Effect of noise on vocal behaviour, physiological systems and reproductive success in birds

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General introduction

Since the dawn of the industrial revolution, the world has experienced an exponential human population increase, and with it a parallel increase in the growth of urbanized areas. Urbanization has an irreversible impact on ecosystems (United Nations, 2012). Indeed, the growth of urban areas is considered one of the biggest causes of contamination (Grimm et al., 2008a), counting chemical, light and noise pollution. Anthropogenic noise is expected to increase immeasurably levels, as urban areas continue to growth. Anthropogenic noise has been related to harmful consequences for humans (United Nations, 2012) and animals (e.g Gil and Brumm, 2013) in cities. Despite the cost of living in a noisy environment, currently, very little is known about the effect of noise pollution on the health and fitness of animals in urban areas.

Bird species are a good model to investigate the impact of noise. Many species of birds have survived and developed different strategies to live in urban habitats (Gil and Brumm, 2013). In fact, many of them are exposed to anthropogenic noise during their whole life. Therefore, increasing the knowledge around the effects of noise on birds not only contributes to progress of basic science but may also have immediate relevance for conservation actions to maintain biodiversity and to create awareness about the current state and consequences of human lifestyle. In addition, birds have similarities to humans, such as the effects of environmental conditions on physiological systems and the vocal learning process, which may allow us to further understand the mechanisms underlying the effects of noise in people exposed to chronic noise.

In this thesis, I investigate the way noise can impact birds through three different mechanistic pathways (Fig. A), by disrupting communication, by impacting physiological systems via stress, and by interfering with development. In turn, each of these systems can be interconnected, and may affect the others, resulting in both direct and indirect effects on the fitness of birds. 1. Noise may have an impact on vocal communication. Survival and reproductive success are highly correlated to successful communication. Noise pollution may affect fitness of the individuals exposed to it by disrupting communication signals that are fundamental to different stages in life.
like: mate attraction, familial bonds, begging calls, preventing rivals to invade territories, perceiving predators and foraging. 2. Noise may have an impact on physiological stress responses in birds. Health, immune function and longevity may be reduced by noise exposure affecting fitness of the exposed individuals. At the same time, the quality of vocal signals is related to physical condition (Ritschard and Brumm, 2012). Therefore, if noise negatively affects health condition in birds it may affect fitness by influencing the song, which is the mating signal. 3. Noise may have an impact on development and learning in birds. Stages of development and learning are crucial moments that can shape adult life in birds, any deficit or delay can have serious consequences affecting fitness. I will expound upon what we already know about each of these three systems, and what we can hypothesize about the way they may be impacted by noise exposure in the sections below.

**Figure A.** A conceptual scheme showing how environmental noise may impact fitness by affecting different biological systems. These systems can, in turn, influence each other in ways that indirectly decrease fitness. Adapted from: Brumm, 2012

1. **The impact of noise on vocal communication**

Acoustic signals in birds are crucial in several contexts directly related to fitness and the survival of individuals. Birds use acoustic signals to attract mates, to defend territories, to communicate with their parents, to maintain social cohesion, share information about food sources, and to prevent or avoid predation (Bradbury and Vehrencamp, 2011; Catchpole and Slater, 2008). These signals can be adjusted according to the constraints of the environment. For example, birds adjust
their signals in response to changes in the social context (e.g. Brumm and Slater, 2006b; Gavassa et al., 2013; Janik, 2000) or when they experience challenging conditions for signal detection (e.g. Goodwin and Podos, 2013; Lengagne et al., 1999). Certainly, environmental constraints on signal detection are considered one of the major forces driving the evolution of animal communication systems across different taxa (Brumm, 2013; Wiley, 2015).

Background noise typically constrained signal detection in acoustic communication (Brumm and Slabbekoorn, 2005a; Klump, 1996). One of the forms of vocal plasticity to overcome background noise is the Lombard effect. The Lombard effect is the regulation of vocal amplitude in relation to noise level (Brumm and Zollinger, 2011) and can occur very fast as a response of background noise (Hage et al., 2013; Hardman et al., 2017; Luo et al., 2017a). The use of Lombard effect has been reported in many bird species, as well as in mammals, including humans (reviewed in Brumm and Zollinger, 2011; Hotchkin and Parks, 2013). In addition, recent evidence showed that two frog species use Lombard effect in response to noise playbacks (Halfwerk et al., 2016; Shen and Xu, 2016). Nevertheless, an anuran species (Love and Bee, 2010), and a reptile (Brumm and Zollinger, 2017) did not show the Lombard effect in the presence of background noise.

The Lombard effect, according to modelling studies is more efficient in increasing signal detection in noisy backgrounds than other frequently observed changes in other signal parameters (Luo et al., 2015; Nemeth and Brumm, 2010). Some species, such as domestic fowl (Gallus gallus) in the presence of noise just adjust the amplitude (Brumm et al., 2009a). In other cases, the Lombard effect is accompanied by changes in other spectral and temporal vocal traits. However, this multiple adjustment varies depending on the species (reviewed in Brumm and Zollinger, 2011). For instance, in humans an increase in fundamental frequency and/or a shift in spectral tilt is associated with the Lombard effect (e.g. Cooke and Lu, 2010; Jessen et al., 2005; Liénard and Di Benedetto, 1999). In birds such as in a tinamou and a parrot an increase in vocal pitch is associated with the Lombard effect (Osmanski and Dooling, 2009) differently to songbirds (Potvin and Mulder, 2013; Templeton et al., 2016; Zollinger et al., 2017). These differences may be explain by a physical coupling of the two parameters during vocal production (Beckers et al., 2003; Elemans et al., 2008) that in songbirds can be decoupled. In other cases, the Lombard effect is associated with changes in both spectral and temporal traits like in the case of a
new world monkey (Hotchkin et al., 2015). Additionally, primates (e.g. Brumm et al., 2004; Hotchkin et al., 2015; Summers et al., 1988), a bat (Luo et al., 2015), a songbird (Leonard and Horn, 2005), and a parrot (Osmanski and Dooling, 2009) showed an increase in the duration of brief vocalizations, which improves detectability (Klump and Maier, 1990; Luo et al., 2015; Pohl et al., 2013). However, in some cases vocal changes, like in echolocating bats, in the presence of noise are independent from the Lombard effect. (e.g. Hage et al., 2014; Luo and Wiegrebe, 2016).

While the Lombard effect has been well studied in many different species, the evolutionary history of the trait remains unclear. Even though all tested bird and mammal species present the effect (reviewed in Brumm and Zollinger, 2013; Hotchkin and Parks, 2013) to establish whether or not it is ancestral in either clade, major lineages still need to be tested. To understand the phylogeny of the Lombard effect, it is important to take into account that extant birds are classified in two groups: Palaeognathae, and Neognathae, the latter contain more than 99% of all extant species (Mayr, 2017). To date the Lombard effect has been reported in a paleognath clade, the Tinamiformes, and in two of the three neognath lineages, the Galliformes and Neoaves (reviewed in (Brumm and Zollinger, 2011). Nevertheless, whether Anseriformes, the third neognath lineage present the Lombard effect is still unknown.

Apart from the relationship between noise and changes in vocal signalling, noise can have negative fitness consequences on signal receivers by masking important acoustic signals and cues, such as alarm signals, noises made by predators or prey, or signals relevant for breeding behaviour and offspring care (Arroyo-Solís et al., 2013; Fernández-Juricic and Tellería, 2000; Fuller et al., 2007; L Quinn et al., 2006; McGiffin et al., 2013; McIntyre et al., 2014; Meillere et al., 2015; Nordt and Klenke, 2013; Templeton et al., 2016). Acoustic masking by noise may be crucial particularly for birds during their reproductive time, since many species use acoustic signals (song) to attract mates and defend territories, to keep bonds, and coordinate offspring feeding and care (Catchpole and Slater, 2008). Therefore, noise disruption may potentially cause major fitness consequences (Brumm and Slabbekoorn, 2005b).

Noise can mask parental alarm calls, decreasing the response of nestlings towards predator threats (McIntyre et al., 2014). Detection of alarm calls by great tits is significantly impaired in
the presence of traffic noise (Templeton et al., 2016), which is likely to increase the risk of predation in habitats with high levels of noise pollution. In the same direction, detection of predators can be affected by noise (Fernández-Juricic and Tellería, 2000; L Quinn et al., 2006; McGiffin et al., 2013; Meillere et al., 2015). Certainly, some species increase vigilance time as a result of it, causing a reduction of feeding rate (Fernández-Juricic and Tellería, 2000; L Quinn et al., 2006). Other species showed differences in anti-predator behaviour but not directly related to reproductive performances (McGiffin et al., 2013; Meillere et al., 2015). Additionally, studies have suggested that noise is related to changes in timing of song for some birds in the city (Arroyo-Solís et al., 2013; Fuller et al., 2007; Nordt and Klenke, 2013).

Cities are becoming important places to study the interactions between noise and changes in vocal signalling and acoustic signals and cues since they provide dynamic environments in which wildlife are living and adapting to a changed environment. However, cities are also characterized by fundamental changes in ecological factors, noise is one of these parameters but it is not the only one (Shanahan et al., 2014). For instance, different studies have shown that light pollution also affects biological rhythms, reproductive and vocal behaviour in birds, e.g timing of dawn chorus (Bergen and Abs, 1997; Da Silva et al., 2015; Dominoni and Partecke, 2015; Dominoni et al., 2013a, 2014; Kempenaers et al., 2010; Miller, 2006).

Birds have a peak of singing activity just before sunrise, well-known as the dawn chorus (Catchpole and Slater, 2008). Several hypotheses have been proposed to explain the adaptive value of the dawn chorus; however the function is still debated. For example, the microclimatic conditions before sunrise may favour sound transmission due to low acoustic attenuation (Henwood and Fabrick, 1979). It may also be the movement of invertebrates at that time is reduced because of low temperatures and light levels, therefore singing might be more profitable than foraging before sunrise (Hutchinson, 2002; Kacelnik, 1979; Kacelnik and Krebs, 1983). Additionally, studies have proposed that the onset of dawn singing may be an indicator of age (Kempenaers et al., 1997) or male quality (Barnett and Briskie, 2007; Thomas and Cuthill, 2002) and thus could be related to female choice. Certainly, several studies found that males that started singing earlier in the morning had more extra-pair offspring (Kempenaers et al., 2010; Poesel et al., 2006; Sexton et al., 2007) highlighting the importance of the dawn chorus in reproduction. Supporting evidence arises from a study that experimentally demonstrated that a delayed dawn
song was associated with a decreased number of extra-pair offspring in great tits (Parus major) (Greives, 2015).

Artificial light during the night has been related to an advance in the onset of the dawn chorus in the morning (Bergen and Abs, 1997; Dominoni et al., 2013a; Kempenaers et al., 2010; Miller, 2006) and earlier in the year (Da Silva et al., 2015). In addition, some evidence showed that noise pollution also has an impact on the timing of the dawn chorus. For instance, blackbirds (Turdus merula) sing earlier in places with high noise levels (Nordt and Klenke, 2013). Additionally, in another study starlings (Sturnus unicolor) and house sparrows (Passer domesticus) in response to noise playback started to sing earlier at dawn (Arroyo-Solís et al., 2013). Furthermore, European robins (Erithacus rubecula) were found to show night singing activity in urban areas, likely to avoid high levels of diurnal background noise (Fuller et al., 2007).

Together, all these studies clearly showed that noise has serious repercussions concerning vocal communication in birds. However, there are still some gaps regarding the impact of noise on vocal communication. For instance, this field has been little studied in tropical areas, where uncontrolled urbanization is growing and the majority of bird’s biodiversity is present (MacArthur and MacArthur, 1961; Wilson, 1999). In the tropics the effects of noise on vocal behaviour may change because day length varies only slightly and some species seem not to rely on day length cues to time their reproductive stages (Moore, 2005). Although some tropical species respond to photoperiodic changes (Hau et al., 1998; Quispe et al., 2017), it may be that some other species do not use it because variations in day length are too small, or because day length is not a reliable predictor of environmental conditions. Therefore, it is unclear how urbanization, specifically noise and light pollution affects the timing of reproductive behaviours, such as song. In addition, the vast majority of the studies investigating the impact of noise on birds have focused on oscine songbirds, and thus very little is known about the strategies used by birds of other taxonomic groups, such as ducks, one of the species that are also common inhabitants of cities. Besides, investigating the effect of noise in different species and different places in the world will give insights of how the patterns of urban ecology are functioning across species and latitudes. Finally, the mechanisms underlying the changes on communication due to noise are still unknown, thus controlled experiments are needed to investigate them.
2. The impact of noise on physiological systems

Exposure to high levels of ambient noise has been shown to have different physiological effects on mammals including humans as well as in galliform birds. Noise can reduce health, immune function and longevity interfering with fitness of the exposed individuals. Chronic noise in humans has been correlated with high cortisol, adrenaline and noradrenaline, as well as with high-pressure levels, increased glucose and cholesterol levels in the blood (Stansfeld and Matheson, 2003). Additionally, noise has been linked to impairment in performance in memory and cognitive tasks during adulthood. Children exposed to chronic noise have presented reading deficit, impaired speech perception and problems with long and short-term memory (Clark and Stansfeld, 2007; Cohen et al., 1973; Haines et al., 2001; Hygge et al., 2002; Klatte et al., 2013; Stansfeld and Matheson, 2003). Non-acoustic effects of noise have been found in animals. In mice, cellular and humoral immune responses have been affected by noise exposure (Zheng and Ariizumi, 2007). In the same vein, noise has been found to have an effect on levels of corticosterone and catecholamine in rats (De Boer et al., 1989).

Previous studies have suggested that there may be a link between chronic noise and reduced fitness, expressed in reproductive success, in free living birds. Ovenbirds (*Seiurus aurocapilla*) living in boreal forest in Canada close to compressor sites showed reductions in pairing success, as well less experienced young-ovenbirds males were found to live close to natural gas and oil stations (Habib et al., 2007). Habib et al., (2006), attributes the decrease in pairing success to the disruption of vocal communication that may happen close to the compressors. In the same direction, great tits breeding close to highways, where traffic noise levels are higher, laid smaller clutches and had fewer fledglings compared to individuals away from the roads (Halfwerk et al., 2011). In addition, hatching rates and fledging success is reduced for Eastern bluebirds (*Sialia sialis*) living in noisy places (Kight et al., 2012). Also house sparrows breeding close to large generators produced fewer young, of lower body mass, fewer recruits and parents provision their young less often compared to breeding sites away from the generators (Schroeder et al., 2012). All these field studies point to a correlation between noise pollution and reduced fitness. There is one very recent experimental study that found that embryo mortality was higher in birds exposed to traffic noise and mothers with higher corticosterone levels were affected in laying latency and
clutch size (Potvin and MacDougall-Shackleton, 2015). However, carefully pair designed experimental studies using real levels of traffic noise under controlled conditions are still needed.

Up to now, learning impairment and somatic development as physiological stress responses in developing birds have been associated to different stressors (e.g. weather, agonistic encounters, food deprivation and short-term noise exposure). However the link between chronic anthropogenic noise and physiological stress responses at early stages in birds is still unknown. Stress response is known as the physiological processes activated by a stimulus (stressor). During the stress response the activation of the hypothalamic-pituitary-adrenal (HPA) axis plays a key role including the release of glucocorticoid hormones (review in Dawson et al., 2002). Stress exposure through early stages like deficit in nutrition, brood size manipulation or experimental administration of glucocorticoids have been found to impact vocal behaviour, in some cases, related to song learning. Specifically birds exposed to stressors in early life showed shorter song motifs (Spencer et al., 2003), decreased song complexity (Nowicki et al., 1998, 2002), song learning impairment (Brumm et al., 2009a) and delayed development in song control regions of the brain (MacDonald et al., 2006; Nowicki et al., 2002).

The response to regular stress exposure can be different for some species. Commonly, stress exposure causes an increase in baseline levels of glucocorticoids. In some cases, birds exposed to a severe stressor exhibited temporally higher levels of plasma corticosterone. In the same direction, birds exposed to chronic stress may persistently elevate their corticosterone levels. On the other hand, there are cases in which birds that have been exposed to stressful conditions showed decreased levels of glucocorticoids (Rich and Romero, 2005). For instance, European starlings (*Sturnus vulgaris*) seem to reduce activity in the hypothalamic-pituitary-adrenal (HPA) axis in response to chronic stress. Chronically stressed birds had similar levels of plasma glucose and triglyceride than birds unexposed to stressors. In addition, stressed birds showed a reduction in baseline levels of plasma corticosterone. This counter-intuitive response may be a mechanism to avoid the negative pathological effects linked to chronically high glucocorticoid concentrations (Cyr and Romero, 2007, 2009).

Despite the apparent non-response to chronic stress in European starlings, it is important to take into account that this result does not necessarily suggest that chronic stress won’t have serious
and harmful consequences nor that is a sign of habituation. Reproductive success was affected by chronic stress exposure (Cyr and Romero, 2007, 2009) as well as body weight and blood chemistry (Awerman and Romero, 2010). In addition, individuals showed depressed responses to novel stressors (Rich and Romero, 2005). All these together were signs that habituation to stressors did not occur (Cyr and Romero, 2009).

Telomere shortening has also been associated with stress in mammals and some birds, likely as a result of increased oxidative stress (Epel et al., 2004; Haussmann et al., 2012; von Zglinicki, 2002). Telomeres are the non-coding, repetitive DNA sequences that cap the ends of eukaryote chromosomes that have the function of marking chromosome ends and protecting them from degradation (Monaghan, 2014). Their length correlates with longevity (Heidinger et al., 2012), and their attrition is thought to play a role in cell senescence and ultimately in increased risk of mortality (reviewed in Haussmann and Marchetto, 2010; Monaghan et al., 2009). Therefore, the analysis of telomere dynamics has become an important molecular tool to understand the effects of chronic stress exposure in animals (Heidinger et al., 2012; Monaghan, 2014; Monaghan et al., 2009).

Physiological stress responses may be linked to learning impairment and delayed in the presence of stressors like noise. Thus, exposure to noise during learning phases may be associated to physiological stress responses. Laboratory mammals have been found to suffer from disruptions to the learning processes and delayed neural development in the presence of noise. For example, industrial noise exposure caused various cytological modifications in the adrenal cortex, which through the production of mineralcorticoids and glucocorticoids mediate stress responses, of rats (Oliveira et al., 2009). Mice that were experimentally exposed to chronic noise had increased levels of oxidative stress and cellular damage in three regions of the brain: the inferior colliculus (midbrain nucleus of the auditory pathway that receives input from the auditory cortex and from several peripheral brainstem nuclei in the auditory pathway), auditory cortex (part that processes auditory information) and hippocampus (fundamental for consolidation of information from short-term memory to long-term memory and spatial memory) (Cheng et al., 2011). Additionally, mice exposed to noise showed learning and cognitive impairment in cognitive tasks (Cheng et al., 2011).
3. The impact of noise on development and learning

The external conditions during early developmental stages can be crucial to the long-term development and fitness of animals. For instance, high levels of environmental noise at prenatal stages has been correlated with deficit of calcium in bones and teeth, premature births, slow growth rates and egg mortality in mammals, fish and shrimps (reviewed in Kight and Swaddle, 2011). In birds, the consequences of prenatal noise exposure are still unknown. However, noise potentially, could interrupt pre-hatch communication in birds.

Auditory stimuli are important while birds are still in the egg (Höchel et al., 2002). Several bird species synchronize hatching time in response to calls emitted before hatching (Woolf et al., 1976). Also zebra finches showed a phenotypical response to calls from their parents indicating high ambient temperature (Mariette and Buchanan, 2016). Any interference during this communication period could have serious consequences in the lifetime reproductive success and thus decrease fitness. In addition, exposure to high levels of white noise has been shown to affect negatively vocal learning and behaviour of nestlings and fledgling songbirds, in some cases even used to experimentally deafen birds. Birds exposed to extreme high levels of noise produced atypical songs that are similar to songs of deafened birds (Funabiki and Funabiki, 2009; Funabiki and Konishi, 2003; Leonardo and Konishi, 1999; Zevin et al., 2004). Noise experienced in cities, usually, is not high enough to cause temporary or permanent hearing loss in birds (Dooling, 2005). However, chronic noise even at moderate levels can potentially negatively impact auditory development and learning.

Rat pups were found to have a significant delay in the development of the auditory cortex when they were exposed to continuous and moderate level of noise during development (Chang and Merzenich, 2003). In birds, the impact of traffic noise in vocal learning has been little explored. There is one study that showed that the syntax of the song in zebra finches is affected by traffic noise, as well as the size of brain regions associated with song learning (Potvin et al., 2016). It is still unknown whether song crystallization is delayed by noise or not. In a study by (Zollinger et al., 2017), great tits were exposed to moderate levels of filtered high or low frequency noise for the first year. They found that in the birds exposed to noise that most heavily overlapped the tutor
song frequencies, they crystallized song nearly a month later then the group with noise that did not significantly overlap their tutor songs (Zollinger, personal communication). This suggests that noise might delay song ontogeny. However, whether this delay in learning was the product of slower brain development, such as that found in rats (Chang and Merzenich, 2003), or of a different mechanistic pathway, is still unclear.

**Thesis outline**

The aim of the first chapter was to investigate the impact of noise on vocal behaviour. In the chapter I.1, I tested whether and how an anseriform bird, the mallard duck (*Anas platyrhynchos*), can compensate for elevated levels of noise in its environment. In addition, I examined if a potential Lombard effect in ducks might be accompanied by other call parameters. These results will help contribute to the phylogenetic reconstruction of the evolution of the Lombard effect and related aspects of vocal plasticity in birds.

In the chapter I.2, the goal was to disentangle whether anthropogenic noise after dawn or artificial light during the night better predicts the onset of the dawn chorus in rufous-collared sparrows (*Zontricha capensis*) in Bogota, Colombia, one of the largest cities in tropical South America. As far as I know, this is the first study on this topic conducted in the tropics. These results will contribute to understand how urban ecology function in tropical cities. In tropical areas rufous-collared sparrows do not rely on photoperiod to regulate their life stages, instead they breed when local conditions and weather are favourable (Class, 2009; Moore, 2005). Additionally, day length varies only marginally in our study site. Therefore, I predict a stronger impact of traffic noise than light pollution on the onset of the dawn chorus of rufous-collared sparrow.

Chapter II and III are focused on the effects of traffic noise on stress physiology. To understand how chronic noise exposure affects physiology and well-being of birds is important to design integrative studies that allow us to link proximate aspects, such as physiology and ultimate aspects like reproductive success, as a proxy for fitness. Thus, I investigated these topics with a series of experiments in breeding zebra finches and their progeny. Specifically, in chapter II, I experimentally investigated the direct and cross-generational effects of traffic noise exposure at
different developmental stages on telomere lengths at 21 and 120 days post-hatch in zebra finches bred in aviaries in our laboratory. In chapter III, I investigated the impact of traffic noise on the immune system, baseline corticosterone levels, reproductive success and extra-pair paternity in captive zebra finches. I predicted that traffic noise would act as stressor. Therefore, I expected to find higher levels of baseline corticosterone associated to a deficit on the immune system and a low performance in reproductive success. Additionally, since song is modified by noise (reviewed in Brumm and Zollinger, 2013) and could have an impact on breeding performance (Halfwerk et al., 2011; Kight and Swaddle, 2011; Potvin and MacDougall-Shackleton, 2015; Schroeder et al., 2012), I predicted an effect on extra-pair paternity.
Chapter I. Impact of noise on vocal behaviour in birds

I.1 Vocal plasticity in mallards: multiple signal changes in noise and the evolution of the Lombard effect in birds


Abstract

Signal plasticity is a building block of complex animal communication systems. A particular form of signal plasticity is the Lombard effect, in which a signaler increases its vocal amplitude in response to an increase in the background noise. The Lombard effect is a basic mechanism for communication in noise that is well-studied in human speech and which has also been reported in other mammals and several bird species. Sometimes, but not always, the Lombard effect is accompanied by additional changes in signal parameters. However, the evolution of the Lombard effect and other related vocal adjustments in birds are still unclear because so far only three major avian clades have been studied. We report the first evidence for the Lombard effect in an anseriform bird, the mallard (Anas platyrhynchos). In association with the Lombard effect, the fifteen ducklings in our experiment also increased the peak frequency of their calls in noise. However, but they did not change the duration of call syllables or their call rates as has been found in other bird species. Our findings support the notion that all extant birds use the Lombard effect to solve the common problem of maintaining communication in noise, i.e. it is an ancestral trait shared among all living avian taxa, which means that it has evolved more than 70 million years ago within that group. At the same time, our data suggest that parameter changes associated with the Lombard effect follow more complex patterns, with marked differences between taxa, some of which might be related to proximate constraints.
Chapter I. Impact of noise on vocal behaviour in birds

Introduction

Signal plasticity is a key feature of derived animal communication systems that allows individual signal adjustments in response to changes in the environment. Animals use this capacity in situations such as changing social contexts (e.g. Brumm and Slater, 2006b; Gavassa et al., 2013; Janik, 2000) or coping with challenging conditions for signal detection in fluctuating environments (e.g. Goodwin and Podos, 2013; Lengagne et al., 1999). Indeed, there is growing evidence that signal detection constraints are one of the major forces driving the evolution of animal communication systems across different taxa (Brumm, 2013; Wiley, 2015).

In acoustic communication, signal detection is particularly constrained by background noise (Brumm and Slabbekoorn, 2005a; Klump, 1996). One efficient form of signal plasticity that mitigates masking by noise is the Lombard effect, i.e. the regulation of vocal amplitude in relation to the noise level (Brumm and Zollinger, 2011). This phenomenon is found in many birds and mammals, including human speech (reviewed in (Brumm and Zollinger, 2011; Hotchkin and Parks, 2013). Recently, the Lombard effect has also been reported in two frog species (Halfwerk et al., 2016; Shen and Xu, 2016) but it is absent in another anuran species (Love and Bee, 2010), and it is also absent in a non-avian reptile (Brumm and Zollinger, 2017).

Several studies have addressed how the strength of the Lombard effect is affected by noise characteristics (e.g. Garnier et al., 2010; Kobayasi and Okanoya, 2003). Others have investigated the neural substrate underlying the effect (Eliades and Wang, 2012; Hage et al., 2006; Nonaka et al., 1997). In echolocating bats and singing canaries (Serinus canaria), the Lombard effect is triggered at least 30-320 milliseconds after noise onset (Hage et al., 2013; Hardman et al., 2017; Luo et al., 2017a), which means that vocal amplitude can track noise levels more or less in real time.

Sometimes the Lombard effect is accompanied by changes in other vocal parameters, although such multiple adjustments are not universal (reviewed in Brumm and Zollinger, 2011). For instance, primates (e.g. Brumm et al., 2004; Hotchkin et al., 2015; Summers et al., 1988), a bat (Luo et al., 2015), a songbird (Leonard and Horn, 2005), and a parrot (Osmanski and Dooling,
2009) also increase the duration of brief vocalizations, which further increases detectability (Klump and Maier, 1990; Luo et al., 2015; Pohl et al., 2013). In contrast, domestic fowl (Gallus gallus) do not increase call syllables duration with Lombard-induced increases in amplitude (although, like other birds, they show the Lombard effect) (Brumm et al., 2009a). Likewise, spectral signal changes may also accompany the Lombard effect in some species but not in others. In human speech, for instance, the Lombard effect is often associated with a concurrent increase in fundamental frequency and/or a shift in spectral tilt (e.g. Cooke and Lu, 2010; Jessen et al., 2005; Liénard and Di Benedetto, 1999). Both phenomena have also been found in the calls of a New World monkey (Hotchkin et al., 2015). In contrast, echolocating bats may adjust their call frequencies in noise but this response appears to be independent from the Lombard effect (e.g. Hage et al., 2014; Luo and Wiegrebe, 2016). In birds, however, Lombard-related increases in vocal pitch were observed in a tinamou and a parrot (Omsanski and Dooling, 2009) but not in songbirds (Potvin and Mulder, 2013; Templeton et al., 2016; Zollinger et al., 2017). These differences between bird species cannot be explained by the fact that songbirds are vocal learners because parrots also acquire their calls through vocal production learning. It may be that the concurrent increase of amplitude and pitch in some species reflects a physical coupling of the two parameters during vocal production (Beckers et al., 2003; Elemans et al., 2008) that can be decoupled in songbirds. The fact that the Lombard effect is widespread in birds and mammals, while the suite of associated parameter changes differs between species, parallels findings from modelling studies suggesting that the Lombard effect is more efficient in increasing signal detection in noise than the other frequently observed changes in other signal parameters (Luo et al., 2015; Nemeth and Brumm, 2010).

Despite the wealth of studies on the Lombard effect in many different species, the evolutionary history of the trait remains unclear. Though the effect is present in all tested bird and mammal species (reviewed in (Brumm and Zollinger, 2013; Hotchkin and Parks, 2013) it is not possible to establish whether or not it is ancestral in either clade because in both groups major lineages have not yet been investigated. Extant birds are divided into Palaeognathae, and Neognathae, with the latter comprising more than 99% of all extant species (Mayr, 2017). The Lombard effect is present in a paleognath clade, the Tinamiformes, and in two of the three neognath lineages, the Galliformes and Neoaves (reviewed in (Brumm and Zollinger, 2011). However, the character state is not documented in the Anseriformes, the third neognath lineage. To fill this gap, we tested
whether an anseriform bird, the mallard (*Anas platyrhynchos*), exhibits the Lombard effect. We also examined if a potential Lombard effect is accompanied by other call parameter changes in this species. The results will help reconstructing the phylogenetic origin of the Lombard effect and other related vocal changes in birds and they will also allow testing the hypothesis that an uncoupling of amplitude and pitch during the Lombard effect is a derived feature of songbirds.

**Methods**

*Animals and housing*

The experiment was conducted at the Max Planck Institute for Ornithology in Radolfzell, Germany. We used 15 mallard ducklings, aged between two to ten days post hatching. The birds came from three different clutches and all subjects were kept with their parents and siblings in family outdoor aviaries (3.95 x 2.90 x 2.60 m), equipped with a pond and roof. The birds were fed on an ad libitum diet. As in many precocial birds, vocalizing in mallard ducklings can readily be elicited by separating them from their brood (Evans and Gaioni 1986; Gaioni and Platte 1982). Typical mallard separation calls consist of series of several of relatively short, frequency modulated call elements (Fig. 1).

![Figure 1. Typical separation call of a mallard duckling. In this example the call series consists of 7 elements. In our sample the number of elements per series ranged between 6-23.](image)

*Experimental set-up*

The subjects were placed singly in a wire cage (48x36x60 cm) in the centre of a test arena (2.55 x 1.64 m) which was surrounded by sound absorbing materials. An omnidirectional microphone (Sennheiser ME66) connected to a digital recorder (Marantz PMD660) was suspended 40 cm above the cage to record the calls of the ducklings. Recording with the microphone above the
animal and facing downwards reduces variation due to the orientation of the animal and the
directional pattern of vocal sound radiation (Brumm, 2002; Brumm and Zollinger, 2011). Filtered
white noise (bandwidth 0-12kHz) was broadcast from a PC, fed through an amplifier (Technics
SU-V300M2) and then to four loudspeakers (JBL PRO III N108) that were placed in the middle
of each of the four sides of the arena. Experimental treatments consisted of noise played at two
levels, 60 and 70 dB SPL (measured with a Voltcraft SL 400 sound level meter in the center of
the test cage at the height of the ducklings’ heads) and no noise in random order. The mean
ambient noise level, when no experimental noise was broadcast, was 44 dB(A) SPL. After a
duckling had produced at least 15 call elements, which took on average about one minute, the
noise treatment were changed.

Acoustic analysis and statistics
The calls were recorded as WAV files with a sampling rate of 44.1 kHz and 16-bit resolution. All
acoustic analyses were done using the software using Avisoft SASLab Pro version 5.2.0.8
(Avisoft Bioacoustics, Germany). Call rates were determined from spectrograms (FFT size 1024
points, Hamming window) and the peak frequencies and durations of the call elements were
measured using the automatic measurement function of Avisoft (durations were measured at 10
dB below the peak, frequency resolution: 43.1 Hz). The maximum rms amplitude of each element
was measured with an integration time of 50 milliseconds and then the background noise value
was subtracted following published procedures (Brumm and Zollinger, 2011). The amplitude
measurements were calibrated with a recording of white noise of known sound pressure level
recorded with the experimental recording set-up (70 dB SPL, measured at the position of the
recording microphone with a Voltcraft SL 400 SPL meter).

All statistical analyses were performed with R 3.1.1 (R Core Team 2013). We fitted linear mixed-
effects models and general linear models using the “lmer” and “glmer” functions (R package
lme4). Additionally, we used the “sim” function (package arm) to simulate the posterior
distribution of the model parameters, values were extracted based on 2000 simulations (Gelman
and Hill, 2007). The statistical significance of fixed effects and interactions were assessed based
on the 95% credible intervals (CI) around the mean (estimate). We considered an effect to be
“significant” in the frequentist’s sense when the 95% CI did not overlap zero (Nakagawa and
Cuthill, 2007). We fitted one model for each call parameter, our response variables were the
parameters and background noise (dB SPL) was fitted as fixed effect and individual ID as random effect. To examine effects of noise on call rate and the number of elements per series we fitted general linear models (glm) with a poisson distribution. In the call-rate model we fitted the number of calls as the response variable, taking into account in the model duration of the series as a covariate. Since all the individuals were tested with the three treatments, all the models were run taking into account the order in which the treatment were presented to each individual, as well as the age of the individual (days-post hatched). However we did not find any significant correlations with the order of treatment nor with age. Thus, we present only the reduced models without these variables. A general correlation between the peak frequency and the amplitude of call elements was investigated with a Spearman rank correlation. For this test, we randomly selected 45 elements from each individual, irrespective of the noise treatment. (for five birds 45 elements was the total number of elements recorded during the experiment).

**Results**

On average we recorded 3 call series comprising in total between 15 and 30 elements per bird and treatment (median: 17 elements). The amplitude of the call elements increased significantly in response to an increase of background noise (Table 1, Fig. 2). Thus, the subjects tested showed the Lombard effect, with an average increase of a 4.8 dB between no-noise (when ambient noise was approx. 44 dB(A) SPL) and the 70dB noise conditions. The 10-day old birds showed a much stronger Lombard effect than the younger birds, with individual increases of up to over 20 dB (Fig. 3).

In addition to the Lombard effect, the ducklings also increased the peak frequency of their call elements in noise (Table 1, Fig. 2). Between the no-noise and the 70dB conditions call peak frequencies increased on average by 227 Hz. Peak frequency and call level were significantly correlated independent of the noise treatment (Pearson correlation: r = 0.44; N = 675, p< 0.001; Fig. 4).

In contrast, increases in noise level resulted in no significant changes in call element duration, call rate or serial redundancy, measured as the number of elements per call series (Table 1, Fig. 2).
Table 1. Outcomes of models testing the effects of three noise treatments on acoustic characteristics of mallard separation calls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimate (β)</th>
<th>95% CrI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Call level (lm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixed effects</td>
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<td></td>
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<tr>
<td>(Intercept) 45db</td>
<td>79.72</td>
<td>77.36,82.20</td>
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<tr>
<td>60db</td>
<td>3.14</td>
<td>0.47,5.89</td>
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<tr>
<td>70db</td>
<td>4.82</td>
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<tr>
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<td>Std. Dev (σ²)</td>
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</tr>
<tr>
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<td></td>
</tr>
<tr>
<td><strong>Peak frequency (lm)</strong></td>
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<td></td>
</tr>
<tr>
<td>Fixed effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Intercept) 45db</td>
<td>3825.20</td>
<td>3667.39,3972.68</td>
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<tr>
<td>60db</td>
<td>117.63</td>
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<tr>
<td>70db</td>
<td>227.41</td>
<td>103.83,359.73</td>
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<td>Random effects</td>
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<td>Individual ID</td>
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<td></td>
</tr>
<tr>
<td><strong>Call rate (glm)</strong></td>
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</tr>
<tr>
<td>Fixed effects</td>
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<tr>
<td>(Intercept) 45db</td>
<td>1.23</td>
<td>1.11,1.34</td>
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<td>-0.15,0.17</td>
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<tr>
<td>Random effects</td>
<td>Std. Dev (σ²)</td>
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<tr>
<td>Individual ID</td>
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<td>0</td>
</tr>
<tr>
<td><strong>Call duration (lm)</strong></td>
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<td>Fixed effects</td>
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<tr>
<td><strong>Number of call elements per series (glm)</strong></td>
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<tr>
<td>70db</td>
<td>0.02</td>
<td>-0.19,0.15</td>
</tr>
<tr>
<td>Random effects</td>
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<tr>
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<td>0.33</td>
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</table>
Figure 2. Change in call characteristics as a function of noise level. Data points give the mean estimates of the models with 95% credible intervals. Call level and call peak frequency varied significantly with the noise treatment, whereas the other variables did not (see Table 1 for details).
Figure 3. The strength of the Lombard effect in mallard ducklings as function of age in mallard ducklings. The Lombard strength is measured as the average increase in call level (in dB) between the quiet control and the 70 dB(A) SPL noise playback.
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Figure 4. Relation between peak frequency and call level in mallard separations calls. Colours denote different individuals. Across all individuals, the two variables were significantly correlated with each other (see results for details).

Discussion

We found that mallards exhibited the Lombard effect and that this noise-dependent regulation of call amplitude was associated with an increase in call peak frequencies. In contrast, the tested birds did not adjust their duration of call elements, their call rates or the serial redundancy of call series. The presence of the Lombard effect in an anseriform bird corroborates the notion that the common problem of communicating in noisy environments has led to the common solution of the Lombard effect in birds (Brumm and Zollinger, 2013).
Multiple signal changes in noise

Concurrent with the Lombard effect, the ducklings also increased the peak frequency of their call elements. Such additional parameter changes are often associated with the Lombard effect, but which parameters exactly are adjusted differs between species and contexts (reviewed in (Brumm and Zollinger, 2011). In some cases, a whole suite of spectral and temporal traits changes along with the Lombard effect, e.g. in human speech, but not every signal change is necessarily adaptive in the context of signal transmission (Brumm and Zollinger, 2011). For instance, a rise in vocal pitch, like the one that we observed in our ducklings, typically occurs during the Lombard effect irrespective of any release from signal masking (Lu and Cooke, 2009; Osmanski and Dooling, 2009; Schuster et al., 2012).

Like tinamous (Schuster et al., 2012) and parrots (Osmanski and Dooling, 2009), but unlike songbirds (Potvin and Mulder, 2013; Templeton et al., 2016; Zollinger et al., 2017), the Lombard-induced increase in call amplitude led to a concurrent increase in vocal pitch in the ducklings in our study. This finding supports the hypothesis that a decoupling of amplitude and frequency during the Lombard effect may be a derived trait of songbirds. The increase in call frequency in noise in ducks and other birds is most likely a passive by-product of the Lombard effect, due to a physical coupling of amplitude and frequency during phonation (Beckers et al., 2003; Elemans et al., 2008). Perhaps the more derived syrinx anatomy of songbirds (King, 1989) allows them to control amplitude and frequency of their vocalizations independently and thus enables them to uncouple both parameters during Lombard-induced increases of vocal amplitude. Such capacity might be related to the sophisticated fine-tuning of air pressure by the songbird syrinx that controls the modulation of fundamental frequency (Amador and Margoliash, 2013).

The lack of noise-dependent adjustments of call element durations in our ducks is in line with previous findings in domestic fowl (Brumm et al., 2009a). However, birds more distantly related to ducks, such as parrots (Osmanski and Dooling, 2009), as well as primates (e.g. (Brumm et al., 2004; Hotchkin et al., 2015; Summers et al., 1988) and bats (Luo et al., 2015) do increase call element durations in Lombard-induced vocalizations and it remains to be investigated why domestic fowl and mallards do not. The absence of this trait deserves particular attention because an increase in element duration increases the detectability of the calls (Klump and Maier, 1990; Luo et al., 2015; Pohl et al., 2013) and thus helps maintain communication in constant broad-
band noise as it was used in our and other experiments. Both ducks and domestic fowl belong to an early divergent bird clade, the Galloanserae, and the lack of duration adjustments in noise might be indicative that this capacity is a synapomorphy of Neoaves (and a convergent trait in mammals).

We also did not find evidence for noise-related adjustments of call rate nor serial redundancy in the tested ducklings. This is in contrast to previous studies on galliform birds (the sister taxon of the Anseriformes): Japanese quail (Coturnix coturnix japonica) increased both their call rates and the number of elements per call series in response to experimentally elevated noise levels (Potash, 1972). Domestic fowl also increase their call rates along with increasing noise levels but at noise amplitudes above 70 dB they decrease call rates again (Brumm et al., 2009b). Further evidence for a noise-related adjustment of serial signal redundancy comes from correlative field studies in other neoaves: as the environmental noise levels rises, king penguins (Aptenodytes patagonicus) produce more call syllables (Lengagne et al., 1999) and chaffinches (Fringilla coelebs) repeat song types more often (Brumm and Slater, 2006a). Taken together, the evidence shows that birds do not exercise an increase in call rates or redundancy for communication in noise as generically as the Lombard effect. Maybe the separation of the ducklings in our experiment already triggered maximum call rates and thus the tested birds could not further increase their call rates in noise. Considering that the variation in call rates did not differ between individuals, this might well be the case. Alternatively, the lack of increased call rates in noise may be due to the fact that some species utilize call element rates to encode information (Bradbury and Vehrencamp, 2011) and therefore rates cannot be adjusted to increase signal transmission without compromising the information content of the signal.

Interestingly, some of the changes that may be associated with the Lombard effect can also occur in species that do not exhibit a Lombard effect. For example, Cope’s grey tree frogs (Hyla chrysoscelis) increase their call duration in masking noise, even though call amplitude does not change (Love and Bee, 2010). Likewise, tokay geckos (Gekko gecko) do not exhibit the Lombard effect, but, like the tree frogs, do increase the duration of their call syllables in noise (Brumm and Zollinger, 2017). These findings demonstrate that some noise-related signal changes may occur independently from the Lombard effect and are perhaps phylogentically older than it.
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Ontogeny of the Lombard effect

Although we did not design our experiment to investigate the ontogeny of the Lombard effect in mallards, our observation that it is already present in birds as young as 2 days after hatching has implications for the development of this vocal trait. Previous studies have also documented the Lombard effect in young animals: in domestic chicken at four weeks after hatching (Brumm et al., 2009b), in nestling tree swallows (Tachycineta bicolor) nine to ten days post-hatch (Leonard and Horn, 2005), and in pale spear-nosed bats (Phyllostomus discolor) two weeks after birth (Luo et al., 2017b). These and our findings demonstrate an early developmental origin of the Lombard effect, suggesting a fast maturation of the neural circuits responsible for auditory-vocal feedback in birds and mammals. The neural circuits underlying the Lombard effect are thought to be located in the brainstem (Hage et al., 2006; Nonaka et al., 1997) but higher cortical areas may also be involved (Eliades and Wang, 2012). The early ontogenetic emergence of the effect suggests rather basic brain mechanisms that do not require extended maturation after birth. This basic control of the Lombard effect may then be modified by higher-level processes that may fully develop later in life. In addition, the magnitude of the Lombard effect will also be affected by the general development of the vocal apparatus, and thus may increase when animals increase their vocal power capabilities during ontogeny (Brumm and Hultsch, 2001; Leonard and Horn, 2006). Such a developmental change may explain why the magnitude of the Lombard effect in young animals increased with age (Luo et al., 2017b) because older and larger animals may be able to produce louder vocalizations and therefore can use a wider range of amplitudes. It could also be that the vocal motor control of amplitude improves with age. The latter may be accounted for our observation that ten-day old ducklings showed a much stronger Lombard effect than the younger birds not because the older birds produced higher call amplitudes in noise but rather they produced lower ones in the control treatment, suggesting greater control over amplitude modulation rather than increased power capabilities. However, the three ten-day old individuals in our experiment were all from the same clutch and thus we cannot exclude potential, yet unknown, genetic effects.

Evolution of the Lombard effect

Our results are in line with the hypothesis that the Lombard effect is present in all extant birds (Brumm and Zollinger, 2011). With the new data on mallards the Lombard effect is now documented for all three major lineages of the Neognathae (Fig. 5). Therefore, we may conclude
that the trait is probably ancestral in Neognathae, which comprise more than 99% of all extant bird species. The remaining species belong to an early diverging clade, the Palaeognathae, in which the Lombard effect is present in Tinamiformis (Fig. 5). If the effect is also a shared trait of the Palaeognathae, then it is most likely a synapomorphy of all birds. To test this hypothesis, the character state needs to be documented in other paleognathous species, especially ostriches and rheas, because the presence of the Lombard effect in these groups would strongly suggest that the trait was present in the common ancestor of the Palaeognathae (Fig. 5).

Figure 5. The evolution of the Lombard effect in birds. Plus (+): Lombard effect present, question mark (?): character state not documented. The cladogram is based on Prum et al. (2015).

Presuming that the Lombard effect is ancestral in birds, we can proceed to consider broader patterns of its evolutionary history. The absence of the Lombard effect in a lizard (Brumm and Zollinger, 2017) suggests that the noise-dependent regulation of vocal amplitude is either a convergent trait in birds and mammals or that it is a plesiomorphy that has been lost in Lepidosauria. Further Lombard experiments in Testudines and Crocodilia would be a critical test of these two alternative hypotheses. Both tortoises and crocodiles vocalize (Colafrancesco and Gridi-Papp, 2016) and if, like geckos, they do not regulate their vocal amplitudes in relation to noise, it is likely that the Lombard effect has evolved independently in birds and mammals.
Published Lombard tests in frogs are still scarce and the overall picture remains unclear. Tungara frogs, *Engystomops pustulosus* (Halfwerk et al., 2016) and concave-eared torrent frogs, *Odorrana tormota* (Shen and Xu, 2016) have been reported to adjust their call levels depending on the level of background noise. However, the Lombard effect is absent in Cope’s grey tree frogs, *Hyla chrysoscelis* (Love and Bee, 2010). Thus the evolutionary history of the Lombard effect in frogs is still largely unresolved. In birds and mammals, the number of species for which the character state has been documented is also low, but all evidence supports the hypothesis of the Lombard effect being ancestral in both clades, whereas the current evidence in frogs does not. Some frogs may not show the Lombard effect because they always call close to their physical limitations and thus have little room for amplitude adjustments (Schwartz and Bee, 2013). Such a mechanistic constraint might also explain the lack of amplitude adjustments in lizard calls (Brumm and Zollinger, 2017). The occurrence of the Lombard effect in birds and mammals, on the other hand, suggests that these animals are either under less intense selection to maximize signal transmission or that the evolution of motor control over vocal amplitude enabled them to trade off the costs and benefits of high-amplitude signals (Zollinger and Brumm, 2015).

**Conclusions**

Our study confirms an early appearance of the Lombard effect in terms of evolutionary history and ontogeny. The presence of the Lombard effect in an anseriform bird is in line with the hypothesis that the effect is a shared trait of all extant birds. If the Lombard effect is ancestral in birds, then, according to the phylogeny of (Prum et al., 2015), it has evolved at least 70 million years ago. In addition to it being likely a shared character in birds and, probably convergently, in mammals, the Lombard effect in both groups appears to already be present in very young individuals, a few days after hatching or birth, respectively. These findings highlight how important the maintenance of vocal communication in changing environments is, and hence that the need to overcome noise should be regarded as a powerful driver of the evolution of animal communication systems.
I.2 Anthropogenic noise, but not artificial light levels predicts song behaviour in an equatorial bird


Abstract

Birds in cities start singing earlier in the morning than in rural areas; commonly this shift is attributed to light pollution. Some studies have suggested that traffic noise has a stronger influence on singing activity than artificial light does. Changes in the timing of singing behaviour in relation to noise and light pollution have only been investigated in the temperate zones. Tropical birds, however, experience little seasonal variation in day length and may be less dependent on light intensity as a modifier for reproductive behaviours such as song. To test whether noise or light pollution has a stronger impact on the dawn chorus of a tropical bird, we investigated the singing behaviour of rufous-collared sparrows (*Zonotrichia capensis*) in Bogota, Colombia at two times during the year. We found that birds in places with high noise levels started to sing earlier. Light pollution did not have a significant effect. Birds may begin to sing earlier in noisy areas to avoid acoustic masking by traffic later in the morning. Our results also suggest that some tropical birds may be less sensitive to variations in day length and thus less sensitive to light pollution.

Introduction

The world is facing rapid environmental changes as a result of ever increasing urbanization. The growth of urban areas is projected to attain unprecedented levels in the next few decades, which will have dramatic effects on ecosystems; including the extinction of species (United Nations, 2012). In order to adjust to urban environments animals often need to change a whole suite of traits, including behaviour, physiology, and morphology. This is particularly true for birds, since many avian species colonize urban areas (Gil and Brumm, 2013). Due to anthropogenic activities these areas are characterized by fundamental changes in ecological factors, e.g. habitat
fragmentation, changes in micro-climate, limitation of resources, alteration of resources flow, changes in species interactions, and pollution (Shanahan et al., 2014).

Indeed, urbanization is considered one of the major causes of pollution around the world (Grimm et al., 2008a), including chemical contamination and interference by noise and light emissions. In birds, light pollution is often associated with changes in biological rhythms (Dominoni and Partecke, 2015; Dominoni et al., 2013b, 2014), which can ultimately affect breeding behaviours and fitness. For example, blue tits (*Cyanistes caeruleus*) exposed to artificial light during the night lay eggs earlier than individuals living in darker areas (Kempenaers et al., 2010). Changes in avian reproductive success have also been linked to noise pollution; it has been observed that birds in noisy areas have reduced numbers of offspring (Habib et al., 2006; Halfwerk et al., 2011) or show lower rates of provisioning for their young (Schroeder et al., 2012). Bird reproduction is particularly susceptible to noise because most species rely on acoustic signals (songs) to defend territories and attract mates (Catchpole and Slater, 2008). Therefore, acoustic masking by noise is likely to have major fitness consequences (Brumm and Slabbekoorn, 2005b). Likewise, there is a growing body of evidence showing that birds adjust their song characteristics and their song performance to mitigate interference from noise (Gil and Brumm, 2013). For instance, in response to increases in the background noise level birds increase the amplitude of their songs, and additionally they may also adjust the redundancy, the duration, the frequencies, and the timing of their vocalizations (reviewed in Brumm and Zollinger, 2013).

The daily singing activities of many songbirds have a peak just before sunrise, a behaviour that is commonly referred to as the dawn chorus (Catchpole and Slater, 2008). While the function of the dawn chorus is still debated, several hypotheses suggesting its adaptive value have been proposed. For instance, singing may be more profitable than foraging before sunrise since the movement of invertebrates at that time is reduced due to low temperatures and light levels (Hutchinson, 2002; Kacelnik, 1979; Kacelnik and Krebs, 1983). It may also be that the microclimatic conditions before sunrise create conditions that are particularly favourable for sound transmission because of low acoustic attenuation (Henwood and Fabrick, 1979). In addition, some studies have proposed that the onset of dawn singing may be an indicator of male quality (Barnett and Briskie, 2007; Thomas and Cuthill, 2002) or age (Kempenaers et al., 1997) and thus could be linked to female choice. Indeed, the significance of the dawn chorus for
reproduction is highlighted by several studies that found that males that started singing earlier in the morning had more extra-pair offspring (Kempenaers et al., 2010; Poesel et al., 2006; Sexton et al., 2007). Corroborating evidence for this notion comes from a recent study demonstrating that an experimentally delayed dawn song was associated with a decreased number of extra-pair offspring in great tits (*Parus major*) (Greives, 2015).

The onset of the dawn chorus is affected by light pollution: birds start to sing earlier in the morning (Bergen and Abs, 1997; Dominoni et al., 2013a; Kempenaers et al., 2010; Miller, 2006) and earlier in the year (Da Silva et al., 2015) when they are exposed to artificial light during the night. Some authors have argued that noise pollution also has a crucial impact on the timing of the dawn chorus. For example, Nordt and Klenke (2013) found that blackbirds (*Turdus merula*) started the dawn chorus earlier in areas with high noise levels. In another study, starlings (*Sturnus unicolor*) and house sparrows (*Passer domesticus*) advanced their dawn singing in response to noise playback (Arroyo-Solís et al., 2013). Furthermore, European robins (*Erithacus rubecula*) were found to shift their singing activity to the night in urban areas, presumably to avoid high levels of diurnal background noise (Fuller et al., 2007). All these studies have been conducted in temperate zones, where the breeding stages of birds are tightly linked to photoperiod. In the tropics, however, the effects of light and noise pollution on bird behaviour may be different because day length varies only marginally and some species seem not to rely on day length cues to time their reproductive stages (Moore, 2005). Even though some tropical species have the capacity to respond to photoperiodic changes in principle (Hau et al., 1998), it may be that they do not use it in their natural habitats either because variations in day length are too small, or because day length is not a reliable predictor of environmental conditions. Therefore, it is unclear whether light pollution affects the timing of reproductive behaviours, such as song, in tropical birds as it does in temperate birds. This issue is important because it will reveal whether the patterns of the urban ecology of bird song observed so far reflect general processes or whether they are specific to temperate conditions where photoperiod triggers reproduction. The neglect of studies on tropical birds is particularly profound since not only are many of the world’s largest urban areas found in the tropics, but the tropics are also the regions with the highest bird biodiversity (MacArthur and MacArthur, 1961; Wilson, 1999).
The main goal of this study was to disentangle whether anthropogenic noise after dawn or artificial light during the night better predicts the onset of the dawn chorus in rufous-collared sparrows (*Zontricha capensis*) in Bogota, Colombia, one of the largest cities in tropical South America. Rufous-collared sparrows are widespread in the Americas, ranging from Southern Mexico to the temperate zone in Tierra del Fuego (Chapman, 1940; Davis, 1971). In tropical areas they do not rely on photoperiod to regulate their life stages, but they breed when local conditions and weather are favourable (Class, 2009; Moore, 2005). In the Colombian Andes, rufous-collared sparrows reproduce throughout the entire year, with breeding peaks in mid-January and mid-June in certain areas (Miller, 1961). In Bogota, rufous-collared sparrows are present within the city and its surroundings, and their dawn chorus can be heard throughout the year (Rodríguez-Rocha et al., 2012). We chose different sites in the metropolitan area to include habitats with different combinations of light and noise pollution and we related these variables to the onset of the dawn chorus. Since we assumed a weaker link between diel behaviours, such as the onset of dawn singing, and photoperiod under the almost invariable day length conditions in our study population, we expected noise to have a stronger impact on the onset time of dawn singing than light pollution.

**Methods**

*Study site*

We studied 33 urban sites in public parks and gardens in the city of Bogota, Colombia (4° 35' 53" N 74° 4' 33" W). Bogota, Colombia is located in the tropical zone; around 500 km North of the equator and sunrise times vary by about 21 min throughout the year. Buildings or houses surrounded all the places of the study, creating green islands inside the urban area. Eighteen of these were visited in May-June 2013 and 15 in November-December 2013. In addition, six of the 18 May-June sites were visited again in November 2015. All of the sites were chosen based on a previous monitoring study (Rodríguez-Rocha et al., 2012) that indicated where rufous-collared sparrows are present. The two sampling seasons were chosen because they both coincided with the end of the rainy season in the particular years, during which time similar precipitation and temperature conditions were experienced. We selected these sites with particular attention to cover a broad range of different combinations of daytime noise and light pollution levels (see Fig.
Chapter I. Impact of noise on vocal behaviour in birds

1 and 3). The average distance between urban sites was 1.5 km (minimum 100 m). At every site we recorded the onset of the dawn chorus of rufous-collared sparrows, the levels of light pollution and the levels of daytime noise. Daytime noise was mainly due to traffic and the light pollution was due primarily to street lamps. To compare the onset of the dawn choruses of urban birds to those of rural birds, we also collected the same data from five rural areas in May-June. The rural sites were located between 120-125 km from Bogota, in areas with very low levels of noise and light pollution. The average distance between them was 1.5 km (minimum 500 m).

Figure 1. Noise levels and light pollution levels at the sample sites. We systematically chose locations with different combinations of light and noise and the two measures were not correlated.
Figure 2. Bogota is the capital of Colombia, located at the centre of the country (map on the right lower corner). The black lines denote the limits of the city. The red points show to the sites visited in May-June, the blue points correspond to sites visited in November-December, and the green points show the places that were visited in May-June 2013 and November 2015. Map made by Ana Maria Bastidas-Urrutia.
Onset of dawn chorus, light and noise pollution levels

Each morning, at 3:50am or earlier (i.e. at least 10 minutes earlier than the earliest record of the onset of the dawn chorus of this species in Bogota), we visited sites and recorded the time when we heard the first individual singing (see Fig. 3 for rufous-collared sparrow song and exemplary traffic noise recordings). We collected data on weekdays from Monday to Friday to ensure comparable levels of noise due to morning commuting traffic. We marked the position where the bird first sang and then took the measurements of light and noise as close as possible to this position. The height of the song perches ranged approximately between 0.5 m and 4.0 m from the ground. Night time light levels were measured with a digital Luxmeter (Voltcraft MS 1500, Germany) between 80 and 160 minutes after sunset. Five light measures were taken for ten seconds each, four of them with the lux meter held with a stretched out arm in four directions separated by 90 degrees and one measurement with the meter pointing upwards. With the same protocol, noise levels were taken, using a sound pressure level (SPL) meter (Voltcraft SL 400, Germany) during the peak traffic hours after sunrise, from 06:00 to 08:30. This time period was chosen based on a traffic census made by the Secretaria Distrital de Mobilidad of Bogota. We measured noise levels every 10 seconds for one minute pointing the SPL meter in a different direction. Noise levels were measured as dB(A) SPL, a procedure that measures the noise in decibels relative to the standard reference of sound pressure in air, 20µPa. Twenty µPa corresponds to 0 dB SPL, which is the threshold of human hearing. The frequency range of the SPL meter was 31.5 Hz - 8 kHz and the reading range was 30 – 130 dB(A). The SPL meter and the Luxmeter were held approximately 1.25 meters above the ground.
Figure 3. Rufous-collared sparrow song and exemplary traffic noise recorded in the city of Bogota. (a) spectrograms of three rufous-collared sparrows songs (I,II & III) and (b) power spectra of the average of the song of the three individuals (grey line) and the traffic noise (red line). The recordings were made with a Zoom H1 Ultra-Portable Digital Audio Recorder with a sample rate of 44.1 kHz and 16 bit accuracy.

Data analyses
The statistical analyses were carried out using R 2.11.0 (Team, 2013). We confirmed the normality of the data by conducting a residual analysis, plotting the residuals against the fitted values, the normal quantile-quantile (qq)-plot of the residuals, the square-root of the absolute values of the standardised residuals vs. the fitted, and the residuals against the leverages. We used linear models from the R package “arm” (Iman and Hill, 2007) to examine the relationship between noise and light levels as well as the relationship between onset time of the dawn chorus
and light and noise. The onset time of the dawn chorus was calculated using the difference between the time of sunrise and the time of singing at each site. Our response variable was the onset time of the dawn chorus and the independent variables were noise levels (dB(A) SPL), light levels (lux) and period of the year (May-June; November-December). We ran the same model including daily temperature and precipitation (extracted from a meteorological data base (IDEAM, 2013) but we did not find a statistically significant relationship between the weather variables and the dawn chorus onset nor a significant interaction with the other variables. We also did not find an interaction between noise and light variables when running the model with the interaction included. Thus we only present models excluding weather and the interaction between noise and light. In addition, we ran a linear model to compare the onset of dawn chorus in rural areas and urban areas in May-June.

Results

The city sites covered a wide range of different levels of artificial light at night (0.7-9.4 lux; Mean: 3.8 ±2.5) and of daytime noise levels (46-75 dB(A) SPL; Mean: 57±10). In this sample, the noisiest site was located about 20 meters from a dual carriageway and the quietest site was located in a garden in a suburban area (see Fig. 2 for recording locations). The most heavily lighted site was illuminated by several street lights within about two meters distance, whereas the darkest site had no artificial lights in direct view. Often light and noise levels co-vary in cities because busier streets tend to have more lighting. However, as we systematically chose locations with different combinations of light and noise, the two measures were not correlated in our data set (Pearson correlation: 0.14; N=33, p= 0.41). Also there was no significant interaction between light and noise levels according to the initial model (t=-0.278; p=0.78317; r²= 0.73; df=27, Fig. S2).

The onset of the dawn chorus was significantly related to daytime noise levels but the strength of this relationship depended on the season (t=-3.42; p=0.001; r²= 0.73; df=27; Table 1; Fig. 4a). In November-December, birds began to sing earlier in the morning in noisier places, with an average advancement of 10 minutes for every 5 dB increase in noise. During the May-June period, however, the same increase in noise yielded only an average advance in the dawn chorus of 2.5 minutes. The much stronger effect in November-December was linked to a general
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difference in singing behaviour between the two seasons, as birds started singing later in November-December compared to the May-June period (t=3.77; p<0.0001; r²= 0.73; df=27; Table 1). This seasonal difference between sites was also confirmed within sites: when we re-sampled seven of the sites that had initially been visited in May-June. Birds did not occupy three of them and in four of them we found that birds started singing later in relation to sunrise in November than in May-June (mean November: 67.75±27.62 min before sunrise; mean May-June: 91.75± 9.60 min before sunrise). In contrast to the observed effects of daytime noise, the onset of the dawn chorus in urban areas did not vary significantly with light pollution levels (t =0.22; p = 0.82; df=27; Table 1; Fig. 4b).

In comparison to the urban birds in our study, the birds in rural areas started to sing later in relation to sunrise (t=3.85; p=0.001; r²= 0.79; df=19, Fig. 5). On average, the rural dawn chorus began 50 minutes before sunrise in May-June, which is 10 minutes later than the latest dawn chorus onset in the city in the same period. As expected, rural levels of noise (mean =39 ± 7 dB(A) SPL) and light (mean = 0 ± 0 lux) were low compared to the ones measured in the city (Fig. 5) but this difference was not statistically significant (Noise levels: t=-1.14; p=0.27; df=19; Light levels: t=-0.34; p=0.73; df=19 ).

**Table 1.** Outcome of a linear model testing the effects of light pollution, daytime noise level, and the period of the year (Nov-Dec vs. May-June) on the onset of the dawn chorus in rufous-collared sparrows.

<table>
<thead>
<tr>
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<th>Estimate</th>
<th>Std. Error</th>
<th>t-value</th>
<th>p-value</th>
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<td>&lt;0.001</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Light:period of the year</td>
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<td>1.827</td>
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<td>0.621</td>
</tr>
<tr>
<td>Noise:period of the year</td>
<td>-2.037</td>
<td>0.594</td>
<td>-3.426</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Chapter I. Impact of noise on vocal behaviour in birds

Figure 4. Relation between onset time of the dawn chorus of urban rufous-collared sparrows and (a) noise levels and (b) light pollution levels, May-June (circles) and November-December (triangles). Regression lines denote linear model estimation with 95% confidence intervals (dotted lines).

Figure 5. Onset time of the dawn chorus of rufous-collared sparrows in May-June in the city of Bogota (black) and rural areas in the Colombian Andes (green). (a) Relationship with noise levels and (b) light pollution levels.
Chapter I. Impact of noise on vocal behaviour in birds

Discussion

We found that the onset of the dawn chorus of rufous-collared sparrows in a tropical city varied with the level of noise pollution. At sites with high levels of daytime traffic noise, birds started to sing earlier in the morning. In contrast, the onset of dawn singing was not correlated with levels of artificial light.

The impact of light and noise pollution on animal behaviour has been studied almost exclusively in temperate zones. Here we report for the first time how the usage of mating signals (song) varies with these anthropogenic factors in the tropics, where the photoperiod varies very little across the year. In the temperate zones with the marked seasonal changes in day length, photoperiod is a cue for the timing of reproductive stages of birds in general and for the onset of the dawn chorus in particular (Dawson et al., 2001; Gwinner, 2003; Wingfield and Farner, 1993; Wingfield and Kenagy, 1991). On a seasonal level, light at night can advance the timing of breeding (e.g. gonad development (Dominoni et al., 2013b), and the likely underlying mechanism is a perceived increase in day length (Dominoni and Partecke, 2015). Related to this, dawn singing would emerge earlier in the season through an earlier entry into the reproductive stage in areas with artificial light at night (Da Silva et al., 2015). In addition, light also directly influences the onset time of dawn chorus in the morning, as demonstrated for several songbird species in the temperate latitudes (Dominoni et al., 2013a; Kempenaers et al., 2010; Miller, 2006). However, it has been suggested that anthropogenic noise may also affect the daily onset of the dawn chorus. Birds would increase the periods for undisturbed singing by shifting their song activity to earlier hours (Arroyo-Solís et al., 2013; Nordt and Klenke, 2013). In line with this notion, it has been demonstrated that two of six species tested, advanced their dawn chorus when exposed to noise (Arroyo-Solís et al., 2013). On the other hand, a recent study (Da Silva et al., 2015) proposed that the observed shifts in singing activity in urban birds is due to light pollution rather than traffic noise; although the study did not measure noise levels directly.

Our evidence pointed to a stronger impact of noise over light pollution on the singing behaviour of an urban bird in the tropics. Rufous-collared sparrows probably sang earlier to avoid the peak of traffic noise, which occurred after dawn. Birds and other animals use a whole suite of vocal changes to communicate in noisy conditions (reviewed in Brumm and Zollinger, 2013), most of
them, however, are based on immediate individual plasticity in response to changes in the background noise. The phenomenon described here is different, since the birds adjusted their dawn song onset in anticipation of a change in the environment that occurred roughly two hours later when the morning rush hour set in. This raises the question about the mechanisms by which this adjustment is achieved. This issue can be addressed by experiments on urban and rural birds (Dominoni et al., 2013b) and also by studying the dawn song within cities, comparing the song onset of individual birds during week days with heavy commuting traffic and less noisy weekend days (Brumm, 2004).

In contrast to previous work on temperate birds, we found that the onset of the dawn chorus did not vary with levels of artificial light at night. This finding cannot be accounted for by a difference in light pollution, since the light intensities at night measured in our study are within the range of values observed in a temperate study that found a strong effect of light (Da Silva et al., 2015). Moreover, in our data set from November-December, city birds in the least illuminated places started their dawn singing at comparable times as their conspecifics at non-illuminated rural sites.

Our findings could be explained if sunrise is not a strong seasonal cue for some birds close to the equator. In the tropics, the photoperiod changes very little across the year, unlike in the temperate zones where a marked increase in day length during spring triggers the breeding season of birds. Similarly, it has been found that changes in day length are not used as cues to trigger reproductive behaviours in tropical rufous-collared sparrows (Moore, 2005). Close to the equator, some birds may be generally less entrained with the photoperiod. Therefore, disturbances of natural light regimes through light pollution may have less of an effect on their behaviour. However, to generally establish such a difference between birds in the tropics and the temperate zones, more species close to the equator need to be studied.

Interestingly, we found a seasonal difference in the onset of the dawn chorus: birds started singing later in relation to sunrise in November-December than in May-June. We could corroborate this finding by re-sampling some of the sites that were initially sampled in May-June again in November. The later song onset in November-December means that the birds had less time for their displays before the rush hour set in. Our data suggest that the rufous-collared
sparrows shifted the onset of their singing activity to earlier periods to gain more time before their songs were masked by the traffic noise later in the day. As a result, in November-December the birds in the noisiest city areas started singing on average about 70 minutes earlier than those in the quietest places. In May-June, however, when birds started singing earlier in relation to sunrise and noise had a much weaker effect on the onset of the dawn chorus, the maximum difference in singing onset time was only 40 minutes. Thus, our data suggest that anthropogenic effects can reduce the behavioural synchronisation of bird populations.

The observed difference in the onset of the dawn chorus between seasons was unexpected and we can only speculate as to why there was such marked seasonal effect. Singing behaviour, and specifically the dawn chorus in birds, is associated with territory defence and mate attraction and retention (Catchpole and Slater, 2008). Thus, there is often a correlation between breeding stage and singing behaviour. Rufous-collared sparrows in the equatorial tropics use cues such as food availability and weather to time their breeding season (Class, 2009; Moore, 2005). There was no clear difference in meteorological conditions between the two sampling seasons (IDEAM, 2013). In addition, we have no information about the breeding status of the recorded individuals. Anecdotal observations from the city of Bogota indicate more fledgling rufous-collared sparrows in Bogota in February and June than at other times of the year, suggesting potential breeding peaks in mid-January and mid-May (Mateo Hernandez, personal communication,). A similar pattern has been observed in other parts of the Colombian Andes (Miller, 1961). A breeding peak in May could indeed be accounted for our observation that urban sparrows started to sing earlier in relation to sunrise in May-June compared to November-December.

In conclusion, our study highlights that general ecological patterns, such as latitude, can have fundamental effects on how animals respond to urbanization. Bird species like rufous-collared sparrows, which are present in both tropical and temperate zones, are suitable candidate species for further comparative studies addressing the effects of seasonal variation in day length on mating behaviours and how anthropogenic disturbances may differentially affect populations at different latitudes.
Chapter II. Timing matters: traffic noise accelerates telomere loss rate differently at different developmental stages


Abstract

Noise pollution is one of the leading environmental health risks for humans, linked to a myriad of stress-related health problems. Yet little is known about the long-term effects of noise on the health and fitness of wildlife. We experimentally investigated the direct and cross-generational effects of traffic noise on telomeres; a measure of cellular ageing that is predictive of disease and longevity in humans and other organisms. Zebra finches (Taenopygia guttata) experienced three different treatment groups: 1) parents were exposed to traffic noise before and during breeding, together with their nestling young, 2) fledged juveniles but not their parents were exposed to traffic noise, and 3) control group birds were never exposed to traffic noise. Although there was no significant effect of traffic noise exposure at early (pre-fledging) stages of offspring telomere length or loss rate, traffic noise exposure accelerated telomere loss in older (post-fledging) juveniles. The age-dependent differences found in this study in telomere loss could occur if parents buffer younger offspring against the detrimental effects of noise exposure and/or if younger offspring are less sensitive to noise exposure. Telomere length during early life has been shown to be positively related to lifespan and the observed noise-induced increase of telomere attrition rate could reduce the fitness of the affected birds and potentially alter the population dynamics of birds in noise polluted areas. Our data highlight the need to consider the developmental stage of an organism to better understand the ecological consequences of anthropogenic change.
Introduction

Rapid environmental change due to urbanization, can be detrimental for many organisms, including humans (Gil and Brumm, 2013; Habib et al., 2006; McKinney, 2002). The growth of urban areas is linked to severe environmental contamination, including chemical, light, and noise pollution and these anthropogenic changes can often be regarded as environmental stressors (Grimm et al., 2008a). For example, in humans and other mammals, noise is related to delays in brain development, impaired cognitive function and deficits in learning and memory as well as high blood pressure, hyperglycemia, and elevated cholesterol levels (Chang, 2003; Cheng et al., 2011; Cui et al., 2009; Haralabidis et al., 2008; Jensen et al., 2010; Kight and Swaddle, 2011; Oliveira et al., 2009; Zheng and Ariizumi, 2007). However, to understand the causal effects of urbanization on organisms it is important to identify model systems that can be experimentally manipulated. Such investigations are necessary to disentangle the impacts of different environmental factors associated with urban habitats on the development, health, behaviour, and fitness of exposed individuals.

Moreover, the mechanisms that underlie the negative effects of increased noise exposure on health and fitness are poorly understood. In diverse organisms, environmental conditions experienced during early life often have delayed impacts on phenotypic development and fitness (Metcalfe and Monaghan, 2001; Monaghan et al., 2009). Telomeres are a emerging as a mechanism that may provide a link between early stress exposure and longevity (Monaghan, 2010). Telomeres are non-coding, repetitive DNA sequences that cap the ends of eukaryote chromosomes and enhance genome stability (Blackburn, 1995). Telomeres shorten during cell division and limit cellular lifespan (Blackburn, 1995) and telomere length has often been shown to be positively associated with longevity (Heidinger et al., 2012). Telomeres have also been shown to shorten in response to stress in mammals and some birds (Epel et al., 2004; Haussmann et al., 2012; von Zglinicki, 2002).

In humans and in birds, there is also evidence that stress experienced by parents can impact offspring telomere length and loss rate (Boonekamp et al., 2014; Entringer et al., 2011, 2013; Haussmann et al., 2012; Heidinger et al., 2016; Hoxha et al., 2009; Love and Williams, 2008;
Nettle et al., 2014; Park et al., 2015; Tissier et al., 2014). For example, human mothers that reported experiencing stressful conditions during pregnancy produced offspring with shorter telomeres at birth (Entringer et al., 2013) and in adulthood (Entringer et al., 2011). As these studies are necessarily correlative it is difficult to separate cause and effect. But, these findings are also supported by a recent experimental study in birds where experimentally elevated stress hormone levels in the yolk resulted in chicks with shorter telomeres at the end of post-natal development (Haussmann et al., 2012). However, the relative importance of stress exposure experienced by parents and offspring at different developmental stages has rarely been disentangled.

Information regarding effects of urbanization on telomere length or attrition rates in birds is still scarce. However, two recent studies have found links between urbanization and telomere length in juvenile songbirds. In a cross-fostering experiment, it was found that great tits (Parus major) reared from 2 days of age in urban environments had significantly shorter telomeres at 15 days of age than birds reared in rural areas (Salmón et al., 2016). It remains unclear which aspects of urbanization may have contributed to this effect of living in an urban environment on telomere dynamics. In another study, playback of traffic noise in the field resulted in shorter telomeres in 9-day-old house sparrows (Passer domesticus) (Meillère et al., 2015). Yet, neither of these studies investigated whether the effects of exposure to traffic noise or urban habitats had longer-term effects on telomere attrition rates, or how chronic noise exposure affects individuals at different ontogenetic stages.

We experimentally tested for direct and cross-generational effects of traffic noise exposure across developmental stages in zebra finches bred in aviaries in our laboratory. In this experiment we compared telomere lengths at 21 and 120 days post-hatch in (1) birds that hatched to parents that were exposed to noise during courtship, egg-laying, and nestling care periods, with the offspring themselves also exposed to noise until ca. 18 days post-hatch, (2) birds that hatched to non-noise exposed parents, but which were themselves exposed to noise from day 18 to 120, and (3) controls in which neither the parents or the chicks were exposed to noise.
Chapter II. Timing matters: traffic noise accelerates telomere loss rate differently at different developmental stages

Methods

Study system
We bred adult zebra finches (2-3 years old) from the colonies at the Max Planck Institute for Ornithology in Seewiesen, Germany. Each of three experimental rooms consisted of three aviaries (1 x 2 x 2 m), each housing 7-8 pairs of birds. Each aviary was provided with 12 wooden nest boxes and *ad libitum* nesting materials, seeds, commercial finch egg food and water. In addition, birds were provided with fresh vegetables and hard-boiled eggs twice weekly throughout the experimental period. Our experimental birds were the offspring of these breeding adults. Each aviary produced an average of 29 offspring (16 – 42), with a total of 263 offspring from all treatment groups. Offspring that died before they reached 120 days post-hatch were not included in the experiment. Animal housing and care was all in accordance with European and local laws governing the care and use of laboratory animals (Council of Europe Treaty ETS-123). All experimental procedures were approved by and done under license from the Government of Upper Bavaria (Regierung von Oberbayern), licence number 55.2-1-54-2532-51-2013.

Experimental treatment
To determine if typical city traffic noise affects telomere dynamics in juvenile birds, we designed three noise exposure treatments: 1) the parents were exposed to noise during breeding, egg-laying and nestling care periods (PNoise), 2) juvenile birds were exposed to noise exposure from fledging throughout the sensory motor learning period, 18-120 days post-hatch (JNoise), and 3) a control group that was not exposed to noise at any time point (NoNoise) (Table S1). Thus, the offspring in the PNoise treatment group were not exposed to traffic noise after fledging, and the offspring in the JNoise treatment were not exposed before fledging, nor were their parents. The PNoise group had a total of 95 offspring, the JNoise group 59, and the control group (NoNoise) had 109 offspring. The difference in sample size between treatments is because the parents of the treatments PNoise and NoNoise bred twice, once in PNoise and once in NoNoise treatments. To control for potential effects of breeding experience we considered the number of breeding rounds in the statistical analysis (see below).

Noise playback consisted of 80, 5-minute long recordings of street traffic noise, which was recorded at several busy intersections in Munich, Germany during April 2013. During the
daylight hours (06:30 – 20:30), the 80 recordings were played continuously, in randomized order, with playback levels (measured at the position of the nest boxes) averaging between 65 and 85 dB(A) re 20 μPa. Nighttime playback (20:30 – 06:30) consisted of randomized playback of 40 noise recordings, which were less dense in the rate of passing than the daytime recordings and were reduced in overall amplitude, with playback level averages ranging between 45-75 dB(A). Therefore, noise playback mimicked typical urban noise patterns, according to published noise maps (Bayerisches Landesamt, 2000). We played noise from a laptop computer to an array of 12 pairs of amplified portable speakers (Hama Sonic Mobil 400 Alu PS1032), with 4 pairs arranged above each of the three aviaries in the room. Noise playback was run using a script written in MatLab (version 7.5.0; Natick, MA, USA; www.mathworks.com) to randomize playback during day and night. For the PNoise group, playback of noise began 4 weeks before the introduction of nesting materials and nest boxes and continued until the median juvenile in the room had fledged (the date when half of the offspring had fledged). For the JNoise group, the noise playback began when the median juvenile was 18 days post-hatch, and continued until all juveniles had reached 120 days.
Table S1. Overview of aviaries, rooms in which the aviaries were placed, dates of experiments, and treatments. PNoise (the parents were exposed to noise during breeding, egg-laying and nestling care periods, which also meant that nestlings were exposed to noise until they left the nest, ~18 days post-hatch), JNoise (juvenile birds were exposed to noise exposure from fledging throughout the sensory motor learning period, 18-120 days post-hatch) and control (parents and juveniles not exposed to noise at any time point).

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<td>Control</td>
</tr>
<tr>
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<td>Control</td>
</tr>
<tr>
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<td>Control</td>
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<td>Nov 2014-Mar 2015</td>
<td>PNoise</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>Mar 2015- Aug 2015</td>
<td>JNoise</td>
</tr>
<tr>
<td>8</td>
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<td>Mar 2015- Aug 2015</td>
<td>JNoise</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>Mar 2015- Aug 2015</td>
<td>JNoise</td>
</tr>
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Telomere measurement

Blood samples were collected by brachial venipuncture for each bird at 21 and 120 days post-hatch to measure telomere length and loss rate. Telomere length at 25 days has previously been shown to be positively related to lifespan in zebra finches (Heidinger et al., 2012). Blood was collected into heparinized capillary tubes (1.4 x 75 mm), transferred into Eppendorf tubes, and centrifuged to separate the cells from the plasma. The cells were then stored at −80°C until DNA extraction. We analyzed samples for 263 birds in total, 137 females, and 126 males, at both ages. We used the DNeasy Blood and Tissue kit (Qiagen) to extract genomic DNA from the red blood cells following the manufacturer’s protocol. We used a NanoDrop 8000 spectrophotometer (Thermo Scientific) to measure the quantity of the DNA. To measure relative telomere length we
used quantitative PCR (Stratagene MX3000P), as described in (Cawthon, 2002), and adapted to zebra finches (Criscuolo et al., 2009).

The relative telomere length of each sample was measured by calculating the ratio (T/S) of telomere repeat copy number (T) to single control gene copy number (S), relative to a reference sample. As the control gene, we used the Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The following forward and reverse primers were used to amplify the telomere: Tel1b (5’-CGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3’), Tel2b (5’GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3’) and zebra finch-specific GAPDH sequences: GAPDH-F (5’-AACCAGCCAAGTACGATGACAT-3’), GAPDH-R (5’-CCATCAGCAGCAGCCTTCA-3’). The telomere and GAPDH reactions were carried out on different plates, the number of PCR cycles required for the products to accumulate enough fluorescent signals to cross a threshold was determined. The detailed description of the conditions of the PCR can be found in (Criscuolo et al., 2009). A standard curve was included to measure the efficiencies of the reactions on every plate. The reference sample was from a zebra finch that was 21 days old at the time of collection. The efficiencies were within an acceptable range (i.e., 100 +/- 15%) in all cases. All samples, including the standard curve, were run in triplicate, and average values were used to calculate the relative T/S ratios for each sample relative to the reference sample (for details see (Heidinger et al., 2012). All of the samples for an individual were run on the same plate. The average intraplate variation of the Ct values was 1.99±1% for the telomere assays and 0.15±10.09% for the GAPDH assays, respectively. The average interplate variation for the ΔCt values was 3.96%.

**Paternity analysis**

To account for possible genetic effects on telomere loss, we considered the identity of parents in the analysis (see below). Since there is typically a considerable amount of extra pair young in captive zebra finch colonies (Forstmeier et al., 2011), genetic paternity analysis is necessary to reliably assign parentage. To this end, all offspring were genotyped at 11 highly polymorphic microsatellite markers (Forstmeier et al., 2007) and parentage was assigned by exclusion.
Chapter II. Timing matters: traffic noise accelerates telomere loss rate differently at different developmental stages

Statistics
All statistical analyses were performed with R 3.1.1 (R Core Team 2013). We fitted linear mixed-effects models to analyze our data, using the “lmer” function (package lme4). Additionally, we used the “sim” function (package arm) to simulate the posterior distribution of the model parameters and values were extracted based on 2000 simulations (Gelman and Hill, 2007). The statistical significance of fixed effects and interactions were assessed based on the 95% credible intervals (CI) around the mean (estimate). We considered an effect to be “significant” in the frequentist’s sense (p < 0.05) when the 95% CI did not overlap zero (Nakagawa and Cuthill, 2007). Telomere length (log-transformed) was set as the dependent variable, treatment (NoNoise, PNoise, JNoise), age when the sample was taken (21 or 120 days old), sex, mass of every individual at 21 and 120 days (mass) and breeding round as independent factors. Breeding round is the number of times the adults have reproduced. The individual ID, the ID of the genetic parents, and the aviary (to account for effects of the common aviary) were included as random effects. Genetic parentage was determined by exclusion using the R package SOLOMON (Christie et al., 2013). The model used in the paper included interaction between treatment and age and was compared to other models using the Akaike Information Criterion (AIC) (Akaike, 1981).

Results
Telomere length decreased with age in all treatment groups (Table 1, Fig. 1). At 21 days, telomere lengths did not significantly differ between treatments (NoNoise: Mean: 1.40 T/S ratio ±0.55; PNoise: Mean: 1.46 T/S ratio ±0.53; JNoise: Mean: 1.46 T/S ratio ±0.50). The JNoise group experienced significantly greater telomere shortening between days 21 and 120 days than the PNoise and NoNoise groups (Table 1, Fig. 1 and Fig. S1). Consequently, zebra finch juveniles exposed to noise post-fledging (JNoise treatment) had shorter telomeres at 120 days (Mean: 0.87 T/S ratio ±0.33) than the offspring from the PNoise treatment (Mean: 1.17 T/S ratio ±0.42) and NoNoise treatment (Mean: 1.19 T/S ratio ±0.53) groups. The estimate of individual repeatability was 0.17 CI 0.08, 0.27. The estimate of mother repeatability was 0.19 CI 0.07, 0.31 and father repeatability was 0.08 CI 0.05, 0.29. The values of telomere length at 21 days in all three groups were within the range of telomere lengths found in previous studies in zebra finches.
(Heidinger et al., 2012). There were no significant effects of sex, breeding round, or body mass on telomere length (Table 1).

**Table 1.** Outcome of linear models testing the effects of noise on the telomere length of juvenile zebra finches that had parents exposed to noise (PNoise), or that were themselves exposed to noise (JNoise) and a no-noise control group. The asterisks represent “significant” differences in the frequentist’s sense, i.e. when the 95% credible intervals did not overlap zero (Nakagawa and Cuthill, 2007).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimate (β)</th>
<th>95% CI</th>
</tr>
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<td></td>
</tr>
<tr>
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</tr>
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<td>Parents in noise (PNoise)</td>
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<td>-0.28,-0.10*</td>
</tr>
<tr>
<td>Breeding round</td>
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<td>-0.08,0.07</td>
</tr>
<tr>
<td>Mass</td>
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</tr>
<tr>
<td>PNoise x age</td>
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<td>-0.16,0.04</td>
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<tr>
<td>JNoise x age</td>
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<td>-0.47,-0.24*</td>
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<td><strong>Random effects</strong></td>
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<tr>
<td>Group (Intercept)</td>
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</table>
Figure. 1. Predicted telomere length values (with 95% credible intervals) of zebra finches with parents exposed to noise (PNoise), exposed to noise themselves (JNoise) and control (NoNoise).
Chapter II. Timing matters: traffic noise accelerates telomere loss rate differently at different developmental stages

Figure S1. scatter plot of telomere length values of zebra finches at day 120 against day 21. The colours represented the treatments: parents exposed to noise (orange circle), juveniles exposed to noise themselves (red circle) and control (blue circle).

Discussion

This study is the first to experimentally examine the effects of traffic noise on telomere length and attrition rate under controlled laboratory conditions. We found that traffic noise had a direct impact on the rate of telomere loss in post-fledge juvenile zebra finches. However, there was no significant effect transmitted by parents, nor of a direct effect on nestlings exposed at an earlier developmental stage (pre-fledging). At day 21 post-hatch, birds from the three different treatments did not differ significantly in telomere length. Importantly though, we found that the juveniles directly exposed to noise during the post-fledging period (18 to 120 days post-hatch) experienced significantly more telomere shortening and had significantly shorter telomeres at 120 days than birds in the control treatment, or those whose parents were exposed to noise. Therefore,
noise exposure during the later stages of ontogeny (between 18-120 days) resulted in an increased rate of telomere loss. We did not find significant differences in telomere length or attrition rates between sexes. Our data suggest that juveniles that experience traffic noise will have greater telomere attrition, which may serve as a biomarker that predicts reduced longevity with negative consequences for fitness.

The pattern of noise-induced telomere loss in older juveniles may suggest that zebra finches are less sensitive to traffic noise when exposure occurs while they are still in the nest. One explanation is that the expression of telomerase, the enzyme that repairs telomeres, may be expressed during some developmental stages. In zebra finches, telomerase activity is highest during the hatchling age period, when the proliferative demands of most organs are the highest (Haussmann et al., 2007), which may mean that very young birds are buffered against stress-induced telomere loss by higher rates of repair, compared to older juveniles. However, two field studies on other songbird species have found differences in telomere length in response to noise exposure or urbanization as early as day 9 and day 15 (Meillère et al., 2015; Salmón et al., 2016). Alternatively, it could be that the effect was more pronounced during young adulthood in zebra finches because the period between 18 and 120 days post-hatch is a critical period. Zebra finches typically leave the nest around day 18 and continue to be fed entirely or partially by their parents or other adults until approximately 30 days post-hatch when juveniles become independent from their parents. In addition to the stress of weaning from parental nutritional support, this time period (ca. day 20) is approximately when zebra finches enter the song learning period (Roper and Zann, 2006). This could mean that birds are more sensitive to noise or that it is a more potent stressor during this stage, than at earlier life stages. Corroborative evidence for this notion comes from a recent study that found that noise exposure affects the size of brain regions associated with song learning in zebra finches during their song learning period although it did not affect corticosterone levels (Potvin et al., 2016).

That we did not find an effect of early life noise exposure on telomeres might be the result of a different mechanism, for instance if parents in noisy environments somehow buffer their offspring against potential negative effects of noise exposure on telomere loss. Previous studies have found that parental behaviours often change in the presence of noise (Meillere et al., 2015) which can reduce reproductive success (Potvin and MacDougall-Shackleton, 2015; Schroeder et
In our study, it could be that parents exposed to traffic noise found ways to mitigate the effect of noise on their offspring. Such alleviation could have been mediated by increased parental care behaviour or even earlier in the gamete formation stage. For example, if young chicks (<18 days) experienced higher rates of telomere loss than controls, noise-exposed parents may have produced offspring with longer telomeres at hatching, which were then lost at a higher rate during their first 20 days post-hatch. Thus, this rapid change may be indistinguishable from the other treatment groups when the first samples were taken at day 21. However, when the noise treatment ceased at day 18, their rate of telomere loss may have decreased, so that the rate of loss from that point matched that of the control birds. Alternatively, it may be that noise-exposed parents invest more in parental care, which may buffer noise-induced telomere loss, and as a result telomere length in their offspring is not significantly different to offspring from the control group. Such an adjustment of parental care could, at least partly, be triggered by the chicks, e.g., hatchlings increase the amplitude of their begging calls in noise (Leonard and Horn, 2008), which may increase feeding rates by the parents.

Telomere length in zebra finches is thought to be heritable (Atema et al., 2015), and this is supported by our data, wherein parents explain 27% of the variation in our model. However, whether rates of telomere attrition are also heritable is not clear. We found that juveniles exposed to noise post-fledging had higher rates of attrition, even though their telomere length at day 21 was not different from the other groups (Fig. S1). This suggests that environmental conditions, in this case traffic noise, have an impact on the attrition rate of telomeres in juvenile zebra finches, regardless of inherited differences in telomere length. However, the exact mechanisms underlying the differences observed between our groups, and the degree to which heritability may impact rates of telomere loss independent of environmental conditions needs further study.

The first sample in our study was taken when juveniles were 21 days old post-hatch, around 10 days after the sampling day in other studies (Meillère et al., 2015; Salmón et al., 2016). In contrast to prior research (Meillère et al., 2015; Salmón et al., 2016), our results showed that traffic noise exposure did not have an effect on telomere length at 21 days old post-hatch. That our birds did not respond to traffic noise exposure in the same way as the birds in these previous studies may be an indicator of species differences in sensitivity to traffic noise, or may represent a difference between how captive birds and wild birds respond to noise. It may also be that in our
study, parents were exposed to noise continuously (like in the city) and while our playback consisted of randomized, unpredictable fluctuations in noise, parents may have habituated more to this chronic noise condition than to shorter daily periods of playback, such as in Meillère et al. 2015.

Our study contributes critical new data to our understanding of the long-term effects of traffic noise pollution on avian health and fitness. We show that chronic exposure to realistic levels of traffic noise increases rates of telomere loss in older, but not very young juvenile zebra finches. Since telomere length has been shown to be related longevity in zebra finches (Heidinger et al., 2012), a noise-induced increase of telomere attrition rate may serve as a biomarker that predicts reduced long-term survival of the affected birds with potential consequences the population dynamics of birds in noise polluted areas. Additionally, we present here the first data on the effects of noise exposure on rates of telomere loss during ontogeny, rather than just a measure of telomere length at a single point in time. While urbanization consists of a complex suite of ecological changes, our study is a first step towards identifying the causal mechanisms that may underlie differences observed between urban dwellers and their rural conspecifics. As suggested by our findings, it is essential to consider developmental stage and parental effects when studying these mechanisms and how they ultimately affect eco-evolutionary processes.
Chapter III. Traffic noise exposure alone may delay offspring growth, but is not a potent enough stressor to disrupt immune function, stress physiology or reproductive success in breeding birds

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Collaborators: Wolfgang Goymann, Wolfgang Forstmeier, Ulrich Knief, Ana Maria Bastidas and Henrik Brumm
*Paper in preparation*

Abstract

The impact of human activity on the acoustic environment is overwhelming, and anthropogenic noise levels have been steadily increasing with each passing decade. As urbanization and noise levels rise, investigations into the impacts of noise become very important. The World Health Organization has identified noise pollution as one of the leading environmental health risks in humans, and it has been linked to a myriad of non-acoustic, short- and long-term health and psychosocial effects in exposed individuals. However, less is known about the non-acoustic effects of anthropogenic noise exposure on animals. We investigated long- and short-term effects of traffic noise exposure on zebra finches breeding in small group aviaries, using a repeated measures design. Birds were allowed to breed in both noise and no-noise conditions, and we measured baseline plasma glucocorticoid levels before, during and after breeding. In addition, we assayed immune function, measured reproductive success and offspring growth rates, and compared rates of extra-pair paternity of breeding adults. Although we did not find significant differences in any of the traits measured in breeding birds, we did find that offspring of birds exposed to noise were smaller than offspring in control groups. Our results suggest that traffic noise poses a more severe hurdle to developing birds than it does to adult birds. Chronic exposure to noise in urban birds may not, by itself, be a sufficient stressor to cause acute effects on health or reproductive fitness in exposed individuals. However, animals living in urban
habitat is not a potent enough stressor to disrupt immune function, stress physiology or reproductive success in breeding birds.

Introduction

Humans have been dramatically altering the environment for millennia, and as the global population swells, the growth of urban areas is projected to reach exceptional levels in the coming decades. This expansion of human developments will certainly continue the dramatic alteration of ecosystems; even including the extinction of species (United Nations, 2012). Some of the challenges that animals face in cities are habitat fragmentation, changes in micro-climate, limitation of resources, alteration of resources flow, changes in species interactions, and air, water and noise pollution (Grimm et al., 2008b; Shanahan et al., 2014).

Pollution is one of the main consequences of human development, and may take the form of chemical contamination as well as light and noise emissions. The past decade has seen a large groundswell of interest in the effects of anthropogenic noise pollution on birds; primarily this has focused on changes in the vocal behaviour of birds. Many dozens of studies have reported correlations between background noise levels and various song characteristics. For instance, in response to increases in the background noise levels birds increase the amplitude of their songs, and additionally they may also adjust the redundancy, the duration, the frequencies, and the timing of their vocalizations (reviewed in (Brumm and Zollinger, 2013). These observations have let many to suggest that these modifications of vocal behaviour are an attempt of birds to moderate the negative effects of noise on acoustic communication (Gil and Brumm, 2013b), although some strategies are likely to be more effective at mitigating the effects of noise than others (see Nemeth and Brumm, 2010). In addition to its potential to disrupt vocal signalling in birds, noise can have negative fitness consequences on signal receivers by masking important acoustic signals and cues, such as alarm signals, noises made by predators or prey, or signals relevant for breeding behaviour and offspring care (Fernández-Juricic and Tellería, 2000; Quinn et al., 2006; McGiffin et al., 2013; McIntyre et al., 2014; Meillere et al., 2015; Templeton et al., 2016). Birds may be particularly sensitive to noise disruption during their reproductive period, since many species rely on acoustic signals (songs) to attract mates and defend territories, to
Chapter III. Traffic noise exposure alone may delay offspring growth, but is not a potent enough stressor to disrupt immune function, stress physiology or reproductive success in breeding birds

maintain pair bonds, and coordinate offspring feeding and care (Catchpole and Slater, 2008). Thus, acoustic masking by noise is likely to have major fitness consequences (Brumm and Slabbekoorn, 2005b).

There is some evidence that noise exposure can impair or influence avian reproductive success in different ways. For example, great tits (Parus major) breeding in areas with high levels of traffic noise produced fewer fledglings and laid smaller clutches than their conspecifics in quieter areas (Halfwerk et al., 2011). Eastern bluebird (Sialia sialis) nesting in noisy areas also had reduced reproductive success, with lower hatching rates and fledging success than their conspecifics at quieter sites (Kight et al., 2012). House sparrows (Passer domesticus) breeding in noisy sites hatched fewer young, of lower body mass, produced fewer recruits and had lower rates of offspring provisioning than sparrows in quiet sites (Schroeder et al., 2012). Noise can also mask parental alarm calls, reducing the response of nestlings towards predator threats (McIntyre et al., 2014). Detection of alarm calls by great tits is significantly impaired in the presence of traffic noise (Templeton et al., 2016), which is likely to increase the risk of predation in habitats with high levels of noise pollution. In the same direction, detection of predators can be affected by noise (Fernández-Juricic and Tellería, 2000; L Quinn et al., 2006; McGiffin et al., 2013; Meillere et al., 2015). Indeed, some species increase vigilance time as consequence of it, resulting in a reduction of feeding rate (Fernández-Juricic and Tellería, 2000; L Quinn et al., 2006). Other species showed differences in anti-predator behaviour but not directly related to reproductive performances (McGiffin et al., 2013; Meillere et al., 2015).

Noise can also have negative non-acoustic effects, in that it may act as a stressor, and could increase physiological stress responses such as elevated plasma glucocorticoids, which can lead to depressed immune function, and increased oxidative stress in the brain and organs of the immune system (reviewed in Kight & Swaddle 2011). Several species of birds have shown correlations between levels of the stress hormone corticosterone (CORT) and chronic environmental noise (Blickley et al., 2012; Wright et al., 2007). Elevated levels of CORT in mothers has been shown to lead to small body size and slow plumage development in offspring (Saino et al., 2005). Elevated CORT has also been found to reduce the time parents spend incubating eggs, and to increase the instances of nest abandonment (Spée et al., 2011; Thierry et
Parents with high levels of CORT may also reduce feeding rates (Angelier et al., 2009). On the other hand, in some studies increased levels of CORT seemed to help parents adapt to new situations better (Escribano-Avila et al., 2013), resulting in an increase of parental care and fledglings (Bonier et al., 2009, 2011).

To date, most studies on the effect of noise on birds have been done in the field. While field studies are important to identify potential relationships between environmental factors and changes in populations, they can present challenges for understanding causal relationships, as there are often many interacting factors that cannot be controlled for. To understand the causal effects that underlie observed correlations between anthropogenic noise and changes in behaviour or fitness, it is important to identify model systems that can be experimentally manipulated. Investigations with these models are necessary to disentangle the impacts of different environmental factors associated with urban habitats on the development, health, behaviour, and fitness of exposed individuals. So far, just one study in captivity has studied the direct effect of noise in reproductive success related to corticosterone levels in birds (Potvin and MacDougall-Shackleton, 2015). This study found that traffic noise increased embryo mortality and reduced nestling growth. In addition, mothers with high CORT levels were affected in laying latency and clutch size.

In the present study we experimentally exposed breeding zebra finches (Taeniopygia guttata) to realistic levels of traffic noise, and measured whether noise exposure alone was sufficient enough as stressor to affect baseline stress hormones (CORT) levels, reproductive success, levels of extra-pair paternity, or immune function. The experiment was a conducted with a pair-wise design in which birds living in group aviaries were allowed to reproduce twice, once exposed to traffic noise and once without noise playback. We hypothesized that traffic noise would act as stressor. Thus, we expected to find higher levels of baseline corticosterone linked to a deficit on the immune system and a low performance in reproductive success. In addition, since song is modified by noise (reviewed in (Brumm and Zollinger, 2013) and could have an impact on breeding performance (Halfwerk et al., 2011; Kight and Swaddle, 2011; Potvin and MacDougall-Shackleton, 2015; Schroeder et al., 2012), we expected to find an effect on extra-pair behaviour.
Materials and methods

Study system
We allowed 88 adult zebra finches (2-3 years old) from the colonies at the Max Planck Institute for Ornithology in Seewiesen, Germany to breed in 6 aviaries. Each of two experimental rooms consisted of three aviaries (1 x 2 x 2 m), each housing 7-8 pairs of birds. Each aviary was provided with 12 wooden nest boxes and ad libitum nesting materials, seeds, commercial finch egg-food and water. In addition, birds were provided with fresh vegetables and hard-boiled eggs twice weekly throughout the experimental period. Our experimental birds were the breeding adults. Animal housing and care was in accordance with European and local laws governing the care and use of laboratory animals (Council of Europe Treaty ETS-123). All experimental procedures were approved by and done under license from the Government of Upper Bavaria (Regierung von Oberbayern), licence number 55.2-1-54-2532-51-2013.

Experimental treatment
To determine if typical city traffic noise affects baseline plasma corticosterone levels, reproductive success and immune function in adult birds we used a crossed design experiment in which every individual was exposed to noise throughout breeding, egg-laying and nestling care periods during one season, and was not exposed to noise throughout the same period during one season, with half of the birds receiving the noise treatment during the first breeding round, and half the birds receiving noise exposure in the second breeding round.

Daytime noise playback consisted of 80, 5-minute long recordings of street traffic noise, which were recorded at several busy intersections in Munich, Germany during April 2013. During the daylight hours (06:30 – 20:30), the 80 recordings were played continuously, in randomized order, with playback levels (measured at the position of the nest boxes) fluctuating between 65 and 85 dB(A) re 20 µPa. Nighttime playback (20:30 – 06:30) consisted of randomized playback of 40 noise recordings, which were less dense in the rate of passing traffic than the daytime recordings and were reduced in overall amplitude, with playback level averages fluctuating between 45-75 dB(A). Therefore, noise playback mimicked typical urban noise patterns, according to published
noise maps. We played noise from a laptop computer to an array of 12 pairs of amplified portable speakers (Hama Sonic Mobil 400 Alu PS1032), with 4 pairs arranged above each of the three aviaries in the room. Noise playback was run using a script written in MatLab (version 7.5.0; Natick, MA, USA; www.mathworks.com) to randomize playback during day and night. For the noise group, playback of noise began 4 weeks before the introduction of nesting materials and nest boxes and continued until the median juvenile in the room had fledged (the date when half of the offspring had fledged, with an average fledgling age of 17 days post hatch).

**Baseline corticosterone levels**

To determine hormone profiles, we captured birds in the experimental rooms and collected blood samples in under 3 minutes from entering the room. Baseline corticosterone levels collected in less than 3 minutes from the start of the disturbance are not thought to be greatly affected by capture and handling (Romero and Reed, 2005). Each bird was sampled four times during the breeding cycle: 1) pre-treatment, 2) during the courtship period, 3) during the nesting period (incubating eggs, but before hatching) and 4) post-treatment. All blood samples were collected between 11:00 and 12:00 h. We did not take a blood sample during the nestling care period because during a pilot study we found that the disruption of the catching and bleeding of the parents had too large an impact on the wellbeing of the young offspring.

**Blood sampling techniques and hormone assays**

Blood samples were collected by brachial venipuncture for each bird and was collected into heparinized capillary tubes (1.4 x 75 mm), transferred into Eppendorf tubes, and centrifuged (max speed of 10,000 rpm for 10 min) to separate the cells from the plasma. Plasma was stored at −80 °C at the Max-Planck Institute for Ornithology, Seewiesen. Corticosterone concentrations were determined by direct radioimmunoassay following Goymann et al. (2006). All antibodies were obtained from Esoterix Endocrinology, Calabasas Hills, CA. Extraction efficiency (as calculated from tracer amounts of tritiated hormone added to each sample) was 3.7% for 3H-corticosterone (Perkin Elmer, NET 399). The average limit of detection was 4.98pg per tube for corticosterone. We ran a total of seven corticosterone assays, three for the first breeding round and four for the second breeding round. As calculated from extracted chicken pools with known hormone concentrations run within each assay, the intra-assay variation for corticosterone was
8.1 %, 7.6 %, 8.6 %, 11.1%, 4.3%, 3.6%, 2.5%. The inter-assay variation was 4.7 % for the first round and 3.7% for the second round.

**Immune function**

To test the effect of traffic noise on immune function we used two tests: leukocyte profile and bacteria killing essay. During nestling and together with the corticosterone sample, we took blood samples for the leukocyte profile and for the bacteria killing assay.

**a. Leukocyte profile**

We prepared blood smears using a standard two slide wedge procedure. The smears were air dried and then stained using a Differential Quik Stain Kit (Modified Giemsa).

We analyzed two blood smears, each collected during the egg incubation period(?) from the first and second breeding rounds for all adults in the experiment (n=74). Every slide was reviewed under a microscope with the help of oil immersion objective at 100X. For every visual field where a leucocyte was observed, a picture was taken as an evidence of the different forms present in the sample.

We counted the number of lymphocytes and granulocytes (Eosinophils, basophils, heterophils), in the first 50 leucocytes we identified for each slide. We used the granulocyte to lymphocyte ratio as a proxy for Heterophil to Lymphocyte ratio (H/L ratio).

**b. Bacteria killing assay**

We followed the protocol described by (Ardia et al., 2008) based originally from (Millet et al., 2007). This test measured whether an individual has the ability to kill off a pathogen, and for a bird with an active immune system, the resultant agar plates should show reduced bacterial growth relative to controls. We mixed 5ul of whole blood with a known dilution of bacteria (*Esterichia coli* ATCC #51813 Microbiologics #0791E8) in a growth medium made of CO2 independent media (Invitrogen; Gibco media #18045) and L-Glutamine (Sigma). We incubated the samples at 40C for 45 minutes. We placed the samples in labeled petri dishes and incubated them for 12 hours at 40C. We counted the number of bacteria colonies in each plate and
Chapter III. Traffic noise exposure alone may delay offspring growth, but
is not a potent enough stressor to disrupt immune function, stress
physiology or reproductive success in breeding birds

compared these to control plates. Each individual had two plates in each treatment (Noise and No
noise condition, N =85).

Reproductive success
We supplied each aviary with 12 nest boxes and ad libitum nesting materials (coconut fibers and
cotton string). Every other day, nests were checked, the fate of each egg and each offspring noted
and the individuals attending the nests (the social parents) were identified by observation in
person or with video. When eggs hatched, the nestlings were weighed, and if nestlings survived
to day 8, we collected a small blood samples (~10μl) from for parentage analysis (described
below). When nestlings died before day 8, we stored a tissue sample in alcohol for DNA
extraction for parentage analysis. In addition, we opened all eggs that did not hatch and scored
the eggs as infertile or as containing embryos that died before hatch. Tissue from dead embryos
was also collected and used for parentage analysis. For every individual we counted the number
of genetic offspring that reached adulthood (120 days old), the number of embryos that died
before hatch (embryo mortality) and the number of babies that died in the first 2 days post-hatch
(chick mortality). In addition, every offspring was weighed at day 10, 21 and 120 post-hatch.

Paternity analysis
Since there is typically a considerable amount of extra pair young in captive zebra finch colonies
(Forstmeier et al., 2011), genetic paternity analysis is necessary to reliably assign parentage. To
this end, all offspring were genotyped at 11 highly polymorphic microsatellite markers following
(Forstmeier et al., 2007) and (Forstmeier et al. 2010) and parentage was assigned by exclusion.

Statistics
All statistical analyses were performed with R 3.1.1 (R Core Team 2013). We applied linear
mixed-effects and general mixed-effects models to analyze our data for which we used the “lmer”
and “glmer” function (package lme4). Additionally, we used the “sim” function (package arm) to
simulate the posterior distribution of the model parameters and values were extracted based on
2000 simulations. The statistical significance of fixed effects and interactions were assessed
based on the 95% credible intervals (CI) around the mean (estimate). We considered an effect to
be “significant” in the frequentist’s sense (p <0.05) when the 95% CI did not overlap zero
(Nakagawa and Cuthill, 2007). For immune response, H/L ratio was set as the dependent variable, treatment and sex as independent factors. In addition we set corticosterone level (log-transformed) as the dependent variable, and we set treatment (Noise and No noise), time when the sample was taken (pre-treatment, during courtship, during nestling or post-treatment), sex, time when the sample was taken (during the first, second, third minute, or greater than third minute after entering the room) and breeding round as independent factors. For both models, the individual ID and the aviary (to account for effects of the common aviary) were included as random effects.

For our model of reproductive success, we set number of offspring reaching adulthood, chick mortality and embryo mortality as the dependent variables, and treatment, order of treatment and breeding round were independent factors. In the three models, genetic mother ID, genetic father ID and aviary were included as random effects. Additionally, genetic pair ID was included as random effect in the models for offspring reaching adulthood and embryo mortality. For the model of chick mortality social nest and social pair ID were included as random effects. Genetic parentage was determined by exclusion using the R package SOLOMON (Christie et al., 2013).

**Results**

*Baseline corticosterone levels*

Baseline plasma CORT levels were very variable within individuals (Table 1). CORT levels were significantly higher in both treatment and control birds during the courtship phase of the breeding cycle, however we did not find a statistically significant difference in plasma CORT levels between control and treatment birds at any stage of the experiment. However, birds exposed to noise tend to have lower levels of CORT during courtship and nesting than control birds (Fig. 1). Males in both treatment groups had significantly lower levels of CORT in comparison with females. Furthermore, CORT levels in both groups decreased in the second breeding round, irrespective of treatment. Whether the blood collection was made during the first, second or third minute post-disturbance also influenced how high baseline corticosterone levels were.

*Immune system*
Chapter III. Traffic noise exposure alone may delay offspring growth, but is not a potent enough stressor to disrupt immune function, stress physiology or reproductive success in breeding birds

Traffic noise did not have a significant effect on granulocyte/lymphocyte (H/L) ratio in adult zebra finches (Table 1). On average, birds exposed to noise showed a non-significant tendency towards lower H/L ratios (Fig. 1) - birds in noise exposure treatment had a ratio of 0.69 and control birds had a ratio of 0.88. Males had significantly lower H/L ratios than females on average females had a ratio of 0.91 and males of 0.65.

We did not find a significant effect of treatment on the bacteria-killing abilities of whole blood from our adult zebra finches (Table 1). In addition, there was no significant difference between females and males in bacteria killing ability. However, breeding round did have an effect on bacteria killing capacity, as the number of bacteria growing on the plates was greater in the first round than the second. In this assay, individual variation was very large, as was the variation in colony numbers on the positive controls.

Reproductive success and extra-pair paternity

Traffic noise did not have an effect on the number of offspring that reached adulthood (Table 1). The order of treatment, whether noise was presented during the first or second breeding round, also did not have an effect on the results. However, birds had significantly fewer offspring surviving to adulthood in the second breeding round (Table 1).

Birds exposed to noise tended to have higher 0-2 days old chick mortality (Fig. 1), but the effect was not statistically significant (Table 1). Additionally, embryo mortality was not affected by traffic noise, nor by breeding round or order of treatment (Table 1). Finally, we found that the amount of extra-pair paternity was not affected by traffic noise, breeding round or order of treatment (Table 1).

The offspring of birds exposed to noise during courtship, nest-building and the nestling care period were significantly smaller than those born to parents in control groups (Table 1). In addition, there was no significant difference between body mass of female and male offspring at any age category. On average the body mass of the offspring at day 10 was 10.43 +/- 1.37 g in the noise treatment and 10.75 +/- 1.11g in the control treatment; at 21 days 12.23 +/- 0.93 g in noise and 12.25 +/- 1.24 g controls and at 120 days 14.50 +/- 1.27 g in noise 14.62 +/- 1.12 g controls.
Chapter III. Traffic noise exposure alone may delay offspring growth, but is not a potent enough stressor to disrupt immune function, stress physiology or reproductive success in breeding birds.

Table 1. Outcome of linear models testing the effects of noise on H/L ratio, plasma corticosterone levels, reproductive success and extra-pair paternity of adult zebra finches that were exposed to noise during breeding season and a no-noise control group. The asterisk represents “significant” differences in the frequentist’s sense, when the 95% confidence intervals did not overlap zero (Nakagawa and Cuthill, 2007).

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<tr>
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<th>Estimate</th>
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</tr>
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Chapter III. Traffic noise exposure alone may delay offspring growth, but is not a potent enough stressor to disrupt immune function, stress physiology or reproductive success in breeding birds

<table>
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<table>
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</table>
Chapter III. Traffic noise exposure alone may delay offspring growth, but is not a potent enough stressor to disrupt immune function, stress physiology or reproductive success in breeding birds.

<table>
<thead>
<tr>
<th>Aviary</th>
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</table>

Discussion

We found that traffic noise did not have a direct impact on most of the variables we measured, except for the mass of offspring at 10 days post hatch. Although it was not statistically

Figure 1. Effect size of each measured parameter for individuals in noise. Data points give the mean estimates of the models with 95% credible intervals. All the values were scaled according to Gelman and Hill (2007).
significant, birds that experienced noise during the breeding period tended to have lower levels of CORT, lower H/L ratios, and higher levels of chick mortality in addition to having significantly smaller offspring than birds in control group.

**Baseline corticosterone levels**

Contrary to our expectations, baseline levels of corticosterone were not significantly affected by noise. To date, the evidence linking noise exposure and elevated CORT levels is inconsistent. Some studies have found positive correlations between noise and CORT levels (Blickley et al., 2012; Wright et al., 2007), while others did not find such a correlation (Potvin and MacDougall-Shackleton, 2015), and one study even found a negative correlation (Crino et al., 2013). These differences in the evidence could be related to methodology, species or geographic location (Partecke et al., 2013).

The zebra finches in our study tended to have lower levels of CORT when they were exposed to noise, although the trend was not significant. It may be that traffic noise is not a severe enough condition to induce a physiological stress response in birds. It may also be that when noise is chronically present, birds deal with this long-term source of stress with a suppression of baseline CORT levels (Rich and Romero, 2005). Birds may physiologically reduce activity of the hypothalamic-pituitary-adrenal (HPA) axis during periods of chronic stress to elude pathological effects linked with chronically elevated glucocorticoid concentrations, such as weight loss, impaired immune function and hyperglycemia (Cyr and Romero, 2007, 2009). However, it is important to consider that the large individual variation may play a role in our results. In order to test whether birds in chronic noise are down-regulating the activity of the HPA axis, it would be necessary to follow up with further experimental testing, such as measuring acute CORT responses to a second, novel stressor (Romero and Wingfield, 2015).

Another possible interpretation of our results could be that traffic noise is initially a potent stressor, but that the birds quickly habituate to the noise (i.e. the birds learn that the stimulus is not harmful or aversive). In order to rule out habituation, one must look beyond baseline glucocorticoid levels at other measures, such as immune function, behavioural changes, oxidative damage on the cellular level, or secondary responses to additional stressors (Romero and
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Wingfield, 2015). For instance, European starlings exposed to chronic stress had lower CORT levels than controls, but also had reduced reproductive success (Cyr and Romero, 2007), suffered decreases in body weight and altered blood chemistry (Awerman and Romero, 2010), and showed depressed CORT responses to novel stressors (Rich and Romero, 2005), all of which are diagnostic indications that habituation had not occurred (Cyr and Romero, 2009). Therefore, in our study we investigated, not only the plasma CORT levels, but also reproductive success, extra-pair paternity, and two measures of immune function, in order to have a wider perspective on potential physiological consequences of chronic noise exposure in zebra finches.

Immune system

Zebra finches exposed to chronic noise did not have significantly higher H/L ratios, as we had predicted. Previous studies have found that an increase in H/L ratio was a reliable indicator of chronic stress in birds (Campbell, 1995; Fourie and Hattingh, 1983; Gross and Siegel, 1986; Maxwell, 1993; Müller et al., 2011; Ots and Horak, 1996). One study even found a fairly rapid increase in H/L Ratio in chicken when they were chronically exposed to a very loud unfamiliar sound. However, in our study we found an opposite tendency to what we expected a non-significant reduction in the H/L ratio for individuals exposed to noise. It is possible that the increase of lymphocytes could be related with the mating, nesting, hatching and chick rearing periods in which birds need to be in the better condition to resist this demanding process. Also, it is important to take into account that other conditions were stable, such as ad-lib food availability. Thus birds might, for instance, limit negative effects of other stressors on their immune system by changing food intake (Klasing, 2007). Finally, our data did show a differential response in females and males, in that males had lower H/L ratios. This observation suggests that either breeding behaviour or being in reproductive condition may have different physiological consequences for males than for females.

While we did not find a significant effect of noise treatment in the bacteria killing assay, we feel cautious about making very strong conclusions from this aspect of our experiment. As the amount of between-plate, within-bird variation was so high in our samples; we did not feel confident that a difference between treatments would be detectable. It could be that a different methodology,
such as using spectrophotometry to count bacteria density in the growth media would produce less variable results than plating and counting colonies.

Reproductive success and extra-pair paternity

As a measure of reproductive success we counted the number of offspring that reached adulthood, chick mortality and embryo mortality. We did not find any significant correlations between noise and any of these variables. However, chick mortality tended to be higher in birds exposed to noise. In addition to these measures of reproductive success we also measured growth rates in chicks. We measured the body mass of offspring at 10, 21 and 120 days old and we found that the offspring of the noise group had significantly lower body mass at 10 days old than those in the control group.

Several field studies have shown that reproductive success may be affected by urban noise (Halfwerk et al., 2011; Kight et al., 2012; Kuitunen et al., 2003). For instance, Flycatchers (*Ficedula hypoleuca*) showed a decreased number of fledglings per breeding attempt when they live close to roads (Kuitunen et al., 2003). Great tits (*Parus major*) living close to a motorway showed a decreased number of fledglings and females laid smaller clutches (Halfwerk et al., 2011). In addition, Eastern Bluebirds (*Sialia sialis*) exposed to anthropogenic noise showed smaller clutches and decreased in productivity (Kight et al., 2012). Moreover has been shown in many species that the song, an important trait in the reproductive behavior of songbirds, differs in populations that breed in noisy areas compared to songs of congeners in nearby quieter habitats (Brumm and Slater, 2006a; Hanna et al., 2011; Potvin et al., 2011; Slabbekoorn and den Boer-Visser, 2006). Thus, one hypothesis that may explain an observed decrease in reproductive success in urban birds, might be that “urban songs” may be less attractive to females (Halfwerk et al., 2011), or that noise may affect female preferences (Swaddle and Page, 2007), and this, in turn, results in a reduced reproductive output. One way in which we might test whether female preferences were affected by noise exposure is by comparing rates of extra-pair matings between noise and control treatments. However, we did not find a difference in extra-pair paternity between our treatment groups. Together with the tendency for high chick mortality and the significantly lower body mass in chicks exposed to noise, our results may indicate that noise affects parental behaviours (Potvin and MacDougall-Shackleton, 2015), such as feeding rate.
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Supporting this idea, feeding rates in other songbird species have been shown to be lower in birds raising their young in noisy environments, possibly as a result of noise masking the begging calls of young in the nest (Leonard and Horn, 2008; Schroeder et al., 2012). Begging calls are essential in parent-offspring communication since chicks produce begging calls that elicit feeding behaviour in adult (Budden and Wright, 2001; Burford et al., 1998). However, it will be important to do more targeted experiments in the future to work out exactly if and how noise exposure may impact parental feeding behaviours and how that influences reproductive fitness.

In line with our observation that offspring of birds exposed to noise grew more slowly than the offspring from birds in quiet aviaries, previous studies have found that noise during ontogeny can retard growth. For example, noise exposure during egg development resulted in chicks with lower body and brain mass in domestic chickens *Gallus gallus* (Kesar, 2014). Chronic noise exposure impairs normal brain development in the auditory cortex in rats (Chang, 2003), and in areas of the song control system in zebra finches (Potvin et al., 2016). Further, in a related study, we found that juvenile zebra finches exposed to the same noise treatments that were used in this study had higher rates of telomere loss than juveniles from control groups, or even than juveniles whose parents were exposed to noise (Chapter II). Together, this evidence indicates that noise may indeed be an important source of stress-related health effects, and is particular potent during early life.

While our study did not find many statistically significant effects of noise playback during breeding on either the health or baseline stress physiology of the parents, it may be premature to conclude that chronic noise has no negative impact on urban birds. Our experimental birds were housed in climate controlled, parasite- and predator-free aviaries, with unlimited access to food and drink. They were not exposed to chemical pollutants, nor to light pollution at night. It may be that noise in itself is indeed a stressor, but not a potent enough stressor to cause serious effects on the breeding success or health of adult birds. In humans, studies have found that noise effects on health may be augmented by, or in turn may increase the impact of other stressors (reviewed in Stansfeld and Matheson, 2003). Similar results to those we report here have been found in response to other types of stressors in zebra finches breeding in captivity. For example, when breeding pairs were required to spend more time foraging for food, their offspring were lighter at
fledging than offspring from parents with easy access to food (Brumm et al., 2009a). It could be that if birds, already under some pressure, are then also exposed to chronic traffic noise, the effect of noise may become more severe. It will be important to follow up these studies with experiments designed to investigate the combined effects of multiple aspects of urbanization to truly understand the effects of urban environments on bird fitness.

This study is one of the first comprehensive experimental tests of non-acoustic effects of traffic noise on songbirds. While many studies have previously reported correlations between anthropogenic noise levels and differences in reproductive success or stress physiology in urban-dwelling animals, ours is the first to investigate effects of chronic exposure to city traffic noise at realistic levels, and in the absence of other effects of urbanization that typically accompany noisy habitats. We found that noise had an effect on the growth of chicks in noise, but that noise did not, by itself, induce acute negative effects on the health or reproductive success of exposed breeding adults. Further studies into whether noise exposure may have more long-term fitness-related consequences, and if noise interacting with additional stressors leads to more serious acute effects are needed before ruling out noise as a threat to the health and fitness of city wildlife.
General Discussion

The goal of this thesis was to elucidate the impact of noise on vocal behaviour, stress physiology, immune system and reproductive success on birds. In doing so, I investigated how noise may indirectly affect fitness through different pathways and how these pathways may be interrelated (Fig. A). From these studies, we can conclude several things: First, noise has an impact on vocal communication. This conclusion can be drawn from the two studies presented in Chapter I. Mallard ducks reacted to background noise using the Lombard effect in Chapter I.1. This result supports the hypothesis that birds use a common strategy to overcome noise (Brumm and Zollinger 2013). In Chapter I.2, the results of a field study showed that the onset of the dawn chorus of rufous-collared sparrows in a tropical city varied with the level of noise pollution. However, the onset of dawn singing was not correlated with levels of artificial light. Second, noise did not have a significant effect on physiological stress responses, immune function, reproductive success and extra-pair paternity in adult birds during breeding season (Chapter III). However, noise affected significantly cellular ageing of young birds (Chapter II) and body mass of very young nestlings (Chapter III). Consequently, noise may have an impact on development and learning in birds, but this hypothesis is still speculative since data on vocal learning, physical condition, and immune function during the vocal ontogeny stage is still needed. Altogether, these findings suggest that noise may affect fitness through different pathways. In order to understand these effects, it is important to take into account species identity, ecological patterns, developmental stages and individual variation. Although many of the different variables I tested in my thesis were not affected by noise in a statistically significant way, these results are nonetheless important to see the long-term effects on the subsequent generations.

1. The impact of noise on vocal communication

Acoustic signals are the primary means of communication for birds and are extremely important in many situations related to fitness and the survival of species. Birds use acoustic signals for behaviours related to reproduction, to communicate with their kin, to prevent or avoid predators,
to share information about food sources or breeding status, maintain social cohesion and coordinate movement among other things. Avian reproduction is particularly susceptible to noise because most species rely on acoustic signals (songs) to defend territories and attract mates (Catchpole and Slater, 2008). Thus, acoustic masking is likely to have major fitness consequences (Brumm and Slabbekoorn, 2005b) and if this disruption is recurrent it may have an impact on the population or species levels (Bayne et al., 2008; Blickley et al., 2012). Acoustic disruptions can emerge in dynamic environments like changing social contexts (e.g. Brumm and Slater, 2006b; Gavassa et al., 2013; Janik, 2000) or challenging conditions for signal detection (e.g. Goodwin and Podos, 2013; Lengagne et al., 1999). In those environments signal plasticity is an important feature used by animals to adjust their signal in response to a changing environment. Certainly, environmental constraints on signal propagation and detection can be considered one of the major forces driving the evolution of animal communication systems across different taxa (Brumm, 2013; Wiley, 2015).

The confirmation of the Lombard effect in mallards ducks (Chapter I.1), a representative of the Anseriformes, emphasizes the importance of maintaining vocal communication in acoustically challenging environments. This new evidence supports the hypothesis that all extant birds share this trait as a strategy to deal with fluctuating levels of environmental noise (Brumm and Zollinger, 2013), and documents the presence of the Lombard effect in all three major lineages of the Neognathae (Fig. 5. Chapter I.1). According to this, the trait likely emerged early in the evolution of the class Aves as a strategy to deal with changing environments for Neognaths. Whether Lombard effect is a synapomorphy for all birds is still not known, as we lack information about the trait in some groups of Palaeognathae. To date, the presence of the Lombard effect has been found in Tinamiforms (Schuster et al., 2012) but has not been confirmed in ostriches and rheas so far. If the Lombard effect is ancestral in birds, then, according to the phylogeny of (Prum et al., 2015), it has evolved at least 70 million years ago.

In addition, my discovery of the Lombard effect in 2 days old ducks (Chapter I.1) adds to and confirms the evidence that this effect emerges very early in life for birds and mammals (Brumm et al., 2009b; Leonard and Horn, 2005; Luo et al., 2017b). This suggests that the Lombard effect is produced by way of basic brain mechanisms that do not require extended maturation after birth. However, higher-level processes may modify this basic control of the effect. These
processes may not be fully developed until later in life, since the effect was stronger in 10 day old ducks (Chapter 1.1). Alternatively, the magnitude of the effect could be affected by the development of the vocal apparatus, which may change during ontogeny (Brumm and Hultsch, 2001; Leonard and Horn, 2006). In my study, it seems that the motor control of amplitude improves with age since the older ducks produced calls with lower amplitude in control treatment resulting in a higher magnitude of the Lombard effect (Chapter 1.1). However, more studies are still needed that specifically focus on the development of the Lombard effect during the ontogeny combining anatomical, physiological and behavioural evidence, before we have a clear picture of its development and utilization.

In many cases, the Lombard effect is accompanied by changes in other spectral and temporal traits of the calls depending on the species and context (reviewed in (Brumm and Zollinger, 2011). In addition to their noise-dependent regulation of call amplitude, ducks increased call peak frequencies (Chapter 1.1), similarly to tinamous (Schuster et al., 2012) and parrots (Osanski and Dooling, 2009), but unlike songbirds (Zollinger et al., 2017). The increased of call frequency during noise exposure in ducks and other birds could be a passive by-product of the Lombard effect, due to a physical coupling of amplitude and frequency during phonation (Beckers et al., 2003; Elemans et al., 2008). Possibly the syrinx anatomy of songbirds is more sophisticated (King, 1989) and permits them to control amplitude and frequency of their vocalizations independently. Thus, they can uncouple the two parameters, frequency and amplitude, during Lombard-induced increases of vocal amplitude. Such capacity could be linked to the modulation of fundamental frequency due to the controlled fine-tuning of air pressure by the songbird syrinx (Amador and Margoliash, 2013). Taken together, this result supports the hypothesis that a decoupling of amplitude and frequency during the Lombard effect may be a derived trait of songbirds.

Even though the Lombard effect seems to be a general strategy used by birds to overcome noise, many species may also adjust the redundancy, the duration, the frequencies, and the timing of their vocalizations (reviewed in Brumm and Zollinger, 2013). These changes, in general, are based on immediate individual plasticity in response to changes in the background noise. However, there are cases in which it seems that birds can adjust their vocal behaviour in anticipation of a later and constant disruption in the environment. For instance, rufous-collared
sparrows (*Zonotrichia capensis*) living in a tropical city started to sing earlier in the day, probably to avoid masking noise produced by traffic, which occurred approximately two hours after the onset of their dawn chorus (*Chapter I.2*). Cities, in general, are dynamic environments in which animals frequently need to change a whole suite of traits, including behaviour, physiology, and morphology in order to adapt.

Certainly, urbanization is considered one of the major causes of contamination around the world (Grimm et al., 2008a), including chemical, noise and light pollution. Surprisingly, the impact of light and noise pollution in birds had only been studied in temperate zones before the onset of my studies in 2013. In the temperate zones, seasonal changes in day length are very evident. Thus, birds use photoperiod as a cue to start breeding season in a broad context and in particular it is use for the onset of the dawn chorus as well (Dawson et al., 2001; Gwinner, 2003; Wingfield and Farner, 1993; Wingfield and Kenagy, 1991). Light at night has been related to advancement of the breeding time (Dominoni et al., 2013b), and to a change in the onset time of the dawn chorus (Da Silva et al., 2015; Dominoni et al., 2013a; Kempenaers et al., 2010; Miller, 2006). On the other hand, it has been suggested that anthropogenic noise may also affect the singing behaviour in some species in temperate zones (Arroyo-Solís et al., 2013; Fuller et al., 2007; Nordt and Klenke, 2013). The neglect of studies on tropical birds is particularly profound since these regions not only harbour the highest bird diversity (MacArthur and MacArthur, 1961; Wilson, 1999) but also some of the world’s largest urban areas and fastest urban growth (United Nations, 2012). In order to disentangle whether light or noise pollution more strongly affects singing behaviour, I conducted a study in Bogota, Colombia, a tropical city of over 8 million people (*Chapter I.2*). The results suggest that the onset of the dawn chorus of rufous-collared sparrows (*Zonotrichia capensis*) in a tropical city is related to levels of noise pollution. Birds started to sing earlier at places with high levels of daytime traffic noise.

This relation between the onset of dawn chorus and noise levels in rufous-collared sparrows may be related to external cues used by birds to start reproduction. Since singing behaviour, and specifically the dawn chorus in birds, is linked to territory defence and mate attraction and retention (Catchpole and Slater, 2008), it may be affected by external cues. In the tropics, photoperiod varies very little across the year, in contrast to temperate zones where a marked increase in day length during spring triggers the breeding season of birds. Even though some
species located in rain forest and other tropical forest types have the capacity to respond to small photoperiodic changes (Hau et al., 1998; Quispe et al., 2017), it may be that species identity and location (e.g. mountain or tropical forest) are important for the such a response. The fact that rufous-collared sparrow singing behaviour in an Andean mountain city was related to traffic noise could be explained if sunrise was not a strong seasonal cue for some birds close to the equator to start breeding season. In the same direction, previous studies found that changes in day length are not used as cues to trigger reproductive behaviours in tropical rufous-collared sparrows (Moore, 2005). Close to the equator, some birds may be generally less entrained to the seasonal photoperiod, therefore, light pollution may have less of an effect on their behaviour. However, to determine whether this difference in light responsiveness represents a general difference between birds in the tropics and those in the temperate zones, more comparative studies with more species are still needed. One appropriate candidate for such studies is rufous-collared sparrow, which are common and present in both tropical and temperate zones, as well as in a wide range of altitude (from 100 masl to 4200 masl). Studying this kind of widely dispersed species will allow us to address the effects of seasonal variation in day length on mating behaviours and understand how anthropogenic disturbances may differentially affect populations at different latitudes, without having to control for species differences.

In conclusion, birds use different strategies to increase acoustic detectability in changing environments. Acoustic plasticity allows birds to adjust their calls temporally and/or spectrally in response to changing conditions in order to communicate. The Lombard effect seems to be a common strategy evolved in different taxa as a strategy to overcome noise (Chapter I.1). However, the contexts of when and where the species need to communicate play a relevant role in which strategies are used. For instance, for birds living in a tropical city, like rufous collared sparrows in Bogota, Colombia, the adjustment to efficiently communicate is the advancement of the onset of the dawn chorus in noisy areas, as an anticipation of peak traffic noise (Chapter I.2). Therefore, physical constraints, and general ecological patterns, such as altitude, play an important role in how birds respond to masking noise.
2. The impact of noise on physiological systems

Noise not only impacts acoustic communication in birds, but may also have non-acoustic effects on health and physiological systems. These effects can be correlated with changes in behaviour, physical and health state and may vary depending on the needs of the current life stage of the exposed organisms. For instance, levels of the stress hormone corticosterone (CORT) in adult birds has been associated with chronic environmental noise (Blickley et al., 2012; Wright et al., 2007). In addition, levels of CORT in parents has been correlated to physical changes in offspring (Saino et al., 2005) and changes in parental care behaviours (Angelier et al., 2009; Bonier et al., 2009, 2011; Escribano-Avila et al., 2013; Spée et al., 2011; Thierry et al., 2013). In addition, noise has been related to negative effects on reproductive success and physical state of young birds (Halfwerk et al., 2011; Kight et al., 2012; Schroeder et al., 2012). Also, noise is part of urbanization and urbanization has been shown to be related to short telomeres at very early stages in birds (Meillère et al., 2015; Salmón et al., 2016). Up to the present, the majority of studies on the effect of noise on birds have been conducted in the field. Thus, studies to understand the causal effects that underlie observed correlations between anthropogenic noise and changes in physiology or fitness are needed. Additionally, it is important to identify model systems that can be experimentally manipulated. These models allow unraveling the impacts of different environmental factors associated with urban habitats on the development, health, behaviour, and fitness of exposed individuals. In order to contribute critical data to understand the long-term effects of traffic noise pollution on avian health and fitness, zebra finches were experimentally exposed to realistic levels of traffic noise at different life stages. The fact that the living conditions of the birds were stable, except for the presence or absence of traffic noise, gives the chance to provide evidence of a causal link between chronic noise and physiological systems. In chapter II and III zebra finches were experimentally exposed to traffic noise levels during breeding season, before fledging period (from 0 to 18 days old post-hatch) and post-fledging period (from day 18 to 120). Different parameters were measured like CORT levels, immune function, reproductive success, extra-pair paternity, body mass and telomere loss rate.

Impact of traffic noise on adults during breeding season

Birds exposed to traffic noise during breeding season did not show statistically significant difference in the baseline CORT levels compared to the levels of CORT in control treatment. I
expected to find a significant impact of noise on CORT levels, as a stress indicator. However, this study is aligned to previous evidence in which there was no consensus across studies as to how noise affects CORT levels: Some studies suggest that noise and CORT levels are positively correlated (Blickley et al., 2012; Wright et al., 2007), whereas others did not find any correlation (Potvin and MacDougall-Shackleton, 2015) and one even showed a negative correlation (Crino et al., 2013). This inconsistency in the evidence could be explained by differences in geographic location, methodology or species identity (Partecke 2014).

In chapter III, my experiment showed that adult zebra finches in breeding season tend to have lower CORT levels when they were exposed to noise, although the correlation was not significant. One explanation for such a result is that traffic noise might not be a severe condition to induce a significant stress response. On the other hand, it could be that birds deal with this chronic stress condition suppressing the baseline of CORT levels (Rich and Romero 2005). Physiologically, birds may decrease the hypothalamic-pituitary-adrenal (HPA) axis activity throughout times of chronic stress to avoid pathological effects associated with elevated glucocorticoid concentration, like hyperglycemia, impaired immune function and weight loss. Nevertheless, the large individual variation in my data may contribute to the lack of a clear and significant tendency in the results. In the direction to test if birds exposed to chronic noise down regulate HPA axis activity, further experimental testing would be necessary such as the CORT response to a second novel stressor (Romero and Wingfield, 2015).

The long-term exposition to chronic noise can also lead to habituation and can explain why there was not a strong effect of noise in CORT levels in my experiment. However, it is important to rule out habituation checking immune function, behavioral changes, oxidative damage on cellular level or responses to additional stressors (Romero and Wingfield, 2015). For example, European starlings exposed to chronic stress showed that they were not habituated to that condition (Cyr and Romero, 2009) because they had low CORT levels together with reduced reproductive success (Cyr and Romero, 2007), low body weight and altered blood chemistry (Awerman and Romero, 2010) and they showed depressed CORT responses to novel stressors (Rich and Romero, L. M., 2005). Thus, in chapter III baseline CORT levels were tested together with immune function, several measures of reproductive success, and extra-pair paternity in order to widen the perspective of the impact of traffic noise in zebra finches.
I counted the number of lymphocytes and granulocytes (eosinophils, basophils, heterophils) for each individual in control and noise treatments as immune function measurement. I used the granulocyte to lymphocyte ratio as a proxy for Heterophil to Lymphocyte ratio (H/L ratio). According to previous evidence, increase of H/L ratio was a reliable indicator of chronic stress in birds (Campbell, 1995; Fourie and Hattingh, 1983; Gross and Siegel, 1986; Maxwell, 1993; Müller et al., 2011). For example, one study showed a rapid increase in H/L ratio in chicken when they are exposed to a very loud unfamiliar sound (Gross, 1990). In chapter III, birds exposed to traffic noise showed the opposite tendency to decrease H/L ratios even though the correlation was not statistically significant. The fact that the sample was taken during breeding season could indicate that birds need to be in better conditions to cope with this demanding process, thus the lymphocytes possibly increased. Additionally, it is important to consider that immune system can be related to food intake (Klasing, 2007). It could be that the decrease of H/L ratios is related to a behavioral change associated with food intake. Also, it is important to consider that to determine the influence of external variables in the immune system of birds might be difficult since they show individual responses to stress (Vleck et al., 2000). The immune function can also be measured by the bacteria killing assay. I used this method and took several months to standardize the technique. Despite the investment of considerable time and effort, the controls remained too variable for a reliable application of this method to infer the immune state of the birds of the experiment.

To see the whole picture of how traffic noise is affecting adult zebra finches during breeding season, I counted the number of offspring that reached adulthood, chick mortality and embryo mortality as reproductive success measurement. I found that traffic noise did not have any significant correlation to any of these variables. Nevertheless, birds exposed to noise tend to have higher chick mortality than when they were in the control treatment. Reproductive success has been shown to be affected by urban noise in several field studies (Halfwerk et al., 2011; Kight et al., 2012; Kuitunen et al., 2003). Furthermore, it is well known that the song, a crucial mating trait, can be affected by noise (Brumm, 2006; Hanna et al., 2011; Potvin et al., 2011; Slabbekoorn and den Boer-Visser, 2006). Thus, songs modified by environmental noise may be less attractive to females (Halfwerk et al., 2012) or preferences of females may be affected by noise (Swaddle and Page, 2007). One way to see whether preferences of females are potentially affected by noise
is checking extra-pair paternity of breeding birds during noise and control treatments. In chapter III extra-pair paternity did not change according to the treatments. The results suggest that noise may have an effect on parental behaviour related to chick mortality and low mass in chicks.

**Impact of traffic noise on chicks and juvenile zebra finches**

Zebra finches exposed to noise from egg stage until fledging showed statistically significant lower mass than chicks born in control groups at 10 days old (chapter III). I did not take a sample of telomeres from the zebra finches before they were 21 days post-hatch (chapter II). Thus, how parental or very early life exposure to traffic noise impacts telomere dynamics in this species is still missing. Also, even though I found an effect of noise on the mass of 10 days old chicks, this does not necessarily imply an effect on telomere length as well, because, according to my results, telomere length and mass were not related. Current experiments are now addressing this question using a telomere sample at day 8 post-hatch. Nevertheless, sampling at day 21 post-hatch is a good predictor of longevity in zebra finches, as has been shown in other study. Pre-fledging-noise-exposed, post-fledging-noise-exposed and control birds did not differ significantly in telomere length at 21 days old. However, juveniles directly exposed to noise during the post-fledging period (18 to 120 days post-hatch) had significantly shorter telomeres at 120 days than birds in the control treatment, or birds exposed to noise at pre-fledging stage. Therefore, noise exposure during the later stages of ontogeny (between 18-120 days) resulted in an increased rate of telomere loss. Thus, the evidence suggests that juveniles that experience traffic noise will have greater telomere attrition, causing a potential reduction in longevity with negative consequences for fitness.

Previous studies have found that parental behaviours may change in the presence of noise (Meillere et al., 2015) which can reduce reproductive success (Potvin and MacDougall-Shackleton, 2015; Schroeder et al., 2012). In the same direction, in Chapter III, I found a tendency of higher chick mortality for birds exposed to noise. In addition, a change in parental behaviours may explain why there was a negative effect of noise exposure on the mass of 10 days old chicks (Chapter III) and no effect on telomeres of noise exposure of pre-fledging birds (Chapter II). It could be that parents exposed to traffic noise reduced feeding rates as consequence of masked begging calls (Leonard and Horn, 2008; Schroeder et al., 2012) resulting
in low mass 10 days old chicks. It could be that later in their life, when the chicks are 21 days old, parents find ways to mitigate the effect of noise on their offspring. Possibly noise-exposed parents invest more in parental care, which may buffer noise-induced telomere loss, and as a result telomere length in their offspring is not significantly different to offspring from the control group at 21 days. Alternatively, very young birds may be buffered against stress-induced telomere loss by higher rates of repair, compared to older juveniles. At different developmental stages, the expression of telomerase, the enzyme that repairs telomeres, differs. (Haussmann et al., 2007) when the proliferative demands of most organs are the highest. However, the exact mechanisms underlying the differences observed between experimental groups need further study.

Contrary to previous field evidence (Meillère et al., 2015; Salmón et al., 2016), zebra finches exposed to noise did not differ in telomere length at 21 days post-hatch, when the first sample was taken (Approx. 10 days after previous studies) (Chapter II). Time of sampling, species differences in sensitivity to traffic noise or differences between wild and captive birds may explain this result. It may also be explained by experimental design differences. Parents of 21 day olds that were exposed to noise, together with their offspring until 18 days, continuously (like in the city) may have been able to become habituated more readily to the noise when it was continuously present, than to shorter daily periods of noise, such as they experienced in study of Meillère et al. (2015). Additionally, parents exposed to noise in our study may have made changes in their parental care behaviours to compensate for the additional stress experienced by their offspring (Charnov and Krebs, 1974; Williams, 1966).

In zebra finches, young adulthood (between 18 and 120 days post-hatch) is a critical period. This could explain the significant effect of traffic noise on post-fledging young adults. Zebra finches typically leave the nest around day 18 and continue to be fed entirely or partially by their parents or other adults until approximately 30 days post-hatch, at which time juveniles become independent from their parents. In addition to the stress of weaning from parental nutritional support, this time period (ca. day 20) is approximately when zebra finches enter the song learning period (Roper and Zann, 2006). This could mean that birds are more sensitive to noise or that noise is a more potent stressor during this stage than at earlier life stages. Corroborative evidence for this notion comes from a recent study that found that although noise exposure did not affect
corticosterone levels, it did affect the size of brain regions associated with song learning in zebra finches during their song learning period (Potvin et al., 2016).

In conclusion, traffic noise impacted birds differently depending on their life stage. Adults seem to cope physiologically to the noise exposure in other ways than expected, since their immune function and baseline corticosterone levels were not significantly affected by traffic noise. However, the impact of noise was reflected in their offspring, which presented low body mass. The evidence points to the idea that parental care and parent-offspring communication may be affected by noise, resulting in physical changes for their offspring. However, studies specifically addressing those issues are still needed. In addition, juveniles directly exposed to traffic noise showed a significant decrease in telomere attrition rate, which potentially means a reduction in longevity. Thus, studies addressing the long-term consequences of traffic noise exposure for different generations are crucial to understand the physiological mechanisms affected by noise.

3. The impact of noise on development and learning

Zebra finches were affected by traffic noise differently according to their developmental stage (Chapter II and III). Physiological mechanisms such as telomere loss rate and physical condition, such as body mass, were negatively affected by traffic noise at very early stages (Chapter III) and at young adulthood (Chapter II). However, more evidence about how noise affects hormonal levels, immune system and vocal learning during different developmental stages is still missing.

The evidence I present in chapter II and III indicates that a disruption in parent-offspring communication may be causing a negative effect on mass and telomere length of the offspring at crucial moments during ontogeny. In fact, birds that showed a higher rate of telomere loss were exposed to noise during their vocal learning period (Chapter II). Additionally, there is one study showing that the syntax of the song in zebra finches is affected by traffic noise, as well as the size of brain regions associated with song learning (Potvin et al., 2016). In addition, in a pilot experiment, Zollinger et al. (2017), found that great tits showed a delay in the crystallization time of the adult song in a group exposed to moderate levels of white noise during the first year (Zollinger, personal communication). Hence, the hypothesis that the process of vocal learning, immune system and physical condition is affected by traffic noise arises.
To test this hypothesis, I designed an experiment in which I followed vocal development of young zebra finches raised in either no-noise conditions or exposed to traffic noise. I placed pairs of adult zebra finches in sound boxes and allowed them to breed, and then recorded their vocal output, as well as the physiological and physical condition from hatching to young adulthood. Specifically, I collected data of immune system function, body size and mass, plasma corticosterone levels and song development from each individual. Currently the data is still in the analysis phase. Once the results are available, this will amplify the perspective of how traffic noise is affecting individuals at different developmental stages.

**Conclusions**

Generally, the studies in my thesis show how noise differently impacted traits related to fitness in birds depending on species, ecological factors and developmental stages. I found that birds use certain common strategies to overcome the challenges of living in noisy environments, such as the Lombard effect. While it is clear that urbanization provides challenging conditions in terms of noise exposure for birds living in urban areas, my experiments show that physiological systems were not significantly affected in adults birds exposed to noise during breeding season. However, telomere loss rate, as a predictor for longevity, and physical condition of their offspring were affected by traffic noise exposure. Taken together, this evidence points to the importance of approaching questions regarding the effects of urbanization both experimentally and integratively. By using birds as a model system for researching the effect of noise and their consequences in fitness, and combining both laboratory and field experiments, we have moved closer to understanding the complex effects of anthropogenic noise on wildlife. Similarities between birds and humans, such as the effects of environmental conditions on telomeres and vocal learning, mean that research on birds can provide valuable and unique clues to the understanding of the causes and consequences of noise exposure in humans. Finally, these data on the physiological costs and fitness consequences of exposure to anthropogenic noise may provide insights for conservation efforts of birds in the cities.
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Abstract

Urbanization is considered one of the biggest environmental challenges of our times. One of the consequences of the continuing growth in global urbanization is increasing noise pollution. Indeed, anthropogenic noise is predicted to rise considerably levels in the near future. The concern about an increase in anthropogenic noise is that it has been associated with a series of negative effects for humans and animals. For instance, noise is linked to cognitive and auditory problems in humans. In birds, it has been related to changes in vocal signal traits and changes in breeding and parental care behaviour, among others. Despite the relevance and the consequences of anthropogenic noise for humans and wildlife, the effects of noise on health and fitness of the urban fauna are still little known.

In this thesis, I investigate how noise can impact birds through different mechanistic pathways by disrupting communication, by impacting physiological systems via stress, and by interfering with development. I choose birds as model organisms for this topic because many species of birds are common inhabitants of cities and have developed a variety of strategies to adapt to urban habitats. Thus, an investigation about the impact of noise on birds not only contributes to the advancement of basic science, but can have direct relevance for conservation actions aimed at maintaining or increasing biodiversity in urban landscapes. In addition, the similarities between humans and birds such as the effects of environmental conditions on physiological systems and the vocal learning process, may allow us to increase the understanding of the mechanism underlying effects of noise in people exposed to chronic noise.

The disruption of vocal communication can have detrimental consequences for birds since they use acoustic signals for a variety of functions including mate attraction, territorial defense, kinship communication, maintenance of social cohesion, and information sharing about food sources and potential predators. Environmental noise can cause such as disruption. Consequently, birds have developed strategies to overcome the challenge of communicating in noisy environments. One strategy is known as Lombard effect, in which in response to an increase in the background noise, a signaler increases its vocal amplitude. This effect is well-studied in
human speech and has also been reported in other mammals and several bird species and is considered as a basic mechanism for maintaining communication in noise. In some cases, the Lombard effect is accompanied by additional changes in signal parameters, such as increased frequency. To date, only three out of eight major avian clades have been studied, therefore the evolution of the Lombard effect and other related vocal adjustments in birds are still unclear. In chapter I.1, I report the first evidence for the Lombard effect in an anseriform bird, the mallard duck (*Anas platyrhynchos*). Linked to the Lombard effect, ducklings also increased the peak frequency of their calls in noise, however, they did not change any of the others measured parameters. This evidence supports the hypothesis that all extant birds use the Lombard effect to solve the common problem of maintaining communication in noise. Thus, Lombard effect is an ancestral trait shared among all living avian taxa, which strongly suggests that it has evolved more than 70 million years ago within that group. At the same time, our data suggest that parameter changes associated with the Lombard effect follow more complex patterns, with marked differences between taxa, some of which might be related to proximate constraints. In addition to changing certain traits of the vocalizations, birds can also shift the time of singing behaviour in presence of noise. For instance, birds in cities start to sing earlier in the morning than birds in the rural areas. Some studies have attributed these temporal shifts to increased levels of light pollution, while other studies suggest that the shifts are linked to noise pollution. However, all previous studies have taken place in temperate zones. In contrast to temperate birds, tropical birds experience little seasonal variation in day length and may be less dependent on light intensity as a modifier for reproductive behaviours such as song. To test whether noise or light pollution has an impact on the dawn chorus of a tropical bird, in chapter I.2 I investigated the singing behaviour of rufous-collared sparrows (*Zonotrichia capensis*) in Bogota, Colombia, at two times during the year. The results show that birds start to sing earlier in places with high noise levels. On the other hand, light pollution did not have a significant effect on song timing. Birds may begin to sing earlier in noisy areas to avoid acoustic masking by traffic that peaks later in the morning. These results also suggest that some tropical birds may be less sensitive to variations in day length and thus less sensitive to light pollution.

Noise not only can impact acoustic communication, it can also have an impact on short and long-term effects on health and fitness of wildlife. To test how chronic noise affects physiology and well-being of birds, I did a series of experiments in breeding zebra finches (*Taenopygia guttata*)
and their offspring. In chapter II, I experimentally investigated the direct and cross-generational effects of traffic noise on telomeres at 21 and 120 days post-hatch. Telomere length is a measure of cellular ageing that is predictive of disease and longevity in humans and other organisms. Birds were exposed to traffic noise at different developmental stages (pre and post-fledging). Noise did not have a significant effect on telomeres when the birds were exposed pre-fledging. On the other hand, birds exposed to noise during the post-fledging stage exhibited faster telomere loss than pre-fledging exposed and control birds. In chapter III, breeding birds were exposed to noise during courtship and nestling period and I measured the impact of noise on the immune system, baseline corticosterone levels, reproductive success and extra-pair paternity as well as the growth rate of the offspring. Although I did not find significant differences in any of the traits measured in breeding birds, I did find that offspring of birds exposed to noise were smaller than offspring in control groups. These results suggest that traffic noise affects birds differently depending on which the developmental stage they were in when they were exposed.

Taken together, the evidence of this thesis suggests that noise has a varying impact on traits related to fitness, such as vocal behaviour, longevity, reproductive success and growth rate in birds depending on species identity, ecological factors and developmental stages. The combination of laboratory and field approaches is necessary to understand how the different elements of urbanization, such as noise pollution, are impacting wildlife in the urban areas.
Zusammenfassung


Die Störung der vokalen Kommunikation kann für Vögel nachteilige Folgen haben, da sie akustische Signale verwenden, um Partner anzuziehen, Territorien zu verteidigen, mit ihren Eltern zu kommunizieren, den sozialen Zusammenhalt aufrechtzuerhalten, Informationen über
Zusammenfassung

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Record of achievement

Chapter I

I.1 A.M.D.C. carried out the data analysis, drafted the manuscript, and participated in the design of the study and data collection; M.R.R. participated in data collection and helped finalizing the manuscript; H.B participated in the design of the study, coordinated the study and helped finalizing the manuscript. All authors gave final approval for publication. All authors gave final approval for publication.

I.2 A.M.D.C. carried out the data analysis, drafted the manuscript, and participated in the design of the study and data collection; S.Z participated in the design of the study and helped finalizing the manuscript; H.B participated in the design of the study and data collection, coordinated the study and drafted and finalizing the manuscript. All authors gave final approval for publication.

Chapter II

A.M.D.C. carried out the data analysis, drafted the manuscript, and participated in the design of the study and data collection; S.Z. participated in the data analysis, drafted the manuscript, and participated in the design of the study and data collection; B.H. participated in the data collection and analysis and helped finalize the manuscript; H.B. participated in the design of the study, coordinated the study and helped finalize the manuscript. All authors gave final approval for publication.

Chapter III

S.Z. participated in the data analysis, drafted the manuscript, and participated in the design of the study and data collection; A.M.D.C. carried out the data analysis, drafted the manuscript, and participated in the data collection; W.G., W.F., U.K. and A.M.B. participated in the data collection and analysis and will help to finalize the manuscript; H.B. participated in the design of the study, coordinated the study and will help to finalize the manuscript.
Curriculum Vitae

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PUBLICATIONS


