from their home aviary and vice versa. In the novel aviary, three artificial trees were evenly placed, each with one top branch and one bottom branch, positioned in the same way on the trees as had been done for the trees in the parents' novel environment tests. For the offspring, other than for the parents, all the "branches" of the artificial trees were wrapped with white paper to make the novel aviary look different from the home aviary where subjects were housed with the same type of artificial trees and branches. Moreover, a black plastic sheet (80 x 60 cm of surface) was suspended from the top of the aviary such that it hung among the three trees and partially blocked the view from the tunnel, so that birds could land on the first tree, but had to fly around the sheet to get into the aviary to find additional branches to perch on. One food and one water

feeder were placed on the ground in the middle of the aviary. A webcam (Logitech HD Pro Webcam) was positioned at one wall so that all tree branches were visible in the recording.

To start a trial, the experimenter sat outside the room in front of a PC monitor showing the webcam's recording of the entrance to the tunnel and waited until a bird entered the tunnel. The monitor also showed the read-outs of the identification tags. If the passing bird had not been tested before, the experimenter would instantly close the trap door to the home aviary and open the door to the novel aviary. If the bird passing the antenna had been tested already, the door to the novel environment would not open and the experimenter would wait for at least ten minutes. If the bird was still in the tunnel, the experimenter would operate the door and gently encourage the bird to move back into the home aviary. If there were multiple birds in the tunnel, the experimenter would also operate the door in the same way to keep only one bird in the tunnel. When the trap door to the novel aviary opened, the webcam started to record for 90 minutes. After the recordings, the trap door to the home aviary opened again to allow the birds to fly back.

Scoring of video tapes

In the parental generation, the videos were scored using the VLC-Media Player software (Version 2.2.1, with default settings, at normal speed). All events were manually written down by the experimenter (BA). The latency for the bird to leave the transportation box (defined to be the moment when both feet were outside the box) was scored as emergence time. The total number of different branches visited by each bird were also scored. A branch visit was defined as any event where the bird landed with both feet on the branch at least once. Interobserver reliability was very high: the scores for 10 recordings were consistent between two observers (BA, SV, correlation test, intraclass correlation coefficient = 1). For the offspring generation, the first 80 minutes of all video recordings were scored in 'BORIS' (Friard & Gamba, 2016). The scoring method was the same as for the parents except that we used the 'point event' function in BORIS to record the birds' visiting to different branches instead of writing it down. To score the exploration behaviour in the voluntary test, birds were given a score of 1 for entering the novel aviary and for each newly visited branch another 1 was added to the score. Once a branch had been visited, repeat visits to a previously visited branch received no extra scores. After the testing in the novel environment, birds scores could thus range from 1 (bird had entered but not visited any branches) to 7 (birds had entered the new aviary and visited all branches). In addition, birds that did not participate (i.e. did not enter the novel environment) were given a score of 0. The experimenters who scored these videos (KF, CD, QL, intraclass correlation coefficient = 1) were blind to the treatment of the birds.

Chapter 5

Statistical analyses

All statistical analyses were conducted using R (R Core Team, 2019) statistical software (v. 3.5.3). We first tested if total number of song bouts and bout duration of the offspring were affected by rearing noise conditions using mixed linear models. In these models, rearing noise (far/near) and breeding round (first/second) were fixed factors, brood size was a covariate, (experimental) and brood IDs were random intercepts. The model included the interaction between rearing noise and brood size. For the bout duration, bird IDs were also treated as random intercepts. To test if the total singing duration was affected by rearing noise conditions and whether total singing duration was correlated with explorative behaviour, a linear mixed model was created with the logarithmically transformed total singing duration as response variable; rearing noise and breeding round as fixed factors. The model also included the interaction between rearing noise and brood size. Exploration score and brood size were covariates. Brood IDs were random intercepts.

The repeatability of the main response variable (number of different branches visited) in the exploration test of the parental generation was calculated with a generalised linear mixed model (lmer package), with the number of different branches visited as response variable (Poisson distribution); sex (female/male) and test round as fixed factors; emergence time, age, test date and time as covariates (linearly and quadratically, numeric values were scaled to mean = 0, sd = 1); and bird ID as random intercepts. The purpose of this full model was to find the best fitting model to calculate the repeatability of the birds (random intercepts) in this test through model selection (MuMIN package). The model with the most degrees of freedom within $2 \Delta AICc$ was selected as the best model. Finally, the repeatability of the exploration test was estimated (using rptR package) in R(epeatablity) value of the random intercepts (bird IDs) in the best model (Nakagawa & Schielzeth, 2010), following the method used in several other studies (Patrick & Weimerskirch, 2014; McCowan et al., 2015; Wuerz & Krüger, 2015; Thompson et al., 2018).

To test if rearing noise conditions affected offspring's explorative behaviour in a novel environment, a generalised linear mixed model was created: exploration score as response variable (Poisson distribution); rearing noise, breeding round, sex and the housing group ID (a/b/c/d, birds were housed as two male and two female cohorts) as fixed factors; brood size was a covariate; brood ID was random intercept to account for the variance caused by the social parents and the siblings. The model was built through the glmmTMB function from the package 'glmmTMB'.

Results

Singing effort

During the recorded 24 hours, male birds raised in near-distance traffic noise (n=26 males) sung on average 307 ± 18 song bouts with an average duration of 1.79 ± 0.80 s and total duration of 533 ± 788 s. Males raised in far-distance traffic noise (N=19 males) sung on average 360 ± 19 song bouts with an average duration of 1.55 ± 0.70 s and a total duration of 564 ± 717 s. Rearing noise conditions did not affect any of the aforementioned parameters (Figure 3, Table 1).

Table 1: Results of (generalised) linear mixed model analyses of singing activity: a) number of song bouts, b) song bout duration and c) singing duration were not affected by the rearing noise.

Fixed factors	Level	Estimate	S.E.	χ^2	P value
a) Number of song bouts: noise + noise x brood size + breeding round + brood size					
Noise				0.05	0.83
	<i>Far</i>	2.02	1.26		
Breeding round				1.69	0.34
	<i>Second</i>	0.82	0.63		
Brood size	<i>Covariate</i>	0.02	0.26	0.91	0.19
Noise x brood size				1.75	0.19
b) Song bout duration: noise + noise x brood size + breeding round + brood size					
Noise				1.04	0.31
	<i>Far</i>	-0.05	0.48		
Breeding round				0.11	0.86
	<i>Second</i>	0.07	0.20		
Brood size	<i>Covariate</i>	0.03	0.08	0.03	0.73
Noise x brood size				0.09	0.77
c) Singing duration: noise + noise x brood size + exploration score + breeding round + brood size					
Noise				0.00	0.95
	<i>Far</i>	2.23	1.71		
number of different branches visited	<i>Covariate</i>	-0.02	0.14	0.02	0.88
Breeding round				1.76	0.19
	<i>Second</i>	0.91	0.69		
Brood size	<i>Covariate</i>	0.02	0.28	0.88	0.35
Noise x brood size				1.90	0.17

Marginal and conditional R2 for the models are a) 0.13 and 0.13, b) 0.01 and 0.22 and c) 0.13 and 0.13. Brood IDs were random intercepts. For the song bout duration, bird IDs were also treated as random intercepts.

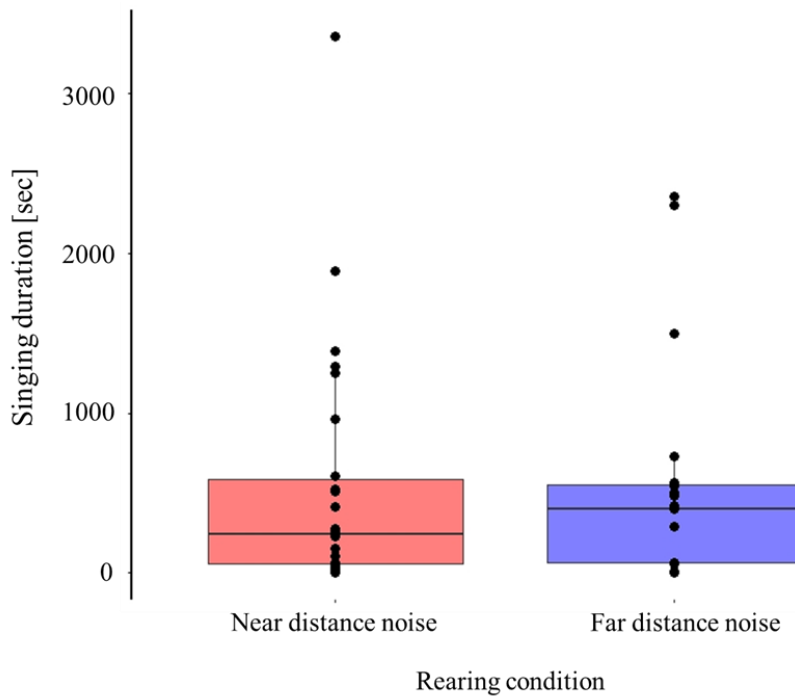


Figure 3: Singing duration of male offspring raised in two types of traffic noise exposure conditions. The Y-axis shows the singing duration in seconds. Each dot represents a single bird. Rearing conditions had no effects on the three quantitative measures: number of song bouts, song bout duration and total singing duration (see Table 1).

Exploration tests: parents

The number of different branches visited by individual birds from the parental generation in the exploration test was repeatable ($R = 0.49$, 95% CI [0.14, 0.63], $n = 48$, $p < 0.001$, see Figure 4) in the best model (Exploration: Emergence²_{scaled}) which only includes quadratically scaled emergence time.

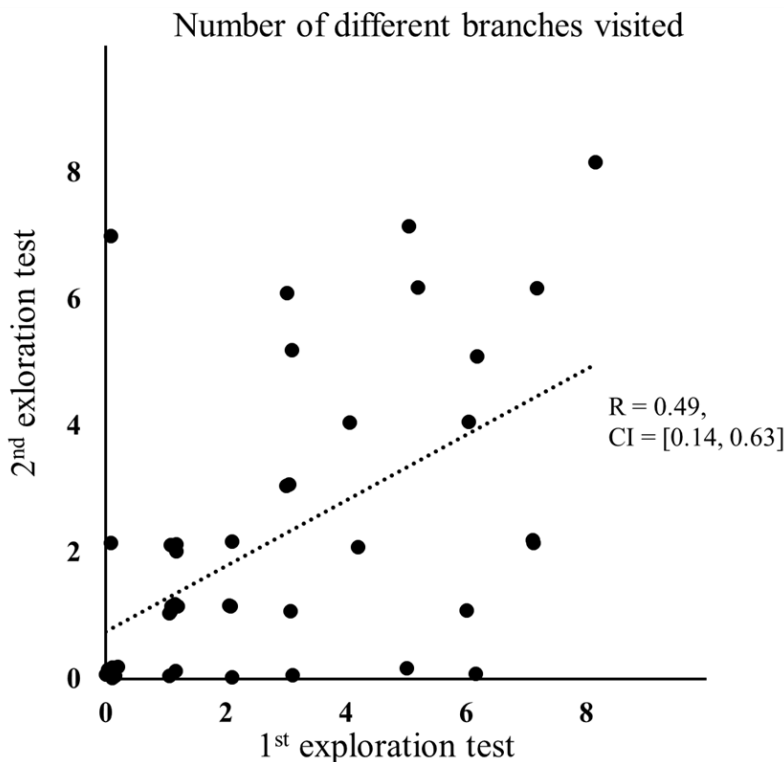


Figure 4: Repeatability of the number of different branches visited by the parents. Every dot represents a single bird. X and Y axes are the number of different branches visited (randomly jittered by 0 - 0.2) during the 1st and the 2nd exploration test.

In the offspring generation, 19 (out of 26) males and 16 (out of 28) females raised from near-distance traffic noise and 14 (out of 20) males and 33 (out of 46) females raised from far-distance traffic noise entered the novel environment. There was no significant difference in terms of participation between birds raised from the two treatment groups (chi-squared test, $p = 0.41$), and between the two sexes (chi-squared test, $p = 0.48$). Comparing the explorative behaviour of birds showed no systematic difference among the birds from the different rearing environment, suggesting that the early rearing noise conditions did not affect the explorative behaviour in the offspring (see Figure 5, Table 2). The brood size, breeding round, sex and the housing group ID also did not affect the exploration score of the offspring in a novel environment.

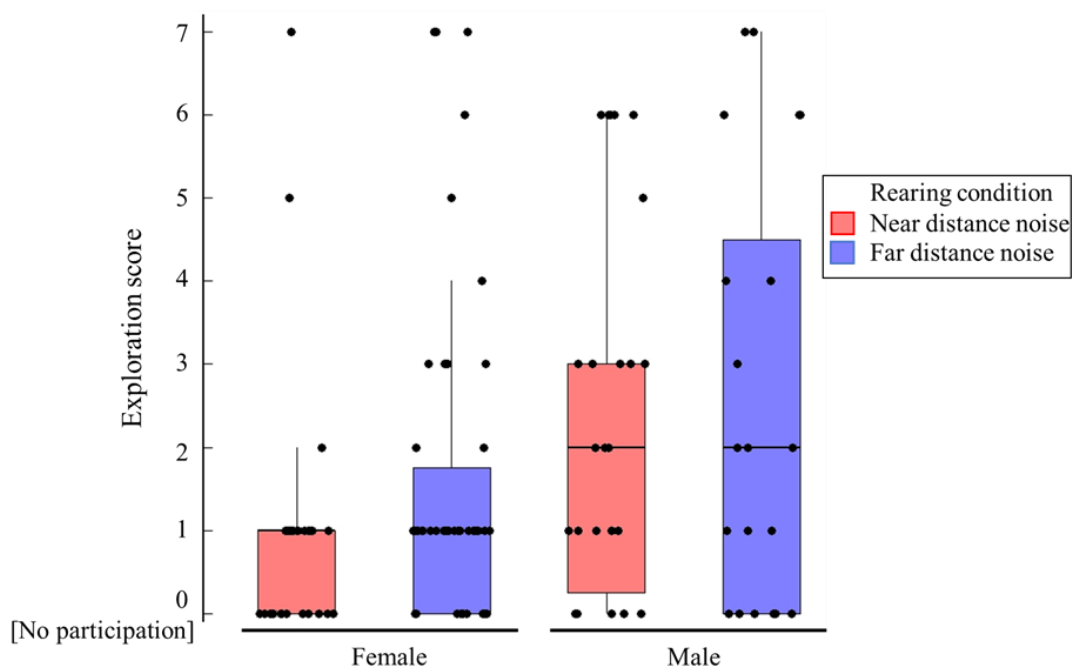


Figure 5: Boxplots showing the exploration score of the offspring generation in the voluntary exploration test plotted by rearing condition and sex. The y-axis shows the exploration score. Zero scores indicate birds that did not participate in the voluntary exploration test. Each dot shows the exploration score of a single individual.

Table 2: Results of the generalised linear mixed model analysis testing the effect of rearing conditions on offspring exploration score. None of the tested factors were statistically significant.

Fixed factors	Level	Estimate	S.E.	χ^2	P value
offspring exploration score: noise + brood size + sex + housing group ID					
Noise				1.56	0.87
	<i>Far</i>	0.35	0.23		
Brood size	<i>Covariate</i>	-0.01	0.08	-0.24	0.81
Sex				0.00	1.00
	<i>Male</i>	0.34	859.71		
Housing group				3.19	0.36
	<i>Group b</i>	-0.05	859.71		
	<i>Group c</i>	-0.10	7.61		
	<i>Group d</i>	-0.39	859.71		

Brood IDs were random intercepts. Marginal and conditional R^2 for this model are 0.13 and 0.40.

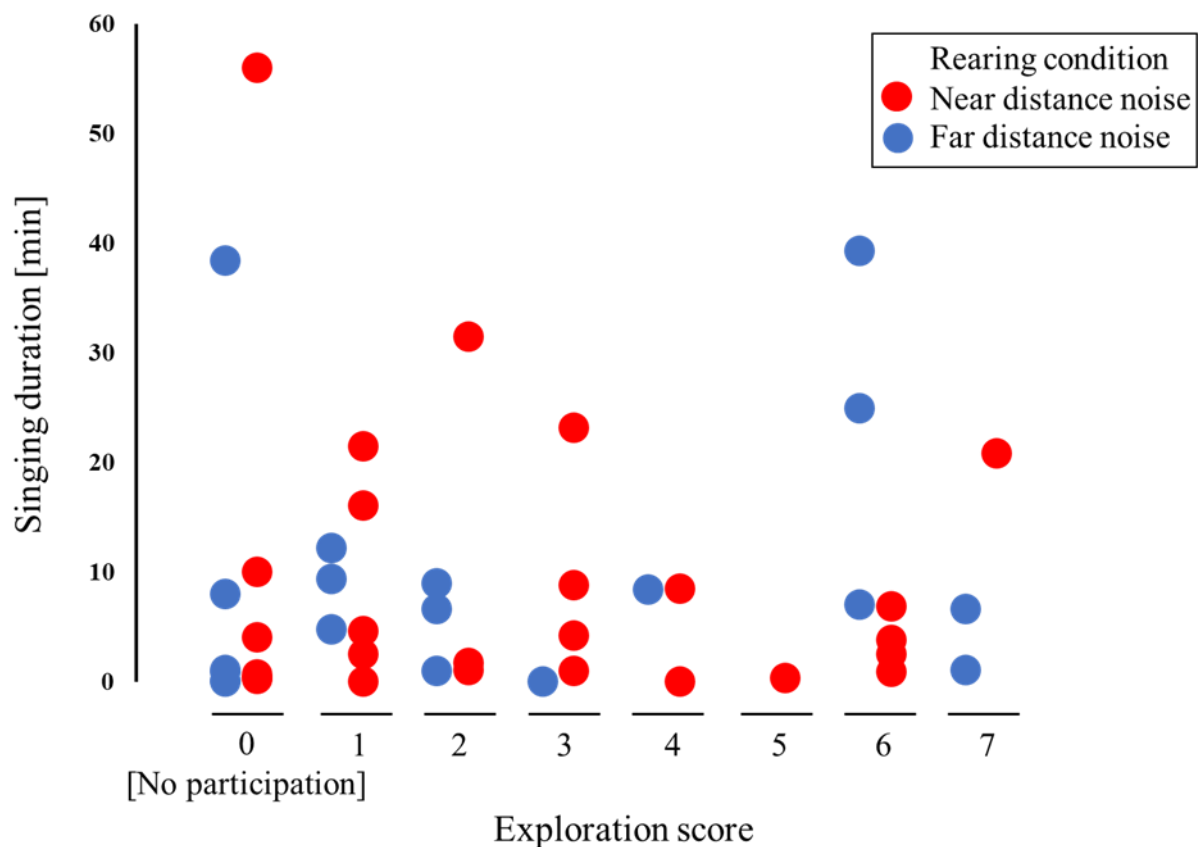


Figure 6: Total singing duration of male offspring plotted against their exploration scores in the novel environment. The y-axis shows the singing duration in minutes. The x-axis shows the novel environment exploration score. Each dot represents a single bird. Treatment had no effect on the plotted variables (statistical analyses in Table 1).

In the tested males, the total singing duration and the exploration tendency did not covary (see Figure 6, Table 1c). Similarly, rearing noise, breeding round, brood size and the interaction between rearing noise and the brood size all did not affect the exploration tendency.

Discussion

We aimed to test if chronic exposure to traffic noise during rearing would affect adult phenotypes by focusing on two behaviours known to show developmental plasticity in the zebra finch: singing effort and explorative behaviour. There was no significant effect of noise treatment on male singing effort, measured as total time singing or number of song bouts, nor on the performance in the exploration test. Exploration behaviour has been shown to be consistent in zebra finches. The parental birds were tested on two different occasions one month apart and they behaved consistently in our exploration test. Such consistency in ex-

ploration has also been found in several other zebra finch studies, indicating the robustness of the exploration test (David et al., 2011; McCowan et al., 2015; Wuerz & Krüger, 2015). We did not test for consistency in singing effort, but we also did not find any correlation between the total singing duration and the explorative behaviour.

Singing effort: body condition and social context

Across songbird species singing effort does show individual differences but is also highly condition dependent (Gil & Gahr, 2002) and this also holds for zebra finches (Riebel, 2009). Developmental stress is known to affect the HPA-stress axis which has been implicated both in individual differences in personality traits (Groothuis & Trillmich, 2011; Spencer, 2017), and song learning (Podos et al., 2009; Spencer & MacDougall-Shackleton, 2011). We had hypothesised that if the chronic noise exposure affected the parents or offspring, singing and exploration might differ between the birds raised in high level, behaviourally aversive noise (see chapter 2 and 3) versus those reared in the control, lower noise level condition. The absence of a difference on singing effort between the two treatment groups could indicate a true absence of an effect, or that our method is not suitable to detect it.

The absence of an effect may also be due to the fact that there were no differences between the birds in both treatments in body condition, which has been most convincingly linked to singing effort. Singing effort shows substantial variation among individuals in the field during breeding seasons (Dunn & Zann, 1996) and this variation has been related to both internal and external factors. Body condition, for example, has been shown to affect singing effort in zebra finches. Food restriction has been found to reduce the rate of song bouts (Ritschard & Brumm, 2012) while supplementary lutein and cholesterol can help zebra finches maintain song output with decreased photoperiod (Casagrande et al., 2016). The birds tested in the present study, however, did not differ in body mass (see Chapter 3), which may be one explanation for the lack of difference in singing effort.

Singing effort in zebra finches may also vary with variation in their social environment. One study compared singing in males housed with one unfamiliar female or a group of 40 - 45 unfamiliar birds of both sexes and found more singing in the more simple social environment, but also a gradual change towards an increase in singing effort in both groups after the change to a new environment (Adar et al., 2008). Males also differ in how much they sing when alone or in company (Jesse & Riebel, 2012; Bruno & Tchernichovski, 2019). In our study, the birds were all housed in a similar social environment, which may have led to a homogenous singing effort, but this seems not very likely as other studies also reported systematic individual differences in song output when monitoring males' singing effort during comparable durations of solo housing (Jesse & Riebel, 2012; Bruno & Tchernichovski, 2019).

Chapter 5

In summary, singing effort has been used in most studies to date to assess current condition, or effects of social context, making it more difficult to link this literature to the studies that report differences in singing effort between birds in noisy and quiet habitats in the field. It is currently discussed whether these differences arise from the current or the recent exposure of noise versus long-term effect of being reared in a noisy condition (Potvin, 2017). Bermúdez-Cuamatzin et al. (2020) recorded great tit (*Parus major*) singing at 11 paired urban-rural sites and found the urban birds sang fewer songs outside the dawn chorus compared to their rural counterparts. Also urban and rural red-winged blackbirds (*Agelaius phoeniceus*) distributed their singing effort differently over the day (Cartwright et al., 2014). As our negative results could arise from a true absence of an effect but also from using an unsuitable method, currently our result cannot support or dismiss that such differences in singing effort are long-term effects of rearing traffic noise exposure.

Explorative behaviour: developmental stress, parental effects and social environment

Explorative behaviour in the offspring was not significantly affected by the rearing noise conditions in our study. We had picked this parameter as several studies had already shown that this behaviour can systematically covary with manipulations of the rearing environment and in particular in the context of developmental stress. Martins et al. (2007) scored how many feeders in a novel cage were visited by zebra finches from three lineages of different baseline corticosterone levels and found that the birds from higher baseline corticosterone lineages visited more feeders, and among the birds from lower baseline corticosterone lineages, the more plasma corticosterone they had, the more feeders they visited. Spencer & Verhulst (2007) experimentally administered exogenous corticosterone to developing zebra finches and observed the birds' behaviour with either a toy moose or a furry ball at 30 and 50 days old. They found males in the treatment groups had shorter latency to approach the perch with the object. We did not measure the corticosterone levels in the subjects of our study, but did weigh the offspring at 11, 65 and 120 days old but found no differences between the two groups (see Chapter 3). Although this is only an indirect way to measure stress, lack of a difference in weight between groups could be in line with no difference in stress, although we suggested that parental behaviour may also have compensated for offspring stress (see Chapter 3). Interestingly, the studies exposing developing birds to anthropogenic noise report very different outcomes, ranging from increased (Crino et al., 2011) to decreased baseline corticosterone (Kleist et al., 2018). However, the majority of studies to date have not found noise exposure affecting baseline corticosterone (Crino et al., 2013; Angelier et al., 2016; Casasole et al., 2017; Injaian et al., 2019; Flores et al., 2020).

Group housing and a voluntary test can affect the exploration behaviour of zebra finches. In

our voluntary test, not all birds participated but this might change by opening more entrances in the future or reducing the total number of birds in the aviary as some birds started to monopolize the access to the tunnel by chasing other birds away (personal observations). This highlights social influences on exploration of an area (Templeton et al., 2014), which we need to understand better as most studies on individual differences and behavioural syndromes test birds individually for exploration. In zebra finches, the association between exploration and other personality traits in individual versus paired situations vary (Schuett & Dall, 2009) or can be absent. Emmerson & Spencer (2017) reported that their corticosterone treatment affected young zebra finches' behaviour in a novel environment test, featuring several novel objects. However, in social testing conditions, birds from all treatments spent similar time perching near novel objects. This indicates the importance of considering social environment in behavioural tests.

Link between singing effort and explorative behaviour

We also tested for a potential covariance between singing behaviour and behavioural traits related to exploration that have been reported in several bird species. In wild collared flycatchers (*Ficedula albicollis*) the males that explored more in a novel environment were more likely to sing at lower song posts (Garamszegi et al., 2008) than their less explorative conspecifics. In great tits, exploration score, normally measured as the number of movements between different features in an exploration room, has been found to correlate with the number of songs in different stages of breeding (prior to egg laying, Naguib et al. 2010; during main periods of fertility and maternal investment, Naguib et al. 2016) and territory defence (Amy et al., 2010; Snijders et al., 2015). However, we did not find any correlation between exploration and singing effort in our subjects. This may be due to species and/or methodical differences (Carter et al., 2013; Jacobs et al., 2014).

The function(s) and context of singing differ among species and this may also affect the correlation between singing effort and exploration. The most prominent findings on correlation between singing effort and exploration so far have been reported in great tits (e.g. Amy et al., 2010; Jacobs et al., 2014; Snijders et al., 2015) as this is also one of the species where these questions have been most intensively studied in the field. Great tits sing seasonally and an important function of their song is territorial defence and higher exploration is associated with more aggressiveness (Verbeek et al., 1996; Carere et al., 2005). In great tits, the relationship between exploration and singing effort could be mediated by other personality traits (e.g. aggressiveness). In zebra finches, singing is year-long and has the function of pair bond maintenance (Dunn & Zann, 1996). Singing probably also has a social function in the large flocks (Elie et al., 2011). In this context, singing effort should perhaps be expected to be pos-

itively correlated with sociality, as what has been demonstrated in a few studies (Adar et al., 2008; Jesse & Riebel, 2012).

Conclusions

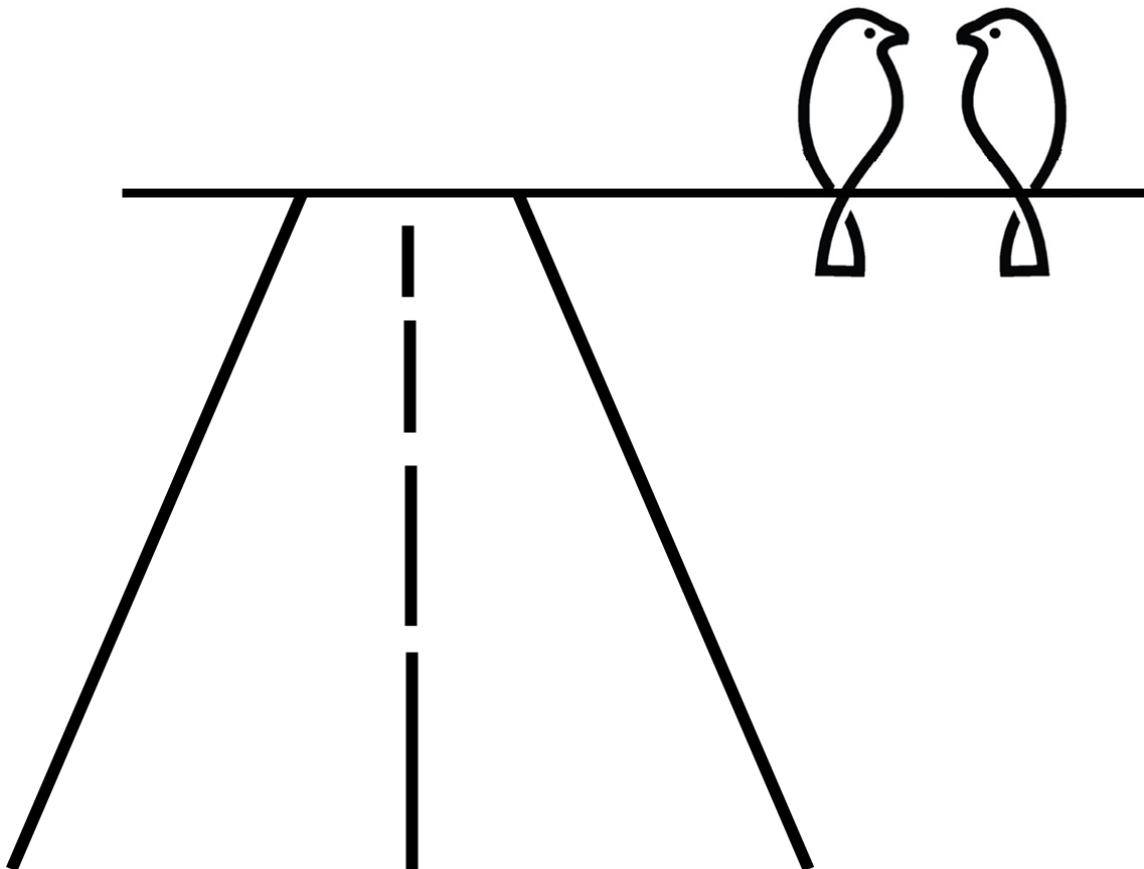
For the experimental subjects raised with chronic noise exposure of near-distance (actively avoided by birds) and far-distance traffic noise, singing efforts did not differ during a 24h assessment of song output in a standardised situation in a (novel) recording chamber. The birds' explorative behaviour, did not show measurable covariance with the two noise rearing treatments. The total singing duration in an unfamiliar recording chamber was not correlated with the birds' explorative behaviour in a novel environment. However, as a negative result is not proof of absence of an effect this is difficult to interpret: there might have been no effect, but given that the birds were tested several months after the end of the treatment, the observed behavioural responses in singing and exploration might also have been influenced by current or relatively recent exposure to noise or social factors. With just two types of behaviour investigated we cannot draw firm conclusions on the effects of noise on behavioural development. This is an understudied area, and we hope the insights here can contribute to the design of future studies to further choose and develop methods to address the important questions of potential long-term effects of noisy rearing environments on behavioural phenotypes.

Acknowledgements

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Chapter 6

Thesis Summary & General Discussion



The importance of studying the effects of noise on animals

Anthropogenic noise negatively affects wildlife in a wide range of taxonomic groups, from molluscs to mammals (Slabbekoorn et al., 2018a; Kunc & Schmidt, 2019). Especially for birds, a substantial number of observational studies have now shown negative associations between noise pollution and abundance and diversity along roadsides (Reijnen et al., 1996; Francis et al., 2009; Halfwerk et al., 2011). Birds might be more studied in general than other species, but as they rely on sound for communication, they may also be particularly susceptible to masking effects of noise. Consequently, researchers investigating birds' behavioural responses to high level noise to date have mostly focused on the immediate adjustment of vocal signalling behaviour. However, there is more than one mechanism by which birds might cope with increasing noise levels (reviewed by Potvin, 2017). They may show immediate behavioural reactions, such as spatial avoidance and/or vocal adjustment, but also more ontogenetic adjustments with long-term consequences like changes in sensory and personality traits (Dooling & Popper, 2007; Carrete & Tella, 2011).

Natural noise can lead to an array of adaptations in acoustic signalling in animal populations (Brumm & Naguib, 2009). However, given the current speed of urbanisation and expansion of infrastructure, the numbers of anthropogenic noise sources like highways increase at a higher pace than populations previously not exposed to such noise can evolve. Consequently, birds have to rely on mechanisms acting within an individual's life time to cope with noisy conditions, such as spatial avoidance or habituation. The potential extent of behavioural plasticity and flexibility is discussed widely in this context but has seen little experimental testing, aside from studies on song adjustment (reviewed in Potvin 2017). It is important to understand the underlying mechanisms for a proper interpretation of the many observational field studies showing declining bird numbers near roads. If we can disentangle the effects of noise *per se* from other factors related to traffic (increased pollution, moving vehicles, habitat destruction and human presence), we may be better able to understand, predict and mitigate detrimental effects of anthropogenic noise.

It is often argued that animals habituate to noise, or that growing up in noise might change sensory thresholds. However, to my knowledge there have been no experimental studies to date that have addressed whether rearing noise levels lead to more noise tolerant phenotypes in birds. Gaining insight regarding these issues requires an experimental approach to disentangle noise impact from confounding factors, and exposing developing birds to long-term noise testing them at different stages towards and into adulthood.

On the impacts of traffic noise on zebra finch behaviour and development

Testing noise avoidance

In Chapter 2 of this thesis, I developed a method to test whether traffic noise (rather than a combination of all traffic and road associated factors) itself is avoided. By offering birds a choice between two identical aviaries with different levels of highway noise playbacks, I was able to ‘ask’ them whether they prefer a quiet over a noisy area. Previous studies, using experimental noise exposure, typically exposed birds to one type of noise and compared it to a non-exposed control group. An important methodological difference of the approach I chose was that I not only compared the birds’ reactions to sound with a control group but also that I used two types of traffic noise. This method not only revealed that different levels of noise from the same site can be treated differently by the birds, but also provided an opportunity to disentangle the effects of traffic noise from the presence of any novel sound. In the tests, birds exhibited clear preferences, but they only preferentially moved to the quiet aviary during playback of traffic noise recorded at near distances, not during playbacks of noise recorded at far distances. When the near- and far-distance recordings were played back simultaneously, the birds likewise avoided the aviary where they would be exposed to near-distance traffic noise recordings and preferred staying in the aviary with playback of the far-distance recording. These results demonstrate that birds avoid traffic noise once it has a certain level and intensity, and given that the two aviaries were identical apart from the noise, the avoidance must have been caused by high levels of (traffic) noise *per se*. A neophobic response can also be excluded as explanation as the birds were not deterred by the far-distance traffic noise. It is important to point out that all experimental noise levels were recorded in open rural landscape near real highways, where wild and farm animals are continuously exposed to these sound levels. The non-avoided noise was recorded at 200 - 400 meters away from the highway at 55 dB(A), a level that is classified as noise pollution and annoys humans (Nugent et al., 2014). The avoided noise was much louder around 70 dB(A), but was recorded at a distance (5 - 15 meters from the highway) where birds can be observed albeit that their numbers are often reported to be lower near roads across many studies and habitats (Benítez-López et al., 2010). The experiments in Chapter 2 only tested short-term avoidance, but the results demonstrated that different levels of noise can affect spatial distribution of birds.

In the future, the method adopted in Chapter 2 could be used to test additional levels of traf-

Chapter 6

fic noise to investigate whether noise avoidance behaviour is triggered by any noise above a threshold noise level or whether noise avoidance proportionally increases with noise levels, meaning the birds would show stronger avoidance towards louder noise. Besides the intensity of the noise, its characteristics, i.e. its spectral content could also play an important role in deterrence as different bird species differ in how sensitive their hearing is in specific frequency bands (Dooling, 1992). Using the two-choice tests I established in Chapter 2, future experiments could help to identify the dosage and noise characteristics affecting noise avoidance behaviour in different species. For now, the results from Chapter 2 identified one avoided and one non-avoided type of traffic noise for our laboratory population of zebra finches. Having thus identified a level at which these birds avoided space exposed to traffic noise in favor of more quiet space when given a choice, allowed me to ask whether breeding in avoided versus non-avoided levels of traffic noise would affect the birds' breeding decisions and breeding success.

Testing effects of noise on parental behaviour and breeding success

In the experiment described in Chapter 3, the same birds that had been tested for noise avoidance (Chapter 2), were now given the opportunity to breed while exposed to noise to test the potential effects of traffic noise on parental care, breeding success and offspring mass. In a cross-over design, all breeding pairs were given two breeding opportunities where they were exposed to continuous traffic noise recorded at the same site as (and including) the 30-min stimuli used in the experiment in Chapter 2. Pairs were exposed once to playbacks of the avoided (near-distance) and once to the non-avoided (far-distance) traffic noise. In the (previously avoided) near-distance high level traffic noise, breeding pairs spent more time attending the nests than when exposed to the lower level, far-distance traffic noise. The results not only showed that traffic noise can affect nest attendance and feeding rate, but also provided additional validation of the noise avoidance test: the previously avoided noise levels that were recorded near highways were also the levels that affected subjects' behaviour during breeding. However, the noise exposure did not affect the number of eggs or hatchlings parents produced. Future work will have to investigate whether the higher nest attendance resulted from impaired intra-pair communication and nest relief, or whether it was a compensatory strategy by the parents to overcome detrimental masking effects on intra-pair communication or to respond to potentially increased nutritional needs by the chicks. The latter could arise from higher begging rates (Leonard et al., 2015) and/or changed corticosterone levels that have been observed in several other studies that exposed nestlings to noise (Crino et al., 2011; Kleist et al., 2018; Injaian et al., 2019).

Does growing up in noise alter birds' reactions to noise?

In the breeding experiment described in chapter 3, parents successfully raised chicks under two levels of traffic noise. This allowed me to test whether birds that had grown up in noise would be more tolerant to noise. Using the same experimental setup that was used to test noise avoidance in their parents (that had been raised without noise playbacks), the offspring from both treatments were now tested in the same way and with the same stimuli as previously used to test their parents. During the spatial preference tests, the subadult birds of both treatments did not show a preference for quiet space: they used both aviaries regardless of the noise playbacks and showed no evidence of noise avoidance. In contrast, birds of the same age reared in the same size cage in another breeding room without any noise playback did avoid the near-distance traffic noise by moving into the quiet aviary. However, when the experimental birds were tested for a second time, now as young adults and after having lived in quiet laboratory conditions for two months, noise reared birds from both treatments now avoided near-distance traffic noise. This change towards noise avoidance after experiencing a quiet environment is particularly interesting as it shows growing up in noise is not necessarily leading to increased tolerance towards noise in the long term.

Are there long-term effects of rearing noise on adult behaviour?

Noise avoidance behaviour is of course but one way the rearing environment could affect the birds. Following the experimentally noise-exposed birds into adulthood, I then addressed the question of potential long-term effects on adult behaviour in Chapter 5. I focussed on two candidate behaviours that often show individual differences and have been linked to behavioural syndromes in birds and that are often listed as being different between urban (noisy) and rural (quiet) populations, namely singing effort (Sierro et al., 2017; Bermúdez-Cuamatzin et al., 2020) and exploration (Lowry et al., 2013). Singing effort was chosen to establish the connection between this laboratory study to field experiments and observational studies that found birds' singing effort to be affected in noisy areas or by noise playbacks. Explorative behaviour is typically measured as how many objects are found or how many features visited in a novel environment. This type of exploration test has been used and validated in the zebra finch (McCowan et al., 2015; Wuerz & Krüger, 2015) and the measurement from this type of test has also been shown to be a trait with phenotypic plasticity in zebra finches (David et al., 2011; Krause & Naguib, 2011). A number of studies in different species have found associations between individual variation in exploration tendency and vocalization (Garamszegi et al., 2008; Amy et al., 2010; Naguib et al., 2010, 2016; Guillette & Sturdy, 2011). To test the birds' exploration, I developed a system where they could voluntarily enter and explore a novel environment. This new system avoids the potential impacts of catching and handling subjects before an exploration test. Comparing the offspring from the two different noise treatments, showed no systematic differences between the

Chapter 6

groups in either test: male offspring did not differ in their 24 hr singing efforts assessed in a standardised testing situation in a recording chamber, and offspring of both sexes did not differ in their behaviour in the voluntary exploration test. It is worth to point out that the birds reared in noise were only tested in a quiet test environment, so it remains to be tested how these birds would behave in noisy conditions or whether other behaviours would be affected by noisy rearing conditions.

Synthesis

The results from the series of experiments reported in Chapters 2–5 provide new insights on potential impacts of noise on birds but also raise some new questions. I have demonstrated that traffic noise *per se* can contribute to spatial avoidance in birds and cause variation in parental behaviour, and that there can be changes in noise avoidance behaviour in the course of a life time. In the subsequent paragraphs, I will interpret my findings in the context of the existing literature and discuss potential broader implications of these findings and try to develop a framework that can be used to study the behavioural effects of traffic noise exposure.

The role of traffic noise in reducing avian populations near roads

Regardless of the increasing number of studies showing associations between increased noise pollution and decreasing wildlife near roads, it has been difficult to establish a causal link between these two. This is because roads are not only very noisy but also associated with additional potentially aversive factors for wildlife such as the presence of vehicles, chemical pollution and general habitat destruction. This makes experimental studies under controlled laboratory conditions valuable to test the effects of traffic noise itself while holding other factors constant. I used such an experimental approach in the research presented in this thesis. This allowed me to collect experimental data that are in line with the hypothesis that traffic noise *per se* is contributing to the spatial distribution pattern of birds along roads (Benítez-López et al., 2010). I also documented that the mere presence of high intensity traffic noise changed the duration of parental nest attendance and the pattern of feeding rate, adding to the parental behaviours that can be affected by noise (Leonard & Horn, 2012; Leonard et al., 2015). These results provide evidence for two proximate mechanisms through which traffic noise (even without the added potentially detrimental effects of traffic in general) can affect avian communities: noise itself might be sufficient in deterring birds from noisy but otherwise suitable habitat and might also affect their behaviour during breeding.

I did not find an effect of the experimental traffic noise exposure on the number of eggs or hatchlings, nor on growth or on the tested behaviour in the adult offspring. A possible explanation would be that noise primarily exacerbates other challenges that can affect offspring quality (i.e. food accessibility) which may not be seen under the benign laboratory conditions. It is also possible that the current noise exposure scheme was not at a level to affect the offspring. The traffic noise was played back at levels that were the same as at the recording sites, which in case of the near-distance noise was just 5-15 meters away from the highways, and projecting realistically high level traffic noise. It is difficult to extrapolate from an absence of an effect in the benign environment in the lab to an absence of an effect in the field, as the same levels might affect breeding pairs more in the field than in the lab. However, it is also possible that breeding near roads does not necessarily reduce reproductive outcome. Instead the major contributor to avian declines along roads, might not be negative effects on breeding but active avoidance of too noisy areas and not settling to breed. This interpretation can only be speculation at this stage, as one cannot generalise from one series of experiments in the laboratory involving only one species to all bird species in all habitats. The type of experiments presented here should therefore be replicated in other species in the future to better understand what causes the observed reproductive reduction in the field (Halfwerk et al., 2011; Schroeder et al., 2012).

Can birds adjust to noise?

Different possible adjustments to traffic noise exposure may help birds cope with the sounds of busy highways. If noise tolerance is a trait with phenotypic plasticity (i.e. the developmental process whereby the same genotype develops into different phenotypes as the result of different genotype-environment interactions), then we would expect to see systematic differences of noise tolerance relating to rearing conditions (Stamps, 2003; Uller, 2008; Snell-Rood, 2013). If behavioural flexibility (i.e. the ability to react differently to the same stimuli in different contexts, at a different age or in a different state, or due to experience related behavioural adjustment) is of more importance in guiding birds' responses, then we would see more influence of recent and current rather than past environmental factors. Mapping the time scale and (ir)-reversibility of noise tolerance could contribute to our understanding of how individual fitness would be affected by noisy environments when avoidance is not an option (reviewed in Potvin, 2017).

There are some insights that arise from the observations during the experiments described in this thesis regarding behavioural changes determined by noise exposure. In Chapter 3, I reported data that showed that breeding zebra finches changed aspects of their parenting behaviour when exposed to aversive noise. However, this did not affect their reproductive out-

Chapter 6

come, suggesting that behavioural flexibility may help exposed birds to adjust to noise. One possible explanation is that the changed parental care may mitigate potentially negative physiological effects of traffic noise on the chicks (Potvin & MacDougall-Shackleton, 2015; Dorado-Correa et al., 2018; Injaian et al., 2019; Zollinger et al., 2020). The observation that the effect of noise exposure on feeding rate was exacerbated by brood size is in line with this interpretation. Overall, it seems that birds under benign lab conditions (sufficient food, no predators) could sufficiently adjust to a noisy environment in two subsequent breeding events but clearly were more affected when additionally challenged by larger brood sizes. This raises the question of how easily parents could accommodate additional challenges from noise in the context of possibly a more challenging environment in the wild and will need to be further researched.

The results of the noise avoidance experiments in Chapter 4 showed that birds exposed to noise during development, other than control birds, did not avoid noise as juveniles which is in line with both developmental plasticity or flexibility in noise avoidance. However, when tested as adults using the same stimuli, they clearly avoided the noise which contradicts the idea that the birds developed into more noise-tolerant phenotypes. The experiment was not set up to identify the underlying mechanisms of the change towards more avoidance, but it might be worth to discuss which short- and long-term processes of behavioural adjustment seem best in line with these results.

While developmental behavioural plasticity emphasizes different developmental pathways and resulting phenotypes arising from environment-gene interactions, behavioural flexibility (also referred as activational behavioural plasticity), on the other hand, emphasizes the importance of more recent or current environmental factors on behaviour, as it is defined as the ability of an individual to directly respond and adjust its behaviour to environmental stimuli throughout life (Coppens et al., 2010). The behaviour exhibited by the birds shows some key features of behavioural flexibility. The exposure to noise up until the test at day 65 led to a lack of noise avoidance behaviour in the experimental birds. After the first test and up until the second test, noise exposure ceased and was absent as environmental input, and in the second test, birds now spatially avoided the playbacks of the noise stimuli. As noise has been found to delay sensory development (Chang & Merzenich, 2003; Funabiki & Konishi, 2003) or cause to shift sensory thresholds (birds: Dooling & Popper, 2007; fish: Nedelec et al., 2014; rodents: Manohar et al., 2017), the difference in the first test could have been caused by noise exposure altering developmental trajectories. Permanent or even temporary auditory threshold shifts are less likely to explain the results, as the exposure noise levels during the breeding were far below the levels to cause hearing damage, permanent or temporary threshold shifts (at least in adult zebra finches, see Dooling & Popper, 2007).

A recent study comparing bird song amplitudes in urban areas several seasons before and then during the 2020 Covid-19 pandemic lockdown found that birds sang at a lower amplitude during the relatively quiet lockdown than before (Derryberry et al., 2020). These observations support the idea that even for birds having grown up in noisy areas, noise related behavioural changes can be a direct response to current noise levels and relatively quickly reversed, instead of a product of altered developmental trajectories. The results in Chapter 5 provided no evidence for different noise exposure levels during development leading to different phenotypes but only tested a limited set of behaviours. The birds raised with different types of noise did not differ in exploration, a trait that can covary with developmental stress in zebra finches (Martins et al., 2007; Spencer & Verhulst, 2007; Emmerson & Spencer, 2017). This suggests that either traffic noise was not a strong developmental stressor or that both groups were equally stressed (albeit at different levels of noise exposure) and that they, therefore, showed no behavioural differences as adults. Future studies exposing noise-reared birds also to noise in adulthood and then testing their noise avoidance tendency would provide insights into which of these processes are underlying the changes in noise avoidance behaviour. For now, the results in Chapter 3 and 4, showing zebra finches changing behaviours with noise exposure, highlight the importance of distinguishing effects of recent and current exposure to noise in behavioural studies (Gil et al., 2015; Sierro et al., 2017; Weaver et al., 2019; Bermúdez-Cuamatzin et al., 2020). While this shows that some adjustment is possible, the underlying mechanisms and time scales of such adjustment need to be better understood.

How far can we extrapolate the effects of traffic noise to anthropogenic noise in general?

Highway traffic noise shares many characteristics with other types of anthropogenic noise like aircraft, urban and industry related sounds. Most anthropogenic noise (partly) overlaps with bird (and other animals') vocalisations, persists for a long period, and shows dynamic fluctuations (Barber et al., 2010). These similarities raise the question as to how the effects of traffic noise on birds reported in this thesis may, to some extent, apply to other types of noise (drilling noise, Habib et al., 2007; compressor noise, Bayne et al., 2008). For this it is also important to appreciate the many pronounced differences between different types of noise. Urban noise and aircraft noise near airports, for example, show larger circadian fluctuations than the relatively consistent highway traffic noise in busy areas (Slabbekoorn, 2013). The unique features of different types of noise may affect birds differently. For example, the observed circadian shifts of vocalization patterns seen in birds in noisy areas like cities and airports (Gil et al., 2015; Sierro et al., 2017; Bermúdez-Cuamatzin et al., 2020) can be caused by the distinct patterns of urban and aircraft noises due to the fluctuations in human activities. This highlights the importance of considering the characteristics of different types of

Chapter 6

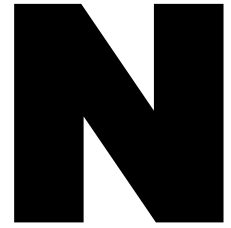
noise when trying to assess its impact, as demonstrated in a number of recent marine noise exposure studies (Shafiei Sabet et al., 2015; Kok et al., 2018; Isojunno et al., 2020).

Anthropogenic noise can affect animals of a wide range of taxa

This study has found that traffic noise deters birds, alters birds' behaviour in the short term, and affects their breeding behaviour. However, birds are not the only animals that can be affected by anthropogenic noise. Invertebrates (Morley et al., 2014), fish (Slabbekoorn et al., 2010), amphibians and reptiles (Simmons & Narins, 2018) and mammals (Weilgart, 2007; Slabbekoorn et al., 2018b) can also be affected by anthropogenic noise. The negative effects of anthropogenic noise on wildlife have increasingly come to the attention of a wide audience over the past decades (Shannon et al., 2016; Slabbekoorn et al. 2018) and this field is witnessing a rapid increase in research effort trying to characterise the relationships between different types of noise exposure and their respective impacts (Morley et al., 2014; Kunc et al., 2016; Bunkley et al., 2017). A recent meta-analysis across multiple taxonomic groups confirms that noise is a form of very wide-spread pollution that affects wildlife from molluscs to mammals (Kunc & Schmidt, 2019). This stresses the necessity to study animals' responses to anthropogenic noise and the mechanisms underlying these responses (or lack of them) to confirm, comprehend, and perhaps, mitigate the detrimental effects of noise on wildlife.

Conclusions

With the experimental work described in this thesis, I set out to investigate the mechanisms underlying the effects of traffic noise on birds, conducted a series of experiments exposing birds to ecologically meaningful stimuli and testing birds of different experimental rearing noise backgrounds. I have provided evidence that traffic noise, even in the absence of traffic, can deter birds from using a particular space and that these behaviourally avoided levels of noise affect parental behaviour in situations where the birds cannot avoid it. Comparing different experimental groups at different ages for noise avoidance behaviour demonstrated substantial behavioural changes in noise avoidance behaviour of birds, which were influenced by their rearing and current noise experiences. These results contribute to our understanding of how avian individuals behaviourally respond to anthropogenic noise exposure and how these responses are shaped by the acoustic characteristics of their rearing environment, which in turn may help to better understand causalities between observed bird distribution patterns near roads and the associated levels of traffic noise.



Nederlandse samenvatting

Tolerantie voor lawaai:

De effecten van snelweggeluid op het gedrag en de ontwikkeling van zebra's

Samenvatting

De afgelopen decennia is er wereldwijd een substantiële toename geweest in de omvang van menselijke populaties, urbanisatie en industrialisatie. Door deze ontwikkelingen is door mensen teweeggebracht geluid wijdverspreid. Antropogeen geluid is niet alleen zorgelijk voor de menselijke gezondheid, maar kan ook invloed hebben op andere dieren. Van alle verschillende vormen van antropogeen geluid is verkeerslawaai in het bijzonder storend. Dit komt doordat verkeerslawaai niet alleen in bewoonde regio's aanwezig is, maar ook in de landelijke gebieden hierbuiten. Over de afgelopen jaren is er steeds meer bewijs dat wilde populaties afnemen in de buurt van wegen. Vooral in het geval van vogels is er duidelijk vastgesteld dat er afnames zijn in diversiteit en dichtheid en dat er veranderingen plaatsvinden in de gemeenschapsstructuur.

Het is nog onduidelijk welke mechanismen ten grondslag liggen aan de afname in vogelaantallen langs wegen en of er wellicht meer tolerantie voor lawaai ontstaat in volgende generaties. In het veld zijn er vrijwel altijd andere factoren die meespelen en ook invloed kunnen hebben op overleving of reproductie van vogels, zoals minder of andere vegetatie, chemische vervuiling of directe impact van auto's, zoals bij botsingen. Daarbij komt dat het ook erg moeilijk is om te controleren voor de omstandigheden waarin de vogels opgegroeid zijn. Deze informatie omtrent de ontwikkeling en de omgeving van de vogels is echter nodig om een goed beeld te krijgen van het daadwerkelijke effect van verkeerslawaai ten opzichte van andere factoren die een effect kunnen hebben op de verspreiding van vogels in de buurt van wegen.

Om te onderzoeken hoe verkeerslawaai reproductie beïnvloedt, zou idealiter een vergelijking gemaakt moeten worden tussen vogels die bloot zijn gesteld aan reguliere geluidsniveaus en vogels die bloot zijn gesteld aan aversieve geluidsniveaus. Ideaal gezien, zouden daarbij beide groepen vogels vergelijkbare achtergronden hebben. Een experimentele aanpak, waarbij er gekwantificeerd kan worden hoe opvoeding onder luidruchtige omstandigheden de volgende generatie beïnvloedt, maakt dit mogelijk. Door het gedrag van nakomelingen die groot zijn gebracht onder luidruchtige omstandigheden te onderzoeken, kunnen we inzicht krijgen in de mechanismen onderliggend aan de invloed van lawaai op vogels en de tijdschalen waarop eventuele veranderingen plaatsvinden.

In dit proefschrift heb ik een serie experimenten uitgevoerd waarbij ik het effect van verkeerslawaai op het gedrag en de ontwikkeling van zebra-vinken op zowel korte als lange termijn heb onderzocht. De zebra-vink is een goede modelsoort voor dit onderzoek, omdat er al veel bekend is over het gedrag en de ontwikkeling van deze soort. Om te onderzoeken of en bij welk geluidsniveau vogels verkeerslawaai actief vermijden, heb ik zebra-vinken de mogelijkheid gegeven om heen en weer te vliegen tussen twee volièrres die enkel in geluidsniveau

Samenvatting

van elkaar verschilden. Gebaseerd op hoe lang de vogels bleven in de verschillende volières waar opnames van verkeerslawaai naast snelwegen werden afgespeeld (er was ook een conditie waarbij er geen verkeerslawaai werd afgespeeld), kon ik blootstelling aan geluidscondities verdelen in neutraal en actief vermeden verkeerslawaai (**Hoofdstuk 2**).

Om te testen of het niveau van blootstelling invloed heeft op broedende ouders en of het leidt tot een verminderde reproductieve prestatie, heb ik ouderlijk gedrag en reproductief succes van zebra-vinken in deze twee geluidscondities vergeleken (**Hoofdstuk 3**). Er is voorheen gesuggereerd dat opgroeien onder luidruchtige condities tot een hogere lawaaitolerantie als aanpassing zou kunnen leiden. Om deze hypothese te testen, heb ik verder onderzocht of en hoe nakomelingen uit verschillende geluidscondities verschilden in hun tolerantie voor lawaai (**Hoofdstuk 4**), zanginspanning en neiging tot exploratie (**Hoofdstuk 5**). In hoofdstuk 2 van dit proefschrift heb ik een methode ontwikkeld waarmee getest kan worden of verkeerslawaai (zonder de andere factoren die gerelateerd zijn aan verkeer) vermeden wordt door vogels in gevangenschap. Door de vogels een keus aan te bieden tussen twee identieke volières die enkel verschilden in geluidsniveau, is de vogels “gevraagd” of ze een voorkeur hebben voor een stille conditie (in plaats van een luidruchtige conditie). In deze test lieten de vogels een duidelijke voorkeur zien. Ze bewogen zich naar de stille volière tijdens het afspelen van hard verkeerslawaai, dat vlakbij snelwegen opgenomen was. Tijdens het afspelen van zacht verkeerslawaai (opgenomen vanaf een grotere afstand van de snelweg) lieten de vogels echter geen voorkeur zien voor de stille ruimte. De vogels vermeden ook hard verkeerslawaai wanneer het tegelijkertijd, elk in een andere volière, werd afgespeeld met zacht verkeerslawaai. Ze gaven hierbij de voorkeur aan de volière met zacht verkeerslawaai. De experimenten in hoofdstuk 2 hebben enkel op de korte termijn getest of vogels verkeerslawaai vermijden. Ze laten echter wel zien dat verkeerslawaai, wanneer dat boven een bepaald niveau is, de ruimtelijke verdeling van vogels kan beïnvloeden. Dat is dus ook het geval wanneer de vogels enkel aan geluid worden blootgesteld, zonder andere bijbehorende factoren van autowegen.

In de experimenten die beschreven staan in hoofdstuk 3 kregen dezelfde vogels, die getest waren op het vermijden van lawaai in hoofdstuk 2, de kans om te paren. Dit terwijl ze ofwel blootgesteld waren aan het gedragsneutrale, lage geluidsniveau, ofwel aan het vermeden, hoge geluidsniveau. Hiermee konden de potentiële gevolgen van lawaai op reproductie worden getest. Alle broedparen kregen twee keer de mogelijkheid om te paren onder continu afgespeeld verkeerslawaai dat op dezelfde locatie was opgenomen als de stimuli in hoofdstuk 2. De paren werden één keer blootgesteld aan playbacks van de vermeden opnames (dichtbij snelwegen/hard verkeerslawaai) en één keer aan de playbacks die ze niet vermeden hadden (ver van snelwegen/zacht verkeerslawaai).

Samenvatting

Tijdens het broeden met hard lawaai brachten ouders meer tijd bij het nest door dan tijdens het broeden met zacht lawaai. Bovendien nam de mate van voeren disproportioneel toe in paren met een grotere leg (vergeleken met paren die een kleinere leg hadden) bij hard lawaai ten opzichte van zacht lawaai. Dit resultaat laat zien dat verkeerslawaai effect kan hebben op de aanwezigheid bij het nest en de inspanning bij het voeren. Blootstelling aan verkeerslawaai had echter geen effect op broedselgrootte of op het aantal uitgebroede jongen. Het ontbreken van dat effect is een resultaat van goed gecontroleerde experimenten die in het lab zijn uitgevoerd, waarbij de vogels voldoende voedsel kregen en niet werden blootgesteld aan predatoren. Het valt niet uit te sluiten dat vergelijkbare geluidscondities wel een nadelig effect hebben in het wild, waar foerageren meer vraagt van de ouders.

In het broedexperiment beschreven in hoofdstuk 3 hebben ouders hun jongen succesvol grootgebracht onder twee verschillende intensiteiten van verkeerslawaai. Hierdoor werd het mogelijk om te onderzoeken of een generatie vogels die opgegroeid is in lawaai ook daadwerkelijk meer tolerant is voor lawaai. De nakomelingen van beide condities zijn getest op dezelfde manier en met dezelfde stimuli als hun ouders, volgens precies dezelfde experimentele methode die eerder gebruikt is om het vermijden van geluid te testen (die zelf opgegroeid waren zonder blootstelling aan lawaai). De vogels van beide geluidscondities lieten geen voorkeur voor de stille ruimte zien als subadulten: ze gebruikten beide volières ongeacht de playbacks en er was geen bewijs dat ze lawaai vermeden. Dat was anders bij een andere groep subadulte vogels die opgroeiden in een andere broedruimte zonder playback van lawaai. Zij vermeden wel het harde verkeerslawaai door naar de stille volière te gaan. Wanneer de experimentele vogels echter opnieuw getest werden als jongvolwassenen, nadat ze twee maanden in stille laboratoriumcondities hadden doorgebracht, vermeden vogels uit beide geluidscondities het harde lawaai wel. Dit suggereert dat opgroeien in luidruchtige gebieden niet per se resulteert in langdurige tolerantie voor lawaai.

Vervolgens adresseerde ik de vraag of er lange-termijn effecten kunnen zijn van verkeerslawaai op het gedrag van volwassen vogels. Dat deed ik in hoofdstuk 5 door de vogels die eerder opgroeiden in luidruchtige condities te volgen tot ze volwassen waren, en vervolgens hun zanginspanning (enkel mannetjes) en neiging tot exploratie (alle vogels) te meten. Zingen en neiging tot exploratie zijn niet alleen cruciale gedragingen van vogels, maar voorheen is ook bewezen dat beiden beïnvloed worden door antropogeen geluid. De zangactiviteit van de mannetjes werd gedurende 24 uur opgenomen. Een script voor de waarneming van zang documenteerde automatisch de hoeveelheid vogelzang en de duur van het zingen voor elk mannetje. Om de neiging tot exploratie van de vogels te testen, heb ik een methode ontworpen waarbij vogels vrijwillig een voor hen onbekende volière, een zogeheten ‘*novel environment*’, kunnen betreden en ontdekken. Door deze methode kon voorkomen worden dat vo-

Samenvatting

gels beïnvloed werden door het vangen en behandelen voorafgaand aan een exploratietest. In beide tests zaten geen systematische verschillen tussen nakomelingen van de twee verschillende geluidscondities: mannelijke nakomelingen verschilden niet in hun zangactiviteit gedurende 24 uur en nakomelingen van beide geslachten varieerden ook niet met hoe veel lawaai ze hadden ervaren tijdens hun ontwikkeling: er was geen significante variatie in hun gedrag bij de vrijwillige exploratietest.

Met het experimentele werk dat in dit proefschrift staat, heb ik bewijs geleverd dat verkeerslawaai op zichzelf (zonder de fysieke aanwezigheid van auto's en de bijbehorende vervuiling) vogels kan verjagen uit een bepaalde ruimte. De geluidsniveaus die vermeden werden hadden ook invloed op ouderlijk gedrag in een situatie waar de vogels het niet konden vermijden. Door het vermijden van geluid door verschillende experimentele groepen op verschillende leeftijden met elkaar te vergelijken, bleek dat dit gedrag beïnvloed wordt door opgroeiomstandigheden en recente ervaringen met betrekking tot blootstelling aan lawaai. De neigingen om veel te zingen of te exploreren waren echter niet verschillend tussen groepen met een verschillende achtergrond in lawaai-omstandigheden, bleek uit de experimenten bij de vogels die getest werden als volwassenen.

Mijn resultaten laten zien dat geluid op zichzelf effecten kan hebben op het gedrag van vogels tijdens ruimtelijke verspreiding en reproductieve inspanning, wat een verklaring kan bieden voor het waargenomen patroon in verspreiding van vogels nabij lawaaiëriëge wegen. Mijn proefschrift laat ook zien dat, zelfs als ze opgroeien in een luidruchtige omgeving, vogels niet noodzakelijk een langdurige tolerantie voor lawaai ontwikkelen. Deze inzichten verbeteren onze kennis over de mogelijke onderliggende mechanismen die leiden tot een negatieve correlatie tussen verkeerslawaai, populatiedichtheid en diversiteit van vogels. Dit kan vervolgens helpen bij het ontwikkelen van beleid en strategieën waarmee de negatieve gevolgen van antropogeen geluid op vogelhabitats bij wegen met veel verkeerslawaai kunnen worden bestreden.

中文概述

C

中文概述

与‘声’俱来：

公路噪音对斑胸草雀行为与发育的影响

在过去的几十年里，随着全球人口剧增，城市化与工业化的加剧，人类产生的噪音已然无处不在。这些噪音不仅仅影响着人类，还影响着动物。在这诸多噪音中，交通工具所导致的公路噪音最为普遍，这些公路噪音不单出现在人类定居的区域，更侵入到了许多人烟罕至的自然区域。近几年，研究发现公路旁的动物数量锐减、种群结构也常常发生变化，这些公路的负面影响在鸟类中最为常见。然而，我们仍然不确定是公路的哪些因素造成了这些影响。在野外研究中，公路噪音通常还伴随着植被退化、尾气污染等因素，甚至是公路上的车辆本身也可能惊扰，甚至撞死鸟类。所以，我们必须要进一步研究到底是噪音，还是这些噪音之外的因素在影响鸟类的生存与繁衍。除此之外，在野外很难控制所观测到的鸟类的品质以及他们的发育历史，如果不能剥离这些干扰因素，我们很难确定噪音到底会如何影响公路旁的鸟类。因此，想要更进一步研究噪音对于鸟类繁殖的影响，我们必须让同一组鸟在不同的噪音环境里各繁殖一次，然后比较这几次繁殖的差异。想要做到这样的条件控制，必须要使用实验的手段来控制各种变量。同时，在上述实验条件下产生的后代，还提供了让我们观测在不同公路噪音环境中长大的鸟类的机会，可以进一步研究噪音是否会对下一代的鸟类也产生行为上的影响。知晓噪音对下一代鸟类的影响，不仅可让我们更好的解决公路噪音对鸟类的危害，还可以加深我们对噪音如何影响整个动物界的理解。

在第二章中，我构建了一种可以在实验室环境下，排除其他干扰，专门测试鸟类对于噪音的逃避反应的方法。在这个方法里，鸟类可以在两个完全相同的鸟室中自由飞翔时，通过改变两个鸟室里的背景噪音，鸟类的选择可以告诉我们它们对不同噪音的逃避程度。在这项测试中，鸟类的表现十分明显：当一间鸟室里播放近距离高速公路噪音时，它们会在另一个完全安静的鸟室呆更久；然而当播放的噪音改为稍远距离高速公路噪音时，它们在两个鸟室里呆的时间则大致相同。当一间鸟室是近高速公路噪音，而另一间是稍远距离的噪音时，这些鸟会逃避播放着近高速公路噪音的房间。虽然这一实验仅测试了鸟类短期的反应，但研究结果充分展示了即使排除其他公路相关的因素，噪音一旦高过阈值，就足够影响鸟类的分布。

在第三章里，为了研究噪音对于鸟类繁殖的影响，之前所测试的斑胸草雀现在可以在两种噪音条件下繁殖：一次是它们所主动躲避的近高速公路噪音，另一次则是在它们并不会躲避的稍远距离的噪音。在繁殖过程中，这些鸟类一直暴露在24小时的高速公路噪音录音里，这些24小时噪音的来源与第二章中所使用的噪音相同。我发现，当暴露在它们所躲避的噪音中时，亲鸟会花费更多的时间打理鸟巢。除此之外，虽然所有亲鸟喂食幼鸟的次数都会随着幼鸟数量的增多而增加，但暴露于所躲避的高

中文概述

强度噪音时，亲鸟喂食的增加更为显著。这一实验结果表明，公路噪音可以影响亲鸟理巢和喂食，却不会影响蛋与幼鸟的数量。此结果可能仅会在实验室环境下出现，在野外，亲鸟需要花费更多的精力去寻找食物，当亲鸟需要同时面对其他环境压力时，噪音可能会降低鸟类的繁殖能力。

第三章实验中，在不同噪音条件下成长的鸟类后代向我们提供了一个研究成长过程中的噪音是否会影响鸟类行为——尤其是对于噪音的忍耐能力——的绝佳机会。在第四章中，我在这些于不同噪音环境中成长的鸟类身上复制了它们父母所经历的‘逃避噪音’实验。当这些后代还是亚成年鸟时，它们不会逃避任何一种噪音：无论两个鸟室里是何种噪音，它们都不会明显地偏好其一。另一批在安静的环境里成长的鸟则会明显地逃避近距离高速公路噪音。然而，当这些在噪音中长大的鸟继续在安静的环境中成长两个月，达到成年之后，它们也会像其父母一样，开始逃避近距离高速公路噪音。这些实验结果表明在噪音环境中长大并不会导致持久的噪音忍耐能力。

在第五章中，我继续研究了噪音成长环境下的鸟类的其他行为特征，特别是对环境的探索能力和雄鸟的歌唱能力。探索与歌唱不仅仅是决定鸟类生存与繁衍的重要行为，而且历往的研究也发现在噪音环境下鸟类的这两种能力有所改变。为了研究唱歌能力，我所编写的程序记录了每一只雄鸟在24小时里鸣唱活动的总数量和总时间；为了研究探索能力，我建造了一个可以允许鸟类主动前往一个新的环境并进行探索的设施。这样的设施可以彻底避免常规实验里抓捕和转移实验对象所造成的影响。通过对比在两种噪音环境下成长的鸟类的实验结果，我并没有发现这些鸟在探索能力和雄性唱歌能力中的系统性差异：雄鸟在24小时内歌唱的总次数和总时间大致相同，性别与成长噪音环境都不能解释探索能力中鸟类的差异。

本论文中的一系列研究成果表明：噪音本身，即使不包括车流、尾气等因素，也足以让鸟类远离公路。这些被鸟类主动躲避的公路噪音也会影响鸟类的繁殖和育幼。对噪音中成长的鸟来说，成长环境与最近所经历的噪音状况共同影响着它们是否逃避某种噪音；成年后，这些鸟的探索能力和（雄鸟的）歌唱能力却没有受到成长时的噪音环境的影响。这一系列研究结果表明，噪音本身会对鸟类的分布与繁殖行为造成影响，这种影响似乎可以解释为什么鸟类在公路旁的种群数量较少。这些研究还发现，即便从小在噪音环境里长大，鸟类也不会永久容忍噪音。这些结果加深了我们对于噪音如何影响鸟类种群数量和物种数量的理解，可以进一步帮助解决公路噪音对于鸟类的负面作用。

B

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Curriculum Vitae

Quanxiao Liu received his secondary education at Dalian No.39 Middle School and Dalian No.24 High School from 2002 to 2008 in Dalian, China. He then moved to the city of Guangzhou in China to attend Sun Yat-sen University for a five-year bachelor program, specializing in *Ecology*. During his bachelor program he participated in an exchange program at the National Cheng Kung University in Taiwan in 2009, taking part in a field project investigating egret populations in South Taiwan. After obtaining his bachelor degree, he started his master program *Ecology, Evolution and Biodiversity* at Leiden University with a *Leiden University Excellence Scholarship*. During this program, he was involved in two projects, *How carcass freshness affects parental care investment in burying beetles* and *The effects of anthropogenic noise on locating conspecific calls in field crickets*. Upon the completion of his master program, he wrote a successful grant application for the Chinese Scholarship Council with Dr. Katharina Riebel. In 2015, he started his PhD. program under the supervision of Prof. dr. Carel J. ten Cate, Dr. Katharina Riebel and Dr. Hans W. Slabbekoorn.