Effects of anthropogenic noise on the behaviour, physiological traits and welfare of two animal models: wild mice (Mus musculus) and Eastern blue tongued lizard (Tiliqua scincoides)

Karen Fabiola Mancera Alarcon
MSc. / BSc. Biol

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School of Veterinary Science
Abstract
Noise is an important source of stress that is able to impact negatively on animals’ welfare. Anthropogenic activities are a major contributor to noise, and their cumulative effects on individuals could have consequences for wildlife populations. Although transportation noise impacts have been evaluated for vocal and charismatic animals, research regarding the effects of other types of anthropogenic noise on non-charismatic animals has not been undertaken. This research explored the effects of mining noise, which is prevalent in the Australian soundscape, on wild mice (Mus musculus) and Eastern blue tongued lizards (Tiliqua scincoides). The first experimental stage focused on developing a reliable methodology to assess behavioural responses in the blue tongued lizard, and suitable noise exposure and acoustic processing techniques for subsequent experimentation. This was achieved through the analysis of the effects of typical transport stressors on lizards’ behaviour. Lizards were exposed to Heat (35°C), Cold (15°C), high or low frequency noise or a Control treatment with no stimulus in a test chamber for a 5 s. The test chamber was connected to an escape chamber, accessible after exposure to the stimulus, and a small hiding chamber opposite the test chamber. Lizard behaviour was monitored during stimulus exposure and then for a further 15 minutes, after which each lizard was removed. Lizards exposed to Cold spent less time in the test chamber and more time inactive in the escape chamber. They also spent longer walking towards the hiding chamber both away from the wall and by the wall and walking in the hiding chamber away from the stimulus. Thus, cold temperatures were noxious for lizards in a simulated transport environment as they reduced activity and increased escape attempts. In a second experiment, noise exposure was studied in more detail and the behaviour of blue tongued lizards when exposed to five combinations of mining machinery noise was evaluated. Lizards were exposed to low and high frequencies (≤ or > 2 kHz) at both low (60-65 dB (A)) and high (70-75 dB (A)) amplitudes, and a Control treatment, following the same exposure technique developed in the first experiment. In the test chamber lizards exposed to any mining machinery noise, but especially high frequencies, spent more time freezing, a typical stress response in reptiles, when compared with animals in the Control treatment. In the hiding chamber, high frequencies at high amplitudes decreased durations of the head being held to the right face downwards, suggesting a lateralized fear reaction. High frequency, high amplitude noise was the most detrimental. Mining noise had negative effects on the lizards’ behavior and welfare. To estimate mining machinery noise effects on the second animal model, wild mice, amplitudes and frequencies were tested separately to differentiate between these two important components of noise. Using the high and low amplitudes previously established, wild mice were exposed to 3 weeks of continuous noise. Effects of noise on their behaviour, organ morphology and fecal corticosterone levels were compared with a control treatment (no extra auditory stimuli, below 55 dB (A)). This was probably due to gender-based
differences in stress activation. Circling behaviour in both clockwise and anti-clockwise directions was increased in animals exposed to high amplitude noise. In mice exposed to low amplitude noise, fecal corticosterone was increased but total circling remained the same as control. These results suggest that dopamine-related stereotypies during high amplitude mining noise were a coping mechanism that prevented excessive physiological arousal. Both noise treatments increased circling to the left, which corresponds to right hemispheric activation of the stress response; however only the high amplitude noise increased circling to the right (left hemisphere activation), which may inhibit stress arousal by right hemisphere. When organ morphology was evaluated, females, housed in pairs, had responses that differed from those of males, which had to be housed individually. Females exposed to high amplitude noise had a smaller adrenal cortex and cortex/medullary ratio compared to controls. This adrenal atrophy and decreased fecal corticosterone in the high amplitude treatment indicates a state of chronic stress during noise exposure that had accentuated effects for females. Females in both the high and low noise treatments had smaller kidneys than males in these treatments, suggesting an epinephrine-mediated vasoconstriction. A similar effect was seen in the males’ spleens exposed to both noise treatments, expected as physiological stress can generate spleen atrophy. Females, housed in pairs, had behaviour responses that differed from those of males, which had to be housed individually. In relation to amplitude therefore, mining machinery noise produced stress response on wild mice that were amplitude dependent and appeared to require the generation of coping mechanism. As high amplitudes were the most noxious for wild mice, we used this energy intensity to evaluate the effects of mining machinery noise at two frequency ranges: high (HF > 2 kHz) and low (LF ≤ 2 kHz) on the behaviour, organ morphology and fecal corticosterone of wild mice, compared with a control treatment (no extra auditory stimuli) using the exposure methodology described for amplitude exposure. High frequency mining noise increased fecal corticosterone and decreased partial hiding and nest activity. Females were the most affected, since they had the highest fecal corticosterone levels and a tendency for spleen atrophy, probably due to a specific high frequency sensitivity. Stereotyped circling anticlockwise, but not clockwise, was increased in female mice exposed to high frequencies compared to low frequencies. Low frequency mining noise increased fecal corticosterone levels in males but not females. Thus, mining machinery noise produces stress responses in wild mice that are also frequency dependent. From both sets of experiments, it is evident that mining noise has adverse effects on the two species, both of which are found in the vicinity of mining sites. These effects are clearly frequency and amplitude dependent, which should encourage environmental policy makers to effectively regulate noise levels in mining areas.
**Declaration by author**

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Journal Publications


Conference abstracts


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**Mancera K** 2013 Effects of mining noise on behavioral and physiological indicators of wild mice (Mus musculus). *College Science Week Scientific Meeting- Australian and New Zealand College of Veterinary Scientists*: July 13th Gold Coast, QLD, Australia.

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Publications included in this thesis


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Contributions by others to the thesis
The design of the experimental methodology of this research was produced by the active collaboration of me and my advisors, Professor Clive Phillips and Associate Professor Peter Murray. The statistical analysis was produced with the professional advice of the Statistical Advisor Allan Lisle. Mining noise recordings were produced with the aid of Miss Sara Rounsifer in consultation with Dr. Don Bridgeman. The personnel of the Native Wildlife Teaching and Research Facility of the University of Queensland were actively involved in the technical aspects and the training necessary for the husbandry of the animals used on this research.

Statement of parts of the thesis submitted to qualify for the award of another degree
The experiment detailed on Chapter one was submitted by Mr. Ying Nan Gao as the core subject of his thesis to obtain the degree of Master of Animal Studies at the School of Agriculture and Food Science at the University of Queensland, 2012, degree awarded in July 2012. Later on, the work published on his thesis was changed and improved on its statistical analysis, the description of the experimental methodology and the final discussion and conclusion. Literature review sections were written independently by each student. The description of the methodology used to generate the mining machinery soundtrack used on the experiments described in Chapters two to four was used by Miss Sarah Rounsifer to obtain her Bachelor's Degree on Ecology and Evolutionary Biology (Neuroscience) from the University of Princeton, 2013, degree awarded on September 2013. Likewise, the raw data obtained of organ and total body weights described on Chapter four were used for her thesis. Her analysis of this data was done independently from this work.
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In addition, none of this work would have been possible without all the helping hands along the way: Ying Nan Gao (aka Brian), the lizard whisperer with incredible working ethics and great cooking skills; Marie Besson, how was not only able to watch hours of behavioural videos, but also improved my work with her great insight and disposition and my life by becoming my friend; Laetitia Duller for her incredible ability to learn wild mice husbandry rapidly and make French toast superbly; and Floriane Facheux and Coline Dupont, who were as reliable as a rock for me and the lizards, during a fast-paced experiment that they carried out smoothly.

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Durante mi ausencia, no solo he encontrado que mi afecto por mis amigos y mi familia se ha vuelto mucho más grande, sino que he comprobado que cuando hay amor, las cosas se preservan indefinidamente. Gracias a mi papá y mi mamá, Eduardo y Lidia. Papá, no solo me proveíste de un techo por muchos años, sino de tus consejos y amor incondicional; no encuentro las palabras para manifestarte lo mucho que significas para mí, lo mucho que eres parte de mí. Mami, tú has representado para mí las lecciones más importantes de mi vida. Admiro tu capacidad de renacer y reinventarte, de tomar solo lo que te sirve y transformarlo en lo que es bueno para ti. Ocupas un lugar primordial en mi corazón y en mi vida. Lalo, no sabes lo mucho que significas para mí. Eres una de mis almas gemelas; vinimos del mismo lugar, vivimos las mismas experiencias, sé que cuento contigo para todo. Te admiro; ser tu hermana es para mí un inmenso orgullo. Amín, eres la viva prueba de que la sangre es más pesada que la distancia. Admiro tu fortaleza y me alegro de tenerte en mi vida. Mis amigos, Denébola, Jazmín, Selene, Erika, Gaby, Grisel, Ana, Pepe, Antonio…son ustedes mi alegría y mi fortaleza. Mi vida es una experiencia maravillosa gracias a ustedes. Esta tesis, como todos mis logros, está dedicada a todos ustedes.
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animal welfare, anthropogenic noise, stereotypy, coping, wildlife, mining, stress, lateralization, blue tongued lizard, wild mice

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ANZSRC code: 060603, Animal Physiology-Systems 30%
ANZSRC code: 050211, Wildlife Conservation and Management, 25%

**Fields of Research (FoR) Classification**
FoR code: 0707, Veterinary Science, 60%
FoR code: 0606, Physiology, 40%
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List of abbreviations used on this thesis

ABR = Auditory Brainstem Response
BLR = Binary Logistic Regression
C = Control
CL = Cold
CFS = Chronic Fatigue Syndrome
CI = Confidence Interval
CITES = Convention on International Trade in Endangered Species of Wild Fauna and Flora
CRH = Corticotrophin Releasing Hormone
dB (A)= A-weighted decibels
DOA = Death on Arrival
E$_2$ = 17β-estradiol
EBT = Eastern blue tongued
EC = Escape Chamber
EIA = Enzyme Immunoassay Technique
ENG = Electronystagmography
EPA = Environmental Protection Act
EPNP = Environmental Protection (Noise) Policy
FCM = Fecal Corticosterone Metabolites
FIV = Feline Immunodeficiency Virus
h = hour(s)
GLM = General Linear Model
H = Heat
HA= High amplitude
HC = Hiding chamber
HF= High frequency
HPA = Hypothalamic-Pituitary-Adrenal
Hz = Hertz
H&E = Haematoxylin and Eosin
kHz = Kilohertz
KW = Kruskal- Wallis test
LA = Low Amplitude
LEM = Linear Effects Model
LF = Low frequency
MW = Mann-Whitney test
NMDA = N-methyl-D-aspartate receptor
OR = Odds Ratio
QASP = Queensland Animal Science Precinct
r = correlation coefficient
RAAS = Renin- Angiotensin-Aldosterone System
ROS = Reactive Oxygen species
RSPCA = Royal Society for the Prevention of Cruelty to Animals
s = seconds
SED = Standard Error of the Difference
SEM = Standard Error of the Mean
SGCP = South Galilee Coal Project
SMR = Standard Metabolic Rate
SPL = Sound Pressure Level
TC = Test chamber
W = Watts
ZF = Zona Fasciculata
ZR = Zona Reticularis
CHAPTER 1: General introduction

1.1. Sound: general characteristics and special considerations

Animals rely on their senses to assess their surroundings. This assessment allows individuals to choose those behaviors that will enhance their adaptability and therefore, increase their chances to reproduce and survive. Amongst these senses, hearing, the one that deals with the perception of sound, is one of the most important as it processes transmitted information that potentially includes one of the most important forms of communication vocal.

Sound is a mechanical vibration and/or mechanical wave that travels on an elastic media (Blauert & Xiang 2009). Its propagation and perception is directly linked with the oscillations that the sound waves produce; the disturbances in molecular density that mechanical vibrations generate in gases or liquids promote pressure changes, which in turn, generate compressions and refractions that travel into the medium. Thus, sound waves are considered both a sequence of pressure disturbances and a large number of vibrating particles in the medium. (Kuttruff 2006).

As with any other mechanical vibration, sound waves can be described in terms of simple harmonic motion. Once energy is applied, a molecule in a system will go from one side of the initial equilibrium point (where the acceleration is zero) to the opposite side (maximum displacement of particles) and back to the initial point for an indefinite amount of time, that is, it will oscillate. Each oscillation is called a cycle. The number of cycles per time interval is called frequency. The maximum displacement of the molecules from the equilibrium point is called amplitude (Franklin, et al. 2010).

The frequency determines the pitch and it’s measured as the number of cycles per second or Hertz (Hz). However, frequency and pitch do not refer to the same phenomenon; frequency is an objective, measurable wave property and pitch describes the subjective, psychological impression (Franklin, et al. 2010). Nevertheless, the higher the frequency (that is, the greater the amount of cycles per second), the higher the perceived pitch.

The sound intensity is determined by the amplitude, which determines in great part the loudness perceived. Perceived loudness in humans does not scale linearly with sound intensity, since the ear response to intensity (which is mainly used to establish sound energy measurements) is logarithmic, being loudness proportional to the logarithm of sound intensity. Therefore, the unit of choice to compare sound intensities is the decibel (dB), which is the logarithm of the intensity of the smallest
pressure change that can be detected by the human ear (20 µPa), in $10^{-12} \text{ W m}^{-2}$ (Franklin, *et al.* 2010, Haughton & Feth 2002).

From a biophysical point of view, sound is not only the name given to the physical vibrations; it also relates to the subjective sensation of hearing (Haughton & Feth 2002). Thus, sound is considered the compression strain waves transmitted through a medium that have sufficient intensity and are of a frequency that can be detected as hearing (Franklin, *et al.* 2010). For instance, the hearing range of humans has been established as 20 Hz to 20 kHz at 60 dB SPL (Heffner & Heffner 2007). Therefore, mechanical waves traveling through air withholding these characteristics are considered sound (Drosopoulos & Claridge 2005, Haughton & Feth 2002). Sound falling outside this range is described as either infrasonic (below 20 Hz) or ultrasonic (above 20 kHz) (Franklin, *et al.* 2010). However, even when infra and ultra sounds are typically undetectable, it has been proven, for example, that infrasound with enough intensity (100-140 dB SPL) can be perceived by healthy human ears (Berglund & Hassmén 1996). Thus, sound detectability is determined by an interaction between amplitude and frequency sensitivity that may vary between individuals and species (Pater, *et al.* 2009).

In addition to relative detectability, the perception of loudness is also influenced by the hearing sensitivity. For example, frequencies between 2-4 kHz are better heard by humans. Therefore, low levels of amplitude are needed to perceive sounds between these frequency values, implying that the perception of loudness will be greater even when little energy is contained into the sound wave. To address this particular phenomenon, the A-weighted decibel scale (dB [A]) has been created to measure the intensity of the sound filtered and corrected for human sensitivity (Franklin, *et al.* 2010, Haughton & Feth 2002). This scale is widely used when sound is measured in the work place, urban areas and other human contexts. Nevertheless, human activities often overlap with different animal species that have varying frequency sensitivities. Despite this, little effort has been done to create decibel weighting methods that take into account such variability, partially because such consideration requires detailed research into most animals’ hearing capabilities (Pater, *et al.* 2009). Thus, sound can be defined as a mechanical vibration that travels through an elastic media, such as water or air, and that is able to produce the sensation of hearing. This implies that the experience of hearing depends greatly on the abilities of the receiver.

Likewise, mechanical vibrations that don’t represent an audible experience and are still present in the medium can be perceived through other means. For example, both insects and reptiles use unheard mechanical waves referred to as ‘airborne vibrations’ to communicate with conspecifics
and search for prey (Drosopoulou & Claridge 2005, Wever 1978, Young 2003). Thus, mechanical waves produce sound and vibrations that may influence the behavior and physiology of animals.

1.2. **Physiological effects of noise**

Sound is one of several tools for organisms to interact with the environment, communicate with conspecifics and avoid predators. However, when a specific sound is *unwanted* in terms of the receiver of the stimulus, it is then classified as noise (Maling 2007). Noise, in contrast with sounds used by animals for communication, is able to produce undesirable physical or physiological effects in an individual due to annoyance. Increases in amplitude are commonly related with increased annoyance, because increased amplitude implies a higher amount of energy contained into the sound wave, thus activating vibrating hearing structures with more strength and power. This fact, combined with the specific hearing range of each animal species, explains a great deal of the annoyance produced by noise (Cone & Hayes 1984).

Noise has other important features that make it a noxious experience for the recipient, such as sound complexity, which is defined as the degree of mixture of different sounds. Discordant mixtures of frequencies (i.e., with excessive complexity) are more noticeable and aversive for animals than pure tones (sounds of a single frequency) (Cone & Hayes 1984). In close relation with sound complexity, noise can be also linked to nonlinearity, which is the presence of desynchronized sound that occur during distress and alarm calls and that are able to generate highly complex sounds that include unpredictable components (Blesdoe & Blumstein 2014, Fitch, *et al.* 2002, Tokuda, *et al.* 2002). Some animals like the white-crowned sparrow (*Zonotrichia leucophry*) or the yellow-bellied marmots (*Marmota flaviventris*) react to non-linear artificial sounds as they would do in response to conspecific distress calls (Blesdoe & Blumstein 2014, Blumstein, *et al.* 2008, Crino, *et al.* 2011, Slaughter, *et al.* 2013) implying that responsiveness to nonlinearity can be generated even in artificial noises (Slaughter *et al.* 2013; Blesdoe and Blumstein 2014). Therefore, depending on the characteristics of the wave, the composition of frequencies and the hearing capacities of the receptor, any sound may be turned into noise.

Once noise is perceived it turns into a source of stress for animals. There has been extensive research into the physiological effects of noise exposure, mostly performed in laboratories with the use of rats, mice and humans and sometimes with other species of interest, such as birds and marine animals. From this volume of research, nine aspects of physiological impact can be recognized as affected by noise exposure (Kight & Swaddle 2011):
1. **Audition and cochlear morphology**: hearing impairment and deafness related to noise at high intensities are a result of damage to the cochlea and the related hearing structures. Injuries related to extreme acoustic traumas from a single event have been well documented [for example in the aquatic environment by Popper and Hastings (2009)]. In contrast, chronic noise exposure has not been well studied (Kight & Swaddle 2011), despite the fact that it exists for humans and free ranging animals (Armstrong 2010, Saha & Padhy 2011). The auditory processing of the brain, in particular in the auditory cortex can also be affected. For example, in rats noise exposure leads to poorer acoustic processing and a delay in neural maturation of neurons in the auditory cortex. However, these effects seem to be reversible over time if the exposure to noise is terminated (Chang & Merzenich 2003, Sun, et al. 2011).

2. **The neuroendocrine system**: it has been observed that cortisol levels either increase [in seahorses (*Hippocampus erectus*) (Anderson, et al. 2011), humans (Evans, et al. 2001) and dogs (*Canis lupus familiaris*) (Gue, et al. 1987)] or decrease [in mice (*Mus musculus*) (Jensen, et al. 2010)] when animals are exposed to loud noise. However a study with California spotted owls (*Strix occidentalis occidentalis*) exposed to chainsaw noise showed no change in fecal cortisol levels (Tempel & Gutierrez 2003). These differences in glucocorticoids levels could be due several causes such as the time of exposure and the species specific capabilities in regards to sound perception. Nevertheless, these studies suggest a disrupted response (i.e. non predictable and abnormal) of the HPA axis due to noise stress. Exposure to noise can also produce physical damage to the adrenal cortex (Pellegrini, et al. 1997), changing the normal stress response.

3. **Reproduction and development**: premature birth has been associated with excessive environmental noise (> 85 dB) in humans (Etzel, et al. 1997). In rats, decreased dental calcium concentrations have been found in pups whose mothers were subjected to significant environmental noise due a disruption in normal calcium transport (Siegel & Mooney 1987). Also, mortality of fish eggs and embryos increased when experiencing noise 15 dB louder than the noise they experience in nature according to (Wenz 1962) (Banner & Hyatt 1973).

4. **Endocrine changes**: Noise has been shown to produce disruptions of hormonal responses that could lead to changes in behavior. In panda (*Ailuropoda melanoleuca*), loud ambient noise increased agitation behavior and urinary cortisol, especially during oestrus and lactation (Owen, et al. 2004). Also, mice exposed to 100 dB (A) of white noise (noise where all frequencies show the same intensity) for 6 weeks for 6 hours/day showed a decrease in testosterone levels (Ruffoli, et al. 2006).
5. **Metabolism**: Rats (*Rattus norvegicus*) exposed to 30 days of noise stress (2640 Hz, 30 W) increased vigilance behavior and the time they spend hiding and reduced their feeding behavior, thus losing weight (Alario, *et al.* 1987). The last phenomenon has also been observed in shrimp (*Crangon crangon*), when they were exposed to 30 dB in a frequency range of 25-400 Hz (Lagardère 1982) and prairie dogs exposed to traffic noise at a Leq over the recording period of 77 dB (A) (re.20 mPa) (Shannon *et al.* 2014). Also, domestic hens (*Gallus gallus domesticus*) exposed to noise stress of 80-100 dB for 10 minutes have shown increases in plasma cholesterol and total protein level, possibly as a result of the necessity of these elements for the creation of hormonal signals to respond to stress (Chloupek, *et al.* 2009).

6. **Cardiovascular health**: under stress the body redirects blood through vasoconstriction to the muscles to prepare for the ‘fight or flight’ response (Herd 1991). Consequently, it is not surprising that loud noise has been related to increases in arterial pressure in humans (Andren, *et al.* 1983). However, it also has been shown that ungulates and birds may habituate to short term noise stimuli (Harms, *et al.* 1997, Weisenberger, *et al.* 1996). Rats exposed to noise have been shown to have mitochondrial damage in their myocardial cells (Gesi, *et al.* 2002).

7. **Cognition and sleep**: noise exposure in industrial workers and people living close to major transportation routes has been associated with depression and aggression (Stansfeld 2003). Fear has been also associated with noise stress in hens, with exhibition of tonic immobility (Chloupek, *et al.* 2009). This means that noise can alter animals’ mood and therefore, the behavior. Also, increased noise levels have been associated with detrimental effects on memory in humans (Lercher, *et al.* 2002), and in rats these effects on memory have been associated with chronic sleep problems produced by noise (Rabat 2007).

8. **Immune system**: due the effects that noise has on the HPA axis, the immune system is also affected, since glucocorticoids have inhibitory effects on the immune system (Spencer, *et al.* 2001). Even more concerning is the fact that these effects have been shown to be detrimental across generations. Female mice exposed to an alarm bell (85-95 dB) while pregnant had pups with smaller thymus and lower levels of serum IgG levels when compared with controls (Sobrian, *et al.* 1997).

9. **DNA integrity and genes**: noise can either stimulate chemical cascades that generate DNA damage or alter gene expression. With regards to chemical signaling, it has been shown that
noise exposure leads to an increase in the number of free radicals, which can cause carcinogenic mutations (Samson, *et al.* 2005), as well as an increase in ROS in the cochlea (Ohlemiller, *et al.* 1999), adrenal gland (Frenzilli, *et al.* 2004) and heart (Lenzi, *et al.* 2003). When disrupting gene expression, noise can reduce the generation of NMDA receptors, which are highly related with synaptic plasticity and memory (Cui, *et al.* 2009), upregulating the expression of CRH and its receptors, related to stress regulation (Du, *et al.* 2010), and increasing the expression of benzodiazepine receptors, which regulate inhibitory responses in the central nervous system (Lai & Carino 1990).

Noise is not only a stimulus with negative physiological effects, but is also a widespread phenomenon that is closely related with human activities. These activities are prevalent in areas inhabited by a wide variety of fauna, therefore making noise an issue for conservation of free-ranging fauna.

1.3. **Anthropogenic noise: characteristics and impacts on individuals and populations**

The mixture of sounds associated with human activities (such as urbanization and resource exploitation) is described as urban or anthropogenic noise (Warren, *et al.* 2006). Some of the most important characteristics are the elevated volume, the abundance of flat surfaces that create sound refraction and the presence of altered frequencies (Warren, *et al.* 2006), as well as the fact that most anthropogenic noises have the strongest intensities at a low frequency range (below 2000 Hz) (Barber, *et al.* 2011, Roberts & Roberts 2009, Slabbekoorn & Peet 2003, Slabbekoorn & Ripmeester 2008). There are two particularly important sources of anthropogenic noise: transportation, which includes aircraft, trains, ships and vehicle; and industrial sources, such as military bases, logging, drilling platforms, and mining (Blickley & Patricelli 2010). Other categories are environmental (all the acoustic energy generated by human activities at a given time, mostly composed by noise of other categories), military (noise produced by noise sonar, explosions, aircrafts, etc.), recreation (generated by activities such as whale watching and air tour helicopters), and other (such as white noise) (Shannon *et al.* 2015).

Anthropogenic noise differs significantly from the acoustic stimuli that most species have evolved to experience (Slabbekoorn & Ripmeester 2008). Hence, animals have had little time to adapt, which implies major changes in short periods of time.

Unsurprisingly, anthropogenic noise affects wild animals’ physiology in the same way as general noise exposure: it generates annoyance, chronic stress and hearing loss (Bowles 1995), as well as
adversely affecting reproductive physiology, energetic consumption (Andersen, et al. 1990, Delaney, et al. 1999, Edge & Marcum 1985) and heart rate (Weisenberger, et al. 1996). In addition, it generates effects on individuals and populations by altering communication (Blickley & Patricelli 2010). When it comes to individuals, the most immediate effect that anthropogenic noise can generate is changes in the hearing threshold, that can be either temporary (TTS) or permanent (PTS) depending on the amount of hearing damage (Blickley & Patricelli 2010, Clark 1991, Kight & Swaddle 2011, Wright, et al. 2007).

Anthropogenic noise can also alter the perception of acoustic signals due to increased background noise, thus reducing the efficacy of acoustic communication on birds, amphibians, marine mammals and other highly vocal species, an effect known as masking (Barber, et al. 2011, Barber, et al. 2010, Blickley & Patricelli 2010, Slabbekoorn & Ripmeester 2008, Warren, et al. 2006). To avoid masking, animals have developed certain strategies. When the sound is predictable on a spatial-temporal dimension, individuals can either migrate to quieter areas, as has been seen with marine mammals, or change the time schedule in which animals chose to communicate, therefor avoiding the noisiest times of the day (Laiolo 2010, Warren, et al. 2006, Wright, et al. 2007). However, much anthropogenic noise occurs as unpredictable events. In these situations, changes to amplitude and frequency in animal calls occur in order to improve communication.

The increase in call amplitude in acoustic signaling in the presence of background noise is known as the ‘Lombard effect’, which consists mainly of an increase in the energy or volume induced (Lombard 1911). This adaptation has been demonstrated in many birds (Cynx, et al. 1998, Manabe, et al. 1998, Pytte, et al. 2003), as well as in marine mammals such as the beluga whale (Delphinapterus leucas) (Scheifele, et al. 2005). Changing the volume of calls has adaptive consequences such as the bestowed energy cost and the fact that some individuals may be unable physically to increase the energy provided to their acoustic signals due to their age, injury or because their anatomy doesn’t allow them to (Warren, et al. 2006, Wright, et al. 2007). In addition, since most anthropogenic noises are composed of low frequency sounds, shifts in the frequency spectrum of calls are a strategy adopted by many animals. For instance, Slabbekeoorn and Peet (2003) found that great tits (Parus major) produce calls of higher frequency when compared with members of the same species living in non-urban areas. However, this tactic may bestow problems, since the acoustic signals may be altered over long distances, defaulting the recognition of predators and conspecifics (Wright, et al. 2007).
The effects of anthropogenic noise at a population level are a direct reflection of the cumulative effects at an individual level. A primary consequence is the change in animal distributions, which will be determined in part by the tolerance to noise of each species, leading to the abandonment of suitable and resourceful areas by some animal groups (Francis, et al. 2009). These changes in species diversity have long-term consequences, since processes such as speciation due to the inability to recognize conspecifics calls could be initiated in extremely short periods of time, generating, for instance, the diminution of the genetic pool and the increased risk of inheritable diseases (Slabbekoorn & Peet 2003). Also, the masking of individual calls leads to low reproductive success, as has been observed, for instance, in the European tree frog (*Hyla arborea*), with males being unable to adjust their calls in traffic noise, thus making them more likely to fail while finding females (Lengagne 2008). Since noise exposure is able to affect individuals’ physiology and communication patterns and in consequence populations, anthropogenic noise exposure turns as well into an animal welfare issue, since individual well-being is able to determine population dynamics (Paquet & Darimont 2010).

When studying anthropogenic noise, special emphasis has been given to transportation noise, since this kind of acoustic input is generally common and widespread (Shannon et al. 2015); however, industrial noise, even when more localized, is related to acoustic inputs of great energies (Blickley & Patricelli 2010). From the industrial noise sources, some industries have been greatly overlooked. Such is the case with mining, which is of great relevance in places where this activity is growing at a fast pace.

1.4. Mining noise as a source of stress for animals

The mining industry in Australia has experienced major growth since the year 2000 (Connolly & Orsmond 2011). Since the country has sufficient reserves of coal, iron, bauxite, copper and gold to sustain production levels for decades (Geoscience Australia), mining is considered an important and fast growing industry. Mineral exploitation and related industries (such as rock crushing and gas and oil exploration) have been proven to be a major risk for wildlife, since they can change the distribution and use of space by birds (Read 2000, Saha & Padhy 2011), elks (Kuck, et al. 1985), elephants (Rabanal, et al. 2010) and potentially bats (Armstrong 2010). The specific role that the noise produced by the mining industry plays on these phenomena is unknown. Within mining, open-cast systems are one of the most important means of mineral extraction (Britt, et al. 2014) and it also has been recognized as a major source of noise pollution (Giardino & Marraccini 1981, Haigh 1993, Tripathy 1999).
Mining noise, as with other anthropogenic acoustic disturbances, has dominant and energetic frequencies below 2 kHz; of the most important pieces of machinery used in open-cast mineral exploitation, the dumper truck has the lowest frequency (0.25 – 0.5 kHz), whereas the cooling fan from bulldozers produces the highest (0.3 - 3.5 kHz) (Vardhan, et al. 2005, Vardhan, et al. 2004). However, some machines such as diamond cutters can generate dominant frequencies between 2 and 4 kHz (Pal, et al. 2006), with the overall the dominant frequency range being from 316 Hz to 8 kHz (Peng, et al. 2010). Thus, even when mining noise is basically low-pitched, it also contains elements that go above the expected anthropogenic noise spectrum. This is also anticipated due the fact that mining is an industry that is related to other human activities such as transportation, blasting, aircraft noise and logging, which could potentially generate greater noise complexity and annoyance.

In terms of amplitude, mining noise in the work place can reach 90 to 110 dB (A) (Ahmad, et al. 2014, Mohapatra & Goswami 2012, Peng, et al. 2010). In commercial and residential areas located close to mining sites, noise amplitudes have been reported to reach 89 and 67 dB (A), respectively (Mohapatra & Goswami 2012). These levels are similar to measurements done close to a rock crushing facility, which is operationally similar to open-cast mining, with measurements of 86 ± 0.42 dB (A) in a forest between 0 and 500 meters from the noise source and lower values (64.4 ± 0.25 dB (A)) at 500 – 1,000 meters (Saha & Padhy 2011).

Even when mining is a source of acoustic disturbance that could potentially affect sensitive animals related to areas where this activity takes place, there are no specific measures that mining companies are required to comply with to prevent annoyance and possible disturbance.

1.5. Noise exposure regulations, mining and wildlife: a short case study in South East Queensland

Mining noise is an environmental concern in Queensland and legislation has been formulated to address it. The Environmental Protection Act (EPA) (Queensland Government 1994) safeguards values related with public amenity in relation to noise. This legislation is also supported by the Environmental Protection (Noise) Policy (EPNP) (Queensland Government 2008), which states the following values for acoustic environment:

i. The qualities of the acoustic environment that are meant to protect the health and biodiversity of ecosystems
ii. The protection of human health and wellbeing by ensuring a suitable acoustic environment

iii. The protection of the qualities of the acoustic environment that are related with the amenity of the community

In order to comply with these values, mining projects are required to perform noise assessments and predictions. To exemplify these assessments is possible to study technical reports regarding proposed mining operations such as the South Galilee Coal Project (SGCP), which has been suggested for establishment in South East Queensland (SGCP 2012). The acoustic quality objectives that this kind of mining operations should achieve are in agreement with both the EPA and the EPNP (Table 1).

<table>
<thead>
<tr>
<th>Location</th>
<th>Time of Day</th>
<th>Acoustic Quality Objectives (Measured at the receptors) dB(A)</th>
<th>Environmental Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$L_{A_{eq}, adj. 1\ hr}$</td>
<td>$L_{A10, adj. 1\ hr}$</td>
</tr>
<tr>
<td>Dwelling outdoors</td>
<td>Daytime &amp; evening</td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td>Dwelling indoors</td>
<td>Daytime &amp; evening</td>
<td>35</td>
<td>40</td>
</tr>
<tr>
<td>Dwelling indoors</td>
<td>Night-time</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Protected area, or an area identified under a conservation plan under the Nature Conservation Act 1992 as a critical habitat or an area of major interest</td>
<td>Anytime</td>
<td>The level of noise that preserves the amenity of the existing area or place</td>
<td>Health and biodiversity of ecosystems</td>
</tr>
<tr>
<td>Marine park under the Marine Parks Act 2004</td>
<td>Anytime</td>
<td>The level of noise that preserves the amenity of the existing area or place</td>
<td>Health and biodiversity of ecosystems</td>
</tr>
</tbody>
</table>

Table 1. Specific acoustic quality objectives during day and night for human populations and for areas related with wildlife (SGCP, 2012). $L_{A_{eq, adj}}$, 1 hr= the equivalent steady state noise level in 1 hour that would contain the same acoustic energy as the time varying noise level during the same period; $L_{A10, adj, 1\ hr}$= The noise level in dB(A) which is exceeded for 10% of 1 hour. During this hour, the noise level is below the $L_{A10}$ for 90% of the time; $L_{A01, adj, 1\ hr}$= the average maximum noise level. The maximum noise level over an hour is the maximum level, measured on fast response (0.125 seconds of averaging time in the sound pressure meter device).

It is important to notice that, while specific dB(A) measurements are acknowledged for human health and wellbeing in the EPP, there are no specific targets to achieve in order to preserve the health of biodiversity and ecosystems, which presents serious problems if sensitive wildlife
surround the area. In this regards, the SGCP states in its technical report that their two main considerations are:

1. Preserving acoustic quality objectives that are conductive of human health and wellbeing, ensuring a suitable acoustic environment to perform human main activities and preserving the qualities of the acoustic environment that are related with the amenity of the community.
2. Controlling background creep which is defined as the gradual cumulative increase in minimum noise levels generated by continuously operating noise sources.

Whereas human health is one of the main concerns, the health and biodiversity of ecosystems is not considered a main goal. In evaluations performed by this project on the nearest populated area, the township of Alpha, which is situated at 7 km from the Mining Lease Application, (i.e., the proposed area for the mining facilities), 14 km from the surface works and 8 km from the railway corridor associated with the mine, the noise experienced by humans is predicted to be below the acoustic quality objectives required by law. This statement is based primarily on computational modeling. While the expected noise levels for nearby humans seem to fall within the health and welfare standards when calculated with this process, these speculations don’t take into account modeling for other species, which would require considerations for different hearing ranges and habitat use.

In relation to this, in a study evaluating bird populations at 0, 500 and 1000 m from rock crushing facility, the noise experienced by the animals was reported to be as high as 81.9 dB(A) in the area adjacent to the cluster of rock crushing machinery (higher than the values established in Table 1). Likewise, noise was measured as 64.4 dB(A) and 63.7 dB(A) at 500 and 1000m from the rock crushing area, respectively. When compared with a similar non-polluted forest, bird diversity was significantly reduced due noise and other pollutants (Saha & Padhy 2011). Although some studies have acknowledged that some animals prefer to live in noisy areas to avoid predators, the physiological and welfare tradeoffs associated with this phenomenon have not been studied (Francis, et al. 2009).

1.6 Animal models for the assessment of anthropogenic noise impact
While the impact of some anthropogenic noises on wildlife communication has been studied on animals with extensive vocal abilities, such as marine mammals, birds and amphibians (see, for example Blickley and Patricelli (2010), Brumm and Slabbekoorn (2005), Rabin, et al. (2003), Wright, et al. (2007)). In addition, little research has been conducted to assess anthropogenic noise
impacts on physiology and individual behaviour of wildlife, due the difficulties of assessing such specific traits on free-ranging populations. Research has also overlooked the effects of noise on those animals that are small, uncharismatic and not obviously vocal, reptiles or some small terrestrial mammals (Fisher 2011). In an analysis of 242 publications on the effects of anthropogenic noise on wildlife, it was observed that between 1990 and 2013 only 4% of publications address reptiles and amphibians and 11% consider terrestrial mammals, compared to a 65% on birds and marine mammals (Shannon et al. 2015). These reptiles and small mammals such as rodents could also be affected, since noise exposure continues to be a stressor that produces physiological impacts regardless of its effects in communication.

In the case of reptiles, only a few studies have been completed, mostly related to transportation noise and addressing the possible effects in their hearing threshold. For instance, off-road motorcycles noise (115 dB (A) for 1 and 10 h) severely damaged the acoustic sensitivity of the desert iguana (Dipsosaurus dorsalis) by affecting the peripheral auditory system (Bondello 1977). Likewise, dune buggy sounds (95 dB(A), 500 sec), which contain sufficient energy below 3 kHz, induced hearing loss in the Mojave fringe-toed lizard (Uma Scoparia) as evidence by decreased responsiveness to sound (Brattstrom & Bondello 1983). In both of these studies, sounds of great amplitude values were used in relatively short periods of exposure. The effects of exposure to sounds of moderate intensity over long periods of time (such as those produced by mining facilities), is unknown. Similar noise exposure experiments have been done in the desert tortoise (Gopherus agassizii), where animals were exposed to simulated subsonic aircraft overflights (20 subsonic aircraft overflights, peak amplitude level of 126.1dB over 40 minutes). Animals froze more during initial exposure (30% of individuals) and also presented behaviours such as head retreat and head movements. Nevertheless, there were no meaningful changes on heart rate, which suggested no meaningful effects of short-term exposure, leaving the effects of chronic exposure to these variables unknown (Bowles, et al. 1999)

When it comes to small terrestrial mammals, some observations have been done regarding effects of interrupted vocalizations. For instance, the California ground squirrel (Spermophilus beecheyi), which uses alarm calls to communicate the presence of predators, increases levels of vigilance and caution (measured by proximity to barrows during alarm sounds playbacks) on animals dwelling close to wind turbines. This was interpreted as a greater perception of risk and possible hearing damage, which made them more visually active during distress calls when compared to animals living in unaffected sites (Rabin, et al. 2006). Likewise the greater mouse eared bat (Myotis myotis), which depends on sounds to find food, showed greater avoidance of forage during background
noise (traffic, vegetation and broadband noise) in which vegetation noise (noise recorded from moving reed vegetation) was more avoided due its similarity with prey acoustic cues (Schaub, et al. 2008). A similar phenomenon has been observed in koalas, which perform avoidance behaviours when exposed to a music festival (Phillips 2016) and prairie dogs which increased vigilance and decrease above ground activity and foraging when exposed to traffic noise (Shannon et al 2014). In the case of the desert kangaroo rat (Dipodomys deserti), when exposed to dune buggy sounds (95 dB (A)) animals experienced a temporary threshold shift and at least 3 weeks were required for their hearing sensitivity to recover. Because their hearing range lies in low frequencies, the effects of high amplitude and low frequency sounds, such as those from dune buggies, could be considered as an obstacle in their ability to avoid predators (Brattstrom & Bondello 1983). When it comes to physiological effects, Chesser, et al. (1975) evaluated differences in total body and adrenal glands weight on feral mice (Mus musculus) from populations near the end of a runway at Memphis International Airport (80 -120 dB SPL background noise level) and 2 km away on a rural field (80-85 dB SPL background noise level). Mice close to the airport runway had bigger adrenals due to stress than those from mice on the rural field. Also, when animals from the rural field were exposed to recorder jet aircraft noise, they also developed greater adrenals when compared to unexposed animals.

In addition to the lack of research on these animal groups and the difficulties in evaluating behaviour and physiological aspects in the field, assessing stress on sensitive and threatened wildlife populations is also an obstacle. For this reason, the use of common, widespread species while evaluating the impacts of man-made noises has been proposed. This would generate two important outcomes:1) animals of high abundance are usually the ones that sustain other populations in the food chain, making the evaluation of their welfare and survival greatly important; and 2) the experimental methodologies developed on abundant individuals may shape further methodologies to be used on threatened species that are also under acoustic pressure (Rabin, et al. 2003). Taking this approach into account, two good models of evaluation of acoustic pressures on mining sites are the feral mouse, (Mus musculus) and the Eastern blue tongued (EBT) lizard (Tiliqua scincoides).

When it comes to sound perception for these animal models for the evaluation of acoustic input in their welfare, the wild mouse has a hearing range between 2.3 kHz and 92 kHz, when measured at 60 dB SPL (Heffner & Masterton 1980, Heffner & Heffner 2007). These animals are known to be acoustically unresponsive to frequencies in between 1-2 kHz at amplitude of 70 to 80 dB SPL (Heffner & Masterton 1980). Thus, it is likely that sound below 2 kHz at amplitude below 80 dB
SPL won’t be perceived by mice as sound. However, mechanical vibrations that don’t represent an audible experience and are still present in the medium can be perceived through other means. For instance, frequencies below 2 kHz produce negative effects in mice as with some other animals, such as a reduction on spleen lymphocytes (Aguas, *et al.* 1999) or an early onset of autoimmune diseases (Aguas, *et al.* 1999).

For the EBT lizard, although its hearing range hasn’t been assessed yet, it is likely between 1 and 3 kHz as some other member of the order Lacertalia, (Saunders *et al.* 2000; Christensen-Dalsgaard 2005). Furthermore, lizards from the same genus such as the bob-tailed lizard (*Tiliqua rugosa*), have hearing ranges of 0.2 to 4.5 kHz, with peak sensitivity to 1.2 kHz at 10 dB Sound Pressure Level (SPL) (Köppl and Manley 1992), which implies a slightly wider range than other reptiles. In addition, lizards as other reptiles and insects can use unheard mechanical waves referred to as ‘airborne vibrations’ to communicate with conspecifics and search for prey (Drosopoulos & Claridge 2005, Wever 1978, Young 2003), therefore being able to communicate by unheard sound waves. Therefore, wild mice and EBT lizards are able to perceive mining noise sound waves. In addition to their hearing capabilities, these animal models have other characteristics that are advantageous.

The wild mouse is found in mining sites as an opportunistic species (Fox & Fox 2006, León, *et al.* 2007). There are also a great number of studies that have observed their behaviour (Denmark, *et al.* 2010, Dielenberg, *et al.* 2001, Grant & Mackintosh 1963, Lumley, *et al.* 2000, McAllister & Dixon 1989, Sluyter, *et al.* 1995, Van de Weerd, *et al.* 1997, Van de Weerd, *et al.* 1998, Van Oortmerssen 1971) and physiological changes related to noise exposure (reviewed by Kight and Swaddle (2011)). Also, techniques to evaluate stress hormones in a non-invasive manner have been validated for this species, such as the measurement of fecal corticosterone (Touma, *et al.* 2004), making the wild mouse a good model to develop laboratory methodologies to observe both behavior and physiology and relate such results with wildlife.

The advantages are similar for the EBT lizard. This reptile is an omnivorous skink that is widespread in Australia, is able to survive in varied habitats (Turner 2010, Wilson & Knowles 1988). Although the number of EBT lizards or other reptiles in the surroundings of open-cast mines in Australia has not been established, its presence in this area is probable due to its wide distribution across north- and south-Eastern Australia (Anon 2012) and their great capacity to adapt to environments with a strong anthropogenic influence, such as urban sites (Konig, *et al.* 1996, Turner 2010). EBT lizards also occupy areas where open-cast mining is expected to develop and in close
association with active mining operations, such as the Upper Hunter Valley in New South Wales, Australia (Cottle & Keys 2014, New South Wales Government 2005). In addition, this lizard is related to vulnerable species, or subspecies that are listed as vulnerable: the species *T. adelaidensis* is endangered, and the subspecies *Tiliqua rugosa konawi* is rare and likely to become extinct (Cogger 2014). Therefore, gathering information about the effects of mining noise on one member of the genus may assist with generating conservation strategies for others.

**Conclusion**

Although the knowledge related to the effects of anthropogenic noise exposure on wildlife is vast, there are specific areas that have not been properly explored; in specific, research on the effects of some industrial noise is lacking. In Australia and other countries where opencast mining is commonplace, mining noise is highly relevant as it has become an important soundscape characteristic in areas that are important for fauna conservation.

In addition, animals that rely on acoustic signals are clearly affected by anthropogenic noise as it interrupts and/or modifies acoustic signaling processes, disturbing their distribution and reproduction success. Nevertheless, the effects of noise on non-charismatic small animals that are not renowned for their vocal capacities have not been studied, even though noise exposure is well known in other species to generate critical changes in physiology and behaviour, which can ultimately decrease animal welfare and impact populations.

Therefore, this research addresses these particular topics through the controlled mining machinery noise exposure of two animal models, wild mice and EBT lizards, in order to evaluate the effects that such acoustic experience generates by the activation of the stress response.

**1.7 Objectives and hypothesis**

The objectives of this research are:

- Explore the effects of anthropogenic noise, with a special focus on mining machinery noise (coal truck, drill, bulldozer, shovel, dumper, rock crusher, dragline and blast) on mammals and reptiles, using the wild mouse (*Mus musculus*) and the Easter blue tongued lizard (*Tiliqua scincoides*) as animal models. With the creation of a novel laboratory methodology, to evaluate:
  - The effects of different, relevant amplitudes, (60-65 dB (A) and 70-75 dB (A))
  - The effects of different, relevant frequencies (> 2 kHz and ≤ 2 kHz) For wild mice, fecal corticosterone levels and tissue morphology of organs were evaluated, but this was not possible on the Eastern blue tongued lizards because of their protected status.
The hypotheses are:

- Mining noise is able to generate stress on animals in the same way that other anthropogenic noises have been proven to produce.
- Such chronic stress can increase stress-related behaviours and dysregulate corticosterone release by the HPA axis, as well as generate changes on organ morphology that can compromise the function of systems, such as the immune system.
- The negative effects produced by mining noise vary depending on the amplitude (the amount of energy contained in the sound wave) and the frequency (the perceived pitch) associated with the stimulus, as animals may perceive this variations in different ways due their hearing range.

1.8 References


Bondello MC 1977 *The Effects of High-Intensity Motorcycle Sounds on the Acoustical Sensitivity of the Desert Iguana, Diposaurus dorsalis*. Final report by the Department of Biological Sciences, California State University, to the Bureau of Land Management, on contract CA-060 CT7-2737.


Cone J, and Hayes S 1984 Environmental Problems/Behavioural Solutions. Cambridge University Press: California, USA.


Francis CD, Ortega CP, and Cruz A 2009 Noise Pollution Changes Avian Communities and Species Interactions. *Current Biology* 19: 1415-1419.


Geoscience Australia 2010 *Australia’s Identified Mineral Resources*. Geoscience Australia: Canberra, Australia.


McAllister KH, and Dixon AK 1989 Reappraisal of the mouse ethogram according to grant and mackintosh - social and aggressive-behavior. *Aggressive Behavior* 15: 86-86.

Mohapatra H and Goswami S 2012 Assessment and analysis of noise levels in and around Ib river coalfield, Orissa, India. *Journal of Environmental Biology* 33: 649-655


Ohlemiller KK, Wright JS, and Dugan LL 1999 Early elevation of cochlear reactive oxygen species following noise exposure. *Audiology and Neurotology* 4: 229-236.


Phillips S 2016 Aversive behaviour by koalas (Phascolarctos cinereus) during the course of a music festival in northern New South Wales, Australia. *Australian Mammalogy*

http://dx.doi.org/10.1071/AM15006


SGCP 2012 South Galilee Coal Project- Section 12: Noise and Vibration.


Slabbekoorn H, and Peet M 2003 Ecology: birds sing at a higher pitch in urban noise—great tits hit the high notes to ensure that their mating calls are heard above the city’s din. Nature 424: 267.


Tripathy DP 1999 Noise Pollution. APH Publishing: Delhi, India.


2. **CHAPTER 2: The effects of simulated transport on the behaviour of Eastern blue Tongued Lizards, *Tiliqua scincoides***

2.1. **Introductory statement**

Evaluating the behavioural effects of anthropogenic noise exposure on reptiles requires the development of novel methodologies, as this topic has not been fully explored in relation to noise exposure. In addition, it is necessary to understand the variables that would affect sound processing and broadcasting. Furthermore, since stress can be produced by other environmental factors apart from noise exposure, creating a methodology to evaluate different stimuli that may occur simultaneously with noise exposure is necessary to explore how other variables affect EBT lizards and how to effectively control them to isolate the effects of acoustic stimulus in particular. Likewise, the contrast of different stressors within the same methodological framework is an advantage, as it allows observation of a greater array of behavioural aspects that could be further used to develop a more complete ethogram of the behaviours exercised by the EBT lizard during stress, which is, as mentioned before, a contribution to the genus *Tiliqua* which contains many sensitive and threatened species.

Thus, to address these necessities, the first experiment of this research evaluated the effects of traffic noise, vibrations and temperature changes. These are variables reptiles face during road transport, which happens in pet trading, both illegal and legal. Evaluating these stressors allowed me not only to design a reliable methodology for the evaluation of the effects of mining noise on EBT lizards’ behaviour, but also to increase knowledge of an important topic that has not been researched enough and that contributes to the resolution of welfare issues that are closely related with noise stress.

2.2. **Abstract**

There is widespread transport of reptiles for the pet trade throughout the world and the ‘dead on arrival’ rates are high. The Eastern blue tongued (EBT) lizard (*Tiliqua scincoides*, Order: *Squamata*; suborder: *Lacertilia*) is particularly popular due to its unusual blue tongue. Noise, vibration and thermal discomfort are known contributors to transport stress. We analyzed the behaviour of EBT lizards (n=9) when exposed to four of these stimuli in a changeover design. Lizards were exposed to Heat (35°C), Cold (15°C), high or low frequency noise or a Control treatment with no stimulus in a test chamber for a 5 s. period. Heating blankets and ice packs were used to create the hot and cold temperature stimuli in the test chamber, and a speaker broadcast noise/vibration from a truck recording. The test chamber was connected to an escape chamber,
accessible after exposure to the stimulus, and a small hiding chamber opposite the test chamber. Lizard behaviour was monitored during stimulus exposure and then for a further 15 minutes, after which each lizard was removed. Lizards exposed to Cold spent less time in the test chamber and more time inactive in the escape chamber. They also spent longer walking towards the hiding chamber both away from the wall and by the wall and walking in the hiding chamber away from the stimulus. Heat and noise treatments had not significant effects. We conclude that cold temperatures are potentially noxious for lizards in a simulated transport environment as they reduce activity and increase escape attempts.

Keywords: animal welfare; Eastern blue tongue lizard; noise; transport; temperature; wildlife trade

2.3. Introduction

The trade in reptiles occurs worldwide. In 2009 it was estimated that between 5.8 and 9.8 million live reptiles were imported into the European Union (EU), comprising the majority of the trade worldwide. An estimated 99% of all live reptiles imported into the United Kingdom originated from outside the EU and most of this transport occurs on land (RSPCA 2010). During the importation process, animals may have to endure poor transport conditions, such as extreme temperatures, noise and vibration from vehicles, and lack of food, water and space. If such conditions are sustained for long periods of time, they can contribute to increased mortality of wildlife after transport, which could reach 100% in species that are especially sensitive to rapid environmental changes (EFSA 2004).

The Royal Society for the Prevention of Cruelty to Animals (RSPCA) in 1992 calculated a Dead on Arrival (DOA) rate of 2% of reptiles imported to the UK (or approximately 30000 individuals), with a further 2-3% mortality within 2 days of importation (or approximately 30-45000 individuals) (Smart & Bride 1993). In Germany an average DOA rate of 3.0% (range 0.1 - 6.4%) was estimated for reptiles imported in 1995/1996, but was as high as 84% in extreme cases (Altherr & Freyer 2001). Another study has confirmed that reptiles imported into Germany have an average transport mortality of 3.1%, and that this is the second highest of all the animal groups imported into that country (Schütz 2003). Of the reptiles, lizards (Order: Squamata; suborder: Lacertilia) had particularly high mortality rates (4.4%) when compared with snakes, turtles, tortoises and crocodiles. Within the lizards, the families scincidae, lacertidae, chamaeleonidae and agamidae all had average mortality rates above 5%. Within these values there was considerable variation, and some shipments had no mortality.
Overall, the mortality rate of all reptiles during transport has been estimated as three times higher than that of birds (Steinmetz et al. 1998). Even more, due the late onset of disease that ectothermic animals such as reptiles have, the mortality rate after arrival is even higher, with a 75% of reptiles surviving less than a year in UK homes (Toland et al, 2012) which can also be a by-product of conditions experienced prior arrival and during transport for both the legal and the illegal market.

Reptiles are highly represented in the illegal trade worldwide; it has been estimated from the bulletins issued by a wildlife monitoring network (TRAFFIC 2013) that from 1996 to 2006, 69% of the total illegal live trading reported on this network were reptiles, approximately 128,000 animals (Rosen & Smith 2010). Reptiles are traded for a number of purposes, in particular for culinary and medicinal uses in Asia and North America, as well as pets, whilst in Europe they are imported mostly for the pet industry (Warwick et al. 2005; Warwick 2006). In 1990, approximately 1 million reptiles were traded around the world, and in 2002 the global value of the live herpetofauna trade was estimated at approximately $ US 6 million (Tapley et al. 2011). Even more, specific species of interest such as the red-eared terrapin (Trachemys scripta elegans) were imported to the EU in numbers as large as 7 million before the European ban to foreign species was imposed in 1997 (Warwick 1986, Warwick et al. 1990). Even though the wildlife trade is growing, the existing evidence suggests that this industry has not yet established a reliable set of animal welfare standards for transported animals (Auliya 2003; Arena et al. 2012).

In order to control the import and export of endangered wildlife, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) came into force in 1975, protecting approximately 5000 species. CITES is an agreement between governments in which selected species of flora and fauna are subjected to special trading controls to assist in their survival. The species controlled by this convention are divided into three groups, depending on the degree of protection they need: the first deals with species threatened with extinction that should only be traded under exceptional circumstances; the second includes species not necessarily threatened with extinction, but in which trade must be controlled in order to avoid overexploitation; and the third contains species protected in at least one country which need the assistance of other parties to control trade (CITES 2013). CITES (2013) has provisions for the welfare of individual living specimens, mostly related to the treatment of animals during shipment. These provisions are included and referred to throughout the text, in particular regarding the application of welfare standards, such as space and food allowance and absence of injuries.
There are several concerns regarding the actual application of such welfare standards. One of the most important is that the guidelines related to welfare are not perceived as mandatory by all countries, for several reasons. Firstly, there are no welfare criteria for transport that could provide grounds to verify and/or evaluate if traders comply with regulations and ultimately apply sanctions when necessary, nor are there any reliable statistical data to aid the assessment of the success or failure of the existing welfare provisions, because, even when mandatory, most of the countries under CITES legislation do not record events of mistreatment, injury and mortality. In addition, there are no specific regulations to require the appointment of appropriate technical staff to apply such guidelines. All of these result in a poor enforcement of welfare during transport (Bowman 1998; Maldonado et al. 2009; Nijman et al. 2012).

Apart from the practical ineffectiveness of welfare guidelines included in CITES legislation, of the approximately 7700 species of reptiles recorded in the wild, only 8% (616 species) are protected by CITES, because only this percentage is regarded as endangered or overexploited (RSPCA 2010). Thus, regardless of the issues related to compliance and effectiveness of the CITES welfare standards, regulations during transport apply to only a small number of reptiles.

For non-CITES species, the situation is especially critical, because it is highly probable that they experience low welfare standards during transport as they are not required to follow CITES guidelines, even taking into account concerns about their application. In this case, animals are subjected only to the regulations applicable in the trading countries, which may not include animal welfare. Some jurisdictions apply legislation of their own to regulate wildlife trading in addition to CITES guidelines, which may benefit non-CITES species. For example, the ‘EU Wildlife Trade Regulations’ (EU, 2013) include some reptile species that are excluded from the CITES annexes. However, it has been recognized that there are national differences in the application and enforcement of these guidelines, as well as in the sanctions applied when transgressed and the quality and amount of statistical information available regarding seizures and confiscations, which could help to detect illegal trading (Auliya 2003). This situation is the probable cause of the disparity observed in the mortality rates for CITES regulated and non-regulated reptiles in EU (1.97 and 3.85%, respectively, Schütz 2003).

Trade in non-CITES species is most prevalent within the EU, especially those species with physical features that are attractive to pet owners and have a restricted distribution in the wild, i.e. species inhabiting limited ecological niches with specific characteristics required for the life cycle of the species. Around 600 non-CITES reptile species have been observed in the EU pet trade (twice the
number of CITES-listed species recorded) (Auliya 2003). Reptiles that are traded usually have to travel by vehicle, with or without air transfers, at some point. Land transport is particularly important to the temporary wildlife trading markets, in which retailers move from town to town to sell animals. When this transportation occurs illegally, traded reptiles are commonly smuggled in between floors of lorries, in the interior or side covering of caravans, behind car seats and doors and inside hidden compartments in luggage, which promotes conditions that are considered to be unacceptable. These conditions are also sustained at the final stages of transport, when both legal and illegal species are taken to the wholesalers’ holding sites, often being poorly handled (Holden 1998; Watson 1998; Arena et al. 2012). Air transport may include reptiles sent by mail or smuggled inside clothes or luggage. Regardless of the legality or type of transport, this situation involves many potential stimuli that could have independent or synergistic influences on reptile mortality, including noise, the associated vibrations and changes in the thermal environment.

Of the reptiles, lizards have the best hearing sensitivity, which ranges from 1-3 kHz (Saunders et al. 2000; Christensen-Dalsgaard 2005). Noise generated by vehicles is typically below 2 kHz (Slabbekoorn & Peet 2003; Slabbekoorn & Ripmeester 2008), and is therefore audible to lizards. Many physiological disorders have been linked with anthropogenic noise in humans, rodents, birds, amphibians and fish (Kight & Swaddle 2011) and the associated mechanical (airborne) vibrations generated by low frequencies (Alves-Pereira & Castelo Branco 2007).

Vibration itself is a potential stressor, even though airborne vibrations are used by reptiles for communication (Wever 1978; Young 2003). Transport vibrations can induce secondary vibrations in animals or the substrates on which they lie (Bowles 1995). In poultry and pigs, vibration during transport has been shown to adversely affect physiological traits such as heart rate and glucocorticoid levels (Scott 1994; Warriss et al. 1997; Perremans et al. 1998). In reptiles, it has been acknowledged that the turtle Pelodiscus sinensis can be affected by vibrations in its tank, causing elevated cortisol and renal abnormalities. This exemplifies the importance that vibratory stimuli, which could occur during transport, potentially have for stress levels of reptiles (Hur & Lee 2010).

The other important source of stress during transport is exposure to abnormal temperatures (i.e., temperatures outside their thermal comfort zone), and being ectothermic, reptiles are particularly susceptible (Barten 2005). An environment with a range of temperature variations in different spatial regions, or thermal gradient, is preferable, providing the lizard with flexible and healthy temperature control (Lillywhite & Gatten 1995). During transport this is hard to achieve and even
small temperature changes can be fatal (Altherr & Freyer 2001; Arena et al. 2012). Extreme low temperatures can produce mechanical damage to cells by freezing, a process in which water crystals are formed within the cell, which can destroy cytoplasmic structures and cell metabolism. Freezing also restricts changes in extracellular osmotic concentrations due to water solidification outside the cell membrane, which in turn promotes dehydration, reduced fluid circulation and delivery of oxygen and nutrients (Zung et al. 2001). These processes can cause necrosis, as well as adversely affecting cardiac activity due to changes in osmotic balance (Li et al. 1992; Tumur et al. 2005).

Extreme high temperatures cause panting, loss of coordination and righting ability and muscle spasm, as well as cessation of breathing and heart function (Heatwole & Taylor 1987). Behaviour is also impacted by temperature; for example, lizard sprint speed is reduced at both low and high temperatures, a characteristic which is important for both escaping from predators and catching prey (Huey & Kingsolver 1993). Basking or hiding under rocks or logs is one of the most common means of regulating body temperature at low (McFarland 1999) and high (Lissone 1999) temperatures in the wild, but is unlikely to be possible during transport.

The Eastern blue tongued (EBT) lizard, *Tiliqua scincoides*, is one of the Australian non-CITES species that is most commonly traded in the pet market, often illegally (Beltz 1996; Auliya 2003). It is no longer listed under EU Wildlife Trade Regulations (EU, 2013) and none of the Australian members of the genus *Tiliqua* are listed by CITES (CITES, 2013), even though, of the six species of blue tongued lizards, two are listed as vulnerable, one as endangered, and the subspecies *Tiliqua rugosa konawi* is rare and likely to become extinct (Wilson & Knowles, 1998).

The EBT lizard is an omnivorous skink that is widespread in Australia, valued in part for its brightly coloured blue tongue. It is able to survive in a variety of habitats, including urban areas, but is mostly characterised as diurnal and terrestrial, spending much of its time sheltering beneath low vegetation, hollow logs and abandoned barrows (Wilson & Knowles 1988). The lizard’s colour, size and flexible diet explain its popularity as a pet. Knowledge is scant about the major welfare issues facing the species during transport.

This study aimed to assess the effects of typical stimuli involved in transportation - noise, sound-induced vibrations and hot and cold temperatures - on the behaviour and welfare of the EBT lizard during a modified open field test. We hypothesised that these transport stimuli can be negative experiences for the lizards that will initiate the stress response and generate behavioural reactions related to aversion and avoidance.
2.4. Materials and methods

Procedures were approved by The University of Queensland’s Animal Ethics Committee (UQAEC Approval Number SAFS/322/11) and by the Queensland Parks and Wildlife Service (Scientific Purpose Permit WISP05075208).

Animals

Nine EBT lizards held in captivity at the Native Wildlife Teaching and Research Facility of the University of Queensland were utilised for the study. All were siblings sourced from a local breeder (Pet City, Brisbane, Australia).

They were permanently housed in individual enclosures, consisting of a plastic frame lined with plastic mesh walls (six of 60 cm long, 39 cm wide and 40 cm high and three of 95 cm long, 52 cm wide and 53 cm high). Each enclosure had two layers of paper as substrate that was replaced when soiled, and was furnished with bricks or irregularly-shaped rocks to facilitate ecdysis, a hollow wooden log for shelter, and a glass water dish. Cages were cleaned weekly using water and detergent (Avicare concentrate, Vetafarm, NSW, Australia).

Diet

The animals were fed twice weekly, as is normal for these reptiles in our facility. On each occasion each lizard was provided with 7 g of fruits and vegetables (grapes, honeydew, watermelon, banana, corn and broccoli), and one live giant mealworm (*Zophobas morio*) or a steamed chicken egg for protein. The giant mealworms were given directly to the lizards; fruits, vegetables and eggs were chopped into pieces and sprinkled with a vitamin, mineral and amino acid supplement (Repti-vite, Aristopet, Australia).

Test enclosure design, habituation and training

We designed a test of evasiveness to each stimulus, which assumed that the lizards would move away from aversive stimuli to seek a hiding place, and that the further or faster they moved, the greater the noxiousness of the stimulus. Thus, a three chamber system was developed. This system was a modified version of an open-field test, where animals had an open area to perform activities after exposure to different stimuli, but with the option of a hiding position. Animals were placed at the beginning of the test in a test chamber that was 20 cm long x 10 cm wide, designed to accommodate a single lizard from head to tail comfortably, while preventing excessive movement or visual stimulation. This test chamber was connected to a larger rectangular space, an escape
chamber (80 cm long x 40 cm wide), which provided an open field area for behavioural responses after exposure to the stimuli. Opposite the test chamber and connected to the escape chamber, a hiding chamber was positioned (20 cm long x 10 cm wide), at the farthest distance from the stimuli that the lizards could reach during the tests. This last chamber had the same measurements as the test chamber in order to provide the same adequate space for the animals to hide, assuming that they would prefer a space where they could fit their whole bodies while exercising minimal movement, imitating the main characteristics of the logs that they use for hiding in the wild or that are provided in their cages. All walls surrounding the enclosure were of plywood and had a height of 20 cm (Figure 1). The overall measurements of the chamber system were set in order to provide an appropriate behavioural setting, but also to be easily manipulated by the researchers and on a scale that allowed the best amount of detail possible in the experimental recordings. The testing apparatus was inside a room at ambient temperature, recorded daily (mean temperature 27.7°C ± 0.05). Noise and vibration were controlled during testing by placing the apparatus on a table in an isolated room away from any vehicle traffic. To apply the stimuli, a channel surrounded the test chamber, allowing the placement of ice packs (Cold Ice INC, Okland, CA, USA) and heating blankets (Gw17 Pet Electric Heating Blanket, Zhejiang, China) to decrease and increase temperature, respectively. Vehicle noise was broadcast from speakers placed underneath and in contact with the test chamber, thereby providing both sound and vibrations (Figure 1).

Lizards were first habituated to the chamber system, and their behaviour recorded throughout the process in order to develop an ethogram for the study. Each lizard was initially placed individually in the test chamber facing the hiding chamber for 15 minutes on five occasions over five days. After this initial habituation, a lure was introduced, in order to train them to traverse the escape chamber to take a food reward which was placed at the entrance to the hiding chamber. Latency to take the food reward was recorded in four repetitions on four separate days, with a maximum time available of 15 min. The reward was garden snails (Helix aspersa) for the first three tests and fruits (mangos and strawberries) for the final test.

**Generation of stimuli**

Aversion to four different stimuli was tested during 15 min periods: Cold (CL), Heat (H), High Frequency Noise (HF) and Low Frequency Noise (LF), with a Control (C) treatment for comparison. Taking into account the recommended temperature zone for EBT lizards (20/25 to 30/35°C) (Turner & Valentic 2001), treatment CL was set at 15°C and treatment H at 35°C, allowing the lizards to experience temperature change from the ambient temperature to which they were accustomed without severely threatening their welfare. Temperature changes inside the test
chamber were created with eight icepacks (treatment CL) or two heating blankets (treatment H), placed inside the channel for 10 to 20 minutes until the required test temperature was reached.

A wooden lid was placed on top of the test chamber to prevent heat/cold loss while the temperature conditions were generated in the test chamber. A thermometer was used to measure changes in temperature inside the test chamber. When the desired temperature was achieved, the wooden lid was removed and the animal placed inside. Immediately after the animal was positioned inside the test chamber, the wooden lid was replaced with a transparent Perspex lid that covered the entire chamber system allowing behaviour recording and preventing further significant changes of temperature (only variations of up to 1°C from the test temperatures were tolerated during the test). Temperatures inside the experimental room and the escape chamber were measured and were similar throughout the experiment (mean 27.7 °C ± 0.05). Background noise levels were verified to be below 50 ± 0.1 dB (A) using a Digital Sound Level Meter (model Q1362, Dick Smith Electronics). To broadcast noise and generate vibrations, two speakers (Multimedia Computer Speakers ACS5, Altec Lansing, California, US) were used. One was placed beneath the test chamber, and the second facing the chamber, thus enabling the transmission of both physical and airborne vibrations to the lizards. A volume of 90dB (A) was selected, in accordance with interior noise amplitude in the cabin of a simple transport vehicle (Soltani & Demneh 2011). This volume is similar to that of a food blender or a garbage disposal unit (an electric shredder of food waste to enable it to enter waste pipes) measured at 1 m (Hendrick, 1998). Sound levels were monitored using the same meter as that used to test background levels. The stimulus was recorded from inside the cabin of a moving truck and was acquired from the internet (source: http://www.wavecn.com/content.php?id=46). The recording was divided into two sets of frequencies (Low Frequency Noise (LF) ≤ 500 and High Frequency Noise (HF) > 500 Hz) using a sound editing software program (Audacity; http://audacity.sourceforge.net). It was anticipated that, because the hearing range of lizards is 1 to 3 kHz (Saunders et al., 2000; Christensen-Dalsgaard, 2005), LFN would not be experienced as sound but as airborne and substrate vibrations. The sound measured as maximum volume in each chamber decreased in a gradient from the test chamber (90dB [A]) to the escape chamber (83.3 dB [A]) to the hiding chamber (82.2 dB [A]).

The vibrations produced by treatments HF and LF were measured using an accelerometer (PCB Piezotronics, Accelerometer Model Number: 51017, New York, USA) connected to a sensor that enabled vertical acceleration patterns to be transformed into voltage (LabView, National Instruments, Austin, Texas). The sensor was coupled to a signal conductor via a USB port (National Instruments NI9233 Compact Daq Signal conductor, Serial Number: 13764CD) that translated
changes in voltage into vibrational patterns measured in m/s$^2$. HFN had no important peaks of vibration induced on the substrate. However, LFN had a major peak at 400 Hz, other medium intensity peaks in between 325-25 and 400 Hz, and lesser peaks at 100, 185 and 215 Hz. The vibration measured decreased in a gradient from the test chamber to the hiding chamber.

**Experimental procedure**

All lizards were exposed to the stimuli over 4 consecutive weeks using a cross over design, where the experimental units were the intersection of individual lizards (rows) on specific days (columns), (Table 1). Each week represented the combination of two 5x5 Latin squares with one row removed, as only 9 subjects were tested. Each animal received the five treatments only once a week (Monday to Friday). The distribution of treatments was randomized within and between weeks.

Lizards were placed individually in the test chamber, facing the escape chamber with the door closed. For a preliminary period of five seconds the lizard was held in the test chamber, so that it experienced the stimuli without any opportunity to escape. Then the door was opened and behaviour recording commenced, whilst the lizards responded to the continued stimulus. The tests were conducted between 1400 and 1600 hours daily.

**Behaviour recording and analysis**

Lizard behaviour was recorded by a camera (model K-32HCF, Kobi CCD, Ashmore, Australia) suspended 100 cm above the translucent roof of the chamber system and connected to a video recorder (Model Lite 900, LG, Yeouido, South Korea). Researchers remained in the same room as the lizards during the experiment and observed their reactions on a monitor connected to the camera; there was no visual contact with the lizards during the test. During replay the frequency and duration of behaviours were recorded for each chamber during the habituation and reward training phases of the project. Data was coded with the aid of the behaviour analysis software Cowlog (Hänninen & Pastell 2009). A more detailed ethogram was defined for the stimulus response tests, which focused on the type of movement and where such movements were performed, as well as behaviours observed in other lizard behaviour studies (Greenberg 1977; Torr & Shine 1994; Langkilde et al. 2003). The behaviours recorded were climbing, hesitating (walking only one or two steps, then stopping), tongue flicking (protruding tongue and then returning it to the mouth), head up and down (either to the left, to the right or straight ahead), scanning (moving the head from side to side while stationary or walking), walking from the test chamber to the escape chamber or to the far end of the test chamber, walking from the escape chamber to the test chamber or to the hiding chamber, walking from the hiding chamber to the escape chamber or to the end of the hiding
chamber, walking in the escape chamber against the walls towards the test chamber or the hiding chamber and inactivity in either the test, escape or hiding chamber. These behaviours have been related to negative responsiveness in reptiles towards other kinds of stimuli and were selected to evaluate aversion and general stress on this study.

**Statistical Analysis**

A General Linear Model was constructed which included the factors lizard, treatment and day. Residuals were tested for normal distribution as above, and if not normally distributed (P < 0.05) data was transformed using square root or logarithm as required. Four specific contrasts were tested in the model: Control (C) vs. all stimuli, Cold (CL) vs. all stimuli, temperature (CL and H) vs. noise (LF and HF), CL vs. H and LF vs. HF. When transformed data did not produce normally distributed residuals, the Kruskal-Wallis test for non-parametric data was performed. For behaviours of low frequency and duration, data was transformed to binomial values and tested with Binary Logistic Regression, comparing the number of lizards that did show this behaviour with those that did not between treatments. Results were considered significant at P ≤ 0.05. All calculations were performed with the program Minitab Statistical Software, version 16.

### 2.5. Results

Lizards spent more time inactive in the test chamber when exposed to heat or noise variations compared with when they were exposed to cold (P < 0.007), (Table 2). Lizards that were exposed to temperature changes spent more time inactive in the escape chamber, compared with when they were exposed to noise (P = 0.01). This difference was attributed to the lizards remaining inactive for a longer period of time when exposed to cold (148 s, P = 0.006). Also, lizards exposed to cold spent more time walking towards the hiding chamber away from the wall than those exposed to heat (P = 0.05).

Exposure to temperature changes caused the lizards to walk more proximal to the wall towards the hiding chamber compared with when they were exposed to low and high frequency noise (P = 0.02). Lizards that had been exposed to cold tended to spend more time walking in the hiding chamber towards the end furthest from the stimulus than those exposed to heat (P = 0.06). When the Cold treatment responses were compared with other stimuli, the lizards in the Cold treatment spent less time inactive in the test chamber (P< 0.001) and more time inactive in the escape and hiding chambers (P = 0.03). They also spent more time walking from the escape chamber to the hiding chamber both close by and away from the wall (P= 0.04 and P= 0.02, respectively) and walking in the hiding chamber towards the end furthest from the stimulus (P= 0.04).
When LF was compared with HF there were no significant differences in any of the behaviours analyzed. Also, there were no treatment effects in any of the chambers on the time that lizards spent with their head up or down, analyzed with the Kruskal-Wallis test (P= 0.45 and P= 0.40, respectively), or other behaviours analyzed as binary variables. The means across all treatments (±SEM) of these behaviours were: scanning 24 ± 1.3 s; climbing 14 ± 1.4 s; hesitating 2 ± 0.1 s; walking from escape chamber to test chamber 8 ± 0.5 s; walking from hiding chamber to escape chamber 5 ± 0.2 s; walking in test chamber to the far end 4 ±0.2 s; walking from the hiding chamber to the escape chamber 5 ± 0.2 s; walking by the wall from the escape chamber to the test chamber 2 ± 0.3 s and walking from the test chamber to the escape chamber 3 ± 0.2 s.

2.6. Discussion
This experiment showed the effects of transport stressors on lizards’ behaviour. Although mostly hypoactive, aversion to cold was evident as animals moved away from the stimulus in different ways. Although lizards appeared mostly unreactive to changes in noise and vibration, it is necessary to conduct further experiments where these variables are further refined.

Inactivity during experimental experience
This study aimed to assess the effects of several stimuli experienced during transport on the behaviour and welfare of EBT lizards. EBT Lizards were generally inactive during the trials, which may have been due to prior experiences, especially the long-term captivity that may have decreased their reactions to stimuli. Hypoactivity is a characteristic of many species of reptiles, including the EBT lizard (Christian et al. 2003), which makes avoidance behaviour difficult to assess (Warwick 1990). Nevertheless, it should still be possible to detect differences in activity between environments that do not allow for the normal locomotion requirements of the species (Warwick 1990). In a previous experiment, EBT lizards spent more time walking in large (140 x 140 cm) than small (70 x 70 cm) cages (Phillips et al. 2011). In our experiment, the chambers were designed to be smaller than the large cage of the aforementioned study to provide an opportunity for the lizards to reach the hiding chamber within the 15 minute time period of each test. Hypoactivity induced by small cage size is only likely to develop over time, as the lizards habituate to the environment.

Another variable that affects mobility in reptiles is the Standard Metabolic Rate (SMR; the energy expended by a resting, fasting, and non-stressed animal). EBT lizards have one of the lowest SMR of any squamate, similar to the related species *Tiliqua (Trachydosaurus) rugosus*, which is believed to be slow moving due the high cost of locomotion and its body shape (Andrews & Pough 1985;
John-Alder et al. (1986). Christian et al. (2003) calculated the seasonal patterns of energy expenditure of EBT lizards during dry and wet seasons. Measures of activity cost parameters, such as the total field metabolism allocated for activity, the average intensity of activity and the sustainable metabolic scope were estimated for free ranging specimens, as well as the SMR for animals in laboratory conditions. All values were high, suggesting that a large proportion of the energy budget is spent on digestion, much more than on locomotion. The lizards used in this study were well fed and it is possible that this resulted in a high energy demand for digestion, which inhibited locomotion.

**Effects of temperature stimuli**

When lizards experienced temperature-related treatments, they spent more time inactive inside the escape chamber when compared with noise treatments. Also, they walked more towards the hiding chamber remaining close to the wall, which is an avoidance or escape behaviour in other species such as the domestic dog (Canis lupus familiaris) (Hydbring-Sandberg, von Walter et al. 2004). When CL and H were compared, lizards were more inactive in the escape chamber when CL was applied. Also, when cold temperatures were contrasted with all other stimuli, lizards spent less time in the test chamber and more time walking away from the cold stimulus, but also more time inactive in both the escape and test chambers. These results are consistent with our knowledge of the preferred temperatures for EBT lizards. Their most active thermal zone is between 30 and 35°C, and they become relatively inactive and prone to seek warm places when temperature drops below 30°C (Koenig et al. 2001). Thus, the cold temperature increased inactivity, but encouraged the lizards to move away from the test chamber. Therefore, cold appears to be an aversive stimulus, which is consistent with them being ectothermic.

In addition, it has been suggested that *T. scincoides* will voluntarily move to cooler places when entering a period of inactivity, such as sleep (Myhre & Hammel 1969). This phenomenon of voluntary hypothermia has been observed in other lizards, such as *Tiliqua rugosa* (Firth & Belan 1998), and linked to circadian rhythms of body temperature where the animal actively chooses cooler areas to start periods of inactivity (Ellis et al. 2007). Furthermore, it has been proposed that for some lizards, such as *Sceloporus occidentali*, this rhythm is not only determined by the environment but also by an endogenously-generated behaviour pattern, because the lizards maintain their nychthelial (temperature rhythm) body temperature variations, even when kept in total darkness (Cowgell & Underwood 1979; Cabanac & Gosselin 1993).
In this experiment, tests occurred without previous assessment of these cycles, which would be expected to vary with laboratory conditions (Myhre & Hammel 1969). Therefore, the inactivity in the escape and test chambers observed in treatment CL should be further studied, taking into account the evidence discussed above regarding the nycthermal rhythm of body temperature.

In ectothermic animals a regular response to arousal related to handling, cage restriction and transport is to increase body temperature by actively seeking a heat source. This response is related to recovery, wellbeing and the control of disease, as in endothermic animals. Reptiles will often prefer heat after feeding to facilitate digestion (Cabanac & Gosselin 1993; Cabanac & Bernieri 2000; Arena et al. 2012). The lizards in this study showed a tendency to remain in the test chamber inactive in the hot treatment, which could be linked to heat seeking behaviour.

The lack of effect of hot temperature is consistent with a previous study, in which it was found that increasing the temperatures of the lizards’ enclosures from 19-24 °C to 29-34°C did not have major effects on behaviour of EBT lizards (Phillips et al. 2011). However, although hot temperatures did not affect behaviour in the previous study, it was still felt necessary to investigate avoidance behaviour to fully appreciate whether there was an effect on EBT lizards’ welfare.

There are several other possible welfare problems associated with cold temperatures and transport. First, chronic hypothermia could lead to decreased gastrointestinal motility, which might be responsible for anorexia in reptiles during transport (Diaz-Figueroa & Mitchell 2005), although this process could be counteracted by reduced energy requirements because of immobility during transport. Also, the cellular and humoral responses of the immune system are impaired at low temperatures (Guilette et al., 1995) which can induce, amongst other effects, infections in the respiratory system and a reduction in digestion rate, which even may result in food decaying in the intestines (Altherr & Freyer 2001). Thus, high standards of biosecurity should be maintained during transport, and an isolation period should be considered when trading reptiles.

**Effects of noise treatments**

Due the hearing range of lizards (1-3 kHz) (Saunders et al. 2000; Christensen-Dalsgaard 2005) and because most of the spectral noise energy experienced inside the cabin of a simple transport vehicle is between 20 and 200Hz (Soltani & Demneh 2011), we expected animals to experience LF as a vibrational stimulus and HF as an auditory stimulus.
LF produces airborne vibrations, which are able to induce further vibrations in animals and substrates (Bowles 1995; Hill 2009). Also, reptiles have been proven sensitive to these stimuli and they use them to catch prey, avoid predators and communicate with conspecifics. For example, in the order Squamata (the taxonomical order of EBT lizards), there are several examples of vibrational sensitivity. Snakes are known to perceive airborne vibrations (Young 2003). Wever (1978) has estimated a frequency range for snakes’ sensitivity of 200-400 Hz, with some species having additional high sensitivity for approximately 100 Hz on either side of this range. For some lizards, like the leopard lizard (Gambelia w. wislizenii), good auditory perception of LF (300-700 Hz) has been acknowledged, as well as perception of vibrations below their hearing range (Wever et al. 1966). For the chameleon (Chamaeleo lyprat), the use of low frequency vibrations in the substrate for intraspecific communication has been observed (Barnett et al. 1999). All of these examples of vibrational sensitivity lay within the range chosen as LF treatment (0-500 Hz).

Nevertheless, noise treatments had no effects on the lizards, which could be for several reasons. Firstly, in our study the airborne vibration induced was purely related to sound stimulation and had several important peaks in a broader spectrum, between 100 and 400 Hz, which could have accounted for a variable reaction of lizards in this study. Secondly, lizards living in captivity are exposed to LF generated by equipment such as air conditioning and lights, which may lead to habituation. This applies as well for HF, which did not have any measurable effects on lizards’ behaviour. Furthermore, because of the lizards’ hearing range (in which the best hearing sensibility lies in low frequencies) and the greatest amount of energy in a vehicle cabin noise lies between 20-200 Hz (as mentioned above), reactions to HF were not observed.

Despite these results, it is not possible to conclude that noise and vibration stimuli from road transport are not noxious for EBT lizards or reptiles in general, as there are a great variety of hearing ranges amongst this taxa. The acoustic stimulus that in this case turned out to be non-significant could be extremely noxious for a different species. Likewise, it is possible that the noise gradient in this study was not significant enough for animals to present avoidance behaviours, as the acoustic stimulus is not greatly different in the different sections of the chamber system. Further research needs to be done to have conclusive results on the effects of noise and vibration.

2.7. Conclusions
The lizards showed a high level of inactivity, which made identification of behavioural responses to potentially noxious stimuli difficult. An apparatus involving three chambers, for exposure to the stimulus, escape and hiding was developed which was able to identify differences in responses to stimuli experienced during transport. Lizards exposed to variation from the ambient temperature
tended to spend less time in the test chamber and more time walking from the escape chamber to the hiding chamber. A temperature of 15 ± 1°C induced both avoidance behaviour (which is linked to the biological need to seek heat and move away from cold to keep a preferred temperature), inactivity in the escape chamber and avoidance in the hiding chamber. However, temperatures of 35 ± 1°C, noise and vibrations did not have measurable effects on behaviour, suggesting that the lizards were able to cope with this for at least a period of 15 min.

2.8. Animal welfare implications
This experiment was designed to allow the lizards to move away from cold and other aversive stimuli. However, in normal transport conditions, the containers where animals are kept have little space, thus diminishing the chance to seek relief. In addition, our trials lasted only for short periods (15 min per day). In trade conditions, animals travel for many hours and may experience repeated transport episodes and layovers (Arena et al., 2012). Therefore, the avoidance of cold demonstrated in this experiment may be amplified under transport conditions.

In preparation for our evaluation of the effects of transports stressors on the EBT, they were observed before and during the trials, which led to the creation of an ethogram comprising the most common behaviours displayed by this colony of lizards. However, it must be emphasized that the behavioural signs of stress measured here are not the only ones known for reptiles. Recently, 31 behavioural and physiological signs related to stress have been listed and linked with possible environmental causes (Warwick et al., 2013). Thus, there may be other relevant signs of stress in different reptile species, which could be used in further studies on reptile welfare.

The avoidance of cold temperatures by the lizards presented in this study was interpreted as an indication of welfare impairment if the ability of EBT to escape from cold is thwarted and the cold endures for a long time. This interpretation was based upon Tiliqua scincoides’ preferred temperature zone (20/25° to 30/35°C), which is 5/10°C higher than the temperature set for treatment CL (15°C), thus making avoidance an expected consequence for treatment CL (15°C).

In contrast, the treatment H was set at 35°C, which is an extreme but still inclusive value within the recommended temperature range for the species, potentially accounting for a greater behavioural tolerance to it. However, even when reptiles do not show avoidance of a hot environment, this does not indicate physiological wellness in every case. For example, when experiencing early stages of bacterial infection, reptiles may seek high temperatures to activate the immune system and overcome disease (Warwick, 1991), thus making the preference for warmth a sign of compromised
welfare. Also, reptiles may select and occupy warm zones in response to stressful environmental situations such as a handling or interspecies competition (Warwick et al., 2013).

Although noise and vibration treatments did not have any effect on lizards’ behaviour, these results are not conclusive, and further research should be done addressing different sets of frequencies at different amplitudes, as well as different sources of vibration apart from sound. Also, long term and short term exposure to these stimuli should be contrasted, as it could be a decisive component when addressing their effects on lizards’ behaviour and welfare.

Thus, our results, even though significant for low temperature, should be regarded as an example for this species under otherwise controlled conditions, because warmth could also be chosen as the least uncomfortable condition, which doesn’t necessarily relate to good welfare. Essentially lizards should be provided with a temperature gradient in their cages during transport, or better still a temperature mosaic providing discrete temperatures that can be selected according to the lizards’ needs (Lillywhite & Gatten 1995). Of potential benefit is a reduction in response to human handling at cold temperatures (Greenberg 1995), which may in turn reduce injury.

2.9. References


Auliya M 2003 Hot Trade in Cool Creatures: A Review of the Live Reptile Trade in the European Union in the 1990s with a Focus on Germany. TRAFFIC Europe: Brussels


Cabanac M and Bernieri C 2000 Behavioral rise in body temperature and tachycardia by handling of a turtle (*Clemmys Insculpta*). *Behavioral Processes* 49: 61-68


Christensen-Dalsgaard J 2005 Directionality of the lizard ear. *Journal of Experimental Biology* 208: 1209-1217


Firth BT and Belan I 1998 Daily and seasonal rhythms in selected body temperatures in the Australian lizard *Tiliqua Rugosa* (*Scincidae*): field and laboratory observations. *Physiological and Biochemical Zoology* 71: 303-11


Hendricks R 1998 *Technical Noise Supplement*. California Department of Transportation. Sacramento, California, USA

Hill PSM 2009 How do animals use substrate-borne vibrations as an information source? *Naturwissenschaften* 96: 1355-1371


Maldonado AM, Nijman V and Bearder SK. 2009 Trade in night monkeys Aotus Spp. in the Brazil–Colombia–Peru tri-border area: international wildlife trade regulations are ineffectively enforced. *Endangered Species Research* 9: 143-149


Rosen GE and Smith KF 2010 Summarizing the evidence on the international trade in illegal wildlife. *Ecohealth* 7: 24-32


Schütz C 2003 *Transport LossesL of CITES Protected and Non-protected Animal Species*. Federal Agency For Nature Conservation: Bonn, Germany


Slabbekoorn H and Peet M 2003 Ecology: birds sing at a higher pitch in urban noise - great tits hit the high notes to ensure that their mating calls are heard above the city’s din. *Nature* 424: 267


Soltani A and Demneh MK 2011 Analyzing of noise inside a simple vehicle cabin using boundary element method. World Academy of Science, Engineering and Technology 73: 630-634


Tapley B, Griffiths RA and Bride I 2011 Dynamics of the trade in reptiles and amphibians within the United Kingdom over a ten-year period. Herpetological Journal 21: 27-34


Torr GA and Shine R 1994 An ethogram for the small scincid lizard Lampropholis Guichenoti. Amphibia-Reptilia 15: 21-34


Turner G and Valentic R 2001 Keeping Blue-Tongue Lizards. Australian Reptile Keeper Publications: Bendigo, Victoria, Australia


Young BA 2003 Snake bioacoustics: toward a richer understanding of the behavioral ecology of snakes. *Quarterly Review of Biology* 78: 303-325

Table 1. Experimental design used to test EBT lizards; aversion to 5 different treatments in a modified open-field test

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Table 2. Behaviour of lizards exposed to simulated transport stimuli. Treatment H = Heat, CL = Cold, LF = Low Frequency Noise, HF = High Frequency Noise, C = control treatment. TC = test chamber. EC = escape chamber. HC = hiding chamber. SED = Standard Error of the Difference.

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</tr>
<tr>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>Walk HC, away from stimulus</td>
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Figure 1. Stimulus aversion experimental apparatus with test, escape and hiding chambers, scale 1:10 cm.

3.1. **Introductory statement**

Through our first experiment, the generation of an ethogram with the most important behavioural reactions to stress in Eastern blue tongued lizard, *Tiliqua scincoides* was achieved and a modified open field testing system was created, which allowed me to measure reactions to stimuli through observed avoidance. The noise exposure methodology was also improved in two main areas: first, changes in the chamber system were made to improve noise exposure and generate a greater gradient of noise over distance, thus making the hiding chamber a different and more calming acoustic environment appropriate for retreat. Second, it was observed that substrate vibrations directly generated by the speakers generated a confounding effect since they cannot be fully controlled. Thus, it was decided to eliminate substrate vibrations generated mechanically by the speaker and examine solely the effects of soundwaves, either experienced as hearing or airborne vibrations, which can later induce vibrations in bodies and substrates but that are a direct effect of sound broadcasting. Using the acoustic processing techniques learned, a mining machinery soundtrack was designed and used as the acoustic stimulus for this and subsequent research.

3.2. **Abstract**

The mining industry is an important source of noise for wildlife, and the Eastern blue tongued (EBT) lizard (*Tiliqua scincoides*) is one animal living in affected areas that may be impacted. We analyzed the behaviour of nine EBT lizards when exposed to five combinations of mining machinery noise frequency and amplitude in a changeover design. Lizards were exposed to low and high frequencies (< or > 2 kHz) at both low (60-65 dB (A)) and high (70-75 dB (A)) amplitudes, and a Control treatment, in a test chamber for a 5 second period. Following exposure lizards’ behaviour was monitored for 15 minutes in an escape chamber, which led to a small hiding chamber. In the test chamber lizards exposed to any mining machinery noise, but especially high frequencies, spent more time freezing, a typical stress response in reptiles, when compared with animals in the Control treatment. In the hiding chamber, high frequencies at high amplitudes decreased durations of head right down positioning, suggesting a lateralized fear reaction, as well as standing and freezing rates of behaviours. We hypothesize that lizards have lateralized behaviour reactions to mining noise, with high frequency, high amplitude noise being the most detrimental.
Our results demonstrate that mining noise has negative effects on EBT lizards’ behavior and welfare.

Keywords: animal welfare; anthropogenic noise; Eastern blue tongued lizard; mining noise; sound mimicking; wildlife

3.3. Introduction

Anthropogenic activities are one of the most important sources of acoustic noise for wildlife (Pijanowski et al. 2011). Such noise has been acknowledged as a major stressor that is able to mask and alter calls between conspecifics, hamper the detection of predators, change the hearing thresholds of individuals and alter animals’ distribution (Blickley and Patricelli 2010). At the same time, noise exposure has negative physiological effects on animals, including hearing impairment and deafness, disrupted responses of the Hypothalamic-Pituitary-Adrenal axis, reproductive problems and suppression of their immune system (Kight and Swaddle 2011).

In Australia, mining is a widespread industry that potentially has impact on wildlife. Open-cast mining is one of the two principal means of mineral extraction (Britt et al. 2014), creating a major source of environmental noise pollution (Giardino and Marraccini 1981; Haigh 1993; Tripathy 1999). There are few analyses of mining noise frequency spectrums and amplitudes at a distance to test their impact on wildlife, even though a possible influence on animal populations and their welfare has been recognized (Pal et al. 2006; Armstrong 2010; Saha and Padhy 2011). Studies that have analyzed mining noise as a workplace hazard have revealed that low frequencies, usually below 2 kHz, are dominant and energetic, as they are in many anthropogenic noises (Slabbekoorn and Peet 2003; Slabbekoorn and Ripmeester 2008; Roberts and Roberts 2009; Barber et al. 2011). Machinery used in open-cast mining and rock crushing emits most energy at low frequencies, with the dumper truck as the lowest frequency (0.25 – 0.5 kHz) and the cooling fan from bulldozers the highest (0.3 - 3.5 kHz) (Vardhan et al. 2004, 2005). However, even though mining noise has most dominant frequencies below 2 kHz, it has a broad frequency range. Some special machines, such as diamond cutters, can generate dominant frequencies between 2 and 4 kHz (Pal et al. 2006), with overall the dominant frequency range being from 316 to 8 kHz (Peng et al. 2010)

In terms of amplitude, mining noise in situ can be as high as 90 to 110 dB (A) (Peng et al. 2010; Mohapatra and Goswami 2012; Ahmad et al. 2014). In commercial and residential areas located close to mining sites, noise amplitudes have been reported to reach 89 and 67 dB (A), respectively
(Mohapatra and Goswami 2012). These levels are similar to measurements close to a rock crushing facility, operationally similar to open-cast mining, with measurements of 86 ± 0.42 dB (A) in a forest between 0 and 500 meters from the noise source (1) and lower values (64.4 ± 0.25 dB (A)) at 500 – 1,000 meters from the source (Saha and Padhy 2011).

In addition to its pitch and volume, mining noise is unusual compared to other man-made noises, in that it originates from a wide range of sources (e.g. industrial machinery, railway transport and construction, vehicle traffic and logging activities). Such a combination can enhance noise properties that increase annoyance and aversion, such as sound complexity (the degree of mixture of different sounds) and discordant mixtures of frequencies (excessive complexity) (Cone and Hayes 1984), which are components of nonlinear acoustic phenomena.

Mining noise may be assumed to be a source of non-linear sound due to the great array of machinery involved, with some large amplitude waves that are governed by non-linear equations and a degree of distortion as they travel. Nonlinear acoustic phenomena are desynchronized sound vibrations that occur, for example, when too much air is expelled from an animal’s vocal system during distress and alarm calls, producing highly complex sounds that include deterministic chaos and appear to be noise (Fitch et al. 2002; Tokuda et al. 2002; Blesdoe and Blumstein 2014). Anthropogenic noise nonlinearity has not been widely researched, even when, for example, the reactions of animals like the white-crowned sparrows (Zonotrichia leucophry) resemble those generated by computer-generated nonlinear sounds, which contain acoustic discontinuities found in animal vocalization (Crino et al. 2011, Blesdoe and Blumstein 2014). Likewise, synthetic nonlinear stimuli also evoke similar responses in yellow-bellied marmots (Marmota flaviventris) exposed to conspecific distress calls (Blumstein et al. 2008), implying that responsiveness to nonlinearity can be generated by any nonlinear acoustic stimulus, including artificial noise (Slaughter et al. 2013; Blesdoe and Blumstein 2014).

Mining machinery has been only evaluated as a noxious stimulus in relation to its impact on human health in the workplace (Roy and Adhikari 2007; Peng et al. 2010). The impacts of mining noise on the behaviour, physiology and welfare of free-ranging animals has been widely overlooked, except that there is evidence that mining and related industries can affect the migration patterns and selection of breeding and feeding grounds in elks (Kuck et al. 1985), elephants (Rabanal et al. 2010) and bird populations (Read 2000; Holloran 2005; Saha and Padhy 2011). Such effects can be used as bio-indicators of the degree of environmental impact produced by mining (Read 2000), but
monitoring is difficult under field conditions. Therefore investigations of the effects of mining noise on captive terrestrial animals may provide indicators of animals’ responses that would not be possible in field studies. Eventually it may be possible to mitigate the impacts of mining noise in Codes of Practice and environmental legislation, which currently ignore this source of noise pollution (for example, in the Environmental Protection Act of Queensland 1994).

It is important that research on the effects of noise pollution on animals evaluates the effect of mining noise on a wide variety of taxa. To date, most research on the effects of noise on wild animals has investigated vocal species, such as marine mammals, birds and amphibians (for example, Rabin et al. 2003; Brumm and Slabbekoorn 2005; Wright et al. 2007; Blickley and Patricelli 2010). There is very little information on how noise affects animals that are not as vocal, such as reptiles (Shannon et al. 2015).

The EBT lizard is an omnivorous skink that is widespread in Australia. It can survive in varied habitats and is mostly diurnal, spending much of its time hidden beneath low vegetation, in hollow logs and abandoned burrows (Wilson and Knowles 1988; Turner, 2010). Although the number of EBT lizards or other reptiles in the surroundings of open-cast mines in Australia has not been established, its presence in this area is probable due to its wide distribution across north- and south-Eastern Australia (Anon 2015) and their capacity to adapt to environments with a strong anthropogenic influence, such as urban sites (Koenig et al. 2001; Turner 2010).

EBT lizards also occupy areas where open-cast mining is expected to develop, in close association with active mining operations, such as the Upper Hunter Valley in New South Wales, Australia (Planning 2005; Cottle and Keys 2014). Some reptiles exposed to mining of heavy metals can develop additional health problems when these enter their blood stream, for instance, the giant sungazer lizards (Smaug giganteus) in gold mining sites in South Africa (McIntyre and Whiting 2012). Since noise can decrease immunocompetence (Kight and Swaddle 2011) and there may be synergistic effects of combined stressors (Deak 2007), there is a need to evaluate lizards’ responses to mining noise, as well as that of other stressors.

As a member of the order Lacertalia, the EBT lizard has good hearing capabilities, being especially sensitive to frequencies between 1 and 3 kHz (Saunders et al. 2000; Christensen-Dalsgaard 2005). Other members of this genus, such as the bob-tailed lizard (Tiliqua rugosa), have hearing ranges of 0.2 to 4.5 kHz, with peak sensitivity to 1.2 kHz at 10 dB Sound Pressure Level (SPL) (Köppl and
Manley 1992). Therefore, mining noises are very likely to be readily perceived by EBT lizards, although their interpretation and the consequent responses to such noise are unknown.

In addition, this lizard is related to vulnerable species, or subspecies that are listed as vulnerable: the species (T. adelaidensis, IUCN 2015) is endangered, and the subspecies *Tiliqua rugosa konawi* is rare and likely to become extinct (Cogger 2014). Therefore, gathering information about the effects of mining noise on one member of the genus may assist with generating conservation strategies for others.

Behaviour is an important tool to evaluate animal welfare in a non-invasive manner. In the case of many captive reptiles, locomotion is naturally low (Warwick 1995). Nonetheless, the evaluation of rates and durations of exploratory behaviours and stress-related inactivity have been acknowledged as an important tool to assess discomfort and stress in these taxa (Warwick *et al.* 2013, Mancera *et al.* 2014). Thus, in order to determine whether there was evidence from their behavioural reactions that mining noise is deleterious to their welfare, we exposed captive EBT lizards to mining machinery noise of different frequencies and amplitudes in a modified open field facility.

Increases in amplitude are commonly related with increased annoyance, as it implies a higher amount of energy contained into the sound wave generating more strength and power (Cone & Hayes 1984). Likewise, changes on frequency can determine the level of sound perception, as each animal species has a specific hearing range, which could make some sounds imperceptible while increasing sensitivity for acoustic stimuli of certain frequencies. Thus, we hypothesized that mining noise would have an adverse effect on lizards’ behaviour with negative impacts on their welfare and that such effects would be frequency and amplitude dependent.

### 3.4. Materials and methods

Procedures were approved by The University of Queensland’s Animal Ethics Committee (UQAEC Approval Number SAFS/104/14) and by Queensland Parks and Wildlife Service (Scientific Purposes Permit WISP05075208).

**Animals**

Nine EBT lizards held in the Native Wildlife Teaching and Research Facility of the University of Queensland were utilised for the study. All were siblings sourced from a local commercial supplier
(Pet City, Brisbane, Australia) and were previously used in exploratory studies related to transport stressors and other research.

They were permanently housed in nine individual enclosures, consisting of a tubular plastic frame supporting plastic mesh walls (six were 60 cm long, 39 cm wide and 40 cm high and three were 95 cm long, 52 cm wide and 53 cm high). Enclosures had two layers of paper as substrate that was replaced when soiled, and were furnished with bricks or irregularly-shaped rocks to facilitate ecdysis, a hollow wooden log for shelter, and a glass dish containing water. Cages were cleaned weekly using water and a commercial detergent (Earth Choice, Nature Organics, Australia). Background noise levels, in both the area where they were kept and that where the testing took place, were always below 55 dB (A) when measured with a Sound Level Meter (Model QM-1589, Digitech, California, USA).

**Diet**

In accordance with normal husbandry procedures for these animals, they were fed twice weekly. On each occasion each lizard was provided with 7 g of fruits and vegetables (grapes, honeydew, watermelon, banana, corn and broccoli), and one live giant mealworm (Zophobas morio) or a steamed chicken egg for protein. The giant mealworms were given directly to the lizards; fruit, vegetables and eggs were chopped into pieces and sprinkled with a vitamin, mineral and amino acid supplement (Repti-vite, Aristopet, Australia) prior to being fed to the lizards.

**Test enclosure design, habituation and training**

We designed a test of aversion to mining noise which assumed that the lizards would move away from an aversive auditory stimulus to seek a hiding place. A three chamber facility (Figure 1), that had been previously developed to assess aversion to various stressors that included road traffic noise in lizards (Mancera *et al.*, 2014), was used:

- a test chamber (TC) (57 cm long x 12 cm wide), in which they were exposed to the noise. This accommodated a single lizard from head to tail comfortably, while preventing excessive movement or visual stimulation.
- an escape chamber (EC) (81 cm long x 42 cm wide), in which there was sufficient room to perform activities, creating an open field area for behavioural responses after exposure to the noise stimuli.
- a hiding chamber (HC) (50 cm long x 12 cm wide). Positioned opposite the TC and connected to the EC, the HC was located at the farthest distance from the source of the noise.
stimuli. It provided adequate space for the animals to hide, assuming that they would prefer a space where they could fit their whole bodies while exercising minimal movement, imitating the main characteristics of the logs that they use for hiding in the wild or that were provided in their normal enclosures.

The walls (20 cm high) surrounding the chamber system were made of plywood and lined with insulating material (Reflecta, GID Double Layer, Insulation for sale, NSW, Australia) as well as sound proofing foam (Broadband Studio Acoustic Foam, Swamp Industries Pty Ltd, NSW, Australia) to avoid noise reverberation and isolate the interior of the chamber system from any external sounds. External noise and vibration were controlled during testing by placing the chamber system on a table in an isolated room away from any vehicle or person traffic. The room was kept at ambient temperature, which was recorded daily (mean temperature 26.4 ± 2.02 °C, range 24.4 - 28.4°C). Lizards were first habituated to the chamber system by placing them individually into the TC facing the HC for 15 minutes on three occasions over three days prior to the experiment.

Creation of mining noise and sound processing

Based on the characteristics described for mining noise in the literature (Utley 1980; Pathak et al. 1999; Read 2000; Roy and Adhikari 2007; Camargo et al. 2009; Nanda et al. 2009; Nanda et al. 2011; Scott et al. 2010; Saha and Padhy 2011) and in consultation with a mining geologist, a mining machinery noise soundtrack was created. We utilized online sources specialized in the creation of sound effects (http://sounddogs.com; http://hark.com) and recordings of mining machinery made by a mining equipment company (Caterpillar ®, Peoria, Illinois, USA) https://www.youtube.com/user/catmining) in order to sequence the best acoustic examples of a coal truck, a drill and a bulldozer, which are typical pieces of equipment used in open-cast mining. These sounds were overlapped using the software ‘Audacity’ (http://audacity.sourceforge.net/) to generate a soundtrack lasting 258 minutes. From this recording, a 15 min section containing the simultaneous noise of all three pieces of equipment was selected at random and further processed using the high-pass and low-pass filter functions of the Audacity software to create two tracks of Low Frequency noise (LF), ≤ 2 kHz, and High Frequency noise (HF), > 2 kHz, respectively (Figure 2). This recognized that anthropogenic noise has most of its energy output below 2 kHz (Slabbekoorn and Peet 2003; Slabbekoorn and Ripmeester 2008; Roberts and Roberts 2009; Barber et al. 2011). Since lizards have hearing sensitivity ranging from 1-3 kHz (Saunders et al. 2000; Christensen-Dalsgaard 2005) and are also sensitive to LF airborne vibrations below 1 kHz (Wever et al. 1966; Wever 1978; Young 2003), it was hypothesized that 2 kHz would be the most appropriate division in relation to animal perception and therefore behaviour.
Experimental treatments and procedures

Auditory treatments were further established at two different levels of amplitude, recorded in the Test chamber: High Amplitude (HA), mean 73.90 ± 0.83 dB (A), range 70-75 dB (A), and Low Amplitude (LA), mean 62.94 ± 0.91 dB (A), range 60-65 dB (A). Mean values for amplitude ranges were calculated using recordings of the high and low frequency noises at high and low amplitudes and extracting decibel values from successive samples using the function ‘Sample Data Export’ from the software Audacity®. An increase of 10 decibels is an increase in (noise) power by a factor of 10 (Goelzer et al. 2001).

Since mining noise has only been studied as a health hazard in humans, sound volumes in mining facilities have only been reported using A-weighted decibels, which takes into account human sensitivity to specific frequencies (Möser 2009). Thus the use of dB (A) in this study allowed us to be consistent with the current knowledge of sound energy levels experienced on mining sites. Information on noise pollution in areas surrounding open-cast mining operations was not widely available. The study assessing noise levels in a forest close to a rock crushing facility (Saha and Padhy 2011) referred to in the introduction was used to predict exposure amplitude levels in this experiment, which we consider valid due to the close relationship that rock crushing has with procedures performed in open-cast mining.

With the combination of the two sets of frequencies (HF and LF) and the selected amplitudes (HA and LA), four noise treatments were created: 1) [HF + HA], 2) [HF + LA], 3) [LF + HA], and 4) [LF + LA]. A speaker (output power: 2.5W x 2; Respond Frequency: 40 Hz - 20 kHz; Signal-to-Noise Ratio: 90 dB; Resolution: 85 dB; Punch Box Bluetooth Speaker, Xoopar, China), placed above the entrance of the test chamber (20 cm) and directed to the front of the lizard’s body in its initial position, was used to broadcast the mining noise (Figure 1). A Control treatment (C), where the speaker was turned on and no sound was played was also included (Table 1).

The amplitude of the mining noise was measured in each chamber using the first 15 seconds of the sound recording, which was considered to be a representative fragment due the simultaneous and continuous presence of the three types of machinery noises. It decreased in both the HA and LA treatments by a gradient of 10 dB (A) ± 2 dB (A) from the test chamber to the mid-point of the escape chamber, and by a gradient of 15 dB (A) ± 2 dB (A) from the test chamber to the end point.
of the hiding chamber. Before each test, the sound level meter was used to assign and monitor the correct amplitude to the appropriate frequency, depending on the treatment to be tested.

All lizards were exposed to the stimuli over 3 consecutive weeks using a cross over design, where the experimental units were the intersection of individual lizards (rows) on specific days (columns), (Table 2). Each week represented the combination of two 5x5 Latin squares with one row removed, as only 9 subjects were tested. Each animal received the five treatments only once a week (Monday to Friday). The distribution of treatments was randomized within and between weeks.

At the beginning of the trials, animals were placed individually inside the test chamber facing the escape chamber. Immediately after the animal was positioned, a transparent Perspex lid was used to cover the chamber system to prevent animals escaping and intrusion of exterior sounds. Then, for a preliminary period of five seconds after initiation of the mining noise the lizard was held in the test chamber by a wooden removable door (Figure 1), so that it experienced the noise without any opportunity to escape. The door was then opened and behaviour recording commenced, whilst the lizards responded to the continued stimulus. Each test lasted for a 15 minute period. The tests were conducted between 0900 and 1200 h daily.

**Behaviour recording and analysis**

Lizard behaviour was recorded by four cameras (model K-32HCF, Kobi CCD, Ashmore, Australia) suspended 50 to 60 cm above the translucent Perspex roof of the chamber system and connected to a video recorder (Model Lite 900, LG, Yeouido, South Korea). Researchers (CD and FF) remained in the same room as the lizards during the experiment but observed their behaviour through a television monitor connected to the cameras to avoid any observer effect on lizard behaviour. They recorded the rates and durations of behaviours performed in each chamber using behaviour analysis software (Cowlog, Hänninen and Pastell 2009). A minimum of 3 s of a new behaviour was required to indicate a new bout had been initiated. This duration was selected taking into account previous experimental experiences with the animals (Mancera et al. 2014) and with knowledge of behavioural measurement criteria for bout determination (Martin and Bateson 1993).

An ethogram was defined which focused on the type of movement and where it occurred, as well as behaviours that had been observed in other lizard behaviour studies (Greenberg 1977; Torr and Shine 1994; Langkilde 2006; Mancera et al. 2014). The behaviours recorded were walking, climbing, standing (remaining in the same position while exploring the environment visually and/or
by tongue flicking), freezing (remaining in the same position without any other movement than those related to breathing), tongue flicking (protruding tongue and then returning it to the mouth), sneezing (expelling air from their nostrils in a sudden manner with a jerk of the body) and head position (up and down, as well as facing to the left, right or straight ahead).

**Statistical Analyses**

A General Linear Model was constructed which included the following factors: lizard, treatment, frequency, amplitude, presence or absence of noise, week and day. Residuals of the model were tested for normal distribution, and if not normally distributed (P < 0.05) data was transformed using square root or logarithm10, whichever most effectively returned residuals to a normal distribution. After preliminary exploration of the data using the aforementioned model, five specific contrasts were further tested:

1) Comparison of all treatments: standard comparison where all five treatments are contrasted against each other.

2) Control vs. all noise treatments combined: for this contrast, all noise treatments were joined in one group and compared against Control.

3) HF+HA treatment vs. all other treatments: HF HA treatment contrasted with all the other treatments combined (including Control).

4) HF treatments vs LF treatments: the two high frequencies treatments were combined and compared with the two low frequencies treatments.

5) HA treatments vs LA treatments: the two high amplitudes treatments were combined and compared with the two low amplitudes treatments.

Kruskal-Wallis test for non-parametric data was performed to test time of occupancy in each chamber. For behaviours of low frequency and duration, data was transformed to binomial values and tested with Binary Logistic Regression, comparing the number of lizards that did show this behaviour with those that did not between treatments. Results were considered significant at P ≤ 0.05 and are presented + SEM. All calculations were performed with the program Minitab Statistical Software, version 16.

**3.5. Results**

*Occupation of the chambers*
Following release of the restraining door, animals spent most time (358.5 ± 20.7 s) in the EC, with similar amounts of time spent in the TC (266.7 ± 20.5 s) and HC (291.1 ± 26.4 s).

Both high frequency and high amplitude treatments extended the time spent in TC (HF mean = 260.2 s/900s, LF mean = 160.8 s/900s, P < 0.01; HA mean = 245.9 s/900s, LA mean = 172.4 s/900s, P = 0.05). In the EC and HC, the time animals spent in the chamber was not affected by treatment (Table 3).

**Lizard behaviour in the three chambers**

As a proportion of time spent in the chamber, lizards spent much more time with head down and to the left when they were in the TC, compared with EC and HC (Table 4). Animals in EC spent most of their time with their heads down to the front, and also more time standing, and no time digging. Lizards in the HC spent more time with their heads up (left or right). Animals froze in both the TC and EC, but rarely in the HC, (Table 4).

**Behaviour in the Test Chamber**

Lizards tended to spend more time moving their heads in the high frequency treatments than lizards in the low frequency treatments (P = 0.07), particularly in the HF HA treatment (P = 0.03) (Table 4). They spent more time and were seen freezing more regularly when exposed to high frequency treatments when compared with low frequency treatments (P < 0.001), again this was most evident in the HF HA treatment (Table 5).

**Behaviour in the Escape chamber**

Lizards in the low amplitude treatments spent more time with their head turned right than those in the high amplitude treatments (P = 0.02) (Table 6).

**Behaviour in the Hiding chamber**

Overall, lizards in the hiding chamber spent more time turning their heads to the left, rather than the right, when exposed to noise treatments compared with the Control (P = 0.04) (Table 7). There was a decrease in the rate at which lizards had their heads directed to the front and upwards in all noise treatments compared with the Control (Noise = 0.45 s/900s, C = 1.06 s/900s, P = 0.007), Lizards in the HF HA treatment spent less time and had a reduced frequency of turning their heads down and right when compared with all other treatments combined (P = 0.01 and 0.04, respectively). They also spent less time and were less frequently observed standing than those in the other treatments (P
They also froze less frequently compared with the other treatments ($P = 0.05$) and (Table 7).

**Behaviour in the combined Chamber system**

Lizards spent more time with their head oriented right and downwards when exposed to low amplitude treatments compared with high amplitude treatments ($HA = 43.41 \text{s}/900\text{s}, LA = 81.92 \text{s}/900\text{s}, P = 0.02$), an effect which tended to be greatest in HF HA ($P = 0.07$) (Table 8).

3.6. Discussion

The aim of this study was to evaluate EBT lizards’ behavioural reactions to mining noise, which is of great significance in Australia, where the EBT lizard is indigenous and mining industry has assumed great importance (Connolly and Orsmond 2011). However, it may also provide a model for measuring reptile responses that are useful outside this domain. During a previous anthropogenic noise exposure experiment, EBT lizards did not show any reactions to noise exposure (Mancera et al. 2014). However in this study, a better control of acoustic variables (i.e. the position, the processing, the direction of the auditory stimuli and the use of acoustic materials to line the walls of the chamber system) is likely to have improved the sound quality and allowed the stimuli to be more effectively controlled, thus making the animals more responsive, even though the amplitude used in this experiment was lower than in our previous study (60-75 dB (A) Versus 90 dB (A)).

Overall, the lizard’s pattern of activities in each chamber demonstrated that when lizards were in TC, they spent much of their time freezing and with their head orientated left and downwards; conversely in HC they spent more time with their heads up and less time freezing. Freezing, which is characterized by tension and immobility, is considered a reaction to inappropriate environments with restrictive or deficient characteristics and a sign of stress and fear (Warwick et al. 2013). It was also considered a sign of aversion in EBT lizards when they were exposed to different transport stimuli (Mancera et al. 2014). The increased freezing in the test chamber and decrease in the hiding chamber suggests that it is an indicator of stress, being alleviated as the animal moves away from the noise source.

Although head turning directionality could potentially be difficult to relate to the sound source position, in reality the lizards moved mostly forwards and therefore it was possible to make
deductions about the lizards’ intentions when consistently turning their heads in one direction. Furthermore the responses we observed could be related to the properties of noise itself or to the particular traits of the mining noise, such as its broad frequency range when compared to other anthropogenic noises (Peng et al. 2010).

**Head positions as indicators of visual lateralization and fear**

In the hiding chamber, for each second the lizards held their heads to the right, they spent 1.75 seconds with their heads to the left when exposed to any of the noise treatments, in contrast to the control treatment in which they spent only 0.74 s looking to the left for each second they looked to the right. Such behavioural responses are likely related to the neural interpretation of stimuli and its relationship with the asymmetry of brain functions (Bisazza et al. 1998). Brain asymmetry is a central tenet in neuroscience, indicating the specialization of the two hemispheres (which are also related to differences in size and anatomical structure) to control different tasks (Csermely and Regolin 2012). Lateralized behavior in response to emotional input is regarded as a direct consequence of brain asymmetry; the left hemisphere controls the right side of the body and is linked to attention and learning, whereas the right hemisphere (controlling the left side of the body) is activated in threatening situations that entail fear and aggression as well as escape responses (Ocklenburg and Gunturkun 2012; Ocklenburg et al. 2013).

In reptiles, lateralization has been well established in several species of lizards, for example, *Podarcis muralis* (Bonati and Csermely 2013), *Ctenophorus ornatus* (Robins et al. 2005), *Anolis carolinensis* (Deckel 1995) and *Sceloporus virgatus* (Hews et al. 2004). These species have been regarded as good examples of this neural process due to their brain anatomy, in which the absence of the corpus callosum (that is, the connector and communicator between hemispheres) allows a true independence between the right and the left brain hemispheres (Deckel 1995). Consequently, several studies have assessed visual lateralization in lizards when exposed to predatory and anti-predatory stimuli. For example, the common wall lizard (*Podarcis muralis*) shows a left eye/right hemisphere preference when inspecting predators, which is regarded as lateralized fear processing (Bonati et al. 2010), whilst it exhibits a right eye/left hemisphere inclination when exposed to prey, a task that requires attention and the exercise of established behaviours (Bonati et al. 2008). This feeding-related response is also observed in the ornate dragon (*Ctenophorus ornatus*), which increases its right eye preference as prey become more familiar to them (Robins et al. 2005).
Along with the left eye/right hemisphere response to fear, there is a similar response for behaviours related to aggressiveness. For example, the Carolina anole (*Anolis carolinensis*) prefers to bite, threaten and perform aggressive movements to a conspecific using its left eye as guidance (Deckel 1995). Likewise, striped plateau lizards (*Sceloporus virgatus*) present the same lateralized response when gravid females are exposed to males placed in different fields of vision, with the left visual field being the one that enables more reactions, almost all of them aggressive (Hews *et al.* 2004).

In our experiment, noise-exposed EBT lizards remained mostly facing the left side wall once in the hiding chamber, which was the farthest point they could reach with their heads. Since the noise source was positioned posterior to them, exercising a preference for left head turns allowed left side of the head exposure (right brain hemisphere) to the stimuli source, implying a fear-related reaction (Figure 3). These results are similar to those of Bonati *et al.* (2010), in which lateralized escape reactions in the common wall lizard were stimulated by beating a brush against a transparent tube containing the animal and afterwards, allowing them to decide the direction of their flight in an open field test. While measuring the direction of the escape, discontinuous locomotive performance was noticed due to frequent pauses that allowed the animal to visually survey the environment, as reported previously in other studies (Avery 1993; Brana 2003). During such breaks, there was a significant preference to turn their heads to the left, allowing the left eye to survey the stimulus behind them (i.e. brush beating).

The preference of the lizards in the hiding chamber to face the left during noise exposure was probably visual rather than motor lateralization since animals threatened by possible predators with laterally positioned eyes, such as lizards, initiate visual monitoring of the environment before they decide to flee (Cooper 2008). This visual lateralization is also similar to that encountered in the same species when exposed to threatening stimuli in the wild, where lizards would prefer to initiate surveillance with the left eye when first emerging from the refuge selected after they have fled (Martín *et al.* 2010). In view of the positioning of the ear canal behind the head, it seems unlikely but possible that left head turns functioned to reduce the sensory input to the ear by effectively closing the canal.

In addition to increases of head left movements in the hiding chamber, when exposed to low amplitudes lizards spent less time with their heads right and down when evaluated in all the chambers combined, compared with high amplitude noise. This relative increase in right head
preference at low amplitudes could indicate an analytical rather than fearful response by the lizard, since amplitude is one of the most distressing characteristics of noise (Cone and Hayes 1984).

**Effects of high frequency mining noise on EBT lizards’ acoustic processing: does mining noise could produce sound mimicking?**

In addition to the lateraled behavioural responses to noise in general, EBT lizards also presented specific lateraled head responses to high frequencies in the TC, which have implications when considered together with their standing and freezing behaviours.

Head motions in different directions were one of the most important behavioral reactions observed in the EBT lizards as a result of exposure to different frequencies of noise. Such motions have been studied before when evaluating reptile behavior and have been previously categorized as posture changes, i.e. adjustments in posture that are not associated with locomotion or alter the body’s center of gravity, and are predominantly head movements that allow visual surveillance (Greenberg 1985, 1993).

In this study, lizards increased the time spent moving their heads in the TC when exposed to the HF HA treatment compared with all other treatments. Similarly, in a study of the exploratory behavior of the Carolina anole (*Anolis carolinensis*), an iguanid lizard, posture changes increased with perturbations of the environment (e.g. foliage- and air-induced movements) and were positively correlated to handling or exposure to new surroundings (Greenberg 1985, 1993). Thus our observations in the TC, where animals were initially exposed to noise, point to a stress-related reaction at high frequencies with high amplitudes.

In addition, in the hiding chamber, EBT lizards decreased the time spent with their heads right while exposed to HF HA. Therefore, animals in the HC not only preferred a left eye/right hemisphere fear reaction in relation to all noises, this was particularly associated with high frequency high amplitude sound.

Whilst the arousal by high amplitude sound is expected, that of high frequency is not. Lizards hearing sensitivity is best within 1 to 3 kHz (Christensen-Dalsgaard 2005; Saunders *et al.* 2000) and for some species such as the brown anole (*Anolis sagrei*) it can reach up to 7.7 kHz under favourable temperature and high sound pressure levels (SPL) (Manley and Gallo 1997). There is no specific research about the hearing sensitivity of the EBT lizard, however a close taxonomical
relative, the bobtail lizard (*Tiliqua rugosa*), is known to have a hearing range of 0.2 to 4.5 kHz with a peak sensitivity of 1.2 kHz at 10 dB SPL (Köppl and Manley 1992). Therefore, both HF and LF treatments contained acoustic components that were likely to have been perceived as a hearing experience by the EBT lizards.

Anthropogenic noise distorts and masks hearing cues in wildlife (Blickley and Patricelli 2010). If a noise replicates frequency, amplitude or design of a specific call, it hampers the chances of the receiver discriminating between signals and noise, which in turn can increase false alarms and misinterpretation (Wiley 1994). Hence, it is possible that some mining noise components resembled animal calls of specific frequencies, eliciting similar behaviours to those observed when lizards react to non-anthropogenic acoustic cues related to their survival. Of relevance is the fact that sounds used by reptiles to convey aggression and stress are in the high frequency range. Hissing is one of the most representative distress calls and it has been defined as white noise of several types produced by the massive expulsion of air (Gans and Maderson 1973). Many lizards produce this vocalization when afraid, being handled and during escape attempts, as well as aggression, accompanied with the deliberate inflation of the body (Warwick *et al.* 2013). EBT lizards produce hissing sounds when fearful or when displaying agonistic behaviour (Carpenter and Murphy 1978; Turner 2010). Hissing has been recorded when emitted by the lizard *Pristidactylus volcanensis* and the analysis of the frequency spectrum of this call confirmed frequency ranges between 2.3 and 3.6 kHz (Labra *et al.* 2007), that is, frequencies above 2 kHz.

Additionally, calls emitted by predators have similar characteristics. EBT lizards’ most avid predators are large elapid snakes (Family: Elapidae), such as the Eastern brown snake (*Pseudonaja textilis*) (Turner 2010). Within this family, the cape cobra (*Naja nivea*) is known to produce hissing sounds that range between 3 and 13 kHz (Young 1991). In addition to hissing, rattling or tail vibrations are also a recurrent sound used as aggressive behaviours by snakes, ranging between 2 and 20 kHz (Young 2003).

These acoustical signals related to predator risk and aggression overlap with the high frequency peaks observed in this experiment (Figure 2). Therefore, it is possible that the high frequency component of mining noise was misinterpreted as an alarm call or predation risk due the close resemblance of frequencies from both acoustic stimuli. Such deception could explain the visual lateralization observed in high frequency high amplitude exposure, in which lizards particularly decreased head right positioning as a response to a perceived fearful stimulus.
The decrease in standing behaviours with HF HA in the hiding chamber has similar connotations. Standing was defined in this experiment as maintaining the same body position (keeping the chosen centre of gravity unchanged) while exploring the environment visually (head movements) and/or engaging in tongue flicking. As both tongue flicking and head motions constitute exploratory behaviours for many lizards (Greenberg 1985, 1993, 2002), we can attribute an exploratory function to standing. Exploration activities are useful when evaluating stress in reptiles. They have been recognized as a sign of mild stress when increased (Greenberg 2002), and a good welfare sign when present with unhurried body movements and locomotion (Warwick et al. 2013), but a sign of pronounced stress when severely decreased or absent (Warwick et al. 2013). Decreased freezing in the EC and particularly the HC suggests an overall reduction of stress levels, even though there are still remains of the supposedly stress-related reaction from the left eye/right hemisphere head responses discussed above.

Thus, in this experiment, since high amplitudes seemed to elicit stress and such stress could be related to the communality that exist between sounds that suppose a threat to the lizards, there exist the possibility of overlapping and sound mimicking of meaningful acoustic signals by mining noise frequencies, resulting in erroneous interpretations of the environment and leading to specific behavioural reactions from the EBT lizards. Signal mimicking as a possible consequence of noise pollution has been little explored, although there is evidence that some animals do increase their vigilance patterns with anthropogenic noise (Quinn et al. 2006, Shannon et al. 2014), which could be related to acoustic deception. Nonetheless, it has been recently reported that the endangered species Dipodomys stephensi (Stephens’ kangaroo rat) responds with alertness and foot-drumming (a behaviour used in territorial and mating contexts) to traffic noise playbacks, an anthropogenic noise that can mimic specific auditory signals when overlapped extensively with meaningful frequencies for some animals, thereby deceiving them to engage in false responses that can be energetically costly and decrease their survival (Shier et al. 2012).

Whether this phenomenon is possible in reptiles and other taxa should be the subject of further research, including the study of the frequency spectrum and design of different noises, the analyses of distress and aggression calls and acoustic predatory cues of several species, as well as in-depth knowledge of animals’ behavioural ecology and hearing patterns.
3.7. Conclusions
The assessment of avoidance of different frequencies and amplitudes contained in mining noise was achieved through the development of a three-chamber system with acoustic insulation which enabled exposure to the stimulus, as well as the opportunity to escape and hide. When exposed to noise treatments, lizards spent 1.7 seconds with their head positioned left for every second that they positioned it right and decreased the amount of head orientation to the right with high amplitude exposure in the whole chamber system, suggesting a lateralized fear-response. Also in the test chamber, they increased the time and occasions they spent freezing, a sign of chronic stress. Frequency appeared to have a greater effect than amplitude, suggesting the possibility of frequency-dependent sound mimicking, which generates stress-related behaviours when frequencies overlap with important acoustic cues for lizards. This could engage animals in behavioural responses that in the long term may deplete their energy and reduce their welfare.

3.8. Animal Welfare implications
Animals showed complex behavioural responses to mining noise during short-term exposure in a controlled environment, with the opportunity to mitigate exposure through distance. Such mitigation is well exemplified by the fact that freezing in the HC was reduced when compared with TC.

Nevertheless, in a natural environment, exposure to noise may be constant and greatly prolonged. The choice to move away to quieter areas is determined by a variety of factors such as the risk of predation (which can be reduced, unless the predators relocate), the density of competitors (since noise sensitivity is also bound to individual resistance), the quality of the current area in use and the availability of resources, the distance to other sites that may have the appropriate characteristics to sustain an individual, and the overall investment that an animal has made on a specific habitat (such as gaining territory, establishing dominance amongst peers) (Gill et al. 2001, Wright et al. 2007).

The effects observed in our study could be greatly enhanced in the field, which in turn can generate long term stress related to immune suppression and reproductive malfunction, diminished body condition and accelerated aging (Romero and Butler 2007). Moreover, noise is not the only stressor wild animals face in the wild and it has been shown that when many negative stimuli are combined, a stronger physiological response is generated, which can carry consequences such as the proliferation of inflammatory factors which increase sickness behaviours in acute illness (Deak 2007).
Nevertheless, animals that are subjected to long-term noise stress have been said to habituate (Bowles et al., 1999; Samson et al. 2014), learning that certain stimuli are, in fact, neutral (Bejder et al. 2009). The great majority of studies on anthropogenic noise and wildlife assess instantaneous reactions due the inability to evaluate the same wild individuals repeatedly for long periods of time and without affecting animals’ welfare (Nisbet 2000). Thus, tolerance (the stimulus intensity that an individual is able to endure without responding in a defined way) rather than habituation is often tested (Nisbet 2000). Hence, habituation cannot be assumed as a consequence for long-term noise exposure (Bejder et al. 2009). In addition, exposure to anthropogenic noise may seem neutral if animals have lost their hearing or if they are too energetically challenged to respond (Wright et al. 2007).

The EBT lizards showed great responsiveness to high frequencies, particularly if associated with high amplitude. We have hypothesized that such a behavioural pattern may be the result of signal mimicking, which could have costly consequences for EBT lizards responding to apparent distress contribute to depleted energy resources and distract them from real acoustic signals that are crucial for their survival.

3.9. References

Ahmad AF, Sharma Harendra K, Ahmad, RM and Rao R 2014 Impact of Mining Activities on Various Environmental Attributes with Specific Reference to Health Impacts in Shatabdipuram, Gwalior, India. International Research Journal of Environmental Sciences 3: 81-87


Brana F 2003 Morphological correlates of burst speed and field movement patterns: the behavioural adjustment of locomotion in wall lizards (*Podarcis muralis*). *Biological Journal of the Linnean Society of London* 80: 135-146


Carpenter CC and Murphy JB 1978 Tongue Display by the Common Blue tongue (*Tiliqua scincoides*) Reptilia, Lacertilia, Scincidae. *Journal of Herpetology 12*: 428-429

Cone J and Hayes S 1984 *Environmental problems/ behavioural solutions*. Press syndicate of the University of Cambridge: California, USA


Christensen-Dalsgaard J 2005 Directionality of the lizard ear. *Journal of Experimental Biology 208*: 1209-1217

Deak T 2007 From classic aspects of the stress response to neuroinflammation and sickness: implications for individuals and offspring. *International Journal of Comparative Psychology 20*: 96-110


Fristrup KM 2011 Anthropogenic noise exposure in protected natural areas: estimating the scale of ecological consequences. Landscape Ecology 26: 1281-1295


Giardino DA and Marraccini LC 1981 Noise in the mining industry: An overview. Mine Safety and Health Administration, Pittsburgh Health Technology Center: Pittsburgh, PA (USA)


Greenberg N 1985 Exploratory behavior and stress in the lizard, Anolis carolinensis. Zeitschrift fur Tierpsychologie 70: 89-102

Greenberg N 1993 Central and endocrine aspects of tongue-flicking and exploratory behavior in Anolis carolinensis. Brain, Behavior and Evolution 41: 210-218


Hetherington TE 1992 Behavioural use of seismic cues by the sandswimming lizard Scincus scincus. Ethology, Ecology and Evolution 4: 5-14

Hews DK, Castellano M and Hara E 2004 Aggression in females is also lateralized: left-eye bias during aggressive courtship rejection in lizards. Animal Behaviour 68: 1201-1207


Mcintyre T and Whiting MJ 2012 Increased metal concentrations in Giant Sungazer Lizards (Smaug giganteus) from mining areas in South Africa. *Archives of Environmental Contamination and Toxicology* 63: 574-585

Milne T, Bull CM and Hutchinson MN 2003 Use of burrows by the endangered pygmy blue-tongue lizard, Tiliqua adelaidensis (Scincidae). *Wildlife Research* 30: 523-528

Mohapatra H and Goswami S 2012 Assessment and analysis of noise levels in and around Ib river coalfield, Orissa, India. *Journal of Enviornmental Biology* 33: 649-655


Nisbet IC 2000 Disturbance, habituation, and management of waterbird colonies. Waterbirds 23(2): 312-332


Rabanal LI, Kuehl HS, Mundry R, Robbins MM and Boesch C 2010 Oil prospecting and its impact on large rainforest mammals in Loango National Park, Gabon. Biological Conservation 143: 1017-1024


Saha DC and Padhy PK 2011 Effect of air and noise pollution on species diversity and population density of forest birds at Lalpahari, West Bengal, India. *The Science of the Total Environment* 409: 5328-5336


Slabbekoorn H and Peet M 2003 Ecology: birds sing at a higher pitch in urban noise—great tits hit the high notes to ensure that their mating calls are heard above the city’s din. *Nature* 424: 267.


Slaughter EI, Berlin ER, Bower JT and Blumstein DT 2013 A Test of the Nonlinearity Hypothesis in Great-tailed Grackles (*Quiscalus mexicanus*). *Ethology* 119: 309-315


Torr GA and Shine R 1994 An ethogram for the small scincid lizard *Lampropholis guichenoti*. *Amphibia-Reptilia* 15: 21-34
Tripathy DP 1999 Noise pollution. APH Publishing: New Delhi, India


Vardhan H, Rao Y and Karmakar N 2004 Assessment of machine generated noise in opencast mines and development of suitable maintenance guidelines for its attenuation. *Journal of the Institution of Engineers (India), Mining Engineering Division* 84: 25-37


### 3.10. Tables and figures

**Table 1.** Experimental sound treatments and their frequency and amplitude components.

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>SOUND COMPONENTS</th>
</tr>
</thead>
</table>
| HF + HA    | HF= high frequency noise (≥ 2000 Hz)  
             | HA= high amplitude noise (70-75 dB (A)) |
| HF + LA    | HF= high frequency noise (≥ 2000 Hz)  
             | LA= low amplitude noise (60-65 dB (A)) |
| LF + HA    | LF= low frequency noise (< 2000 Hz)   
             | HA= high amplitude noise (70-75 dB (A)) |
| LF + LA    | LF= low frequency noise (< 2000 Hz)   
             | LA= low amplitude noise (60-65 dB (A)) |
| CT         | Ct= control treatment, where the speaker remained turned on while no sound was played, below 50 ± 0.1 dB (A). |
Table 2. Experimental design used to test EBT lizards; aversion to 5 different noise treatments in a modified open-field test

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Table 3. Time spent by EBT lizards in each chamber. HF = High Frequency, LF = Low Frequency, HA = High Amplitude, LA = Low Amplitude C = Control Treatment. SED = Standard Error of the Difference (all treatments)

<table>
<thead>
<tr>
<th>TIME</th>
<th>MEANS</th>
<th>SED</th>
<th>P VALUE</th>
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<tr>
<td></td>
<td>HF HA</td>
<td>HF LA</td>
<td>LF HA</td>
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<tr>
<td>Test chamber (√s/900s)</td>
<td>17.04</td>
<td>15.2</td>
<td>14.3</td>
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<tr>
<td>Test chamber (s/900s)</td>
<td>290.4</td>
<td>231.3</td>
<td>205.1</td>
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<td>Escape chamber (√s/900s)</td>
<td>16.3</td>
<td>18.5</td>
<td>16.4</td>
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<td>Escape chamber (s/900s)</td>
<td>266.01</td>
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<td>12.9</td>
<td>14.5</td>
<td>15.1</td>
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<td>Hiding Chamber (s/900s)</td>
<td>167.2</td>
<td>209.4</td>
<td>228.3</td>
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Table 4. Proportion (%) of time in each chamber spent in the different behaviours. SED = Standard Error of the Difference between Two Treatments. *Behaviours compared using Kruskal-Wallis test.

<table>
<thead>
<tr>
<th>Behaviour (% total time/ total time per chamber)</th>
<th>Test chamber</th>
<th>Escape Chamber</th>
<th>Hiding Chamber</th>
<th>SED</th>
<th>F value</th>
<th>P Value</th>
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<td>Head up left (median % total time per chamber)*</td>
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<td>0</td>
<td>1.77</td>
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<tr>
<td>Head up left (mean % total time per chamber)</td>
<td>2.95 ± 0.54</td>
<td>2.94 ± 0.46</td>
<td>9.91 ± 1.33</td>
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<tr>
<td>Head up front (median % total time per chamber)*</td>
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<td>8.902</td>
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<td>0.14</td>
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<tr>
<td>Head up front (mean % total time per chamber)</td>
<td>12.2 ± 1.53</td>
<td>14.7 ± 1.58</td>
<td>12.6 ± 1.64</td>
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<tr>
<td>Head up right (median % total time per chamber)*</td>
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<td>0</td>
<td>-</td>
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<td>&lt;0.01</td>
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<tr>
<td>Head up right (mean % total time per chamber)</td>
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<td>1.86 ± 0.36</td>
<td>8.36 ± 1.22</td>
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<td>Head down left (median % total time per chamber)*</td>
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<tr>
<td></td>
<td>93.9 ± 73.2</td>
<td>11.6 ± 1.48</td>
<td>6.7 ± 1.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Activity</td>
<td>Mean % Total Time Per Chamber</td>
<td>Median % Total Time Per Chamber</td>
<td>Walking % Total Time Per Chamber</td>
<td>Standing % Total Time Per Chamber</td>
<td></td>
<td></td>
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<tr>
<td>--------------------------------</td>
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<td>---------------------------------</td>
<td>----------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head down left</td>
<td>5.84^a</td>
<td>6.37^a</td>
<td>4.30^b</td>
<td>1.447</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head down front</td>
<td>34.1</td>
<td>40.6</td>
<td>18.5</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head down right (median)</td>
<td>0</td>
<td>3.83</td>
<td>0</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head down right (mean)</td>
<td>6.03 ± 1.40</td>
<td>11.75 ± 1.67</td>
<td>5.78 ± 1.17</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking (median)</td>
<td>1.16</td>
<td>2.51</td>
<td>1.19</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking (mean)</td>
<td>3.42 ± 0.85</td>
<td>2.62 ± 0.17</td>
<td>1.82 ± 0.17</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing (log(_{10}) % total</td>
<td>0.29^b</td>
<td>0.42^a</td>
<td>0.31^b</td>
<td>0.095</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing (% total time)</td>
<td>1.95</td>
<td>2.63</td>
<td>2.04</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>Median % Total Time (per chamber)*</td>
<td>Mean % Total Time (per chamber)</td>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
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<td>-------------------------</td>
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<td>---------------------------------</td>
<td>---------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freezing</td>
<td>0.41</td>
<td>0.59 0.31 ± 0.03</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digging</td>
<td>0</td>
<td>0.75 ± 0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Climbing</td>
<td>0</td>
<td>0.21 ± 0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flicking (√times/900 s)</td>
<td>3.49b 7.06a 3.84b</td>
<td>1.123 86.36 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a, b indicate significant differences between treatments.
Table 5. Behaviour of lizards exposed to mining machinery noise in the test chamber. HF = High Frequency, LF = Low Frequency, HA = High Amplitude, LA = Low Amplitude C = Control Treatment. SED = Standard Error of the Difference.

<table>
<thead>
<tr>
<th>BEHAVIOUR</th>
<th>MEANS</th>
<th>SED</th>
<th>P VALUE</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HF HA</td>
<td>HF LA</td>
<td>LF HA</td>
<td>LF LA</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration all head movements (√s/900s)</td>
<td>17.1</td>
<td>14.9</td>
<td>14.2</td>
<td>13.01</td>
<td>13.8</td>
<td>3.01</td>
<td>0.702</td>
</tr>
<tr>
<td></td>
<td>291.4</td>
<td>221.1</td>
<td>202.5</td>
<td>169.3</td>
<td>189.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration all head movements (s/900s)</td>
<td>10.99</td>
<td>8.8</td>
<td>6.1</td>
<td>5.1</td>
<td>8.2</td>
<td>2.73</td>
<td>0.63</td>
</tr>
<tr>
<td>Duration freezing (√s/900s)</td>
<td>120.1</td>
<td>77.4</td>
<td>37.8</td>
<td>25.8</td>
<td>66.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate freezing (√times/900s)</td>
<td>1.26</td>
<td>1.04</td>
<td>0.74</td>
<td>0.84</td>
<td>0.94</td>
<td>0.287</td>
<td>0.18</td>
</tr>
<tr>
<td>Rate freezing</td>
<td>1.60</td>
<td>1.08</td>
<td>0.55</td>
<td>0.71</td>
<td>0.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(times/900s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Table 6. Behaviour of lizards exposed to mining machinery noise in the escape chamber. HF = High Frequency, LF = Low Frequency, HA = High Amplitude, LA = Low Amplitude C = Control Treatment. SED = Standard Error of the Difference.

<table>
<thead>
<tr>
<th>BEHAVIOUR</th>
<th>MEANS</th>
<th>SED</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HF HA</td>
<td>HF LA</td>
<td>LF HA</td>
</tr>
<tr>
<td>Duration head right (√s/900s)</td>
<td>4.7</td>
<td>6.5</td>
<td>4.8</td>
</tr>
<tr>
<td>Duration head right (s/900s)</td>
<td>22.5</td>
<td>41.9</td>
<td>23.1</td>
</tr>
</tbody>
</table>

† Up and down
Table 7. Behaviour of lizards exposed to mining machinery noise in the hiding chamber. HF = High Frequency, LF = Low Frequency, HA = High Amplitude, LA = Low Amplitude C = Control Treatment. SED = Standard Error of the Difference

<table>
<thead>
<tr>
<th>BEHAVIOUR</th>
<th>MEANS</th>
<th>P VALUE</th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HF HA</td>
<td>LF HA</td>
<td>LF LA</td>
</tr>
<tr>
<td>Duration all head left/all head right</td>
<td>0.203</td>
<td>0.31</td>
<td>0.18</td>
</tr>
<tr>
<td>Duration head down right (log(_{10}) s/900s)</td>
<td>0.29</td>
<td>0.65</td>
<td>0.82</td>
</tr>
<tr>
<td>Duration head down right (s/900s)</td>
<td>0.95</td>
<td>3.47</td>
<td>5.61</td>
</tr>
<tr>
<td>Duration standing (√s/900s)</td>
<td>6.4</td>
<td>9.3</td>
<td>8.9</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Duration standing (s/900s)</td>
<td>40.9</td>
<td>86.1</td>
<td>80.5</td>
</tr>
<tr>
<td>Rate head up front (√bouts/900s)</td>
<td>0.62</td>
<td>0.58</td>
<td>0.64</td>
</tr>
<tr>
<td>Rate head up front (bouts/900s)</td>
<td>0.38</td>
<td>0.34</td>
<td>0.41</td>
</tr>
<tr>
<td>Rate head down right (√bouts/900s)</td>
<td>0.39</td>
<td>0.73</td>
<td>0.68</td>
</tr>
<tr>
<td>Rate head down right (bouts/900s)</td>
<td>0.15</td>
<td>0.53</td>
<td>0.46</td>
</tr>
<tr>
<td>Rate combined† head left (√bouts/900s)</td>
<td>1.04</td>
<td>1.36</td>
<td>1.32</td>
</tr>
<tr>
<td>Rate combined† head left (bouts/900s)</td>
<td>1.08</td>
<td>1.85</td>
<td>1.74</td>
</tr>
</tbody>
</table>

† Up and down
Table 8. Behaviour of lizards exposed to mining machinery noise in the entire chamber system. HF = High Frequency, LF = Low Frequency, HA = High Amplitude, LA = Low Amplitude C = Control Treatment. SED = Standard Error of the Difference.

<table>
<thead>
<tr>
<th>BEHAVIOUR</th>
<th>MEANS</th>
<th>SED</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HF HA</td>
<td>HF LA</td>
<td>LF HA</td>
</tr>
<tr>
<td>Duration head down right (√s/900s)</td>
<td>6.1</td>
<td>9.7</td>
<td>7.1</td>
</tr>
<tr>
<td>Duration head down right (s/900s)</td>
<td>37.2</td>
<td>94.9</td>
<td>50.1</td>
</tr>
</tbody>
</table>
Figure 1. Auditory stimuli aversion experimental apparatus with test, escape and hiding chambers, scale 1:12 cm.
Figure 2. Frequency spectrums of High frequency noise (left) and Low frequency noise (right) obtained with the program Audacity. The vertical axis corresponds to frequencies in kHz while the horizontal axis corresponds to time in minutes. The grey scale represents the amount of acoustical energy contained in the correspondent frequency; the darker the area, the greater the energy component in that area of the spectrum.
**Figure 3.** Conceptualised visual lateralization and information processing in right and left brain hemispheres in response to mining noise from a posterior source (based on Bonati *et al.*, 2010).
4. CHAPTER 4: The effects of mining machinery noise of different amplitudes on the behaviour and fecal corticosterone of wild mice (*Mus musculus*)

4.1. Introductory statement

The experiments conducted on EBT lizards set further acoustic parameters for the wild mice studies. First, it was evident that the combinations of frequencies and amplitudes generated a complicated analytical model that could be simplified by the separate analysis of these acoustic properties, thus generating a deeper understanding of the effects of mining noise characteristics. In consequence, the following experiment tested different levels of amplitude from the unfiltered mining noise soundtracks. Likewise, the use of wild mice gave us the opportunity to analyze noise in chronic and continued exposure due the different husbandry procedures set for the animals in this colony. I was also able to include organs and hormonal analysis, which further complemented the behavioural observations done on this study. As the information generated was extensive, the findings of this experiment are divided into changes on behavioural patterns (Chapter 3) and changes in organ morphology (Chapter 4).

4.2. Abstract

Mining noise is a source of stress for wildlife and its physiological effects on individuals are unknown. This study evaluated the effects of exposing wild mice to mining machinery noise at two amplitude ranges: high (HA; 70-75 dB (A)) and low (LA; 60-65 dB (A)) for 3 weeks on their behaviour and fecal corticosterone levels, compared with a control treatment (no extra auditory stimuli, below 55 dB (A)). Paired females behaviour differed from that of isolated males, probably due to gender-based differences in HPA activation. Circling behaviour in both clockwise and anti-clockwise directions was increased in animals exposed to high amplitude noise, with a tendency for fecal corticosterone to decrease. In mice exposed to low amplitude noise, fecal corticosterone was increased but total circling remained the same as control. These results suggest that dopamine-related stereotypies during high amplitude mining noise were a coping mechanism that prevented excessive physiological arousal. Both noise treatments increased circling to the left, which corresponds to right hemispheric activation of the stress response; however only the high amplitude noise increased circling to the right (left hemisphere activation), which may inhibit stress arousal by right hemisphere. In conclusion, mining machinery noise produces stress response on...
wild mice that are amplitude dependent and may require the generation of coping mechanism.

Keywords: anthropogenic noise, circling, coping, mining, rotation, stereotypy, stress

4.3. Introduction

Anthropogenic noise has been established as a source of stress for wildlife (Blickley & Patricelli 2010, Rabin, *et al.* 2003, Wright, *et al.* 2007). Amongst anthropogenic noises, transportation noise has been extensively studied (see, for example, Barber, *et al.* (2010)) but other noises that could have a significant impact on wildlife have been largely overlooked. For instance the effects of noise produced by open-cast mining machinery are unknown, even though it has been acknowledged that it could potentially affect both birds’ communities (Read 2000) and bat populations (Armstrong 2010).

Mining noise has however been evaluated as a potential workplace hazard to humans; its characteristics include a high energy, low frequency spectrum, in common with other anthropogenic noises (Barber, *et al.* 2011, Roberts & Roberts 2009, Slabbekoorn & Peet 2003, Slabbekoorn & Ripmeester 2008) and, in accordance with observations at the working face of some coal mining sites, frequency ranges are as high as 0.3 to 8 kHz (Peng, *et al.* 2010). In terms of amplitude, mining noise measured on site can reach 110 dB (A) (Ahmad, *et al.* 2014, Utley 1980) and in neighboring commercial, residential and industrial areas, amplitudes above 80 dB (A) have been recorded (Mohapatra & Goswami 2012, Saha & Padhy 2011).

Noise exposure has negative effects on immunocompetence, reproductive systems, hearing structures and DNA integrity and expression (Kight & Swaddle 2011). Likewise, it is able to affect emotional states. For example, noise generates depression and feelings of aggression in humans (Ising & Kruppa 2004, Stansfeld 2003); in male rats, sub-chronic noise exposure (4 h, 100 dB for 15 days) produces anxiety and depressive behaviours evaluated through an elevated plus-maze (Naqvi, *et al.* 2012); in pandas, exposure to approximately 72 dB ambient noise is related to increased urinary corticoids, locomotion, distress vocalizations and escape attempts (Owen, *et al.* 2004).

Of relevance to stress responses, there are reports of stereotypic behaviour induced by noise exposure. Stereotopies have been defined as ‘repetitive behaviour induced by
frustration, repeated attempts to cope, and/or central nervous system dysfunction’ (Mason & Rushen 2008). This implies that even when repetitive behavioural patterns can be part of a process unrelated to stress or to stress as an adaptative process (in the case of courtship), it can also arise as a by-product to generate relieve (Mason & Rushen 2008). In the case of noise as a noxious stimulus, casual observations suggest that unpredictable noises in laboratory facilities increase the prevalence of stereotypies as a coping mechanism in primates (Patterson-Kane & Farnworth 2006). In a study with rodents, exposure to high intensity noise (140 dB broadband noise for 15 min/day/14 weeks) greatly increased the amount of time animals spent grooming (Anthony, et al. 1959). Similarly, a male panda exposed to construction noise increased the time it spent doing stereotyped pirouettes (Powell, et al. 2006).

Apart from stereotypies, the stress response can also induce variations on other important behaviours observed in rodents. Hiding, for instance, may increase as a response to threat (Hugie 2003) and in females, nesting rates tend to increment as it favors their security and that of their offspring (Taylor, et al. 2000). Freezing is also a measurement of fear and a reaction to perceived threats without chance to escape (Blanchard & Blanchard 1988, Blanchard, et al. 2001). Likewise, maintenance behaviours such as eating and feeding can be effectively suppressed when rodents face environmental stressors (Morley & Levine 1985, Aguilera, et al. 1995).

Although most of these behavioural responses have not been specifically linked to anthropogenic activities, such effects could be observed on wildlife in the vicinity of mining noise. In many mining sites individuals are not able to move away if the risk of predation or the density of competitors is greater elsewhere, the quality of resources is high in the mining sites or if they are intrinsically bound to specific features only encountered in that location (Gill, et al. 2001, Wright, et al. 2007) . Therefore the assessment of the behavioural effects that mining activities have on animals is of great significance, and the appropriate choice of an animal model that could generate valuable information is critical. In that sense, Rabin, et al. (2003) have suggested that a useful approach could be the use of a common, widespread species, since highly abundant species are the base of the food chain, making its health and welfare of great importance. Moreover, the experimental methodologies developed for such animals
may be used to evaluate the effects of acoustic pressure on sensitive species (Rabin, et al. 2003).

One of the commonly found animals around the world is the feral mouse, *(Mus musculus)*, which is regularly encountered in mining sites due its opportunistic nature (Fox & Fox 2006, León, et al. 2007). The wild mouse is a good species for research not only for its abundance, but for the many studies on their behaviour (Denmark, et al. 2010, Dielenberg, et al. 2001, Grant & Mackintosh 1963, Lumley, et al. 2000, McAllister & Dixon 1989, Sluyter, et al. 1995, Van de Weerd, et al. 1997, Van de Weerd, et al. 1998, Van Oortmerssen 1971), physiological changes during noise exposure (see review by Kight and Swaddle (2011)) and also because of the abundance of non-invasive techniques to evaluate stress in rodents, such as the measurement of fecal corticosterone (Touma, et al. 2004). In addition, it is certain that wild mice can perceive at least the higher frequencies of mining machinery noise due their elevated hearing range (2.3 to 92 kHz, measured at 60 dB SPL (Heffner & Masterton 1980, Heffner & Heffner 2007).

In this experiment, the effects of mining machinery noise at two levels of amplitude were examined. The hypotheses were that mining noise would disrupt wild mice behavioural patterns and secretory corticosterone levels and that an increase in amplitude would result in increased magnitude of these changes.

4.4. **Materials and methods**

Procedures were approved by The University of Queensland’s Animal Ethics Committee (Research Approval Number SVS/145/12; colony approval number SAS/071/10/BREED (NF))

**Study animals**

Thirty-six wild mice (24 females and 12 males) from the School of Veterinary Science of The University of Queensland were used. These animals belonged to the 7th generation of a wild mouse *(Mus musculus)* colony of animals captured in the wild and bred in captivity. All animals were born between 4 and 26 April 2012, and were aged approximately 4 months at the beginning of the experiment.
Diet and animal housing

Mice were offered pelleted food (Rat and Mouse Pellets, Specialty Feeds, Glen Forrest, Western Australia) *ad libitum*. Males were necessarily individually caged because of potential aggression and females were caged in pairs for companionship in plastic cages of dimensions (cm) 41 long x 27 wide x 16 high, with metallic grid lids. A 12:12 light-dark cycle was established with light hours 0600 to 1800 h and a temperature range of 21-25 °C. Each cage was supplied with bedding (Sanichip, PJ Murphy Forest Products, USA) which could be used for nesting or hiding, a wide plastic tube to provide both additional nesting and retreat (5 cm diameter, 10 cm long), a narrow plastic tube for hiding when frightened (2 cm diameter, 10 cm long), as well as shredded paper to provide enrichment and nesting material.

Experimental treatments and generation of simulated mining noise

Based on the characteristics of mining noise described in the literature (Camargo, *et al.* 2009, Nanda, *et al.* 2009, Nanda, *et al.* 2011, Pathak, *et al.* 1999, Read 2000, Roy & Adhikari 2007, Saha & Padhy 2011, Scott, *et al.* 2010, Utley 1980) and in consultation with a mining geologist, seven pieces of mining machinery were chosen to recreate the soundscape of a typical open-cast mine: coal truck, drill, bulldozer, shovel, dumper, rock crusher and dragline. A blast was added in order to recreate the sound of explosions that occur on mining sites. Specialized sound effect sources and on-field recordings were used to select the best acoustic samples of machinery (sources listed in Table 1). Once acquired, noise samples from individual machinery were mixed using appropriate software (Audacity: http://audacity.sourceforge.net/). This process generated seven mining noise soundtracks (Table 2), based on the processes that take place under open-cast mining conditions. During the playing of the soundtracks in the experiment, the seven sections were randomly shuffled using appropriate software (Windows Media Player, 2009), in order to avoid habituation to a specific pattern. The blast sequence was played at a random time once a week, consistent with the normal blasting schedule on open-cast mining sites.

Since mining and related noises have been only studied as a work hazard for human health, amplitude has previously been measured as A-weighted decibels, which take into account human sensitivity to certain frequencies (Möser 2009). We used the same amplitude scale to be consistent with previous literature. After one week of habituation
to the experimental rooms and procedures, three groups of four males and four pairs of females were continuously exposed for three weeks to three experimental treatments in an independent measures design: Control (C) with animals exposed to no extra auditory stimulation apart from the normal sounds of daily laboratory activities, which was kept to < 55 dB (A) (mean value = 53.46 ± 0.44 dB (A)); a Low Noise (LA) treatment which had a range of mining noise amplitudes of 60-65 dB (A) (mean value = 63.75 ± 0.93 dB (A)) and a High Noise (HA) treatment which had a range of mining noise amplitude of 70-75 dB (A) (mean value = 72.41 ± 1.12 dB (A)). These Low and High amplitude ranges were set to relate to the sound intensity measured at 0-500 (LA) and 500-1000 m (HA), respectively, from stone mining and crushing operations (Saha & Padhy 2011), as well as the levels of noise registered in residential and commercial areas adjacent to mining facilities, where loudness can reach 67 dB(A) and 89 dB (A), respectively (Mohapatra & Goswami 2012). These increases of approximately 10 decibels, from Control to Low and Low to High, represent an increase in (noise) power by a factor of 10 (Goelzer et al. 2001). Mean values for amplitude were verified during the experiment using a sound level meter (Digital Sound Level Meter, Q1362, Dick Smith Electronics, Australia). In addition, in order to verify the effectiveness of soundproofing materials and the noise to which the animals were exposed, mining noise soundtracks were recorded in the actual treatment rooms using a microphone (Sennheiser ME66 condenser shotgun, Germany) connected to a sound data recorder (Tascam DR100 MkII DAT). Then, decibel values were extracted from the recordings in successive samples using appropriate software (Audacity: http://audacity.sourceforge.net/). Examples of spectrograms from these recordings are shown in Figure 1.

Experimental enclosures

The study took place at the Queensland Animal Science Precinct (QASP) in the University of Queensland, Gatton Campus. A hexagonal facility, with six identical rooms was used to separate the animals into three treatment rooms, each separated from the next by an empty room (Figure 2). Rooms containing mouse treatments were soundproofed using noise and temperature-isolating materials (Reflecta, GID Double Layer, Insulation for sale, NSW, Australia), as well as soundproofing foam (Broadband Studio Acoustic Foam, Swamp Industries Pty Ltd, NSW, Australia). Animals were placed in their cages at distances of 80-266 cm from the speakers (System Frequency...
response: 35 Hz-20 KHz, Output Power (Total) 200 Watt, Speaker system z623, Logitech, Switzerland) (Figure 2).

**Video recordings and analysis of videos**

Mice behaviour was recorded by 12 surveillance cameras (1 camera/ 2 cages ) (model K-32HCF, Kobi CCD, Ashmore, Australia) suspended 60 cm above the cages and connected to a video recorder (Model Lite 900, LG, Yeouido, South Korea). Researchers were only present in the experimental rooms between 0900 and 1100 h for cleaning duties and collection of fecal samples. Animals were recorded continuously throughout the experimental period. From the videos gathered during the experiment, 10 representative days were selected as suitable for analysis, and from these days, 120 of 240 possible cage-day combinations were selected to analyze 24 h periods of activities, maintaining a balance between observations across treatments and gender. Observations were made during the first 5 minutes of each of 24 h.

Taking into account behaviours that are associated with stress in mice (Denmark, *et al.* 2010, Dielenberg, *et al.* 2001, Grant & Mackintosh 1963, Lumley, *et al.* 2000, McAllister & Dixon 1989, Suyter, *et al.* 1995, Van de Weerd, *et al.* 1997, Van de Weerd, *et al.* 1998, Van Oortmerssen 1971), ethograms were created to assess individual behaviour of all mice and social behaviour for the females caged in pairs. Individual behaviours recorded were full body hiding/retreat (completely inside either the wide or the narrow tube), partial hiding/retreat (leaving the head outside the tube), mouse active inside a shredded paper nest, mouse inactive inside the nest (with no detectable movement of paper), nest building (activities related to constructing of the nest, such as gathering and rearranging of paper), moving on the grid (moving upside down on the bars of the metallic grid which formed the lid, but not circling), circling to the left or to the right on the grid (animal moving in circles anticlockwise or clockwise), drinking, feeding, freezing (mouse remaining still in one position, but not in the next, with the only detectable movement being breathing), grooming.

Social behaviours recorded were pushing under conspecific (one mouse moves under the other led by their snout), sniffing each other’s snout, sniffing each other’s anal area, chasing (pursuing conspecific), allogrooming, mounting (mouse moves on top of conspecific either in a copulation-like manner or aligning snout with tail), being inactive
socially but at close proximity (mice remain engaged in individual behaviours while
touching each other or remaining within one body width), touch and go (one individual
touches its partner briefly and runs away), squire (walking while follower keeps its
snout close to the leader’s anal area) and push away (one mouse pushes the other in an
aggressive manner). During replay, the duration and rates of these behaviours were
recorded using the software ‘Cowlog’ (Hänninen & Pastell 2009). In order to record
rates and start points of durations, a change of behavioural state was determined by an
animal spending at least 3 seconds performing a new behaviour. This system was based
on preliminary observations of the videos from this experiment, taking into account
recommended methods of measuring behaviour frequency (Martin & Bateson 1993).

**Fecal sampling and processing**

Samples were collected daily between 0900 to 1100 h by removing mice from their
cages using the narrow tube as a container in order to avoid direct handling. Fresh feces
were selected taking into account moisture and color, whilst discarding those
contaminated with urine to ensure that collection occurred within 20 h. Fecal samples
from females were from pairs of individuals housed together, while fecal samples from
males were from a single animal; thus for both genders the cage was considered the
appropriate replicate. All samples were frozen at -20 °C until further processed. For
analysis, faecal samples from periods of 4 consecutive days were pooled, generating 7
pooled samples per replicate. Samples were freeze-dried for 5 h, homogenized with a
mortar, weighed (to the nearest 0.05±0.0015 g) into a glass scintillation vials and 1 ml
of 80% methanol was added. They were then centrifuged at 800 g for 10 min and the
extract decanted and frozen at -20 °C until required for analysis.

**Fecal corticosterone measurement**

The concentration of fecal corticosterone metabolites (FCM) was determined by a
corticosterone enzyme immunoassay (EIA) technique described previously, but with
minor modifications (Keeley, *et al.* 2012). Microtitre plates pre-coated with goat anti-
rabbit globulin (Arbor Assays, USA; A009) were used for this purpose, and
corticosterone antibody (stock dilution: 1:200) and horse-radish peroxidase (stock
dilution: 1:200) (C Munro, UC Davis, CA, USA) at 1:120,000 and 1: 250,000 dilution
rates, respectively, 100 µl per well. Fecal samples were diluted in assay buffer prior to
analysis (1:7 for females, 1:6 for males) and a serial dilution of a pool of randomly-
selected fecal samples demonstrated parallelism with the standard curve. The intra-
assay and inter-assay coefficients of variation were 2.65% and 7.09%, respectively.
Crossreactivities for the corticosterone EIA antibody were corticosterone 100%,
deoxycorticosterone 14.3%, tetrahydrocorticosterone 0.9%, cortisol 0.2%, progesterone
2.7%, testosterone 0.6% and <1% for all other steroids tested. The sample color
absorbance values were determined using a microplate spectrophotometer reader
(Epoch, Winooski, VT, USA) and appropriate software (Gen 5, Biotek, USA). Test and
reference filters of 405 and 630 nm, respectively, were used.

**Statistical analyses**
Fecal corticosterone was analyzed using a Linear Effects Mixed Model (LEM)
including the factors mouse, treatment, sex and period of time. Data was transformed
using logarithm_{10}, to return residuals to a normal distribution (P < 0.05). When LEM
was significant, a post-hoc analysis with Bonferroni corrections was used to compare
means. Results were considered significant at P ≤ 0.05.

To observe individual females caged in pairs, during video replay one mouse was
initially selected for behaviour recording by the observer, then the video was replayed
and the behaviour of the remaining mouse was recorded. Rates and durations of
behaviours for pairs of females were analysed as the means of the two animals per cage.
A LEM was constructed which included the factors mouse, treatment, sex and day.
There were no ‘treatment x day’ interactions so this was not included in the final model.
If residuals were not normally distributed (P < 0.05) data was transformed using square
root or logarithm_{10}, whichever most effectively returned residuals to a normal
distribution. When LEM was significant, a post-hoc analysis with Bonferroni
corrections was used to compare means. Results were considered significant at P ≤ 0.05
For social behaviors, data was sparse and was therefore transformed to binomial values
(present or absent) and tested with Binary Logistic Regression (BLR), comparing the
presence and absence of behaviours between pairs of treatments. Results were
considered significant at P ≤ 0.05.

All analyses were conducted using IBM SPSS, version 20.
4.5. Results

**Fecal corticosterone metabolites (FCM)**
Mice in treatment LA had increased FCM compared with C, with mice in treatment HA at intermediate levels (P = 0.003), (Table 3). Females had higher levels of FCM than males (P < 0.001). FCM concentrations were elevated in the first period (day 1-4) of the study (P < 0.001), (Figure 3).

**Treatment differences in behavior**
Mice exposed to both noise treatments tended to spend less time hiding inside the wide tube (P < 0.0001). LA mice spent less time hiding in the narrow tube compared to control, with HA as intermediate (P = 0.02). LA exposure decreased the time animals nest built (P = 0.02) compared with control, with HA as intermediate. Mice in both noise treatments spent more time moving on the grid (not circling) (P = 0.001) and circling to the left (P = 0.001) than controls. There was a tendency for circling left to be higher in HA than LA, however, only HA increased the time animals spent circling to the right (P < 0.0001), compared with LA and Control. There was a tendency for grooming to be reduced in the noise treatments, particularly HA (P = 0.095), (Table 3).

Mice in treatment LA had greater frequencies of freezing compared to those in HA, with C treatment as intermediate (P = 0.019). Mice in treatments HA and LA increased the number of times that they were moving on the grid (P = 0.008) and circling left (P < 0.0001). HA exposure increased the number of bouts of right circling (P < 0.0001), (Table 4).

**Gender differences in individual behavior**
Female mice spent more time than males hiding in the wide tube (P = 0.01) and hiding partially in the narrow tube (P = 0.003). Female mice also spent more time moving in the grid (P = 0.025), freezing (0.001) and circling to the left (P < 0.0001) than their males counterparts. Males spent more time active in the nest (P = 0.003), feeding (P = 0.022) and grooming (P = 0.007) than female mice, (Table 3).

Females were more frequently observed moving on the grid and freezing than males (P = 0.02 and P < 0.0001, respectively), (Table 4).

**Combined gender and treatment differences in individual behavior**
Females exposed to LA and males exposed to HA spent less time hiding in the wide tube than C females and males, respectively. (P = 0.007). For the narrow tube, HA males spent less time hiding inside it than HA females (P = 0.014). Females in HA spent more time partially hiding in the wide tube, compared with females and males in LA (P = 0.004). There was a tendency for males in HA to drink for less time than females in HA (P = 0.008). Females in both noise treatments spent more time in total circling than females in C and males in HA spent more time in total circling than males in LA (P = 0.0001), (Table 3).

HA and C females hid more frequently in the wide tube than females in LA and males in C (P = 0.003) (Table 4). HA males hid less frequently in the narrow tube (P = 0.003). Partial hiding tended to be most frequent for females in HA (P = 0.001). Males in treatment HA tended to drink least frequently (P = 0.008). Bouts of moving on the grid were least frequent in C males than males in HA and LA, but no such effect was observed in females (P = 0.003). Females more regularly circled to the left (P = 0.024) and total circling (P < 0.0001) than C females, whereas no difference was seen in males. In the males total circling was more frequent in treatment HA than for males in treatment LA.

**Social behaviour**

Only two social behaviours in females were apparently different between pairs of treatments. Exposure to HA decreased the frequency of allogrooming compared with mice in C (number of positive responders/total count: HA = 4/20, C = 10/20; Coefficient (r): -1.4; Odds Ratio (OR): 0.25; Confidence Interval (CI): 0.061-1.02; P = 0.05). Exposure to LA tended to decrease frequency of touch and go compared with mice in C (number of positive responders/total count: LA =5/20 C =11/20; r: -0.4; OR: 0.67; CI: 0.19-2.33; P= 0.06).

**4.6. Discussion**

Noise exposure generates stress that produces disorders relating to motivation, aggression, learning and behaviour (Kight & Swaddle 2011, Prasher 2009). Anthropogenic noise could be affecting behavioural traits in wildlife that are critical for survival of individuals unable to avoid noise due their inability to migrate to areas with
the same availability of resources. In this study, mining noise generated stress as demonstrated by related behaviours and changes in hormonal profile.

**Fecal corticosterone levels under different levels of intensity: differential response of the HPA axis**

Fecal corticosterone levels were higher in females than males, regardless of the treatment. Gender differences in the stress response were confounded with housing system as we had to house the males in isolation due the risk of aggressive confrontations, whereas females could safely be housed in pairs. However, the effects observed are believed to be an effect of gender rather than housing system, because other studies with rodents have demonstrated that HPA axis stimulation increased glucocorticoid levels in females more than in males, with the latter protected by a testosterone-mediated inhibition of corticotrophin releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus, thus reducing the initiation of the stress response (Haleem, et al. 1988, Heinsbroek, et al. 1991, Kant, et al. 1983, Yoshimura, et al. 2003). Likewise, studies have confirmed that for female mice, social housing is likely to reduce stress, whereas males benefit from their own space allowance (Brown & Grunberg 1995). Thus, even though paired housing may have reduced HPA arousal in females in this experiment, males’ space availability might also provide the same stress-reducing effects, thus equilibrating the impact of housing. Similarly, it has been observed that even when housed in pairs, female mice reduce but do not avoid the effects of stressors such as chronic foot shock (Westenbroek, et al. 2005). Therefore, in our study HPA inhibition due social housing would have been only partial for females and it is more likely that the changes observed in fecal corticosterone are sex-related.

When treatments were compared, the highest levels of secretory corticosterone were seen in the LA treatment when compared with control, whereas HA tended to decrease, especially for females. Activation of the HPA axis and the consequent increases in glucocorticoids is necessary to initiate a physiological stress response (Romero & Butler 2007, Tsigos & Chrousos 2002). Thus the increments in corticosterone in the low amplitude treatment are related to stress arousal. In addition, it has been observed that initial fecal corticosterone increases reduce overtime if the stressor is of certain severity and the exposure is chronic (Dallman 1993, Lightman & Harbuz 1993, Martí & Armario 1998, Miller, et al. 2007). On this experiment, stress intensity played an
important role, as low noise intensity was associated with the highest levels of corticosterone compared with control, whereas high amplitudes remained intermediate. Similar results have been observed after chronic restraints stress (2 h/day) of either high (four limb prone restrain) or low (immobilization in a tube) intensity, where the high intensity restrain group showed a decrease on corticosterone response ([pre-treatment levels] - [post- treatment levels]), whereas the low stress group presented a consistently higher response over 21 days, which was regarded as a result of highly variable corticosterone response in the low restrain group (Pitman, et al. 1988). Other authors have found no change in corticosterone levels during mild stress (Hennessy & Levine 1977). Similarly, when white noise pulses (45min/h, 12 h /day, during 8 days) were broadcasted to rats either at 95 dB or 105 dB, the lower intensity generated a two-fold increase in plasma corticosterone in rats, whereas the higher generated no increase but a major decrease in body weight and food intake compared to 95 dB exposure, which strongly suggest that higher noise intensities were more noxious than the lower, even though corticosterone seemed unaffected (Bijlsma, et al. 2001).

Given this evidence, we hypothesize that chronic and continuous high intensity stressors, as in the high amplitude treatment used on this experiment, will not necessarily elevate corticosterone levels as much as low intensity stress. It is possible that greater intensities initiate the appearance of coping mechanisms to decrease physiological stress. Smaller intensities may require less coping if the physiological arousal has not reached levels where physical damage appears. The behavioural evidence obtained in this study supports this hypothesis as it confirms the activation of the stress response during noise exposure and the subsequent generation of behavioural coping of greater magnitude in the high amplitude treatment.

Gender effects on individual behaviour of mice exposed to mining noise
There were significant differences between males and females in the behaviours observed. Females increased the rates and time they were seen hiding in the narrow and the wide tubes compared to males. Hiding is an antipredatory response in which the animals seek refuge to avoid life threatening interactions (Hugie 2003). Additionally, LA females spent less time hiding in the wide tube both partially and completely and HA females increased the time spent there compared to HA males. The wide tube was use for hiding, but also for nesting element. An increase in nesting behaviour is in
agreement with coping strategies by gender, in which females are more inclined to increase those behaviours that will favor their security and that of their offspring, such as nesting (Taylor, et al. 2000). Thus, the fact that HA females were more inclined to occupy the wide tube in relation to LA females and HA males may implicate a difference on the occupancy of the wide tube in relation to nesting and a necessity to cope.

Likewise, HA females spent more time hiding in the narrow tube than males of the same treatment. It is known that a constant and homogeneous squeeze to the body or deep-touch pressure reduces anxiety for pigs (Grandin, et al. 1989), dogs (King, et al. 2014), humans and cattle (Grandin 1992), amongst others. The characteristics of the narrow tube used on this experiment were such that allowed a similar experience and were even used to reduce handling, as the animals would prefer to hide inside it to avoid contact. Thus, it is likely that the differences observed in narrow tube occupancy for females and males exposed to high amplitudes are also related to a greater necessity of female animals to cope.

Females froze more often and more repeatedly than males. Freezing is measurement of conditioned fear and is seen when a close threat is perceived without the opportunity to escape (Blanchard & Blanchard 1988, Blanchard, et al. 2001). This behaviour also supports a greater activation of the HPA axis of females compared to males (Haleem, et al. 1988, Heinsbroek, et al. 1991, Kant, et al. 1983, Yoshimura, et al. 2003). Likewise, they tended to groom less than males. The suppression of grooming has been observed before in rats exposed to a cat, which was also related to high levels of corticosterone and was considered a stress response that did not produce habituation (Blanchard, et al. 1998).

Females also spent less time feeding than males. Food intake can decrease as a consequence of CRH secretion during the stress response (Morley & Levine 1982). Males spent more time active inside the nest. Even though the specific activities inside the nest are unknown, the unchanged rates of other observable stress behaviours suggest that males experienced less stress than females and that this was also the case inside the nest.
Females drank for longer than males in the HA treatment. Water consumption is affected by stress via the Renin-Angiotensin-Aldosterone System (RAAS), where increased levels of glucocorticoids stimulate the sympathetic nervous system, allowing renin, an enzyme secreted by the kidney, to initiate the RAAS (Aguilera, et al. 1995). Renin stimulates the production of angiotensin I, which transforms into angiotensin II, allowing vasoconstriction and increasing liquid ingestion during noise exposure. For example, under impulse noise exposure (intervals of 9.5 seconds, 127 dB SPL) angiotensin endogenously produced through noise-induced stress elevated blood pressure and water consumption in female rats (Morseth, et al. 1985). Increased water intake is also mediated through a direct action of renin and angiotensin II on the central nervous system, which provokes thirst (Fitzsimons 1972). As females in this experiment had a higher corticosterone level than males, it is possible that increased angiotensin II levels augmented water consumption compared to males, thus affecting observed drinking rates.

Females circled more the left than males. Noise exposed females had the greatest total circling durations and circling left rates. Circling is a behaviour described as the active motion of animals in a circular direction and is considered an stereotypy (Löschter 2010, Pycock 1980). The behaviour ‘moving on the grid’ had the same general tendencies as circling, being increased in females. The increase in stereotypic circling for females exposed to noise was another sign of greater stress responsiveness. Circling is closely related with dopamine release and coping during stress (Reference).

**Stereotypic behaviour and stress**

Circling is a stereotype that is normally found in the caged mouse ethogram (Weber 2005) and as well as excessive self-grooming or gnawing, it becomes a welfare issue when exacerbated due frustration, anxiety or stress (Mason & Turner 1993). Stereotypical circling is closely related to stress and dopamine release. When stress is perceived, glucocorticoids increase to activate the HPA axis (Cabib 2006) and can mediate the effects of dopamine, a catecholamine involved in the regulation of motor control and responsiveness to sensory stimulation, memory, learning and motivated behaviour (Mason 1991, Mason & Turner 1993, Vallone, et al. 2000). Dopamine controls locomotion through the activation of the nigrostriatal dopaminergic pathway, which relies on the synthesis of dopamine in neurons of the midbrain nucleus.
(substantia nigra compacta) and is further innervated into the dorsal striatum (caudate-putamen) (Vallone, et al. 2000). These areas are part of the cerebral basal ganglia, which are involved in various aspects of psychomotor behaviour (Parent & Hazrati 1995). Nigrostriatal degeneration is related to the development of Parkinson’s disease, disabling appropriate control of motor responses (Lang & Lozano 1998).

Due its widespread involvement in locomotion, nigrostriatal dopamine has been related to the generation and maintenance of stereotypies. It has long been known that dopamine or dopamine agonist injections into the striatum produce stereotypical behaviours in rats (Ernst & Smelik 1966). Dopaminergic drugs such as amphetamines also induce stereotypical behaviour in rodents and primates via the nigrostriatal pathway (Lewis, et al. 2006, Mason 1991). Although there is some controversy about whether drug-induced stereotypies correspond to the mechanisms for spontaneous stereotypies (Mason 1991) and whether dopamine is directly involved in all motor dysfunctions (Vandebroek & Ödberg 1997), there is extensive evidence of stress and isolation increasing nigrostriatal dopaminergic activity (Dantzer 1989, Jones, et al. 1989, Robbins & Sahakian 1981) and decreasing thyroxin hydroxylase (which is essential for dopamine synthesis) in the striatum and substantia nigra. This causes the development of stereotypical behaviour in socially-isolated rhesus monkeys (Macaca mulatta) (Martin, et al. 1991). Similarly, circling behaviour is mediated by imbalances in the nigrostriatal dopaminergic pathway (Carlson & Glick 1996, Ishiguro, et al. 2007, Löschler 2010, Schirmer, et al. 2007).

Stress-related stereotypies mediated by dopamine arise through the effects that glucocorticoids have on dopaminergic neurons. Striatal neurons produce dopamine and the neuropeptides enkephalines and tachykinines (Reiner & Anderson 1990), which increase following glucocorticoid release; in adrenalectomized rats, levels of striatal proenkephalin and protachykinin are suppressed but can be restored by small doses of corticosterone (Chao & McEwen 1991). Striatal neuropeptides also increase nigrostriatal dopamine and locomotion in rats (Baruch, et al. 1988, Biggio, et al. 1978). This evidence, along with the high density of type II glucocorticoid receptors in the striatum (about 90%) (Zoli, et al. 1989), suggest a glucocorticoids control of dopamine release by the increased release of striatal neuropeptides, with further effects on locomotion (McEWEN, et al. 1994). This has been demonstrated in adrenalectomized
rats, which decrease dopaminergic-induced exploratory behaviours, which are restored after a corticosterone injection (Veldhuis, et al. 1982).

As well as glucocorticoids, the level of stress alters the activation of dopaminergic pathways. Extreme stress such as that experienced following severe electric shock to the foot (Dunn 1988, Herman, et al. 1982, Uylings, et al. 1990) or cold stress (Dunn & File 1983) activate the mesocortical, mesolimbic and the nigrostriatal dopaminergic pathways directly, whereas lesser stressors such as a mild electric shock or psychological stress (such as observing a conspecific receiving foot-shock) activate the mesocortical system (Deutch, et al. 1985, Herman, et al. 1982, Kaneyuki, et al. 1991), and the nigrostriatal pathway via the frontal cortex through glutamate projections (Druce, et al. 1982, Glick & Greenstein 1973). Frontal cortex connectivity is an important modulator of the magnitude and direction of circling behaviour (Carlson & Glick 1996, Carlson, et al. 1987), implying that changes in this area affect dopaminergic locomotion.

Therefore, the evidence suggests that stress, even when mild, is likely to increase circling behaviour due to dopaminergic activation via corticosterone release. In this experiment, mining noise augmented circling more in females, particularly circling to the left. Right circling was reserved mainly for the high amplitude treatment, which is the part of a stereotypical response that is more stress-dependent. In support of these results Ravindran, et al. (2005) have proven that noise stress (100 dB SPL broadband white noise, 4 h /15 days) increases the amount of dopamine in the corpus striatum.

Females in both noise treatments spent more time in total circling than females in C and males in HA spent more time in total circling than males in LA. Females more regularly circled to the left (P = 0.024) and total circling (P < 0.0001) than C females, whereas no difference was seen in males. In the males total circling was more frequent in treatment HA than for males in treatment LA.

The fact that circling behaviour increased specially for mice exposed to high amplitudes while fecal corticosterone was increased most in the LA mice suggest the emergence of behavioral coping mechanisms mediated by stereotypies. Stereotypical behaviours, which have been defined as ‘a repeated relatively invariant sequence of movements that has no obvious function’ have a tendency to occur in circumstances where the
individual lacks control of the environment (Broom & Fraser 2007) and are expressed
without moving away from the stressful environment (Odberg 1989). It has also been
proposed that stereotypies reduce physiological and emotional arousal by lowering
responses to external stimuli (Broom 1987, Odberg 1978) and increase predictability in
an unpredictable environment (Broom 1983, Broom 1981, Forrester 1980), which
promotes a sense of reward and dissociation from negative experiences (Wiepkema
1987).

The idea of stereotypic behaviours as a means to reduce HPA arousal has been widely
contended since the theory does not explain every type of repetitive behavior and
because not every stereotypy has beneficial effects on all individuals (Mason 1991,
Mason & Turner 1993, Rushen 1993, Rushen & Mason 2006). There is evidence of
stereotypic behaviours decreasing glucocorticoids levels under certain circumstances;
for example, pacing associated with decreased corticosterone in domestic fowls
(Duncan 1970). Similarly, non-stereotyping young tethered sows have higher
corticosterone levels than their stereotyping older peers (Cronin, et al. 1985). In bank
voles (Clethrionomys glareolus), the reduction of jumping stereotypies by lowering the
cage ceiling resulted in increases of corticosterone in previously stereotyping animals.
The increases observed were decreased later only if a new stereotypy was developed
(Kennes & De Rycke 1988, Odberg 1989). In rhesus macaques (Macaca mulatta) self-
directed stereotypies (such as digit-sucking) are negative correlated with fecal
corticosterone levels (Pomerantz, et al. 2012).

Thus, stereotypies generated by stress, such as circling, can also participate in the
inhibition of physiological arousal in situations where stress is intense, unavoidable and
continuous, such as mining noise exposure at high amplitudes. This research confirms
that different stress degrees will generate different adaptive responses, in which more
intense stress generates a greater amount of stereotypies to cope and decrease greater
physiological activation. The increase in stereotypic behaviour confirms an HPA
arousal which required the emergence of behavioural coping in mice exposed to mining
machinery noise especially at high volumes, in agreement with observations where
prefrontal cortex dopamine release (the circling regulating area) functioned as a coping
strategy during severe stress (Sullivan 2004). In addition, although circling left was
increased on both noise treatments, only high amplitude treatment increased
significantly the amount of right circling, which is a phenomenon related to lateralized responses during stress.

*Differential lateralization of stereotypical circling due to mining noise stress at different amplitudes*

The direction of rotational behaviours is determined by hemispheric differences in dopaminergic activity; animals will turn to the side opposite to the hemisphere with greater dopaminergic action (Carlson & Glick 1996, Ishiguro, *et al.* 2007, Löscher 2010, Schirmer, *et al.* 2007). This process is related to brain lateralization, a central theory in neuroscience where the two brain hemispheres are specialized to control different tasks, which is related to their differences in size and anatomical structure (Csermely & Regolin 2012). Lateralized behavioural responses are a direct consequence of brain lateralization; the left hemisphere controlling the right side of the body and primarily regulating communication, attention, learning and established behaviours, and the right hemisphere controlling the left side and regulating responses to threatening situations, social interactions and novelty (Ocklenburg & Gunturkun 2012, Ocklenburg, *et al.* 2013, Rogers 2010).

Lateralization is a process influenced by stress. Several studies have proven that the HPA axis of numerous species will selectively activate the right hemisphere during stress (Rogers 2002). For example, Perez-Cruz, *et al.* (2009) analyzed the apical dendrites form the rat prelimbic cortex, which are known to contract during stress. After a week of chronic restraint stress (6 h/day), the right prelimbic cortex had a reduction in length of the basal dendrites compared with the left hemisphere, suggesting a preferential action of the right brain. Similarly, when cats were separated into high and low cortisol groups after transport stress and new environment exposure, animals in the high group had higher right tympanic temperatures than those in the low (Mazzotti & Boere 2009), probably due the strong relationship between defensive emotional activation and temperature (Oka, *et al.* 2001). Also in mice grouped by paw preference, left-pawed individuals had higher corticosterone levels compared to right-pawed after 1 h of restraint stress, suggesting that HPA axis activation is right-biased and greater in mice with this lateral preference (Neveu & Moya 1997).
Right hemisphere activity during stress is also seen in dopamine release. When rats are exposed to either controllable or uncontrollable electrical foot-shock, uncontrollably stressed rats had increased dopamine production in the right prefrontal cortex compared with those able to escape and controls (Carlson, et al. 1993). Activation of the prefrontal cortex during stress has been demonstrated to upregulate nigrostriatal dopamine, increasing circling behaviour (Carlson, et al. 1987). Likewise, animals exposed to bright novel environments, without the possibility to chew, show increased dopamine turnover in the right prefrontal cortex, nucleus accumbens and striatum when compared with controls (Berridge, et al. 1999). In the same way, after 60 minutes of restraint stress rats increased dopaminergic metabolism in the right hemisphere (Carlson, et al. 1991).

The increments in left circling observed in both noise treatments suggest that mining noise exposure at both amplitudes activates the stress response and increases right hemisphere activation, thus generating left circling stereotypies when compared with control. In addition, the fact that HA animals mainly increased the amount of right circling suggests a shift to left hemisphere dominance during intense stress. In this regard, even though the stress response is traditionally linked to the right brain, some evidence suggests that the left hemisphere is able to inhibit stress arousal by down-regulating right hemisphere activation (Denerberg 1981). For instance, left-hemisphere lesioned rats exhibited higher rates of muricide (mouse killing) than those with right lesions, which had the same level of muricide than non-lesioned rats, thus inferring that an intact left brain inhibits right-hemisphere mediated aggressive responses (Garbanati, et al. 1983). Also in handled rats (a process that increases right hemisphere laterality), when the corpus callosum was lesioned rates of muricide increased when compared with shams, suggesting that not only the left brain is necessary for inhibition but that the corpus callosum, which allows communication between the left and right brain, is essential for interhemispheric communication during this process (Denerberg, et al. 1986). Thus, an increase on right circling during high amplitude mining noise exposure would suggest an activation of the left hemisphere with measurable behavioural effects in an attempt to decrease right hemispheric activation and stress arousal during higher sound intensities.

In addition to interhemispheric inhibition, it has been established that certain type of stressors, such as 24-hrs food deprivation (Carlson, et al. 1988) or 15 min restrain
(Carlson, et al. 1991), require an analytical approach that benefits from left hemisphere activation. Left-biased cerebral activation has been related to stimuli that require analytical, less emotional and motor dominant coping attempts (Sullivan 2004, Sullivan & Dufresne 2006). The great majority of stressors faced in daily life, including chronic noise exposure, are manageable and not an immediate threat, even when energetically intense. Thus, left hemisphere activation would confer an intrinsic advantage, since the negative emotional attachment and the energy expenditure related to right hemisphere activation would be avoided and would prevent controllable stress from becoming unbearable (R. Sullivan, 2004). Therefore, chronic HA noise exposure generates left hemispheric bias, which is not present during low amplitudes, probably due to differences in noise intensity. Whereas HA mining noise generates behavioural responses to inhibit physiological arousal mediated by the right hemisphere, low amplitude noise appears as a stimulus that, even though stressful, does not reach stimulation levels where inhibition is required.

4.7. Conclusions

Mining machinery noise had detrimental effects on wild mice. Paired females reacted differently to noise stress than isolated males, presenting more fear-related behaviours. Increases in stereotypic circling in both directions during high amplitude mining noise exposure suggest a stress response mediated by glucocorticoids that influenced dopamine release. Likewise, the tendencies to reduce corticosterone levels with the increases in stereotypies during HA exposure suggests the emergence of a coping mechanism in which intense stress generates behavioural responses to reduce physiological arousal. Left circling responses were present in both noise treatments as a consequence of a right hemisphere mediated stress response. However, intensified right-biased circling in HA mice suggest an adaptative response during chronic noise exposure, in which greater stressor intensities activate the left hemisphere to initiate interhemispheric inhibition. Left brain activity also suggests a more analytical and motor based response, which is advantageous during uncontrollable and non-life threatening stress exposure. Overall, mining machinery noise produced effects that decrease wild mice welfare and could impact on species with similar characteristics or greater sensitivity to noise stress in the wild.
4.8. Animal Welfare implications

This experiment replicated the noise exposure regime of a 24 hour mining operation, an increasingly common practice in Australia for coal and joint metal (Houghton 1993; Meredith et al. 2014). Although animals have the ability to move away from noxious auditory stimuli in the wild, a reduction in competition in noisy areas and avoidance of predators may contribute to individuals continuing to inhabit areas with high noise levels (Gill et al., 2001; Wright et al., 2007). Therefore, the behavioural and physiological effects observed on this experiment are likely to be experienced in the wild, such as the effects observed in the spleen which could have a major effect on overall immunity.

In this study, females were more sensitive to mining noise, as they presented more stress-related behaviours. Research regarding the relation of individual features such as gender and the stress response generated by anthropogenic noise has not been undertaken to this date. However, this research proved that it is an important area of study, as this interaction can determine the extent of anthropogenic noise damage on individuals. Gender differences in noise-related stress could become a greater welfare concern during sensitive periods such as reproductive seasons of females. Prolonged stress reduces reproduction and compromises immunity (Romero and Butler, 2007). Mining noise and other anthropogenic noises could increase such effects in females, damaging not only their welfare but affecting population dynamics and communities.

Other studies have proposed that animals can habituate to long-term stress, possibly through learning that certain stimuli are in fact, neutral (Bejder et al. 2009; Bowles et al., 1999; Samson et al. 2014). However, in our study habituation (which implies a null effect of stimuli on animals’ well-being) did not occur after 28 days of continuing mining noise exposure. Instead, behavioural responses that were energy and time consuming, such as circling, were generated as a possible attempt to inhibit the stress response. Long-term studies are not a common occurrence in anthropogenic noise research (Nisbet 2000). Therefore, this study provides novel evidence on the effects of noise and the coping mechanisms associated during chronic exposure.

Moreover, wild mice presented behavioural lateralization during circling, which could be attributed to left brain inhibition of a right hemisphere stress response. The change in
circling direction indicated that high amplitudes generated severe stress that needed the appearance of behavioural mechanisms for attenuation. This response could occur in species with similar hearing sensitivity, such as other wild rodents, and may contribute to changes in behaviour that may be maladaptative. This hypothesis needs to be tested in the field.

Overall, mining noise resulted in changes in behaviour and physiology that need to be further explored as they represent an animal welfare concern for wild mice and other small mammals dwelling in mining areas.

4.9. References


Baruch P, Artaud F, Godeheu G, Barbeito L, Glowinski J, and Chéramy A 1988 Substance P and neurokinin A regulate by different mechanisms dopamine release from...


Deak T 2007 From classic aspects of the stress response to neuroinflammation and sickness: implications for individuals and offspring. *International Journal of Comparative Psychology* 20: 96-110


Deutch AY, Tam S-Y, and Roth RH 1985 Footshock and conditioned stress increase 3, 4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. *Brain Research* 333: 143-146.


Keeley T, O’Brien JK, Fanson BG, Masters K, and McGreevy PD 2012 The reproductive cycle of the Tasmanian devil (Sarcophilus harrisii) and factors associated


**Löscher W** 2010 Abnormal circling behavior in rat mutants and its relevance to model specific brain dysfunctions. *Neuroscience & Biobehavioral Reviews* **34**: 31-49.

**Löscher W** 2010 Rat Mutants with Lateralized Rotational Behavior for Studying Disturbances in Cerebral Asymmetries and Their Involvement in Brain Disorders. In: Kalueff AV and Bergenr CL (Eds) *Transgenic and Mutant Tools to Model Brain Disorders* pp 33-64. Springer: New York, USA.


Mazzotti GA, and Boere V 2009 The right ear but not the left ear temperature is related to stress-induced cortisolaemia in the domestic cat (*Felis catus*). *Laterality* 14: 196-204.

McAllister KH, and Dixon AK 1989 Reappraisal of the mouse ethogram according to Grant and Mackintosh - social and aggressive-behavior. *Aggressive Behavior* 15: 86-86.


Neveu PJ, and Moya S 1997 In the mouse, the corticoid stress response depends on lateralization. *Brain Research* 749: 344-346.


Peng Y-d, Guo Y-f, Wu L-t, Li X, Xie W-h, and Dhillon BS 2010 The main projects and development status of noise forecasting and controlling on working face in coal mine. Journal of Coal Science and Engineering (China) 16: 198-205. 
Pomerantz O, Paukner A, and Terkel J 2012 Some stereotypic behaviors in rhesus macaques (Macaca mulatta) are correlated with both perseveration and the ability to cope with acute stressors. Behavioural Brain Research 230: 274-280. 


Slabbekoorn H, and Peet M 2003 Ecology: birds sing at a higher pitch in urban noise—great tits hit the high notes to ensure that their mating calls are heard above the city’s din. *Nature* 424: 267.


Windows Media Player 2009 Windows 7 (Home premium package for PC). Microsoft Windows, Seattle, USA.


### 4.10. Tables and figures

**Table 1.** Machinery acoustically exemplified and sound sources

<table>
<thead>
<tr>
<th>TYPE OF MACHINERY</th>
<th>SOUND SOURCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coal truck</td>
<td><a href="https://www.youtube.com/watch?v=Ph3Mv_19Kb4&amp;feature=related">https://www.youtube.com/watch?v=Ph3Mv_19Kb4&amp;feature=related</a></td>
</tr>
</tbody>
</table>
| Drill             | http://www.sounddogs.com/sound-effects/2107/mp3/322330_SOUNDDOGS__in.mp3  
                    http://www.youtube.com/watch?v=KQH61GQUxSQ |
| Bulldozer         | http://www.sounddogs.com/sound-effects/2665/mp3/1127796_SOUNDDOGS__la.mp3 |
| Shovel            | http://www.youtube.com/watch?v=4x6AV8F11FU&feature=related |
| Dumper            | http://www.sounddogs.com/sound-effects/64/mp3/881485_SOUNDDOGS__in.mp3 |
| Dragline          | https://www.youtube.com/watch?v=nNm6cH6wD0g |
| Blast             | http://www.hark.com/clips/vrfnjkgkyp-explominingcharge-cm046201 |
Table 2. Combinations of machinery and durations of experimental mining noise

<table>
<thead>
<tr>
<th>MACHINERY COMBINATION AND SIMULATED SOUNDSCAPE</th>
<th>DURATION (hours: minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coal Truck, Drill and Bulldozer (heavy duty transport of rock, rock drilling and soil removal)</td>
<td>04:18</td>
</tr>
<tr>
<td>Bulldozer (movement and excavation of soil)</td>
<td>04:13</td>
</tr>
<tr>
<td>Shovel, Bulldozer, Dumper and Crusher (rock collection, transport, rock dumping and crushing)</td>
<td>00:35</td>
</tr>
<tr>
<td>Drills and Dumper (rock drilling and ongoing rock transport)</td>
<td>04:00</td>
</tr>
<tr>
<td>Rock Crusher (crushing of rocks and minerals after collection)</td>
<td>04:16</td>
</tr>
<tr>
<td>Blast (machinery removal progressing into silence (2 h), blast, silence progressing into machinery reactivation (1 h))</td>
<td>03:20</td>
</tr>
<tr>
<td>Dragline (soil digging and dumping)</td>
<td>04:03</td>
</tr>
</tbody>
</table>
Table 3. Durations of individual behaviours and corticosterone concentration in the faeces of mice exposed to mining noise at different amplitudes contrasted by treatment and gender. HA = High noise treatment, LA= Low noise treatment, C= Control treatment. SED = Standard Error of the Difference. Differences between treatments are stated with numbers, whereas differences on treatment*sex interactions are stated by letters. Means that do no share a number/letter are statistically different. *No differences between groups when calculated with Tukey’s post-hoc test

<table>
<thead>
<tr>
<th>HORMONE/BEHAVIOUR</th>
<th>MEANS</th>
<th>SED</th>
<th>P AND F VALUES</th>
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<td></td>
<td>FEMALES</td>
<td>MALES</td>
<td>Treatments</td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td>LA</td>
<td>C</td>
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<tr>
<td>Fecal corticosterone (log₁₀ ng/ml)</td>
<td>2.417</td>
<td>2.470</td>
<td>2.419</td>
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<tr>
<td>(ng/ml)</td>
<td>259.4</td>
<td>299.9</td>
<td>260.02</td>
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<tr>
<td>Hiding in wide tubc (log₁₀+1 s/2h)</td>
<td>1.95&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.43&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.27&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>(s/2h)</td>
<td>88.7</td>
<td>26.1</td>
<td>183.1</td>
</tr>
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<td>Mean (s/2h)</td>
<td>1.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.28&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
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</tr>
<tr>
<td>Hiding in narrow tube</td>
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<tr>
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<td></td>
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<tr>
<td>(log&lt;sub&gt;10&lt;/sub&gt;+1 s/2h)</td>
<td>72.6</td>
<td>18.3</td>
<td>52.6</td>
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<td>Partial hiding wide tube</td>
<td>19.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.08&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>(s/2h)</td>
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<td>Partial hiding narrow tube</td>
<td>1.99</td>
<td>2.27</td>
<td>2.78</td>
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<td>(√s/2h)</td>
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<td></td>
<td></td>
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<tr>
<td>(s/2h)</td>
<td>3.96</td>
<td>5.14</td>
<td>7.78</td>
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<tr>
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<td>289.8</td>
<td>253.8</td>
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<td>(s/2h)</td>
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<td>Nest inactive</td>
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<td>4558</td>
<td>4144</td>
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<td>Activity</td>
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<td>Mean 2</td>
<td>Mean 3</td>
</tr>
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<td>Nest building (s/2h)</td>
<td>8.23</td>
<td>7.62</td>
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<td>Drink (s/2h)</td>
<td>28.19</td>
<td>18.97</td>
<td>35.99</td>
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<td>Feed (s/2h)</td>
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<td>280.04</td>
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<td>Freeze (s/2h)</td>
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<td>13.88</td>
<td>15.61</td>
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<td>Move on grid (s/2h)</td>
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<tr>
<td>(log_{10}+1 s/2h)</td>
<td>1.87 1.48 0.86 0.44 0.36 0.05 0.179</td>
<td>1.33 0.61 0.4 1.35 0.47 0.56 0.220</td>
<td>2.19^a 1.87^a 0.75^{bc} 1.47^{ab} 0.52^c 0.9^{bc} 0.204</td>
</tr>
<tr>
<td>(s/2h)</td>
<td>73.3 29.4 6.16 1.75 1.27 0.11 -</td>
<td>20.47 3.03 1.53 21.43 1.94 2.62 -</td>
<td>152.1 73.5 4.57 28.4 2.3 6.89 -</td>
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<tr>
<td>P = 0.001</td>
<td>F = 7.80</td>
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<td>F = 1.54</td>
<td>F = 0.300</td>
<td>F = 11.9</td>
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†Superscripts are used only when differences between means are present.
**Table 4.** Rates of individual behaviours of mice exposed to mining noise at different amplitudes contrasted by treatments. HA = High noise treatment, LA= Low noise treatment, C= Control treatment. SED = Standard Error of the Difference. Differences between treatments are stated with numbers, whereas differences on treatment*sex interactions are stated by letters. Means that do no share a number/letter are statistically different. *No differences between groups when calculated with Tukey’s post-hoc test

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<tr>
<th>BEHAVIOUR</th>
<th>FEMALES</th>
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<th>MALES</th>
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<th></th>
<th>SED</th>
<th>P AND F VALUES</th>
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<td>HA</td>
<td>LA</td>
<td>C</td>
<td>HA</td>
<td>LA</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hiding in wide tube (bouts/2h)</td>
<td>3.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.46&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.54&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.238</td>
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<td>9.68</td>
<td>4.31</td>
<td>10.43</td>
<td>6.03</td>
<td>6.43</td>
<td>4.81</td>
<td>-</td>
<td>P = 0.05, F = 4.10</td>
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<tr>
<td>Hiding in narrow tube (bouts/2h)</td>
<td>7.14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.64&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.964</td>
<td>P = 0.06, F = 2.93</td>
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<td>4.06</td>
<td>1.99</td>
<td>2.85</td>
<td>1.84</td>
<td>3.33</td>
<td>1.92</td>
<td>0.51</td>
<td>P = 0.58, F = 0.550</td>
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<tr>
<td>Partial hiding</td>
<td>16.45</td>
<td>3.97</td>
<td>8.14</td>
<td>3.37</td>
<td>11.06</td>
<td>3.92</td>
<td>-</td>
<td>P = 0.17, F = 1.93</td>
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<td>(bouts/2h)</td>
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<td>P = 0.001*</td>
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<td>F = 8.64</td>
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*No differences between groups when calculated with Tukey’s post-hoc test.*
<table>
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<tr>
<th>Partial hiding (narrow tube) (√bouts/2h)</th>
<th>0.76</th>
<th>0.73</th>
<th>0.87</th>
<th>0.54</th>
<th>0.63</th>
<th>0.57</th>
<th>0.14</th>
<th>P = 0.89</th>
<th>P = 0.09</th>
<th>P = 0.75</th>
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<tr>
<td>(bouts/2h)</td>
<td>0.58</td>
<td>0.53</td>
<td>0.76</td>
<td>0.29</td>
<td>0.39</td>
<td>0.33</td>
<td>-</td>
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<tr>
<td>Nest active (bouts/2h)</td>
<td>10.67</td>
<td>9.54</td>
<td>10.83</td>
<td>11.46</td>
<td>11.46</td>
<td>8.88</td>
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<tr>
<td>Nest inactive (bouts/2h)</td>
<td>18.85</td>
<td>19.86</td>
<td>18.37</td>
<td>19.42</td>
<td>19.60</td>
<td>18.89</td>
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<td>P = 0.31</td>
<td>P = 0.95</td>
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<tr>
<td>Nest building (√bouts/2h)</td>
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<td>1.74</td>
<td>2.12</td>
<td>1.85</td>
<td>1.83</td>
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<td>P = 0.75</td>
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<tr>
<td>(bouts/2h)</td>
<td>3.06</td>
<td>3.02</td>
<td>4.49</td>
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<td>3.36</td>
<td>4.41</td>
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<tr>
<td>Drink (bouts/2h)</td>
<td>3.35_{ab}</td>
<td>2.93_{ab}</td>
<td>3.9_{a}</td>
<td>1.81_{b}</td>
<td>3.55_{ab}</td>
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<td>Feed (log_{10}+1)</td>
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<td>0.55</td>
<td>0.67</td>
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<td>3.27&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.04&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>Move on grid</td>
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<td>4.47&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.96&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.30&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.93&lt;sup&gt;c&lt;/sup&gt;</td>
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Figure 1. Spectrogram of the coal truck, drill and bulldozer soundtrack broadcast in the experimental rooms corresponding to HA (70-75 dB(A), image A), LA (60-65 dB (A), image B), in comparison to C (no soundtrack played, overall noise ≤ 55 dB (A), image C). Y axis = sound frequency; X axis = time in seconds. The grey saturation along the spectrogram represents the amount of energy contained in the sound wave with greater saturation indicating a greater energy input (observed grey saturation: A>B>C). Measurements were taken at 85 cm from the sound source.
Figure 2. Experimental rooms. HA= High noise treatment; LA=Low Noise treatment; C=Control treatment.
Figure 3. Fecal corticosterone metabolite changes over the seven time periods of the experiment (4 consecutive days per period). P < 0.0001, F = 6.95, SED = 0.062.
5. **CHAPTER 5: The effects of mining machinery noise of different amplitude on tissue morphology and fecal corticosterone of wild mice (Mus musculus)**

5.1. **Introductory statement**

This chapter is the continuation of the results encountered after exposure to different levels of amplitude from the unfiltered mining noise soundtracks. During this experiment we were able to address the changes on organ morphology after chronic and continuous noise exposure, which a broadcasting regime that has not been greatly used on noise exposure studies and that allows the understanding of physiological changes on soundscapes that resemble more the temporal characteristics of anthropogenic noise. Hence, the results obtained here are also a contribution to noise stress research in general.

5.2. **Abstract**

Mining noise is a source of stress for wildlife; however, its physiological effects on individuals are unknown. This study evaluated the effects on organ morphology of exposure of wild mice to mining machinery noise at two amplitude ranges: high (HA; 70-75 dB(A)) and low (LA; 60-65 dB(A)), with a control treatment (below 55 dB (A)) for 3 weeks. LA exposure showed increased levels of fecal corticosterone when compared with C and HA indicating a differential response to chronic stress depending on the stress intensity. Females exposed to high amplitude noise had a smaller adrenal cortex and cortex/medullary ratio compared to controls. This adrenal atrophy and decreased fecal corticosterone in the HA treatment indicates a state of chronic stress during HA exposure that had accentuated effects for females. Females in the high and low noise treatments had smaller kidneys than males in these treatments, suggesting an epinephrine-mediated vasoconstriction. A similar effect was seen in the males’ spleens exposed to both noise treatments, where physiological stress can generate spleen atrophy.

Keywords: anthropogenic noise, mining noise, stress, spleen, adrenal glands, cortex, medulla

5.3. **Introduction**

Anthropogenic noise is a potential source of stress for wild animals (Blickley & Patricelli 2010, Kight & Swaddle 2011, Rabin, *et al.* 2003, Wright, *et al.* 2007). Once perceived, it may disrupt acoustic communication, reproductive success, community dynamics and behavioural patterns (Blickley & Patricelli 2010, Rabin, *et al.* 2003, Wright, *et al.* 2007). While the effects of transport-related noise on animals has been extensively studied (see, for example, Barber, *et al.* (2010)),
other noises with significant damage potential have been overlooked. This is the case for mining machinery noise, which has been recognized as potentially dangerous for bats (Armstrong 2010) and is likely to affect birds’ community dynamics (Read 2000) in the same way as some other mining-related operations (rock crushing) (Saha & Padhy 2011). Hence, mining machinery noise is a research priority for animal impacts.

Even though the effects of mining noise on wild animals have never been explored, it has been studied as a workplace hazard. Mining noise has an energetic low frequency spectrum, in common with other anthropogenic noises (Barber, et al. 2011, Roberts & Roberts 2009, Slabbekoorn & Peet 2003, Slabbekoorn & Ripmeester 2008). However, special machinery such as the rock-cutting drills can generate dominant frequencies between 2-4 kHz (Pal, et al. 2006). Thus, at the workface of some coal mining sites the frequency spectrum can be as ample as 0.32 kHz to 8 kHz (Peng, et al. 2010), which means that mining noise can be perceived by a wide spectrum of animals with different hearing ranges. Mining noise amplitude on site can exceed 90 dB (A) and even reach 110 dB (A) (Ahmad, et al. 2014, Utley 1980). In neighboring commercial areas, it can exceed 80 dB (A) (Mohapatra & Goswami 2012, Saha & Padhy 2011).

Chronic noise exposure in mining areas can diminish fitness through an increased stress response (Romero & Butler 2007). Noise exposure experiments that are not necessarily related to anthropogenic noises have demonstrated negative effects, such as immunosuppression and reproductive dysfunction (Kight & Swaddle 2011). Long term exposure to anthropogenic noise could generate similar responses in free-ranging animals. Such effects could be greatly increased by their inability to move away from affected areas due the increased risk of predation, the density of competitors in other places and the quality of resources in the current area (Gill, et al. 2001, Wright, et al. 2007). Furthermore, the stress responses generated by noise exposure can be moderated by the actions of sex hormones, which can affect stress susceptibility (Haleem, et al. 1988, Heinsbroek, et al. 1991, Kant, et al. 1983, Yoshimura, et al. 2003) and certain traits of the immune response (Klein 2000).

Therefore, in order to evaluate the effects of mining noise on stress it is important to select a model species in which enough physiological information has been generated to facilitate interpretation of the changes observed. A good model can be found in the feral mouse (Mus musculus), which is a common opportunistic species encountered in mining sites (Fox & Fox 2006, León, et al. 2007). The significant number of studies that have observed its behavioral traits (Denmark, et al. 2010, Dielenberg, et al. 2001, Grant & Mackintosh 1963, Lumley, et al. 2000, McAllister & Dixon 1989,
physiological changes related to noise exposure (see review by Kight and Swaddle (2011)), and the existence of techniques to evaluate stress hormones in a non-invasive manner, for example through fecal samples (Touma, et al. 2004), are useful validated methods to test the effects of anthropogenic noise in controlled settings. In order to evaluate anthropogenic noise damage on animals, the use of a common, widespread species is also beneficial because these individuals are the base of the food chain, thus sustaining more susceptible populations, and could also assist in the development of experimental methodologies that may be further used on threatened species (Rabin, et al. 2003).

Wild mice are believed to perceive at least some mining machinery noise by virtue of their hearing range from 2.3 kHz to 92 kHz, measured at 60 dB SPL (Heffner & Masterton 1980, Heffner & Heffner 2007). Although they are acoustically unresponsive to frequencies of 1-2 kHz at 70 to 80 dB SPL (Heffner & Masterton 1980), mice, as with some other animals, experience negative effects when exposed to frequencies below 2 kHz (Alves-Pereira & Castelo Branco 2007), for example a reduction on spleen lymphocytes (Aguas, et al. 1999) or an early onset of autoimmune diseases (Aguas, et al. 1999). This means that they can react adversely to airborne vibrations, even if sound waves cannot be heard. Perception of acoustic stimuli is primarily determined by the amplitude of the sound. Increases in amplitude are directly related to the annoyance level due greater energy being contained in the sound wave (Cone & Hayes 1984). Therefore, we decided to observe the effects of mining machinery noise using two levels of amplitude on female and male mice. We measured wild mice secretory corticosterone levels and changes in organ weight and morphology to evaluate the possible consequences that this noise may have on the individuals and that could ultimately affect their welfare. Effects on mice behaviour are reported separately (Mancera, et al. 2015).

5.4. Materials and methods

Procedures were approved by The University of Queensland’s Animal Ethics Committee (UQAEC Research Approval Number SVS/145/12; UQAEC colony approval number SAS/071/10/BREED (NF)).

Study animals

Thirty-six wild mice (24 females and 12 males) held at the School of Veterinary Science of The University of Queensland were utilized for the study. These animals belonged to the 7th generation
of a wild mice (*Mus musculus*) colony of animals that were captured in the wild and bred in captivity. All animals were born between 4 and 26 April 2012 and were aged approximately 4 months at the beginning of the experiment.

**Diet and animal housing**
Mice were fed Rat and Mouse Pellets (Specialty Feeds, Glen Forrest, Western Australia) *ad libitum*. Males were necessarily individually caged because of the risk of aggression, but females were caged in pairs. Both sexes were in plastic cages of dimensions (cm) 41 long x 27 wide x 16 high, with metallic grid lids on top. A 12:12 light-dark cycle was established (light hours: 06:00 to 18:00 hrs.) with a temperature range of 21-25 °C. Each cage was supplied with bedding (Sanichip, PJ Murphy Forest Products, USA), a wide plastic tube to provide optional nesting and hiding place (5 cm diameter, 10 cm long), a narrow plastic tube mainly used for hiding (2 cm diameter, up to 10 cm long), as well as shredded paper to provide enrichment and nesting material.

**Experimental treatments and generation of simulated mining noise**
Based on the characteristics described for mining noise in the literature (Camargo, *et al.* 2009, Nanda, *et al.* 2009, Nanda, *et al.* 2011, Pathak, *et al.* 1999, Read 2000, Roy & Adhikari 2007, Saha & Padhy 2011, Scott, *et al.* 2010, Utley 1980) and in consultation with a mining geologist, seven pieces of mining machinery were chosen to recreate the soundscape of open-cast mining facilities: coal truck, drill, bulldozer, shovel, dumper, rock crusher and dragline. A blast was added in order to recreate sound impact from the explosions that occur on mining sites. Specialized sound effect sources and on-field recordings were used to select the best acoustic samples of machinery (sources listed on Table 1). Once acquired, noise samples from individual machinery were mixed and overlapped using appropriate software (Audacity: http://audacity.sourceforge.net/). This process generated seven mining noise soundtracks (Table 2) which intended to exemplify the processes that take place under open-cast mining conditions. During the playing of the soundtracks in the experiment, the seven sections were shuffled using appropriate software (Windows Media Player, 2009), in order to avoid habituation to a specific pattern. The blast sequence was played at a random time once a week, consistent with the average blasting schedule on open-cast mining sites in Queensland.

Since mining and related noises have only been studied as a work hazard for human health, amplitude has previously been measured as A-weighted decibels, which take into account human sensitivity to certain frequencies (Möser 2009). We used the same amplitude scale to be consistent
with previous literature. After one week of habituation to the experimental rooms and procedures, three groups of four males and eight females were continuously exposed for three weeks to three experimental treatments in an independent measures design: A Control (C) with animals exposed to no extra auditory stimulation apart from the normal sounds of daily laboratory activities, which was kept to amplitudes below 55 dB (A) (mean value = 53.46 ± 0.44 dB (A)); a High Noise (HA) treatment which had a range of mining noise amplitude of 70-75 dB (A) (mean value = 72.41 ± 1.12 dB (A)); and a Low Noise (LA) treatment which had a range of mining noise amplitudes of 60-65 dB (A) (mean value = 63.75 ± 0.93 dB (A)). These amplitude ranges were set taking as main reference the amount of energy reported in areas in between 0-500 (LA) and 500-1000 meters (HA) in stone mining and crushing operations (Saha & Padhy 2011), as well as the levels of noise registered in commercial and residential areas adjacent to mining facilities, where loudness can reach 88.8 dB(A) and 67 dB (A), respectively (Mohapatra & Goswami 2012). Mean values for amplitude ranges were calculated using recordings of the high and low amplitude rooms and the control room while the noise was being broadcasted. Spectrograms of such recordings are exemplified in figure 1. Recordings were made using a microphone (Sennheiser ME66 condenser shotgun, Germany) connected to a sound data recorder (Tascam DR100 MkII DAT). Then, decibel values were extracted from the recordings in successive samples using the function ‘Sample Data Export’ from the software Audacity®. An increase of 10 decibels is an increase in (noise) power by a factor of 10 (Goelzer et al. 2001). All amplitude levels were measured daily with a sound level meter (Digital Sound Level Meter, Q1362, Dick Smith Electronics).

**Experimental enclosures**

The study took place at the Queensland Animal Science Precinct (QASP) in the University of Queensland, Gatton Campus. A hexagonal facility, with six identical rooms was used to separate the animals into three treatment rooms, each separated from the next by an empty room (Figure 2). Rooms containing mouse treatments were soundproofed using noise and temperature-isolating materials (Reflecta, GID Double Layer, Insulation for sale, NSW, Australia), as well as soundproofing foam (Broadband Studio Acoustic Foam, Swamp Industries Pty Ltd, NSW, Australia) as necessary. Animals were placed in their cages at distances of 80-266 cm from the speakers (System Frequency response: 35 Hz-20 KHz, Output Power (Total) 200 Watt, Speaker system z623, Logitech, Switzerland) (Figure 2).

**Fecal sampling and processing**
Samples were collected daily between 900 to 1100 hrs by removing mice from their cages using the narrow tube as a container in order to avoid direct handling. Fresh feces were selected taking into account moisture and color, whilst discarding those contaminated with urine to ensure that collection occurred within 20 h. Fecal samples from females were from pairs housed together, while fecal samples from males were the excretions of a single animal; thus for both genders the cage was considered the appropriate replicate. All samples were frozen at -20 °C until further processed. For analysis, faecal samples from periods of 4 consecutive days were pooled, generating 7 pooled samples per replicate. Samples were freeze-dried for 5 h, homogenized with a mortar, weighed (to the nearest 0.05±0.0015 g) into a glass scintillation vials and 1 ml of 80% methanol was added. They were then centrifuged at 800 g for 10 min and the extract decanted and frozen at -20 °C until required for analysis.

**Fecal corticosterone measurement**

The concentration of fecal corticosterone metabolites (FCM) was determined by a corticosterone enzyme immunoassay (EIA) technique described previously, but with minor modifications (Keeley, et al. 2012). Microtitre plates pre-coated with goat anti-rabbit globulin (Arbor Assays, USA; A009) were used for this purpose, and corticosterone antibody (stock dilution: 1:200) and horse-radish peroxidase (stock dilution: 1:200) (C Munro, UC Davis, CA, USA) at 1:120,000 and 1:250,000 dilution rates, respectively, 100 µl per well. Fecal samples were diluted in assay buffer prior to analysis (1:7 for females, 1:6 for males) and a serial dilution of a pool of randomly-selected fecal samples demonstrated parallelism with the standard curve. The intra-assay and inter-assay coefficients of variation were 2.65% and 7.09%, respectively. Crossreactivities for the corticosterone EIA antibody were corticosterone 100%, deoxycorticosterone 14.3%, tetrahydrocorticosterone 0.9%, cortisol 0.2%, progesterone 2.7%, testosterone 0.6% and <1% for all other steroids tested. The sample color absorbance values were determined using a microplate spectrophotometer reader (Epoch, Winooski, VT, USA) and appropriate software (Gen 5, Biotek, USA). Test and reference filters of 405 and 630 nm, respectively, were used.

**Tissue collection, processing and evaluation**

As part of the normal end-of-year procedures to avoid a surplus of animals in this colony, which was used to provide animals for teaching, these animals were euthanized by cervical dislocation, immediately followed by assessment of total body weight and the dissection and weighing of spleen, adrenal gland, kidney, liver, brain, pituitary gland and thymus. Afterwards, organs were stored in a 10% neutral buffered formalin solution. Subsequently, tissue slides were generated by
routine processing organs with paraffin embedding, 4 micron sectioning and staining with haematoxylin and eosin (H&E).

Using a binocular microscope Nikon eclipse Ci Microscope (Nikon Instruments Inc, Tokyo, Japan), coupled with the software Nikon Nis Elements Basic Research (Nikon Instruments Inc, Tokyo, Japan), the adrenal cortex and medulla thickness were evaluated in µm) and cortex/medulla ratios calculated. Spleen thickness was also measured using the same technique.

To estimate white matter percentages in the spleen, one representative region was chosen and the total area measured by the software after manual outlining (µm²). Afterwards, white matter within the selected area was visually identified by a trained observer, outlined and measured by the software. Percentages of white matter were calculated by contrasting with the total area selected.

**Statistical analyses**

Fecal corticosterone was analyzed using a Linear Effects Mixed Model (LEM) including the factors mouse, treatment, sex and period of time. Data was transformed using logarithm₁₀, to return residuals to a normal distribution (P < 0.05). When LEM was significant, a post-hoc analysis with Bonferroni corrections was used to compare means. Results were considered significant at P ≤ 0.05. Calculations were performed with the program IBM SPSS statistics, version 20.

Tissue morphology and organ weight were analyzed using General Linear Models (GLM). Tissue and organ variables included the factors sex and treatment, whereas fecal corticosterone included sex, treatment and period of time. Residuals were tested for normal distribution as above, and if not normally distributed (P < 0.05) data was transformed using squared values or logarithm₁₀, whichever most effectively returned residuals to a normal distribution. All calculations were performed with the program Minitab Statistical Software, version 16 (GLM).

Pearson partial correlations between organ characteristics and fecal corticosterone levels at the seventh period were calculated for all animals, as well as males and females separately, taking the total body weight as a covariate. Data was transformed to return data to a normal distribution using squared values when necessary. Results were considered significant at P ≤ 0.05. Calculations were performed with the program IBM SPSS statistics, version 20.

**5.5. Results**

*Fecal corticosterone metabolites (FCM)*
Mice in treatment LA had increased FCM compared with C, with mice in treatment HA at intermediate levels (P= 0.003) (Table 3). Females had higher levels of FCM than males (P < 0.001). FCM concentrations were elevated in the first period (day 1-4) of the study (P = 0.003) (Figure 3).

**Organ weights**
All organ weight contrasts were corrected for body weight. Male mice exposed to both HA and LA treatments had lighter spleens than controls, but in the females noise treatment did not affect spleen weight. By contrast, the kidneys of males were bigger in both HA and LA and were larger than those of females. Compared to male mice, female mice had heavier adrenal glands and thymus, after correction for body weight (P = 0.0001 and P = 0.004, respectively). Females weighed less than males (P < 0.0001), (Table 3).

**Tissue morphometry**
There were no significant overall effects of treatment or sex, but sex by treatment interactions were evident in tissue properties detailed in Table 4. There was a tendency for the males exposed to LA and HA to have a reduced white matter % in their spleens (P = 0.08), compared to males in C. Females had no major change in spleen morphology. However, females in HA had a thinner adrenal cortex than those in C, with those in LA intermediate (P=0.04). Female’s adrenal medulla thickness tended to increase in HA compared to C. In males the converse occurred, cortex thickness tended to increase and medulla thickness, to decrease. Consequently the cortex/medulla ratio decreased for females and tended to increase for males in the HA treatment, compared with those of the same sex in LA and C (P= 0.006).

**Correlations between organ values and fecal corticosterone**
When organ weight was correlated with fecal corticosterone in the seventh period, the adrenal glands showed a positive correlation (Correlation Coefficient (r) = + 0.37, P = 0.03). There was also a negative correlation between spleen weight and corticosterone (r = - 0.37, P = 0.03). Similarly, white matter content was negatively correlated with increasing levels of fecal corticosterone (r = - 0.33, P = 0.05), while thickness of the spleen had a tendency on the same direction (r = -0.3, P = 0.08). Body weight also had a negative relationship with corticosterone (r = -0.41, P = 0.01), (Table 5).

The spleen weight of males was negatively correlated to fecal corticosterone levels in the seventh period (r = -0.76, P = 0.006) as well as medulla thickness (r = -0.82, P = 0.002), whereas the
cortex/medulla ratio had a tendency to be positively correlated with increasing levels of fecal corticosterone \((r = 0.59, P = 0.06)\), (Table 5). Female animals did not show any significant correlation with fecal corticosterone levels at the seventh period.

### 5.6. Discussion

Chronic noise exposure can lead to hormonal changes that are of sufficient magnitude to impact on the development of disease states (Wright, *et al.* 2007). Noise-induced stress is implicated in disorders of the cardiovascular system, learning, memory, motivation, aggression, annoyance, and also in detrimental effects on the immune system (Kight & Swaddle 2011, Prasher 2009). Thus, anthropogenic noise exposure in the wild potentially threatens survival, especially for animals that do not have the opportunity to move away due the lack of resources.

Noise of mining machinery generated stress-related effects in this study, which is consistent with the evidence of mining noise as a noxious experience (Kight & Swaddle 2011, Rabin, *et al.* 2003, Wright, *et al.* 2007). Such responses were further modified by sex, amplitude and the interaction of these factors.

**Fecal corticosterone levels under different levels of intensity: differential response of HPA axis**

Fecal corticosterone levels were higher in females than males, regardless of the treatment. This is believed to be a true effect of gender, rather than housing system, because other studies have proved that in rodents glucocorticoid levels after HPA axis stimulation are naturally higher in females than males, with the latter protected by a testosterone-mediated inhibition of corticotrophin releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus, thus reducing the initiation of the stress response (Haleem, *et al.* 1988, Heinsbroek, *et al.* 1991, Kant, *et al.* 1983, Yoshimura, *et al.* 2003). Likewise, studies have confirmed that for female mice, social housing is likely to reduce stress, whereas males prefer space allowance (Brown & Grunberg 1995) and that even in paired housing conditions, female mice reduce but do not prevent the effects of chronic foot shock stress (3 weeks) (Westenbroek, *et al.* 2005). Thus, even when paired housing may have help reduce HPA arousal on females in this experiment, such inhibition would have been partial and it is more likely that the changes observed on fecal corticosterone are sex-related.

Low amplitude mining noise showed the highest levels of secretory corticosterone when compared with control and high amplitude treatments, whereas HA tended to decrease in a non-significant manner compared to LA, while remaining equal to control. Such activation of the HPA axis via

It has been observed that initial fecal corticosterone increments in response to stress tend to reduce if the stressor is severe and the exposure is chronic (Dallman 1993, Lightman & Harbuz 1993, Martí & Armario 1998, Miller, et al. 2007). Two factors, time of exposure and the stressor’s intensity appear to influence corticosterone levels. In relation to the latter, Pitman, et al. (1988) exposed rats to chronic restraining stress (2 h/day) of either high (four limb prone restrain) or low (immobilization in a tube) intensity, rats exposed to the high intensity group showed a decrease on corticosterone response ([pre-treatment levels] - [post- treatment levels]), whereas the low stress group presented a consistently higher response over 21 days (Pitman, et al. 1988). Even when these results were regarded as a result of highly variable corticosterone response in the low restrain, other authors have also found no decrements on corticosterone levels during mild stress (Hennessy & Levine 1977). Furthermore, white noise pulses (45min/h, 12 h/day, during 8 days) were broadcasted either at 95 dB or 105 dB. After exposure, the 95 dB treatment generated a two-fold increase in plasma corticosterone in rats, whereas the 105 dB exposed rats showed no increase in hormone levels but a much stronger decrease on body weight and food intake, which strongly suggest that high noise intensity was more noxious than the lower, even when corticosterone seemed unaffected (Bijlsma, et al. 2001).

Therefore, we hypothesize that chronic high level stress as in our HA treatment will not necessarily elevate corticosterone levels as much as low intensity stress, as in our LA exposure. It is possible that greater intensities tend to initiate a faster adaptation process to decrease HPA arousal, whereas lesser intensities do not, probably due to differential levels of corticosterone released over time. Organ weight and morphology information obtained on this study confirm the activation of the stress response and the generation of mechanisms to adapt to the noxious stimulus.

**Mining noise as a source of stress: gender effects on HPA arousal and derived changes on related organs**

Body weight was negatively correlated with fecal corticosterone. Alario, et al. (1987) observed decreased body weight gain and food intake during chronic noise exposure (2640 Hz, 30 W, 15 minutes/day, during 30 days). Although corticosterone levels were not measured for noise exposure, during the same study dexamethasone injections (a synthetic glucocorticoid) decreased body weight and food intake compared to controls. It has been established that stress is capable of decreasing
food intake as a consequence of CRH secretion during the stress response (Morley & Levine 1982), which could impact body weight as observed on this experiment.

During noise exposure, specific changes occurred in immune-related organs which differed between males and females. Some organs had weight differences that are part of normal gender dimorphism for rodents; for example, thymus weight was greater in females than males, previously seen in a variety of rodent studies (Eidinger & Garrett 1972, Ito & Hoshino 1963, Robertson 1949). The correlation of adrenal weight with secretory corticosterone may have been accentuated by noise-induced stress, which has been related previously with adrenal hypertrophy in rodents (Zheng, et al. 1997). Studies with female mice exposed to 139-143 dB for 15 minutes per day during for 4 weeks have also demonstrated an increase in adrenal glands weight compared with controls (Anthony, et al. 1959). Moreover, further analysis revealed that females’ adrenal glands were larger than those of males, which is a normal feature in mice (Critchlow, et al. 1963, Halberg & Haus 1960, Westenbroek, et al. 2003), that has been related to estrogens’ positive effect on adrenal cortical secretion (Holzbauer 1957). Thus, both thymus and adrenal weight differences can be related to the aforementioned differences on HPA axis activity in female mice (Haleem, et al. 1988, Heinsbroek, et al. 1991, Kant, et al. 1983, Yoshimura, et al. 2003).

A differential activation of the stress response in males and females may account for the reduction in kidney size in females relatively to males in both noise treatments. Epinephrine, released during stress has vasoconstrictive effects (McCarty, et al. 1988, Tsigos & Chrousos 2002), which can reduce kidney size. For example, when exposed to repetitive social stress (confrontation with a dominant peer), subordinate northern treeshrew males (Tupaia belangeri) had smaller kidneys, an effect believed to be a consequence of renal vasoconstriction following activation of the sympathetic nervous system and consequent catecholamine release (Holst 1972). Although on this study epinephrine release was not measured, the fact that females had a greater stress response than males (Haleem, et al. 1988, Heinsbroek, et al. 1991, Kant, et al. 1983, Yoshimura, et al. 2003) could have increased cathecolamine release in females exposed to noise treatments and shrunk their kidneys, relative to males.

Spleen weight was negatively correlated with fecal corticosterone specially for males and was also reduced in both noise treatments in males, but not females. Spleen atrophy has been considered a consequence of increased glucocorticoids, which triggers lymphocyte apoptosis, thus reducing organ size (Shi, et al. 2003). For instance, it has been noticed that in stress paradigms of
corticosterone release stimulated by ethanol consumption in rodents, levels of glucocorticoids that are naturally attainable can cause apoptosis of mature lymphocytes in the spleen (Collier, et al. 1998). Although ethanol alone can directly induce cellular apoptosis in the spleen in vitro (Slukvin & Jerrells 1995), greater effects can occur in vivo, mediated by glucocorticoids (Collier, et al. 1998). Similarly, spleen atrophy has been observed during tube restrain for 18 h, in which both weight and the number of spleen lymphocytes is reduced (Li, et al. 2012). Rats exposed to a variety of laboratory stressors (restrain, electric shock, constant light, titling, wet bedding and food deprivation) also experienced reduced spleen weight (Camp, et al. 2012). Thus, elevations in corticosterone, as part of the stress response in the noise treatments, may result in the noise initiating the death of lymphocytes and spleen atrophy. The effects observed on spleen morphology therefore suggest a glucocorticoid-mediated atrophy. Although glucocorticoids have been proven to be a factor that generates lymphocyte apoptosis by activating enzymes that induce genetic material damage (Brunetti, et al. 1995, Kirsch, et al. 1998, Weyts, et al. 1998), there is evidence of males being more susceptible to infection and a decrease immune response due the ability of androgens to inhibit several aspects of immunity, whereas estrogens enhance them (Klein 2000). In the spleen and other organs involved in the immune response, high-affinity androgen and estrogen receptors have been detected (Alexander & Stimson 1988, Cutolo, et al. 1996, Kawashima, et al. 1992). Such receptors are able to mediate cellular apoptosis; for example, in the thymus it has been observed that androgens facilitate apoptosis when cells are exposed to substances that break up DNA (Chapman, et al. 1996), while estrogens inhibit cellular death (Vegeto, et al. 1999). In cats, it has been observed that males infected with feline immunodeficiency virus (FIV) exhibit greater rates of lymphocyte apoptosis than females (Hofmann-Lehmann, et al. 1998). Thus, it is possible that natural levels of sexual hormones in this study prevented females from developing spleen atrophy even when the levels of circulating glucocorticoids were high.

For the adrenal gland morphology, in the high amplitude treatment female mice had a smaller adrenal cortex and larger medulla, relative to control females. It has been observed in other studies that during chronic noise exposure of similar intensity (6 h/day for 3-5 months; 70 dB (A)), the adrenal cortex had reduced thickness of the zona glomerulosa and zona fasciculata as well as cell disorganization and depletion of lipid in the first layer of the zona fasciculata (Gannouni, et al. 2014). This has been also observed in rats exposed to textile industry noise (8 h/day for 1-7 months; 92 dB (A)) (Oliveira, et al. 2009). This reduction in lipid availability is regarded as a secondary effect of increased corticosterone production in the initial stress response, which requires lipids to be synthesized in the zona fasciculata (Oliveira, et al. 2009). Both studies also recorded an increase
in the zona reticularis, which specializes in the production of androgens and can be increased in the presence of prolactin. Both hormones are augmented by stress (Robba, et al. 1985, Testa, et al. 1993).

In this study, adrenal cortex thickness was evaluated as a whole and not by zones, as the zona fasciculata and reticularis cannot be distinguished visually on HE stains. Although not all the cortical areas of the adrenal glands are reduced by stress (Gannouni, et al. 2014, Oliveira, et al. 2009), it is possible that the total cortical thickness is significantly reduced by the actions of stress in the zona glomerulosa and fasciculata. Similarly, it has been noted that the adrenal medulla increases during stress, along with increased epinephrine release (McCarty, et al. 1988, Tsigos & Chrousos 2002). Thus, the reduced cortex/medulla ratio is a response to chronic mining noise exposure at high amplitude in females, which results in a decrease in corticosterone production during long periods of stress, and is considered as an adaptative response (Oliveira, et al. 2009).

In male mice the medulla was negatively correlated with increases of fecal corticosterone and cortex/medulla ratio following HA exposure tended to be higher than the control group of male mice, which suggests a reduction of the medulla in unstressed animals. Whereas the adrenal cortex produces glucocorticoids, the medulla controls catecholamine production, that is, epinephrine, norepinephrine and dopamine (Padgett & Glaser 2003). It has been noted that male rats exposed to loud noise repeatedly (100 dB, 6 h per day for 21 days) increased norepinephrine content and norepinephrine-containing granules when compared to controls, whereas epinephrine release was reduced as well as the number of associated granules in epinephrine-producing cells (Gesi, et al. 2002). Since epinephrine-producing cells comprise around 80% of the total population of the gland and are three-fold larger in size than norepinephrine storing cells (Coupland 1965, Kobayashi & Coupland 1993, Tomlinson, et al. 1987), the described changes in epinephrine production and granule content could explain the tendency to a larger cortex/medulla ratio for this gender. This could correlate with the bigger kidneys observed when compared to females under noise treatments, since less epinephrine would prevent the selective shrinkage of this organ.

Overall, this study reports great differences in the stress response produced by mining machinery noise in wild mice depending on gender. Although females were more susceptible to activate the stress response and generate adaptive responses, males experienced immunological effects that were likely corticosterone-dependent and androgen-enhanced.
Moreover, the observed tendencies on fecal corticosterone could be accounted to changes provoked by chronic stress, which are not only physiological, but also, behavioural. Repetitive behaviours or stereotypies decrease glucocorticoid levels by activating inhibitory mechanisms (Cronin, et al. 1985, Duncan 1970, Kennes & De Rycke 1988, Odberg 1989, Pomerantz, et al. 2012). Further exploration of such behavioural regulatory mechanisms has been also reported. It was observed that even when both noise treatments increased the amount of stereotypic circling (animals describing circles during locomotion) directed to the left, it was the high amplitude treatment the one that had the highest rates and durations of total circling (left and right rotations combined), as well as a greater amount of right circling when compared to low amplitudes and control, which implies not only a downregulation of the stress response through behavioural mechanisms, but also an attempt to inhibit the stress response mediated by the right-hemisphere due brain lateralization (Mancera, et al. 2015). Thus, higher noise intensities seem to generate quicker adaptative responses.

5.7. Conclusions
Mining machinery noise exposure had detrimental effects on wild mice organ weight and morphology. The high levels of fecal corticosterone in the low amplitude treatment compared with high amplitude exposure suggest a differential response in stress intensity, in which greater sound energy generates a rapid adaptative response that decreases HPA arousal and glucocorticoid release when compared with low intensities. This may have been due to a high level of total stereotypical circling that has been previously recorded on the high amplitude treatment, which is related to inhibition of stress arousal.(Mancera, et al. 2015). Levels of fecal corticosterone, as well as adrenal glands and thymus weight were greater for females due to gender-based differences in the stress response, which may have stimulated greater sympathetic nervous system activation and decreased kidney weight in females. Males showed spleen atrophy which was probably corticosterone-mediated. Changes in females’ adrenal cortex relate with known adaptative responses to chronic noise exposure. HA exposed males tended to decrease the size of the adrenal medulla, which could affect epinephrine release and storage and explain the lack of kidney shrinkage in males. In conclusion, mining noise exposure affected male and female mice differently, which was probably due to the mediating effects that sex hormones have on different systems of the body.

5.8. References


Alexander J, and Stimson W 1988 Sex hormones and the course of parasitic infection.


Cone J, and Hayes S 1984 Environmental problems/behavioural solutions. Cambridge University Press: California, USA.


Cronin GM, Wiepkema PR, and van Ree JM 1985 Endogenous opioids are involved in abnormal stereotyped behaviours of tethered sows. Neuropeptides 6: 527-530.


Hofmann-Lehmann R, Holznagel E, and Lutz H 1998 Female cats have lower rates of apoptosis in peripheral blood lymphocytes than male cats: Correlation with estradiol-17β, but not with progesterone blood levels. *Veterinary Immunology and Immunopathology* 65: 151-160.


McAllister KH, and Dixon AK 1989 Reappraisal of the mouse ethogram according to Grant and Mackintosh - social and aggressive-behavior. Aggressive Behavior 15: 86-86.


Mohapatra H and Goswami S 2012 Assessment and analysis of noise levels in and around Ib river coalfield, Orissa, India. Journal of Environmental Biology 33: 649-655


Slabbe koorn H, and Peet M 2003 Ecology: birds sing at a higher pitch in urban noise—great tits hit the high notes to ensure that their mating calls are heard above the city’s din. *Nature* 424: 267.


Windows Media Player 2009 Windows 7 Home premium package for PC. Microsoft Windows, Seattle, USA.
## 5.9. Tables and figures

**Table 1. Machinery acoustically exemplified and sound sources**

<table>
<thead>
<tr>
<th>TYPE OF MACHINERY</th>
<th>SOUND SOURCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coal truck</td>
<td><a href="https://www.youtube.com/watch?v=Ph3Mv_L9Kb4&amp;feature=related">https://www.youtube.com/watch?v=Ph3Mv_L9Kb4&amp;feature=related</a></td>
</tr>
</tbody>
</table>
| Drill             | http://www.sounddogs.com/sound-effects/2107/mp3/322330_SOUNDDOGS__in.mp3  
                       | http://www.youtube.com/watch?v=KQH61GQUxSQ |
| Bulldozer         | : http://www.sounddogs.com/sound-effects/2665/mp3/1127796_SOUNDDOGS__la.mp3 |
| Shovel            | http://www.youtube.com/watch?v=4x6AV8F11FU&feature=related |
| Dumper            | http://www.sounddogs.com/sound-effects/64/mp3/881485_SOUNDDOGS__in.mp3 |
| Dragline          | https://www.youtube.com/watch?v=nNm6cH6wD0g |
| Blast             | http://www.hark.com/clips/vrfnjkgkyp-explominingcharge-cm046201 |
Table 2. Combinations of machinery and durations of experimental mining noise

<table>
<thead>
<tr>
<th>MACHINERY COMBINATION AND SIMULATED SOUNDSCAPE</th>
<th>DURATION (hours: minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coal Truck, Drill and Bulldozer (heavy duty transport of rock, rock drilling and soil removal)</td>
<td>04:18</td>
</tr>
<tr>
<td>Bulldozer (movement and excavation of soil)</td>
<td>04:13</td>
</tr>
<tr>
<td>Shovel, Bulldozer, Dumper and Crusher (rock collection, transport, rock dumping and crushing)</td>
<td>00:35</td>
</tr>
<tr>
<td>Drills and Dumper (rock drilling and ongoing rock transport)</td>
<td>04:00</td>
</tr>
<tr>
<td>Rock Crusher (crushing of rocks and minerals after collection)</td>
<td>04:16</td>
</tr>
<tr>
<td>Blast (machinery removal progressing into silence (2 h), blast, silence progressing into machinery reactivation (1 h))</td>
<td>03:20</td>
</tr>
<tr>
<td>Dragline (soil digging and dumping)</td>
<td>04:03</td>
</tr>
</tbody>
</table>
Table 3. Organ weights, corrected for body weight and Fecal Corticosterone Metabolite (FCM) levels of wild mice exposed to mining noise at different amplitudes. SED = Standard Error of the Difference. Organ weights were corrected for total body weight for analysis. Differences between treatments are stated with numbers, whereas differences on treatment*sex interactions are stated by letters. Means that do no share a number/letter are statistically different.

<table>
<thead>
<tr>
<th>ORGAN/ HORMONE</th>
<th>MEANS</th>
<th>SED</th>
<th>P AND F VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEMALES</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td>LA</td>
<td>C</td>
</tr>
<tr>
<td>Fecal corticosterone (log₁₀ ng/ml)</td>
<td>12.41</td>
<td>12.48</td>
<td>2.42</td>
</tr>
<tr>
<td>Fecal corticosterone (ng/ml)</td>
<td>259</td>
<td>299</td>
<td>260</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>0.0214&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.0254&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.0189&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney (g&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.0332&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.0303&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0381&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidney (g)</td>
<td>0.179</td>
<td>0.174</td>
<td>0.195</td>
</tr>
<tr>
<td>Tissue</td>
<td>Sample 1</td>
<td>Sample 2</td>
<td>Sample 3</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Adrenal glands (g)</td>
<td>0.00787</td>
<td>0.00648</td>
<td>0.00769</td>
</tr>
<tr>
<td>Thymus (g)</td>
<td>0.0229</td>
<td>0.0245</td>
<td>0.0188</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>0.640</td>
<td>0.627</td>
<td>0.619</td>
</tr>
<tr>
<td>Brain (g)</td>
<td>0.431</td>
<td>0.434</td>
<td>0.406</td>
</tr>
<tr>
<td>Pituitary gland (g)</td>
<td>0.000331</td>
<td>0.000411</td>
<td>0.00106</td>
</tr>
<tr>
<td>Total body weight (g)</td>
<td>14.37</td>
<td>13.89</td>
<td>14.96</td>
</tr>
</tbody>
</table>
Table 4. Morphological characteristics of adrenal glands and spleen of wild mice exposed to mining noise at different amplitudes contrasted by treatments and sex. F= female, M= male HA = High noise treatment, LA= Low noise treatment, C= Control treatment. SED = Standard Error of the Difference. Differences between treatments are stated with numbers, whereas differences on treatment*sex interactions are stated by letters. Means that do no share a number/letter are statistically different. *No differences between groups when calculated with Tukey’s post-hoc test

<table>
<thead>
<tr>
<th>TISSUE PROPERTY</th>
<th>MEANS</th>
<th>SED</th>
<th>P VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEMALES</td>
<td>MALES</td>
<td>Treatments</td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td>LA</td>
<td>C</td>
</tr>
<tr>
<td>Spleen Thickness (µm)</td>
<td>1085.2</td>
<td>1309.9</td>
<td>1204.0</td>
</tr>
<tr>
<td>White matter (%)</td>
<td>44.51</td>
<td>56.13</td>
<td>48.12</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex thickness (µm)</td>
<td>191.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>235.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>289.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Medulla thickness (µm)</td>
<td>327.7</td>
<td>222.5</td>
<td>226.7</td>
</tr>
<tr>
<td>Cortex/medulla ratio (µm/µm)</td>
<td>0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table 5. Organs, morphological characteristics and total body weight of wild mice exposed to mining noise correlated with fecal corticosterone in the seventh period. Organ weights were corrected for total body weight for analysis.

<table>
<thead>
<tr>
<th>ORGAN/TISSUE PROPERTY</th>
<th>CORRELATION COEFFICIENT (ALL ANIMALS)</th>
<th>P VALUE (ALL ANIMALS)</th>
<th>CORRELATION COEFFICIENT (MALES ONLY)</th>
<th>P VALUE (MALES ONLY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen (g)</td>
<td>-0.37</td>
<td>0.03</td>
<td>-0.76</td>
<td>0.006</td>
</tr>
<tr>
<td>Adrenal glands (g)</td>
<td>0.37</td>
<td>0.03</td>
<td>0.31</td>
<td>0.35</td>
</tr>
<tr>
<td>Kidney (g²)</td>
<td>-0.13</td>
<td>0.47</td>
<td>-0.05</td>
<td>0.9</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>-0.104</td>
<td>0.55</td>
<td>-0.48</td>
<td>0.13</td>
</tr>
<tr>
<td>Brain (g)</td>
<td>0.09</td>
<td>0.62</td>
<td>0.32</td>
<td>0.34</td>
</tr>
<tr>
<td>Pituitary gland (g)</td>
<td>-0.26</td>
<td>0.14</td>
<td>-0.35</td>
<td>0.3</td>
</tr>
<tr>
<td>Thymus (g)</td>
<td>0.09</td>
<td>0.62</td>
<td>-0.04</td>
<td>0.9</td>
</tr>
<tr>
<td>White matter in spleen (%)</td>
<td>-0.33</td>
<td>0.05</td>
<td>-0.43</td>
<td>0.2</td>
</tr>
<tr>
<td>Spleen thickness (µm)</td>
<td>-0.3</td>
<td>0.08</td>
<td>-0.41</td>
<td>0.22</td>
</tr>
<tr>
<td>Cortex (µm)</td>
<td>-0.012</td>
<td>0.94</td>
<td>0.41</td>
<td>0.21</td>
</tr>
<tr>
<td>Medulla (µm)</td>
<td>0.04</td>
<td>0.81</td>
<td>-0.82</td>
<td>0.002</td>
</tr>
<tr>
<td>Cortex/medulla ratio (µm/µm)</td>
<td>0.03</td>
<td>0.87</td>
<td>0.59</td>
<td>0.06</td>
</tr>
<tr>
<td>Total body weight</td>
<td>-0.41</td>
<td>0.01</td>
<td>-0.08</td>
<td>0.81</td>
</tr>
</tbody>
</table>
Figure 1. Spectrogram of coal truck, drill and bulldozer soundtrack broadcasted in the experimental rooms corresponding to HA (70-75 dB(A), image A), LA (60-65 dB (A), image B) and C (no soundtrack played, overall noise ≤ 55 dB (A), image C). Y axis = frequencies; X axis = time in seconds. The grey saturation along the spectrogram represents the amount of energy contained in the sound wave with greater saturation indicating a greater energy input (observed grey saturation: A>B>C). Measurements were taken at 85 cm from the sound source.
Figure 2. Experimental rooms. HA= High noise treatment; LA=Low Noise treatment; C=Control treatment.
Figure 3. Fecal corticosterone metabolite changes over time periods (4 consecutive days per period)
6. **CHAPTER 6: The effects of mining machinery noise of different frequencies on the behaviour, fecal corticosterone and tissue morphology of wild mice (*Mus musculus*)**

6.1. **Introductory statement**

The observations obtained in the study contrasting the effects of different amplitude levels concluded that high amplitudes are the most noxious. This observation set the amplitude standards for the following study, in which high and low frequencies were contrasted. In addition, due the success of the noise exposure methodology used for chronic continuous noise exposure, I broadcast mining noise at different frequencies using the same broadcasting regime as in the last experiment. The results obtained on this experiment served to complement the results obtained by the contrast of amplitudes, providing a greater understanding of the effects of mining machinery noise on small rodents.

6.2. **Abstract**

Mining noise is a potential source of stress for wildlife, but it has unknown physiological effects. This study evaluated the effects of mining machinery noise at two frequency ranges: high (HF > 2 kHz) and low (LF ≤ 2 kHz) on the behaviour, organ morphology and fecal corticosterone of wild mice, compared with a control treatment (no extra auditory stimuli). High frequency mining noise increased fecal corticosterone and decreased partial hiding and nest activity. Females were the most affected, since they had the highest fecal corticosterone levels and a tendency for spleen atrophy, perhaps due to oestrogen-dependent high frequency sensitivity. Stereotyped circling anticlockwise, but not clockwise was increased in female mice exposed to high frequencies compared to low frequencies. Low frequency mining noise increased fecal corticosterone levels in males but not females. In conclusion, mining machinery noise produces stress responses on wild mice that are frequency dependent and require further research.

Keywords: anthropogenic noise, circling, low frequency, high frequency, mining, stress.

6.3. **Introduction**

considered. One example is open-cast mining machinery noise, which has been recognized as potentially dangerous for bats (Armstrong 2010) and proven to affect birds’ community dynamics (Read 2000) in comparable ways with similar industries (rock crushing) (Saha & Padhy 2011). Machinery used in open-cast mining and rock crushing emits most energy at low frequencies, with the commonly used dumper truck having a particularly low frequency (0.25 – 0.5 kHz) and the cooling fan from bulldozers a higher frequency (0.3 - 3.5 kHz) (Vardhan, et al. 2005, Vardhan, et al. 2004). As with other anthropogenic noise, most energy is contained below 2 kHz (Barber, et al. 2011, Roberts & Roberts 2009, Slabbekoorn & Peet 2003, Slabbekoorn & Ripmeester 2008). Rock-cutting drills are also commonly used, generating dominant frequencies between 2 and 4 kHz (Pal, et al. 2006). These acoustic outputs generate b frequency spectrums at the workface of some coal mining sites (0.32 kHz to 8 kHz) (Peng, et al. 2010). Due these frequency characteristics, mining noise can be perceived by a wide arrange of species with different hearing capabilities. In terms of amplitude, mining noise can exceed 90 dB (A) and even reach 110 dB (A) on site (Ahmad, et al. 2014, Utley 1980). In neighboring areas such as forests and residential zones, mining and related industries can exceed 80 dB (A) (Mohapatra & Goswami 2012, Saha & Padhy 2011).

Chronic noise exposure during anthropogenic activities can potentially decrease fitness through the activation of the stress response (Romero & Butler 2007). A great variety of noise exposure experiments have demonstrated negative effects on animals, such as immunosuppression and reproductive dysfunction (Kight & Swaddle 2011). The impact of long term exposure to mining noise could be greatly increased by animals’ inability to move away from affected areas due to increased risk of predation or the low quality of resources in alternative habitats (Gill, et al. 2001, Wright, et al. 2007).

Noise exposure also affects animals’ emotional state; for example, in humans it generates depression and aggression (Ising & Kruppa 2004, Stansfeld 2003) and in rats it results in anxiety and depression (Naqvi, et al. 2012). In pandas, noise exposure increased urinary corticoids, locomotion, distress vocalizations and escape attempts (Owen, et al. 2004). There are also reports of stereotypic behaviour induced by noise exposure. Stereotypies, or repetitive behaviours induced by frustration, repeated attempts to cope, and/or central nervous system dysfunction’ (Mason & Rushen 2008), have been observed as coping mechanisms during noise exposure in casual observations on primates (Patterson-Kane & Farnworth 2006), rodents (Anthony, et al. 1959) and pandas (Powell, et al. 2006). Although most of these behavioural responses have not been
specifically associated with anthropogenic noises, similar reactions could potentially emerge on wildlife in the vicinity of mining facilities.

One of the animals commonly found in mining facilities due its opportunistic nature is the feral mouse, \((Mus\ musculus)\) (Fox & Fox 2006, León, et al. 2007). It is expected that wild mice can perceive some high frequency components of mining machinery noise due to their hearing range of 2.3 to 92 kHz (measured at 60 dB SPL, (Heffner & Masterton 1980, Heffner & Heffner 2007). Thus mining noise above 2 kHz may generate a hearing experience which potentially produces the aforementioned physiological effects related to noise exposure. At the same time, wild mice are unresponsive to frequencies of 1-2 kHz at 70 to 80 dB SPL (Heffner & Masterton 1980), making them apparently unaware of sounds below 2 kHz. Nonetheless, humans and rodents have shown negative effects when exposed to low frequency sound waves, such as changes in the immune system function that could compromise health (Aguas, et al. 1999, Aguas, et al. 1999, Alves-Pereira & Castelo Branco 2007).

Although both audible and inaudible mining noise frequencies could affect wildlife welfare, there is no research comparing the effects of this acoustic characteristic. Therefore, in this experiment, the effects of mining machinery noise at two frequency ranges were examined. The hypothesis was that mining noise would disrupt wild mice behavioural patterns, organ morphology and secretory corticosterone levels, and that those effects would vary with the frequency range experienced.

6.4. Materials and Methods
Procedures were approved by The University of Queensland’s Animal Ethics Committee (UQAEC Research Approval Number CAWE/054/13; UQAEC colony approval number SAS/071/10/BREED (NF))

Study animals
Fifty-seven (34 females and 23 males) held at the University of Queensland were utilized for the study. These animals belonged to the 10\(^{th}\) generation of a wild mice \((Mus\ musculus)\) colony of animals that were captured in the wild and bred in captivity. All animals were born between 9 and 24 February 2013 and were aged approximately 4 months at the beginning of the experiment.

Diet and animal housing
Mice were fed Rat and Mouse Pellets (Specialty Feeds, Glen Forrest, Western Australia) *ad libitum*. Males were necessarily individually caged because of the risk of aggression, but females were caged in pairs. Females were in conventional plastic cages of dimensions (cm) 40 long x 24 wide x 14 high, with metallic grid lids on top, and males in plastic cages 31 long x 14 wide x 12 high. A 12:12 light-dark cycle was established (light hours: 0600 to 1800 hrs) with a temperature range of 21-25 °C. Each cage was supplied with bedding (Sanichip, PJ Murphy Forest Products, USA), plastic tubes mainly used for hiding (2 cm diameter, up to 10 cm long; two for females and one for males), as well as shredded paper to provide enrichment and nesting material.

**Experimental treatments and generation of simulated mining noise**

Based on the characteristics described for mining noise in the literature (Camargo, *et al.* 2009, Nanda, *et al.* 2009, Nanda, *et al.* 2011, Pathak, *et al.* 1999, Read 2000, Roy & Adhikari 2007, Saha & Padhy 2011, Scott, *et al.* 2010, Utley 1980) and in consultation with a mining geologist, seven pieces of mining machinery were chosen to recreate the soundscape of open-cast mining facilities: coal truck, drill, bulldozer, shovel, dumper, rock crusher and dragline. A blast was added in order to recreate sound impact from the explosions that occur on mining sites. Specialized sound effect sources and on-field recordings were used to select the best acoustic samples of machinery (sources listed on Table 1). Once acquired, noise samples from individual machinery were mixed and overlapped using appropriate software (Audacity: http://audacity.sourceforge.net/). This process generated seven main mining noise soundtracks (Table 2) which intended to exemplify the processes that take place under open-cast mining conditions. Once generated, the seven soundtracks were individually processed using the high-pass and low-pass filter functions of the Audacity software to generate two tracks per soundtrack: low frequency (LF, ≤ 2 kHz), and high frequency (HF, > 2 kHz) tracks. This recognized that anthropogenic noise has most of its energy output below 2 kHz (Barber, *et al.* 2011, Roberts & Roberts 2009, Slabbekoorn & Peet 2003, Slabbekoorn & Ripmeester 2008). As most of the energy intensity is contained in low frequencies, the amplify function was used to generate similar levels of output energy in high and low frequency tracks. Figure 1 presents the spectrograms of the soundtrack ‘Coal Truck, Drill and Bulldozer’ and the LF and HF tracks generated from this soundtrack. Spectrograms were obtained from recordings made inside the experimental rooms while tracks were played, using a microphone (Sennheiser ME66 condenser shotgun, Germany) connected to a sound data recorder (Tascam DR100 MkII DAT). During the playing of the soundtracks in the experiment, the correspondent seven sections were shuffled using appropriate software (Windows Media Player, 2009), in order to avoid habituation to
a specific pattern. The blast sequence was played at a random time once a week, consistent with the normal blasting schedule on open-cast mining sites.

Since mining and related noises have been only studied as a work hazard for human health, amplitude has previously been measured as A-weighted decibels, which take into account human sensitivity to certain frequencies (Möser 2009). We used the same amplitude scale to be consistent with previous literature. After one week of habituation to the experimental rooms and procedures, mice were continuously exposed for three weeks to one of the following treatments: Control (C) with 12 females and 8 males exposed to no extra auditory stimulation apart from the normal sounds of daily laboratory activities, which was kept to amplitudes below 55 dB (A) (mean value = 54.34 ± 0.45 dB (A)); the HF treatment where 10 females and 7 males were exposed to the HF tracks created, and a LF treatment where 12 females and 8 males were exposed to the LF tracks. HF and LF treatments had a range of mining noise amplitude of 70-75 dB (A) (HF mean value = 72.93 ± 1.01 dB (A); LF mean value = 71.50 ± 0.79 dB (A)). This amplitude range has been previously proven to activate the stress response in wild mice (Mancera, et al. 2015) and was set taking as a main reference the amount of energy reported in areas in between 500-1000 meters in stone mining and crushing operations (Saha & Padhy 2011), as well as the levels of noise registered in commercial and residential areas adjacent to mining facilities, where loudness can reach 88.8 dB(A) and 67 dB (A), respectively (Mohapatra & Goswami 2012). Mean values for amplitude ranges were calculated using recordings in the high and low frequency rooms while the noise was being broadcasted and the control room. Then, decibel values were extracted from the recordings in successive samples using the function ‘Sample Data Export’ from the software Audacity®. An increase of 10 decibels is an increase in (noise) power by a factor of 10 (Goelzer et al. 2001). All amplitude levels were measured daily with a sound level meter (Digital Sound Level Meter, Q1362, Dick Smith Electronics).

**Experimental enclosures**

The study took place at the Queensland Animal Science Precinct (QASP) in the University of Queensland, Gatton Campus. A hexagonal facility with six identical rooms was used to separate the animals into three treatment rooms, each separated from the next by an empty room (Figure 2). Rooms containing mouse treatments were soundproofed using noise and temperature-isolating materials (Reflecta, GID Double Layer, Insulation for sale, NSW, Australia), as well as soundproofing foam (Broadband Studio Acoustic Foam, Swamp Industries Pty Ltd, NSW, Australia) as necessary. Animals were placed in their cages at distances of 80-266 cm from the
speakers (System Frequency response: 35 Hz-20 KHz, Output Power (Total) 200 Watt, Speaker system z623, Logitech, Switzerland) (Figure 2).

**Fecal sampling and processing**
Samples were collected daily between 900 to 1100 hrs by removing mice from their cages using the narrow tube as a container in order to avoid direct handling. Fresh feces were selected taking into account moisture and color, whilst discarding those contaminated with urine to ensure that collection occurred within 20 h. Fecal samples from females were from pairs of individuals housed together, while fecal samples from males were the excretions of a single animal; thus for both genders the cage was considered the appropriate replicate. All samples were frozen at -20 °C until further processed. For analysis, faecal samples from periods of 4 consecutive days were pooled, generating 7 pooled samples per replicate. Samples were freeze-dried for 5 h, homogenized with a mortar, weighed (to the nearest 0.05±0.0015 g) into glass scintillation vials and 1 ml of 80% methanol was added. They were then centrifuged at 800 g for 10 min and the extract decanted and frozen at -20 °C until required for analysis.

**Fecal corticosterone measurement**
The concentration of fecal corticosterone metabolites (FCM) was determined by a corticosterone enzyme immunoassay (EIA) technique described previously, but with minor modifications (Keeley, et al. 2012). Microtitre plates pre-coated with goat anti-rabbit globulin (Arbor Assays, USA; A009) were used for this purpose, and corticosterone antibody (stock dilution: 1:200) and horse-radish peroxidase (stock dilution: 1:200) (C Munro, UC Davis, CA, USA) at 1:120,000 and 1: 250,000 dilution rates, respectively, 100 µl per well. Fecal samples were diluted in assay buffer prior to analysis (1:7 for females, 1:6 for males) and a serial dilution of a pool of randomly-selected fecal samples demonstrated parallelism with the standard curve. The intra-assay and inter-assay coefficients of variation were 2.65% and 7.09%, respectively. Cross-reactivities for the corticosterone EIA antibody were corticosterone 100%, deoxycorticosterone 14.3%, tetrahydrocorticosterone 0.9%, cortisol 0.2%, progesterone 2.7%, testosterone 0.6% and <1% for all other steroids tested. The sample color absorbance values were determined using a microplate spectrophotometer reader (Epoch, Winooski, VT, USA) and appropriate software (Gen 5, Biotek, USA). Test and reference filters of 405 and 630 nm, respectively, were used.

**Tissue collection, processing and evaluation**
As part of the normal end-of-year procedures to avoid a surplus of animals in this colony which was used to provide animals for teaching, animals were euthanized by cervical dislocation, immediately followed by assessment of total body weight and the dissection and weighing of spleen, adrenal gland and thymus. Afterwards, organs were preserved in a 10% neutral buffered formalin solution. Subsequently, tissue slides were generated by routine paraffin embedding, 4 micron sectioning and staining them with haematoxylin and eosin (H&E). Using a binocular microscope Nikon eclipse Ci Microscope (Nikon Instruments Inc, Tokyo, Japan), coupled with the software Nikon Nis Elements Basic Research (Nikon Instruments Inc, Tokyo, Japan), the adrenal cortex and medulla thickness were evaluated (in µm) and cortex/medulla ratios calculated. Spleen thickness was also measured using the same technique. To estimate white matter percentages in the spleen, one region was randomly chosen and the total area measured by the software after manual outlining (µm²). Afterwards, white matter within the selected area was visually identified by a trained observer, outlined and measured by the software. Percentages of white matter were calculated by contrasting with the total area selected.

**Video recordings and analysis of videos**

Mice behaviour was recorded by 12 surveillance cameras (1 camera/2-4 cages) (model K-32HCF, Kobi CCD, Ashmore, Australia) suspended 60 cm above the cages and connected to a video recorder (Model Lite 900, LG, Yeouido, South Korea). Researchers were only present in the experimental rooms between 0900 to 1100 h for cleaning duties and collection of fecal samples. Animals were recorded continuously throughout the experimental period. From the videos gathered during the experiment, 12 representative days in three blocks (block 1: d 3, 4, 5, 6; block 2: d 12, 13, 19, 20; block 3: d 25, 26, 27, 28) were selected for analysis. In all of these days, noise exposure was continuous; therefore block three represents the last days of noise exposure. A stratified model was designed for behavioural analysis, in which experimental days were selected equally from these blocks. On each day, a period of six hours (one quarter of the day) was observed per animal, without repeating the same quarter twice (Table 3), adding up to a total of 24h/animal/block. Within each period, behaviours were recorded for the first 5 minutes of each hour.

the tube), partial hiding/retreat (leaving the head outside the tube), mouse active or inactive inside a shredded paper nest, nest building (activities related to constructing of the nest, such as gathering and rearranging of paper), drinking, feeding, freezing (mouse remaining still in one position, with the only detectable movement being breathing), grooming self, moving on grid (moving upside down on the bars of the metallic grid lid, but not circling) and circling to the left or to the right (animal describing circles anticlockwise or clockwise during locomotion on the metallic grid lid or on the cage floor). Nest activity was monitored by observing movement of the exterior components of the nest (i.e., movement of the surrounding shredded paper structure or the nesting tube when it was clearly used for this purpose). Due to the unexpected loss of some video-records, only 71.04% of the selected videos were successfully analyzed, but the missing videos were randomly spread across days.

Social behaviours recorded were pushing under another (one mouse moves under the other led by their snout), sniffing each other’s snout, sniffing each other’s anal area, chasing (one animal pursuing the other), allogrooming, mounting (one mouse moves on top of the other either in a copulation-like manner or aligning snout with tail), being inactive socially but at close proximity (mice remain engaged in individual behaviours while touching each other or remaining within one body width), touch and go (one individual touches its partner briefly and runs away), squire (walking while follower keeps its snout close to the leader’s anal area) and push away (one mouse pushes the other in an aggressive manner). Due the unexpected loss of video recordings referred to above, only 82.8% of social behaviour was successfully analyzed.

During replay, the duration and rates of these behaviours were recorded using the free-access software ‘Cowlog’ (Hänninen & Pastell 2009). In order to record rates and start points of durations, a change of behavioural state was determined by an animal spending at least 3 seconds performing a new behaviour. This system was based on preliminary observations of the videos from this experiment and taking into account standard systems for measuring behaviours (Martin & Bateson 1993).

**Statistical analysis**

Fecal corticosterone was analyzed using a Linear Effects Mixed Model (LEM) including the factors mouse, treatment, sex and period of time. Data was transformed using logarithm₁₀, to return residuals to a normal distribution (P < 0.05). When LEM was significant, a post-hoc analysis with
Bonferroni corrections was used to compare means. Results were considered significant at $P \leq 0.05$. Calculations were performed with the program IBM SPSS statistics, version 20.

Tissue morphology and organ weight were analyzed using General Linear Models (GLM). Tissue and organ variables included the factors sex and treatment with body weight as a covariate, whereas fecal corticosterone included sex, treatment and period of time. Residuals were tested for normal distribution as above, and if not normally distributed ($P < 0.05$) data was transformed using square root or logarithm$_{10}$, whichever most effectively returned residuals to a normal distribution. Organ characteristics were correlated with corticosterone concentrations in the final, seventh, period using Pearson's correlations coefficients. All calculations were performed with the program Minitab Statistical Software, version 16.

To observe individual females caged in pairs, during video replay one mouse was initially selected for behaviour recording by the observer, then the video was replayed and the behaviour of the remaining mouse was recorded. Rates and durations of behaviours for pairs of females were analysed as means per cage. In order to eliminate left side skewness of the data due a large amount of zeros, a preliminary analysis was performed to compare inactivity between quarters of time using a Linear Effects Mixed Model (LEM) which included the factors quarter, block, sex, treatment and mouse. The duration of nest inactivity was squared to generate residuals that were normally distributed ($P \geq 0.05$). Calculations were performed with the program IBM SPSS statistics, version 20.

A preliminary analysis was conducted to determine in which six hour periods or quarters mice were the most inactive. Mean inactivity was significantly different between quarters ($P < 0.0001$, $F = 20.8$). From the total time analyzed in a given quarter (5 min * 6 h = 30 min), quarters 3 and 4 (0500 to 1000 h and 1100 to 1600 h, respectively) were the periods with more inactivity ($73.3 \pm 16.8\%$ and $73.9 \pm 16.9\%$ of inactive time, respectively) with no differences within each other ($P = 0.07$), whereas quarters 1 and 2 (1700 to 2200 h and 2300 to 400 h, respectively) were the most active ($53.1 \pm 24.2\%$ and $61.7 \pm 16.6\%$ of inactive time, respectively) and no significant differences when compared ($P = 0.07$). Quarters 1 and 2 therefore differed significantly from quarters 3 and 4 ($P < 0.0001$). Based on this analysis, quarters 1 and 2 were chosen to analyze individual behaviour variables.
Once the most active quarters of time were defined, a LEM which included the factors mouse, treatment, sex, and block was used. Residuals were tested for normal distribution, and if not normally distributed \((P < 0.05)\) data was transformed using square root, logarithm\(_{10}\) or inverse transformation \((1/(x+1))\), whichever most effectively returned residuals to a normal distribution. When normal residuals were not achieved, variables were analyzed using Kruskall–Wallis test, and, if significant \((P < 0.05)\), Mann–Whitney U tests with Bonferroni correction for multiple comparisons were used to contrast mean ranks. For social behaviors, data from all quarters was transformed to binomial values and tested with Binary Logistic Regression (BLR), comparing the presence and absence of behaviours between treatments. Results were considered significant at \(P \leq 0.05\). Calculations were performed with the program IBM SPSS statistics, version 20.

6.5. Results

**Fecal corticosterone metabolites (FCM)**

FCM in females exposed to HF noise was increased compared with females in LF and C, and FCM in C males was slightly lower than in LF and HF treatments \((P < 0.0001)\). Overall, mice in treatment HF tended to have increased FCM compared with C and LF, and females had higher levels than males \((P < 0.0001)\) (Table 4).

**Treatment differences in behaviour**

HF mice tended to spend less time hiding than those exposed to LF and C (respective means 0.48, 1.02, 0.83 s/30 min, \(P = 0.06\)) (Table 4), and HF mice tended to have fewer bouts of hiding \((P = 0.08)\) than mice in LF and C (Table 7). The time spent in partial hiding was also less in HF than LF \((P = 0.02)\) and tended to be less than C \((P = 0.06)\), (Table 5), and there were fewer bouts of partial hiding in HF compared with LF \((P = 0.02)\) and C \((P = 0.06)\) (Table 7). HF mice also spent considerably less time active inside the nest compared to LF and C \((P = 0.02)\) (Table 4) and had fewer nest activity bouts than LF mice \((P = 0.04)\) (Table 6). There was a tendency \((P = 0.09)\) for mice in the C treatment to spend less time inactive in the nest than mice in HF and LF (Table 4) and C mice also had fewer bouts of inactivity in the nest than mice in LF \((P = 0.03)\) (Table 6). There was no effect of treatment on duration of climbing (Table 4), but C mice had more bouts of climbing than mice in LF or HF \((P = 0.004)\) (Table 6). Similarly, there was no effect of treatment on duration of drinking, but HF mice tended to drink more regularly than mice in C and LF \((P = 0.08)\) (Table 7). Mice exposed to LF groomed themselves less than those in C and HF \((P = 0.04)\), (Table 4).
Animals exposed to HF (P < 0.0001) and C (P = 0.01) treatments spent much more time circling to the left than those in LF (Table 5), and HF mice had more bouts of circling than LF (P < 0.0001) (Table 7). There were no treatment effects on time spent in, or number of bouts of, circling to the right. The total time spent circling was highest for mice in HF (P = 0.03) and tended to be higher in C (P = 0.09) than for mice in LF, (Table 5), and there were more circling bouts in HF mice than LF mice (P = 0.037) (Table 7).

Only two variables in social behaviour between females were apparently different when treatments were contrasted. Exposure to LF tended to increase the frequency of mounting behaviour compared to C (number of positive responders/total count: LF = 14/63; HF = 6/43; C = 6/62; Coefficient (r): 1.03; Odds Ratio (OR): 2.79; Confidence Interval (CI): 0.98-7.91; P = 0.05) and decrease touch and go (number of positive responders/total count: LF = 23/63; HF = 18/43; C = 33/62; r: -0.72; OR: 0.49; CI: 0.23-1.03; P= 0.06).

**Gender differences in behaviour**
Female animals spent more time hiding than males (P = 0.008) (Table 4). Compared with males, females also tended to spend more time in nest building (P = 0.06), circling to the left (P = 0.02), total circling (P = 0.001) and a tendency to circle more to the right (P = 0.09), as well as a tendency to spend less time drinking (P = 0.08) and feeding (P = 0.1) (Table 5). Females also had more bouts of climbing (P 0.008), (Table 6) as well as hiding (P = 0.005), drinking (P = 0.06, circling left (P = 0.004) and total circling (P = 0.002), with a tendency to circle more to the right (P = 0.1) (Table 7), compared with males.

**Organ weights and morphology**
Females were lighter than males (mean body weight female: 19.6, male 20.9 g, P < 0.001) (Table 8) and they had thicker adrenal cortices and higher cortex/medulla ratio (Table 9). Spleen weight, corrected for body weight, was less in the HF treatment than LF and C treatments in females, whereas in males those in the C treatment had lighter spleens than those in the HF and LF treatments (P = 0.049) (Table 8).

**Correlations between organ characteristics and fecal corticosterone**
There was a positive correlation between adrenal cortex thickness and fecal corticosterone concentration (P = 0.03). Body weight was negatively correlated with corticosterone concentration
(P = 0.003). Spleen white matter % tended to be negatively correlated with corticosterone concentration (Table 10).

6.6. Discussion
Noise produces a negative experience that generates a great variety of noxious effects by triggering the stress response. A previous experiment found that mining noise broadcast at the amplitudes used in this experiment (70-75 dB) was related to a pronounced stress response that had detrimental effects on organ size and morphology (Mancera, et al. 2015) and generated coping mechanisms that were related to sound intensity (Mancera, et al. 2015). In this experiment, even though mining noise was broadcast at the same amplitude, the different ranges of frequencies had a strong influence on the effects observed.

**Behavioural and physiological effects of high frequency mining noise**
Mice, and particularly female mice, exposed to HF had the highest levels of fecal corticosterone compared to those in control and low frequency exposure. Likewise, males exposed to this treatment had increased levels compared to control males. The mouse hearing range at 60 dB SPL is from 2.3 to 92 kHz (Heffner & Masterton 1980, Heffner & Heffner 2007). At an amplitude of 70 to 80 dB SPL they are acoustically unresponsive to frequencies between 1 and 2 kHz (Heffner & Masterton 1980). Thus, the HF treatment was likely to have been heard whereas the LF treatment was not likely to have been acoustically perceived. Therefore the high frequency mining noise was more likely to produce hormonal and behavioural responses, which in this case were related to stress, as seen by the rise in fecal corticosterone.

The fact that HF female mice showed the highest levels of corticosterone compared not only with other females, but also compared to males in all treatments suggest an important role of gender in frequency susceptibility. Auditory processing is regulated by sex-steroid hormones, with oestrogens one of the principal modulators (Caras 2013). One of the auditory processes influenced by oestrogens is sound frequency perception; it has been proven, for example, that human females have better perceptual sensitivity for high frequency sounds compared to males (Chung, et al. 1983). Evolutionary processes have stimulated the development of a better auditory sensitivity and enhanced detection of threats in females as an adaptative mechanisms to increase survival (Caras 2013), whereas males have a better performance in spatial auditory tasks for improved navigation (Clint, et al. 2012). In studies with rodents, similar differences have been observed. For instance, changes in oestrogen levels during the reproductive cycle affect the behavioural responses of virgin
female mice to synthetic models and recorded pup wriggling calls (three harmonic tones initiated at zero phase at 3.8, 7.6, and 11.4 kHz, 70 dB SPL). Pup call discrimination and responsiveness was worst when circulating levels of 17β-estradiol (E$_2$) are low and best when these levels start to rise in the oestrous phase (Ehret & Schmid 2009). As these calls are between 2 and 10 kHz (Ehret & Bernecker 1986), it is likely that sensitivity to this range of frequencies increases due to an E$_2$ mediated effect.

Although this pup call discrimination process could be regulated by other physiological changes promoted by E$_2$, it has been noted that the expression of the oestrogen receptor α within the mouse cochlea fluctuates during the oestrus cycle, which is hypothesized to be a modulatory mechanism for auditory function, responding to circulating E$_2$ (Charitidi, et al. 2012). Oestrogen also generates differences in Auditory Brainstem Response (ABR), which is the neural response measured on the scalp within 10–15 ms after the presentation of a sound stimulus, long ABR latencies being interpreted as decreased auditory function (Hall 2007). When evaluating oestrogen-related variations in auditory processing, Coleman *et al.* (1994) observed that 90 d ovariectomized female rats shortened their ABR latencies when treated with oestrogen replacement. Taken together, these findings suggest that high frequency mining noise exposure may generate a greater stress response in females, since they would be more sensitive to this auditory input than their male counterparts due to natural differences in oestrogen levels.

In addition, high frequency sensibility mediated by E$_2$ and the consequential increases in fecal corticosterone could be also related to the effects observed on organ weight, where HF females tended to present the lightest spleens. Spleen weight can be reduced by increased glucocorticoid levels, which stimulate apoptosis (Shi, *et al.* 2003). It has been observed that during corticosterone release stimulated by ethanol consumption in rodents, naturally attainable glucocorticoid levels can cause apoptosis of mature lymphocytes in the spleen (Collier, *et al.* 1998) and that even when ethanol can directly induce cellular apoptosis in the spleen *in vitro* (Slukvin & Jerrells 1995), these effects are enhanced *in vivo* due to the effects of glucocorticoids (Collier, *et al.* 1998). Spleen atrophy has been observed after just 18 h of stress during tube restraint, with reductions in both weight and the number of spleen lymphocytes (Li, *et al.* 2012). Thus, elevations in fecal corticosterone, as part of the stress response generated by high frequency mining noise in females, may result in spleen lymphocyte apoptosis and spleen atrophy, which is also suggested by the observed negative correlation between fecal corticosterone and spleen white matter. In males, the spleen had the opposite tendency, tending to increase in mass in the HF treatment. Spleen
hypertrophy is also an observed reaction to stress in mice, associated with increased immune cells recruitment as a reaction to stress (Savignac, et al. 2011). Thus, our results suggest that noise stress is able to disrupt spleen cellular function in a gender-specific manner, with the more extreme stress caused by high frequency noise in females provoking atrophy and the mild stress in males caused by low or high frequency noise resulting in hypertrophy of this organ.

High frequency noise also changed the behaviour of mice. Nest activity and hiding was decreased compared to low frequencies and control. Mice calls above 2 kHz are important for survival, for instance, wriggling calls of between 2 and 10 kHz are emitted by pups to seek attention and stimulate parental care in adult mice, which implies pup retrieval and seeking (Ehret 2013, Ehret & Bernecker 1986). It is likely that some acoustic components of our high frequency treatment contained similar acoustic elements that increased exploration and reduced time active in the nest and hiding. Such calls generate increased responsiveness in mothers (Geissler & Ehret 2004), although virgin females that have co-parented for 1-5 days also respond (Ehret & Schmid 2009), and even females and males with no parental experience, which can display either maternal, avoidance, aggressiveness or exploratory behaviour (Noirot 1972, Smith 1976). Mice in our experiment had shared space with pregnant females and pups prior to the experiment, so it is likely that all animals were acquainted with this acoustic stimulus. Likewise, distress calls (2-30 kHz at 80-90 dB) and defensive calls from females (2-100 kHz at 80 dB) have also similar high frequency characteristics (Ehret 2013) and are likely to elicit locomotion and exploration. In this regard, an increase in exploratory behaviours following background white noise exposure has been observed in chaffinches (Fringilla coelebs) (Quinn, et al. 2006), and in the Stephen’s Kangaroo rat (Dipodomys stephensi) traffic noise was able to elicit foot-drumming, a high-energy expenditure behaviour reserved for the establishment of territory at mating (Shier, et al. 2012). More research into the interpretation and processing of anthropogenic acoustic signals by wild animals is needed to understand its influence on behaviour.

High frequencies did not significantly modify the amount circling behaviour seen in these animals compared to controls. Circling is considered an stereotypy (Löscher 2010, Pycock 1980). Although seen quite commonly in the caged mouse (Weber 2005), it turns into a welfare issue when exacerbated due to stress (Mason & Turner 1993). Circling is the behavioral byproduct of imbalances in dopamine release; the direction of rotational behaviours is determined by hemispheric differences in dopaminergic activity since animals will turn to the side opposite to the hemisphere with greater dopaminergic action (Carlson & Glick 1996, Ishiguro, et al. 2007, Löscher 2010,
Schirmer, et al. 2007). (Baruch, et al. 1988, Biggio, et al. 1978). Stress can increase circling behaviour due the actions of glucocorticoids on dopamine release, as glucocorticoids increase the secretion of enkephalines and tachykinines, (Reiner & Anderson 1990), which in turn, increase nigrostriatal dopamine and locomotion. In previous experiments performed by our group with unfiltered mining noise of different amplitudes, it was seen that circling behaviour increased as a mechanism to inhibit stress arousal during high amplitude exposure (70-75 dB (A)) (Mancera, et al. 2015), as some stereotypies can help to decrease stress arousal and glucocorticoids levels (see, for examples: Duncan (1970), Kennes and De Rycke (1988), Kennes, et al. (1988), Martin, et al. (1991), Pomerantz, et al. (2012)).

In this study where different sets of frequencies were broadcasted at 70-75 dB (A), the unchanged circling patterns observed during high frequency exposure could be related to the importance of frequencies above 2 kHz in rodents (Ehret 2013), making the maintenance of responsiveness to these and similar acoustic cues essential for survival. Under this assumption, the emergence of coping mechanisms could turn into a non-adaptative trait. Supporting this theory is the fact that distress and alarm calls are more resistant to habituation (Bomford & O’Brien 1990), which would implies a continuance of the stress response generated by important acoustic stimuli. Further research needs to establish the mechanisms in which behavioural coping is generated by individuals.

**Behavioral and physiological effects of low frequency mining noise**

Low frequency noise did not increase corticosterone levels in females but fecal corticosterone increased in males compared to their controls. Little effect of low frequency noise on corticosterone has been observed previously, for instance, Waye, et al. (2003) exposed humans to low frequency noise during the night (recorded ventilation noise with added sound pressure levels in the frequency region of 31.5 to 125 Hz). After exposure, the salivary cortisol after awakening was not increased, which differs from the expected normal values (50-100% of increase in free cortisol); glucocorticoid levels after awakening are regarded as a useful index of basal adrenocortical activity (Clow, et al. 2004, Wust, et al. 2000)). In this case, these attenuated levels of cortisol were related to tiredness and negative mood (Waye, et al. 2003). Although in this study authors argued that salivary cortisol may increase later in the day when the exposure is interrupted, other studies have shown that these effects can prevail overtime. For example, data collected from 9-11 year old children before and after the inauguration of a major new airport over a 2 year period showed that while urinary catecholamines and resting blood pressure increased, urinary cortisol levels remained
unaffected (Evans, et al. 1998). Likewise, when twenty male subjects were exposed to low frequencies (6 to 16 Hz at 95-125 dB for 20 min) cortisol levels did not increased, but increments in diastolic blood pressure were present (Danielsson & Landstrom 1985). Taken together, these facts suggest that low frequency exposure is a promoter of hypoglucocorticoidism. However, the fact that LF males showed increased corticosterone levels compared to their controls (unlike LF exposed females) suggests that the stress generated by low frequency noise is also gender related. In chinchillas (McFadden, et al. 1999) and humans (Chung, et al. 1983), it has been observed that males have slightly increased thresholds below 1 to 2 kHz; thus, the higher levels in corticosterone observed in males exposed to this treatment suggest that as hearing is more possible at this frequency range, the physiological response is also present and it generates stress. This observation is corroborated by the tendencies of LF males to develop spleen hypertrophy, as with HF males, which as discussed above, relates to the ability of stress to alter cellular dynamics in the spleen.

Low frequency noise produced a decrease in grooming in mice exposed to this treatment. The suppression of grooming has been observed in mice exposed to a subchronic mild stress paradigm (combination of unpredictable social and environmental stressors), which was hypothesized to a reaction to stress that results in a display of apathy (Ducottet & Belzung 2004). Therefore, the decrease in grooming also supports stressful effects experienced due the low frequency treatment. In addition, circling left (anticlockwise) was decreased in LF when compared to high frequencies and controls. This could be associated with a physiological unresponsiveness, since other stress-related syndromes where unaffected glucocorticoid levels are the general trend in gender-mixed populations such pain (Geiss, et al. 1997), burnout (syndrome characterized by exhaustion, fatigue, headaches, and disturbed sleep patterns) (Pruessner, et al. 1999) and Chronic Fatigue Syndrome (CFS) (Nater, et al. 2008, Wessely & Powell 1989) have also been related to a decrease in activity. Thus, it is possible that low frequencies generate fatigue and decreased locomotion, affecting their predisposition to increment circling behaviour due stress and probably contributing to the decreased grooming as a gender combined group.

**Gender-related effects of mining noise at different frequencies**

Females had higher fecal corticosterone levels than males, which might have been caused by the differences in group size. However, the social housing of females would be expected to reduce rather than increase stress levels and the males would be expected to benefit from the increased space allowance (Brown & Grunberg 1995). Also, when exposed to stress, paired housing can reduce but not prevent an increased stress response in female mice (Westenbroek, et al. 2005).
Previous research has demonstrated that glucocorticoid levels in rodents are naturally higher in females than males due to a testosterone-mediated inhibition of the corticotrophin releasing hormone (CRH) (Haleem, et al. 1988, Heinsbroek, et al. 1991, Kant, et al. 1983, Yoshimura, et al. 2003), therefore we hypothesize that the increased corticosterone levels in females were more likely due to an endogenous gender difference rather than the effects of the housing system.

Supporting this hypothesis, several physiological effects were observed. For instance, females weighed less than males and body weight was negatively correlated with fecal corticosterone. Decreased body weight gain and food intake during chronic noise exposure (2640 Hz, 30 W, 15 minutes/day, during 30 days) has been previously observed (Alario, et al. 1987). Although corticosterone levels were not measured for noise exposure in Alario et al.’s experiment, during the same study dexamethasone injections (a synthetic glucocorticoid) decreased body weight and food intake compared to controls. Likewise, stress decreases food intake as a consequence of CRH secretion (Morley & Levine 1982), which could have impacted on body weight on this study. When behaviour was assessed, females had a tendency to spend more time building the nest. An increase in nesting behaviour is in agreement with gender-specific coping strategies, in which females are more inclined to increase those behaviours that will favor their security and that of their offspring, such as nest building (Taylor, et al. 2000).

Similarly, females circled more to the left than males, a stereotyped response (Löscher 2010, Pycock 1980). The behaviour ‘moving on the grid’ had the same general tendencies as circling, being increased in females. The increase in stereotypic circling for females exposed to noise is another sign of greater stress responsiveness. Circling behaviour is a stereotypy normally seen in the caged mouse (Weber 2005) and as with any other stereotypy, it turns into a welfare issue when exacerbated due to stress (Mason & Turner 1993). Circling is the behavioral byproduct of imbalances in dopamine release; the direction of rotational behaviours is determined by hemispheric differences in dopaminergic activity since animals will turn to the side opposite to the hemisphere with greater dopaminergic action (Carlson & Glick 1996, Ishiguro, et al. 2007, Löscher 2010, Schirmer, et al. 2007). Stress can increase circling behaviour due the actions of glucocorticoids on dopamine release, as glucocorticoids increase the secretion of enkephalines and tachykinines, (Reiner & Anderson 1990), which in turn, increase nigrostriatal dopamine and locomotion (Baruch, et al. 1988, Biggio, et al. 1978). Thus, the increased circling in females was probably a direct consequence of increased glucocorticoid release and the increase in circling directed to the left is likely related to brain lateralization. Brain lateralization is a central tenant in neuroscience that
states that the two brain hemispheres are specialized to control different tasks (Csermely & Regolin 2012). Lateralized behavioural responses are a direct consequence of brain lateralization; the left hemisphere controls the right side of the body and regulates communication, attention, learning and established behaviours, whereas the right hemisphere controls the left side and regulates responses to threatening situations, social interactions and novelty (Ocklenburg & Gunturkun 2012, Ocklenburg, et al. 2013, Rogers 2010). Lateralization is influenced by stress. Several studies have proven that the HPA axis of numerous species will selectively activate the right hemisphere during stress (Rogers 2002). Right hemisphere activity during stress is also seen in dopamine release. When rats are exposed to either controllable or uncontrollable electrical foot-shock, uncontrollably stressed rats had increased dopamine production in the right prefrontal cortex compared with those able to escape and controls (Carlson, et al. 1993). Activation of the prefrontal cortex during stress has been demonstrated to upregulate nigrostriatal dopamine, increasing circling behaviour (Carlson, et al. 1987). Therefore, as circling behaviour establishes its direction in response to dopaminergic imbalances in the brain hemispheres, an increase in circling left implies a greater right hemisphere stress-related response, which was observed for females in this experiment.

Gender differences were also evident in the thickness of the adrenal cortex, greater in females compared to males. Bielohuby, et al. (2007) found similar results in a study comparing the development of the adrenal gland in female and male mice from the 3\textsuperscript{rd} to the 11\textsuperscript{th} week, where the volume and number of cells of the zona fasciculata (ZF) was consistently higher in females than males. Since this ZF comprises 70-75\% of the adrenal cortex (Bielohuby, et al. 2007), a thicker cortex in females is likely an effect of gender variances in this area. In addition, a thicker ZF has also been related to greater adrenal gland weight (Malendowicz 1987), which was not observed on this experiment; however, the adrenal weight is not only affected by ZF volume. Age, for instance, is an important variable, since the appearance of this difference is related with puberty (Parkes & Deanesly 1966). In Wistar rats, differences in absolute weight of the gland appeared at the 70\textsuperscript{th} day of life, whereas the entire adrenal cortex and the ZF were thicker for females from the 49\textsuperscript{th} day, along with increased levels of corticosterone from day 42\textsuperscript{nd} (Majchrzak & Malendowicz 1983). The age of the mice used in this experiment was approximately 4 months, but there were differences up to 15 days between each individual birth, which could have accounted for the observed cortex differences without apparent effects on adrenal gland weight. Likewise, the fact that cortex thickness and corticosterone levels in our study were positively correlated is in agreement with these findings.
6.7. Conclusions

High frequencies increased fecal corticosterone levels and generated behavioural patterns that could be related to high frequency calls from this species, especially in females that also experienced spleen atrophy. Low frequencies generated fecal corticosterone levels that were equal to control in females, but the higher corticosterone levels of males relative to their controls may relate to better low frequency sensitivity in males. Stress-related behavioural patterns were generated are with different stress responses in females than males, which suggests a greater sensitivity in females than males to noise stress. In conclusion, frequencies below and above 2 kHz had differential effects on wild mice that may have important consequences for their welfare and survival and that could be enhanced in wild animals with a greater sensitivity to environmental acoustic stressors.

6.8. Animal Welfare implications

The effects observed during exposure to different frequencies of mining noise have implications for wild mice welfare. Responsiveness to high frequencies and the sustained release of corticosterone, particularly in females, generated changes in behaviour, as animals appeared to be more exploratory. In the wild, corticosterone has also induced dispersal behaviour (Silverin 1997), and depending on the environmental context, a hiding and waiting strategy or a fleeing reaction (Wingfield and Ramenofsky 1997).

Although flight due to mining noise exposure is possible for wild mice, other factors such as predator density and quality of resources can influence wild animals to stay in noisy areas (Gill et al., 2001; Wright et al., 2007). Furthermore, in our study low frequency reduced grooming and locomotion which could encourage animals to stay in mining sites due a negative energy balance that promotes inactivity. The same may occur for other small mammals exposed to different anthropogenic noises.

Females in mining noise areas with prevalent high frequencies or males exposed to low frequencies may be more prone to developing negative physiological outcomes related to long-term stress response, as it could affect immunological aspects, such as the size of the spleen as observed in this study. Even when few studies have examined how inter-population variations influence anthropogenic noise exposure, this experiment gives evidence of gender as a factor worth of study for mining noise and other anthropogenic noises.
Wild mice have a great ability to adapt to changing environments (Auffray, et al. 2009). In real life settings, other animals that are sensitive to human disturbance may be exposed to increasingly long periods of anthropogenic noise, as mining is becoming a 24h operation to maximize productivity and economic yield (Houghton 1993; Meredith et al. 2014). Currently, there are no specific requirements for noise barriers to protect surrounding fauna from mining noise. Materials such as wave trapping barriers, which have shown around 10 dB (A) of noise reduction and can be structurally altered to contain specific frequencies, could be a possible solution to avoid exposure (Pan, et al. 2004).

In conclusion, this study acknowledged mining noise as a possible risk for wild mice and small mammals with similar characteristics. More research needs to be conducted in the field to validate the results observed and explore further consequences for other animal groups.

6.9. References


Carlson JN, and Glick SD 1996 Circling behavior in rodents Motor activity and movement disorders pp 269-300. Springer, New York, USA


Löscher W 2010 Rat Mutants with Lateralized Rotational Behavior for Studying Disturbances in Cerebral Asymmetries and Their Involvement in Brain Disorders. In: Kalueff AV and Bergenr CL (Eds) *Transgenic and Mutant tools to Model Brain Disorders* pp 33-64. Springer: New York, USA.


Malendowicz L 1987 Sex differences in adrenocortical structure and function XXIV. Comparative morphometric studies on adrenal cortex of intact mature male and female rats of different strains. *Cell and Tissue Research* 249: 443-449.

Mancera K, Lisle A, Allavena R, and Phillips C 2015 The effects of mining machinery noise of different amplitude on tissue morphology and fecal corticosterone of wild mice (*Mus musculus*). *Submitted to Plos One*


McAllister KH, and Dixon AK 1989 Reappraisal of the mouse ethogram according to grant and mackintosh - social and aggressive-behavior. *Aggressive Behavior* 15: 86-86.


Mohapatra H and Goswami S 2012 Assessment and analysis of noise levels in and around Ib river coalfield, Orissa, India. *Journal of Environmental Biology* 33:649-655


**Peng YD, Guo YF, Wu LT, Li X, Xie WH, and Dhillon BS** 2010 The main projects and development status of noise forecasting and controlling on working face in coal mine. *Journal of Coal Science and Engineering (China)* 16: 198-205.

**Pomerantz O, Paukner A, and Terkel J** 2012 Some stereotypic behaviors in rhesus macaques (*Macaca mulatta*) are correlated with both perseveration and the ability to cope with acute stressors. *Behavioural Brain Research* 230: 274-280.


Sapolsky RM 1992 *Stress, the aging brain, and the mechanisms of neuron death*. The MIT Press: Cambridge, USA.


Slabbe koorn H, and Peet M 2003 Ecology: birds sing at a higher pitch in urban noise—great tits hit the high notes to ensure that their mating calls are heard above the city’s din. *Nature* 424: 267.


### Tables and figures

**Table 1.** Machinery acoustically exemplified and sound sources

<table>
<thead>
<tr>
<th>TYPE OF MACHINERY</th>
<th>SOUND SOURCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coal truck</td>
<td><a href="https://www.youtube.com/watch?v=Ph3Mv_L9Kb4&amp;feature=related">https://www.youtube.com/watch?v=Ph3Mv_L9Kb4&amp;feature=related</a></td>
</tr>
<tr>
<td>Bulldozer</td>
<td><a href="http://www.sounddogs.com/sound-effects/2665/mp3/1127796_SOUNDDOGS__la.mp3">http://www.sounddogs.com/sound-effects/2665/mp3/1127796_SOUNDDOGS__la.mp3</a></td>
</tr>
<tr>
<td>Shovel</td>
<td><a href="http://www.youtube.com/watch?v=4x6AV8F1FU&amp;feature=related">http://www.youtube.com/watch?v=4x6AV8F1FU&amp;feature=related</a></td>
</tr>
<tr>
<td>Dumper</td>
<td><a href="http://www.sounddogs.com/sound-effects/64/mp3/881485_SOUNDDOGS__in.mp3">http://www.sounddogs.com/sound-effects/64/mp3/881485_SOUNDDOGS__in.mp3</a></td>
</tr>
<tr>
<td>Dragline</td>
<td><a href="https://www.youtube.com/watch?v=nNm6cH6wD0g">https://www.youtube.com/watch?v=nNm6cH6wD0g</a></td>
</tr>
<tr>
<td>Blast</td>
<td><a href="http://www.hark.com/clips/vrfnjkgkyp-explominingcharge-cm046201">http://www.hark.com/clips/vrfnjkgkyp-explominingcharge-cm046201</a></td>
</tr>
</tbody>
</table>
### Table 2: Combinations of machinery and durations of experimental mining noise

<table>
<thead>
<tr>
<th>MACHINERY COMBINATION AND SIMULATED SOUNDSCAPE</th>
<th>DURATION (hours: minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coal Truck, Drill and Bulldozer (heavy duty transport of rock, rock drilling and soil removal)</td>
<td>04:18</td>
</tr>
<tr>
<td>Bulldozer (movement and excavation of soil)</td>
<td>04:13</td>
</tr>
<tr>
<td>Shovel, Bulldozer, Dumper and Crusher (rock collection, transport, rock dumping and crushing)</td>
<td>00:35</td>
</tr>
<tr>
<td>Drills and Dumper (rock drilling and ongoing rock transport)</td>
<td>04:00</td>
</tr>
<tr>
<td>Rock Crusher (crushing of rocks and minerals after collection)</td>
<td>04:16</td>
</tr>
<tr>
<td>Blast (machinery removal progressing into silence (2 h), blast, silence progressing into machinery reactivation (1 h))</td>
<td>03:20</td>
</tr>
<tr>
<td>Dragline (soil digging and dumping)</td>
<td>04:03</td>
</tr>
</tbody>
</table>
Table 3. Selection model for behavioural observations.

<table>
<thead>
<tr>
<th>BLOCK OF DAYS</th>
<th>Quarter 1 1700-2000 hrs</th>
<th>Quarter 2 2300-400 hrs</th>
<th>Quarter 3 500-1000 hrs</th>
<th>Quarter 4 1100-1600 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental DAY 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental DAY 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental DAY 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental DAY 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Corticosterone concentration in the faeces and durations of individual behaviours of mice exposed to mining noise at different frequencies contrasted by treatment and gender. HF = High frequency treatment, LF= Low frequency treatment, C= Control treatment. SED = Standard Error of the Difference. Means that do not share a number/letter are statistically different.

<table>
<thead>
<tr>
<th>HORMONE/ BEHAVIOUR</th>
<th>MEANS</th>
<th>SED</th>
<th>P VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEMALES</td>
<td>MALES</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>LF</td>
<td>C</td>
</tr>
<tr>
<td>Fecal corticosterone (log₁₀ ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>2.584^a</td>
<td>2.405^b</td>
<td>2.420^b</td>
</tr>
<tr>
<td>LF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal corticosterone (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>385.5</td>
<td>254.7</td>
<td>261.2</td>
</tr>
<tr>
<td>LF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hiding (1/(s/30 min+1))</td>
<td>0.61</td>
<td>0.39</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hiding (s/30min)</td>
<td>0.63</td>
<td>1.52</td>
<td>0.93</td>
</tr>
<tr>
<td>Activity</td>
<td>Mean 1</td>
<td>Mean 2</td>
<td>Mean 3</td>
</tr>
<tr>
<td>----------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Nest active ((s/30) min)</td>
<td>6.83</td>
<td>14.33</td>
<td>11.84</td>
</tr>
<tr>
<td>Nest active (s/30 min)</td>
<td>46.58</td>
<td>205.41</td>
<td>140.21</td>
</tr>
<tr>
<td>Nest inactive (s/30 min)</td>
<td>1077.5</td>
<td>984.2</td>
<td>902.7</td>
</tr>
<tr>
<td>Climb ((s/30) min)</td>
<td>12.69</td>
<td>7.47</td>
<td>9.17</td>
</tr>
<tr>
<td>Climb (s/30 min)</td>
<td>161.21</td>
<td>55.79</td>
<td>84.03</td>
</tr>
<tr>
<td>Groom (log(_{10}) s/30 min+1)</td>
<td>1.38</td>
<td>1.19</td>
<td>1.39</td>
</tr>
<tr>
<td>Groom (s/30 min)</td>
<td>23.04</td>
<td>14.74</td>
<td>23.89</td>
</tr>
</tbody>
</table>
Table 5. Durations of individual behaviours of mice exposed to mining noise at different frequencies contrasted with Kruskal-Wallis test (KW). Means were contrasted using the Mann-Whitney test (MW) with Bonferroni correction. HF = High frequency treatment, LF= Low frequency treatment, C= Control treatment.

<table>
<thead>
<tr>
<th>BEHAVIOUR</th>
<th>MEDIAN (s/30 min ± SE)</th>
<th>PKW AND χ²</th>
<th>P MW HF vs C</th>
<th>P MW HF vs LF</th>
<th>P MW LF vs C</th>
<th>P MW and Z GENDER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(MEAN RANK)</td>
<td>HF VS</td>
<td>LF VS</td>
<td>C VS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial hiding*</td>
<td>0.029±0.029</td>
<td>P=0.02</td>
<td>0.06</td>
<td>0.02</td>
<td>1.00</td>
<td>P=0.1</td>
</tr>
<tr>
<td></td>
<td>(77.59)</td>
<td>χ²=8.18</td>
<td></td>
<td></td>
<td></td>
<td>Z=-1.64</td>
</tr>
<tr>
<td>Nest building</td>
<td>7.25</td>
<td>P=0.19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>P=0.06</td>
</tr>
<tr>
<td></td>
<td>(96.28)</td>
<td>χ²=3.24</td>
<td></td>
<td></td>
<td></td>
<td>Z=-1.87</td>
</tr>
<tr>
<td>Drink</td>
<td>6.01</td>
<td>P=0.18</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>P=0.08</td>
</tr>
<tr>
<td></td>
<td>(96.28)</td>
<td>χ²=3.43</td>
<td></td>
<td></td>
<td></td>
<td>Z=-1.76</td>
</tr>
<tr>
<td>Feed</td>
<td>109.17</td>
<td>P=0.17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>P=0.1</td>
</tr>
<tr>
<td></td>
<td>(95.86)</td>
<td>χ²=3.58</td>
<td></td>
<td></td>
<td></td>
<td>Z=-1.49</td>
</tr>
<tr>
<td>Freeze*</td>
<td>28.91±14.32</td>
<td>P=0.13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>P=0.4</td>
</tr>
<tr>
<td></td>
<td>(16.10±10.67)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (Standard Deviation)</td>
<td>Mean (Standard Deviation)</td>
<td>Mean (Standard Deviation)</td>
<td>Mean (Standard Deviation)</td>
<td>P</td>
<td>Z</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------------</td>
<td>---------------------------</td>
<td>---------------------------</td>
<td>---------------------------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>Circle left</td>
<td>91.25±22.59</td>
<td>4.83±2.76</td>
<td>25.32±7.40</td>
<td>52.87±11.03</td>
<td>P 0.8</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td></td>
<td>(101.29)</td>
<td>(71.13)</td>
<td>(92.26)</td>
<td>(98.17)</td>
<td>&lt;0.00001</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Z -3.05</td>
<td></td>
</tr>
<tr>
<td>Circle right</td>
<td>9.81±3.43</td>
<td>11.57±4.71</td>
<td>8.28±3.21</td>
<td>13.46±3.47</td>
<td>P 0.84</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(89.03)</td>
<td>(88.40)</td>
<td>(85.35)</td>
<td>(92.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Circling</td>
<td>101.06±23.98</td>
<td>16.40±5.46</td>
<td>33.62±7.86</td>
<td>2.55</td>
<td>P 0.02</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>(96.59)</td>
<td>(74.98)</td>
<td>(92.34)</td>
<td>(99.78)</td>
<td></td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Medians were zero, therefore mean values were provided.
Table 6. Rates of individual behaviours of mice exposed to mining noise at different frequencies contrasted by treatment and gender. HF = High frequency treatment, LF= Low frequency treatment, C= Control treatment. SED = Standard Error of the Difference. Means that do not share a number/letter are statistically different. There were no significant treatment x sex interactions (P ≥ 0.11).

<table>
<thead>
<tr>
<th>HORMONE/BEHAVIOUR</th>
<th>MEANS</th>
<th>SED</th>
<th>P VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEMALES</td>
<td></td>
<td>MALES</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>LF</td>
<td>C</td>
</tr>
<tr>
<td>Nest active (log_{10} bouts/30 min +1)</td>
<td>0.41</td>
<td>0.71</td>
<td>0.55</td>
</tr>
<tr>
<td>Neat active (bouts /30 min)</td>
<td>1.55</td>
<td>4.08</td>
<td>2.58</td>
</tr>
<tr>
<td>Nest inactive (bouts/ 30 min)</td>
<td>4.85</td>
<td>5.23</td>
<td>4.09</td>
</tr>
<tr>
<td>Climb (log_{10} bouts/ 30 min + 1)</td>
<td>0.76</td>
<td>0.54</td>
<td>1.19</td>
</tr>
<tr>
<td>Climb (bouts/30 min)</td>
<td>4.81</td>
<td>2.45</td>
<td>14.49</td>
</tr>
<tr>
<td>Feed (bouts /30 min)</td>
<td>1.39</td>
<td>1.21</td>
<td>1.67</td>
</tr>
<tr>
<td>Groom (log_{10} Bouts/ 30 min +1)</td>
<td>0.36</td>
<td>0.42</td>
<td>0.39</td>
</tr>
<tr>
<td>Groom (bouts/ 30 min)</td>
<td>1.29</td>
<td>1.63</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Table 7. Rates of individual behaviours of mice exposed to mining noise at different frequencies contrasted with Kruskal-Wallis test (KW). Means were contrasted using the Mann-Whitney test (MW) with Bonferroni correction. HF = High frequency treatment, LF= Low frequency treatment, C= Control treatment.

<table>
<thead>
<tr>
<th>BEHAVIOUR</th>
<th>MEAN* ( bouts/30 min ± SE) (MEAN RANK)</th>
<th>P_{KW}</th>
<th>P_{MW}</th>
<th>P_{MW}</th>
<th>P_{MW} AND Z</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HF</td>
<td>HF vs C</td>
<td>LF vs C</td>
<td>GENDER</td>
</tr>
<tr>
<td>Hiding</td>
<td>0.88±0.21 (74.89)</td>
<td>1.61±0.32 (93.59)</td>
<td>1.50±0.28 (91.88)</td>
<td>1.78±0.29 (98.74)</td>
<td>1.01±0.16 (78.37)</td>
</tr>
<tr>
<td>Partial hiding</td>
<td>0.010±0.009 (77.59)</td>
<td>0.14±0.04 (32.75)</td>
<td>0.24±0.09 (90.48)</td>
<td>0.11±0.03 (90.99)</td>
<td>0.16±0.06 (84.67)</td>
</tr>
<tr>
<td>Nest building</td>
<td>1.47±0.25 (88.41)</td>
<td>1.82±0.27 (96.31)</td>
<td>1.06±0.19 (78.08)</td>
<td>1.41±0.17 (92.13)</td>
<td>1.48±0.21 (83.74)</td>
</tr>
<tr>
<td>Drink</td>
<td>1.41±0.25 (96.30)</td>
<td>0.74±0.12 (76.57)</td>
<td>1.04±0.13 (91.01)</td>
<td>1.07±0.11 (95.10)</td>
<td>1.02±0.16 (81.33)</td>
</tr>
<tr>
<td>Freeze</td>
<td>0.68±0.27 (91.53)</td>
<td>0.45±0.14 (91.81)</td>
<td>0.14±0.071 (79.94)</td>
<td>0.32±0.09 (89.68)</td>
<td>0.48±0.16 (85.73)</td>
</tr>
<tr>
<td></td>
<td>Circle left</td>
<td>Circle right</td>
<td>Total circling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
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<td>------------------</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>44.26± 11.02</td>
<td>2.39±1.37</td>
<td>8.94± 3.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(101.83)</td>
<td>(71.39)</td>
<td>(91.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23.49±5.21</td>
<td>23.49±5.21</td>
<td>11.72±5.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(97.57)</td>
<td>(79.32)</td>
<td>(79.32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P = 0.6</td>
<td>&lt;0.0001</td>
<td>P = 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>χ² = 15.8</td>
<td>Z = -2.88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.72±5.11</td>
<td>11.72±5.11</td>
<td>11.72±5.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(79.32)</td>
<td>(79.32)</td>
<td>(79.32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P = 0.02</td>
<td>P = 0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>χ² = -0.0077</td>
<td>Z = -1.63</td>
<td></td>
<td></td>
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<td>-</td>
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<td>-</td>
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<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>P = 0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>Z = -3.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All medians were equal to zero, therefore mean values are provided.*
Table 8. Organ weights, corrected for body weight and Fecal Corticosterone Metabolite (FCM) levels of wild mice exposed to mining noise at different frequencies. F= female, M= male, HA = High noise treatment, LA= Low noise treatment, C= Control treatment, SED = Standard Error of the Difference. Differences between treatments are stated with numbers, whereas differences on treatment*sex interactions are stated by letters. Means that do no share a number/letter are statistically different. *No differences between groups when calculated with Tukey’s post-hoc test.

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>MEANS</th>
<th>SED</th>
<th>P AND F VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEMALES</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MALES</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>LF</td>
<td>C</td>
</tr>
<tr>
<td>Total body weight ( √g)</td>
<td>4.29</td>
<td>4.84</td>
<td>4.11</td>
</tr>
<tr>
<td>Total Body weight (g)</td>
<td>18.38</td>
<td>23.49</td>
<td>16.87</td>
</tr>
<tr>
<td>Spleen (log_{10} g)</td>
<td>-1.63</td>
<td>-1.53</td>
<td>-1.50</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>0.023</td>
<td>0.030</td>
<td>0.032</td>
</tr>
<tr>
<td>Adrenal glands (log_{10} g)</td>
<td>-1.73</td>
<td>-1.82</td>
<td>-1.98</td>
</tr>
<tr>
<td>Adrenal glands (g)</td>
<td>0.019</td>
<td>0.015</td>
<td>0.010</td>
</tr>
<tr>
<td>Thymus (log_{10} g)</td>
<td>-1.65</td>
<td>-1.71</td>
<td>-1.65</td>
</tr>
<tr>
<td>Thymus (g)</td>
<td>F = 0.29</td>
<td>F = 1.34</td>
<td>F = 0.01</td>
</tr>
<tr>
<td></td>
<td>0.022</td>
<td>0.019</td>
<td>0.022</td>
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<tr>
<td>-------</td>
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<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Thymus (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 9. Morphological characteristics of adrenal glands and spleen of wild mice exposed to mining noise at different frequencies contrasted by treatments and sex. F= female, M= male, HA = High noise treatment, LA= Low noise treatment, C= Control treatment, SED = Standard Error of the Difference. Differences between treatments are stated with numbers, whereas differences on treatment*sex interactions are stated by letters. Means that do no share a number/letter are statistically different.

<table>
<thead>
<tr>
<th>TISSUE PROPERTY</th>
<th>MEANS</th>
<th>SED</th>
<th>P AND F VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEMALES</td>
<td></td>
<td>MALES</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>LF</td>
<td>C</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness (µm)</td>
<td>1054.3</td>
<td>1255.5</td>
<td>1243.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White matter (%)</td>
<td>56.96</td>
<td>59.44</td>
<td>52.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal Gland</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex (µm)</td>
<td>309.6</td>
<td>324.5</td>
<td>306.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medulla (µm)</td>
<td>317.2</td>
<td>275.2</td>
<td>275.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex/medulla ratio (√(µm/µm))</td>
<td>1.02</td>
<td>1.32</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 10. Organs, morphological characteristics and total body weight of wild mice exposed to mining noise of different frequencies correlated with fecal corticosterone in the seventh period. Organ weights were corrected for total body weight for analysis.

<table>
<thead>
<tr>
<th>ORGAN/TISSUE PROPERTY</th>
<th>PEARSON CORRELATION COEFFICIENT</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>-0.38</td>
<td>0.003</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>-0.07</td>
<td>0.63</td>
</tr>
<tr>
<td>Adrenal glands (g)</td>
<td>0.15</td>
<td>0.302</td>
</tr>
<tr>
<td>Thymus (g)</td>
<td>0.12</td>
<td>0.37</td>
</tr>
<tr>
<td>White matter in spleen (%)</td>
<td>-0.23</td>
<td>0.08</td>
</tr>
<tr>
<td>Spleen thickness (µm)</td>
<td>-0.15</td>
<td>0.27</td>
</tr>
<tr>
<td>Cortex (µm)</td>
<td>0.31</td>
<td>0.03</td>
</tr>
<tr>
<td>Medulla (µm)</td>
<td>0.11</td>
<td>0.44</td>
</tr>
<tr>
<td>Cortex/medulla ratio (µm/µm)</td>
<td>0.22</td>
<td>0.14</td>
</tr>
</tbody>
</table>
Figure 1. Spectrogram of coal truck, drill and bulldozer soundtrack broadcasted in the experimental rooms corresponding to HF (> 2 kHz, 70-75 dB (A), image A), LF (≤ 2 kHz, 70-75 dB (A), image B), C (no soundtrack played, overall noise ≤ 55 dB (A), image C). Image D represents the original unfiltered soundtrack (70-75 dB (A)). Y axis = frequencies; X axis = time in seconds. The grey saturation along the spectrogram represents the amount of energy contained in the sound wave with greater saturation indicating a greater energy input. Measurements were taken at 85 cm from the sound source.
Figure 2. Experimental rooms setting. HF= High frequency treatment; LF=Low frequency treatment; C=Control treatment.
CHAPTER 7: General discussion

Conservation management plans necessary for the protection of small and non-charismatic animals is becoming more affordable and equally important to that for charismatic animals (Fisher 2011), however, little research has been done on the effects that anthropogenic stressors may generate on these kind of species. This research project contributes to the study of mining noise, an anthropogenic noise poorly studied, using two animal models (wild mice and EBT lizard) which allowed the fulfillment of the following goals: 1) the evaluation of the behavioural and physiological effects produced by mining noise 2) the relation of observed effects to distinctive amplitudes and frequencies and 3) the validation of behavioural and physiological markers of stress and individual welfare during anthropogenic noise exposure in a controlled environment.

7.1. EBT lizards and mining noise: a novel methodology for stress assessment and the importance of noise frequency and lateralized behavior responses

The first part of this project (Chapter 1) comprised the development of a novel methodology to evaluate EBT lizard’s behaviour in relation to controlled stress exposure. Although the EBT lizards appeared mostly inactive during testing, the evaluation of behaviours during replay allowed us to analyze in depth the use of space and movement patterns, which facilitated the generation of an ethogram to evaluate stress that has potential use in other areas, such as the development of protocols to evaluate reptile welfare in the exotic pet industry. As the development of an effective methodology required exposure to different kinds of stimuli for comparative purposes, we were able to generate information related to EBT lizards welfare during road transport for the pet industry, which is also a rarely studied topic that can help improve conditions during transport, as well as increase overall welfare standards in captivity.

The most significant avoidance patterns were related to cold exposure, which confirms the importance of temperature for this animal group. However, some factors that also vary in road transport and could influence the behavioural control of temperature, such as time of the day, food and space allowance, were not explored and should be included in further research. Such variables could emphasize the need for accessibility to different temperatures during transport and the creation of a thermal gradient within the cages and final enclosures.
Furthermore, this study was fundamental for the subsequent experiments on mining noise exposure, as it allowed us to learn and integrate sound processing and broadcasting techniques and establish adequate techniques to measure amplitudes and filter specific sets of frequencies for analysis. Once the methodology for reptile behaviour assessment and improved noise exposure techniques were generated, these tools were utilized to determine EBT lizards’ behavioural patterns during mining noise exposure. In addition to the usefulness of the ethogram developed in the first stage of this project, this second experiment revealed the relevance of behavioural lateralization in the evaluation of reptile welfare.

As lizards lack a corpus callosum (the structure in charge of connecting brain hemispheres while allowing communication), their lateralization is considered to be “true lateralization”, since no influence or inhibition of the opposite hemisphere can be exercised when one of them is active (Deckel 1995). Indeed, it was evident to us that left/right hemisphere behaviours were prevalent during noise exposure as a normal feature of the stress response (Rogers 2010) and that high frequencies at high amplitudes tended to be more noxious. Hence, behavioural lateralization is a feature that could be included to assess the effects of other sources of stress in captivity (such as light, temperature, enrichment elements and handling). Equally, lateralization could be used to determine levels of stress in the wild by the development of systems such as collars sensitive to lateralized movements. These kind of devices have been used, for instance, with elephants, in which an accelerometer attached to a collar is able to determine lateral positioning (Soltis, et al. 2012). The inclusion of behavioural lateralization for the evaluation of animals’ well-being is a concept that is already being used in production and domestic animals (Rogers 2010) and that could provide an easy tool to understand the quality of the interactions that reptiles have with their natural environment.

In addition, EBT lizards’ responsiveness to high frequencies is possibly related to the evolutionary and behavioural significance of these acoustic cues, as they are related to distress and predator calls heard in the wild. The possibility of mining noise or any other anthropogenic noise as a generator of the stress response due to sound mimicking is a prospect that needs further evaluation, since it has been observed that other animals, such as the Stephen’s Kangaroo rat (Dipodomys stephensi) behave aggressively to traffic noise playbacks (Shier, et al. 2012) and chaffinches (Fringilla coelebs) increase their exploratory behaviour in presence of background white noise (Quinn, et al. 2006). Thus, anthropogenic noise research needs to compare behavioural reactions to natural and
artificial acoustic stimuli as artificial soundscapes may generate costly behavioural and physiological responses on free-ranging animals.

7.2. Wild mice and mining noise: behavioral coping due noise intensity

For the third stage of this research (Chapters 3-5), wild mice were used as models for small mammals due their abundance and their great capacity to adapt. It is fair to assume that the effects that this species experiences due to mining noise exposure would be magnified in animals that are not as physiologically resilient to sudden changes in the environment and/or are bonded to specific resources, as it is true for other rodents (Auffray, et al. 2009). In Australia, many wildlife species such as the sandhill dunnart (*Sminthopsis psammophila*), which is listed as vulnerable, have habitats that overlap with mining exploitation sites (Churchill & Australia 2001, McLean, et al. 2014). Thus, the knowledge provided by this research can be a tool to increase conservation efforts for this and other endangered species under similar pressures.

In addition, an aspect of our wild mice studies that is unique amongst other noise exposure experiments is the chronic, continuous and randomized noise exposure in laboratory settings. Most of the noise exposure studies in the literature are performed during short periods of time or lack continuity when chronic (for examples see, Kight and Swaddle (2011)). As we wanted to replicate the soundscape of Australian mining sites, we designed an exposure model that also included the temporal component of mining operations, which is also relevant to other man-made soundscapes. Furthermore, unlike other anthropogenic noise studies conducted in the wild, we were able to control acoustic characteristics, as well as factors that could generate additional stress (such as space allowance and animal handling), thus enabling us to perform an in depth evaluation of the contribution of different levels of perceived intensity and pitch.

Unlike the mining noise exposure experiment performed on EBT lizards and in order to simplify the number of variables to control during long term exposure, we generated two experiments to evaluate the effects of amplitudes and frequencies separately. Our first wild mice study exemplified the amplitude levels that have been recorded up to 1000 meters from mining operations and similar industries. It was observed that even when both amplitudes generated stereotypic circling behaviour, the high amplitude treatment increased it the most while tending to reduce fecal corticosterone; thus evidence indicates that circling stereotypies may be used as a mechanism to reduce stress arousal. Although the idea of stereotypical behaviour as a means to reduce
glucocorticoid levels has been suggested before (Mason 1991), this research confirms that in fact, certain kind of stereotypies have a function in animals’ physiology that could be adaptative and beneficial. As dopamine plays an important role by mediating the intensity and direction of circling, further research should focus on the assessment of dopamine release and the expression of its receptors in the brain to confirm the role of this catecholamine in the stress response generated by mining noise. As well, the interaction of glucocorticoids and dopamine in the generation of rotating stereotypies should be further tested with the use of other stress paradigms to elucidate if this behavioural pattern is exclusive for noise exposure or could be also seen during other kinds of environmental stress. Since dopamine is related to motor control disorders such as Parkinson’s disease (Lang & Lozano 1998), the possibility of mining noise generating the development of similar symptomatology deserves further research, especially as it is known that low frequency exposure can generate imbalance in rodents (Tamura, et al. 2012) which may be related to deficiencies in dopamine release.

Similarly to the EBT lizards’ experiments, behavioural lateralization played an important part in the stereotypies observed. As the right hemisphere controls stress responses, the increases in left circling detected for both noise treatments were a ratification of mining noise as a noxious experience, as it confirmed a stress response/right hemisphere relation. Moreover, the fact that only high amplitude noise increased right circling and presumed left hemisphere activation asserts the complexity of lateralization in mammals, as the interhemispheric communication mediated by the corpus callosum (in contrast with reptiles) allows the inhibition of the right hemisphere responses through left hemispheric action (Denenberg, et al. 1986). The need for these mechanisms to reduce stress arousal is evidence of the magnitude of the annoyance experienced at different intensities that can also be used to assess stress under diverse environmental variables, where the degree of interhemispheric inhibition is relevant.

7.3. **Lateralization and motivational approach: emotions as part of acoustic information processing**

The left hemisphere/ right circling activation observed has as well has some other interesting connotations that require further research. It has been widely accepted that negative emotions and stress arousal are processed by the right hemisphere while the left brain controls positive feedback (Davidson 1995, Rogers 2010). Nonetheless, new findings suggest that the left hemisphere rather negotiates approach-related responses while the right hemisphere regulates withdrawal, regardless
of the associated emotional valence (Davidson, et al. 2000, Sutton & Davidson 1997). For instance, aggression is a behaviour closely related to anger, which is regarded as an emotion of negative valence (Harmon-Jones, et al. 2013). In humans, extensive evidence suggests that anger leading to aggression activates the left hemisphere (Carver & Harmon-Jones 2009, Harmon-Jones 2003, Harmon-Jones, et al. 2013), for example in verbal attacks (Harmon-Jones & Sigelman 2001) or during physical aggression delivered by conspecifics or computers delivering high pressure air blasts (Verona, et al. 2009).

In animals, aggression could determine if motivational approach (and possibly, left hemisphere dominance) is present (Harmon-Jones, et al. 2013). Offensive aggression involves physical attack without escape attempts as the primary option (Moyer 1976), and is consistent with motivational approach. Rats engaged in conspecific aggression will even cross an electrified grid to attack, portraying high motivation (Lagerspetz 1969). Likewise, offensive aggression is also related with more locomotion, with high-exploratory mice being more active and offensively aggressive when exposed to an intruder than low-exploratory individuals (Kazlauckas, et al. 2005). Such increased locomotion can be dopamine mediated, since offensive animals increase their dopamine release during confrontations (Ferrari, et al. 2003) as this catecholamine, along with other neuropeptides, mediates aggressiveness (Narvaez & de Almeida 2014). Likewise, motivational approach can be triggered by anxious apprehension, defined as a sense of uncontrollability and expectancy where a negative event is anticipated and the individual prepares for coping (Barlow 1991). In humans exposed to a narrative task to stimulate anxious apprehension, left frontal hemisphere activity is greater compared with controls (Heller, et al. 1997). In non-human animals, stress can generate reactions similar to anxious apprehension. For instance, rats conditioned to expect negative outcomes by exposure to an attractive odour followed by LiCl injections, increased dopamine levels in the left core of the nucleus accumbens, expanding to the shell in a second exposure, thus exhibiting left hemisphere dominance due to conditioned stress (Besson & Louilot 1995).

Aggressiveness and anxiety can potentially be activated through anthropogenic noise. Follenius, et al. (1980) have argued that in non-human animals, where there is no informational process that assigns meanings to noise as would happen for humans in experimental settings, noise exposure can generate negative emotions such as anxiety, fright and even hostility, as well as defined behavioural and endocrinological changes. Moreover, the appearance of aggressive responses due to anthropogenic noise (foot-drumming in the Stephen’s Kangaroo rat (Shier, et al. 2012)) suggest the
possibility of acoustic components contained in mining noise that could stimulate motivational approach and increase dopaminergic stereotypies (and overall locomotion). Therefore, the emotional dimension of the lateralized behavioural responses observed during mining noise exposure should be also subject of further study.

7.4. **Noise stress and gender: the influence of sex-related frequency sensitivity**

Gender based differences were also observed in the stress responses observed during high amplitude exposure. Females were more reactive to stress and had the highest levels of stereotypies observed as well as a reduction in adrenal cortex thickness. In contrast, males showed a decreased spleen weight, similarly a sign of stress. Having these gender-based responses, the final stage of this research (Chapter 5), where animals were exposed to frequencies below and above 2 kHz at the same amplitude, contributed to elucidate the role of sex-based differences in wild mice stress responses to mining noise exposure.

High frequencies resulted in higher levels of corticosterone for mice exposed to this treatment, probably due the species hearing range in which these range of frequencies are favored. Nonetheless, high frequency exposed females presented the highest levels of corticosterone due a heightened sensitivity that is probably oestrogen-dependent, which also led to the reduction of spleen weight. This indicates that, although high frequencies are detrimental for both genders in general, females are more affected, perhaps differentially over time as the oestrogen levels vary within the reproductive cycle. This implies that in the wild, mining noise exposure may generate intense stress in female rodents during the proestrus, when the highest levels of estrogen occur, as well as the highest sensitivity for high frequency calls (Ehret & Schmid 2009). At the same time, males exposed to this treatment experienced spleen hypertrophy, thus confirming that high frequency mining noise is a mechanism that alters spleen cellular dynamics, not necessarily generating a tendency to increase or decrease of cellular content, but a disruption of normal splenic functions. In addition, as the behaviours observed during high frequency exposure are related to a tendency to explore, it is possible that noise is interpreted as natural calls in the same frequency range. Hence, in close similarity with the conclusions reached on the EBT lizards study and the results obtained with high amplitude exposure, the possibility of sound mimicking emerges as an area in which research is greatly required.
Likewise, the unchanged circling behaviour during high frequency exposure also aligns to the possibility of sound mimicking, since it implies that the generation of stereotypies is a mechanism to decrease physiological stress that is exclusive for circumstances in which physiological arousal reaches threatening levels. During circumstances where continued responsiveness to acoustic cues of certain characteristics is essential, the development of coping mechanisms inhibiting stress-arousal may be non-adaptative. As high frequency calls are an important component for wild mice to assess their surroundings and engage in appropriate behavioural responses (Ehret 2013), the absence of behavioural coping during high frequency mining noise may be related to the preservation of reactivity to these acoustic cues. In order to understand this process, the study of anthropogenic noise in relation to acoustic signal interpretation is a research area of great importance.

On the other hand, the effects of low frequency stress were especially observed in male animals, which increased their corticosterone levels when compared to controls and showed a tendency for spleen hypertrophy. As some male rodents have proven to have low frequency sensitivity compared to females, our results suggest that a difference in auditory perception due gender-based differences is crucial when evaluating the effects of different ranges of frequencies contained in anthropogenic noise. As low frequencies are more likely to elevate blood pressure and catecholamine levels (Evans, et al. 1998, Schust 2004, Waye, et al. 2003), further research should include these measurements to evaluate the effects of anthropogenic noise, as most man-made noise includes an important low frequency component that could be affecting vascular health. As the generally unchanged glucocorticoid levels with low frequencies in gender-mixed populations is related to disorders such as Chronic Fatigue Syndrome or chronic pain (Geiss, et al. 1997, Nater, et al. 2008, Wessely & Powell 1989), research into the similarities that low frequency exposure has with these kinds of diseases is essential to understand the effects, which is relevant for every other anthropogenic noise that is dominant in the low frequency spectrum. In fact, research on human exposure to these noises has amply shown that there are clear relationships between fatigue and tiredness after increasing low frequency noise (Schust 2004), thus making low frequency exposure a possible generator of physiological fatigue that could affect wild animals and decrease their chances of survival.

It is also worth noting that, if estrogen may have played an important role in the sensitivity to certain frequencies it could also diminish the severity of acoustic trauma, as several lines of
evidence support the fact that estrogenic regulation of brain-derived neurotrophic factor and estrogen receptor \( \beta \) have protective capacities in the auditory system (Caras 2013). Therefore, it is fair to assume that while female males are more susceptible to high frequencies, they could also be better protected to changes in intensity. Further research should take into account the part that sex-steroid hormones play while counteracting the effects of auditory trauma when evaluating the effects of anthropogenic noise exposure.

The possibility of mining noise or other anthropogenic noises with similar characteristics having gender related effects has further implications for reproduction and fitness, as many of the alterations mentioned can generate an inability to reproduce, leading to population declines of important species. Another aspect that could be potentially affected is parental care, which could also reduce the ability of certain species to reach reproductive age. Clearly, these concerns have to be addressed by further research where intra-population factors such as gender are taken into account.

7.5. **Mining noise and potential damage to the vestibular system arising from dopaminergic stereotypies**

Additionally, an aspect of noise exposure that was not evaluated in our experiments is the possible effects of anthropogenic noise on the vestibular system. Noise exposure can damage directly animal’s hearing structures, which can also be a cause for circling behaviour. Nearly all the laboratory strains of rodents that present spontaneous increments in circling behaviour are also linked to vestibular impairments. The vestibular system provides animals with their sense of balance and spatial orientation (Angelaki & Cullen 2008) and in animal models such as the Bronx-Waltzer (bv) mouse (Deol 1981) or the ci2 rat (Schirmer, et al. 2007), hair cell degeneration in the vestibular macula is present. Given this relationship, it has been suggested that vestibular defects during postnatal development, which could be inherited or induced, produce secondary changes in the dopaminergic systems from the basal ganglia, such as the nigrostriatal dopaminergic system (Schirmer, et al. 2007). For instance, Shima (1983) studied circling behaviour in relation to the function of lesions of the vestibular nuclei and suggested that unilateral interruptions of the ascending vestibular pathway can increase dopamine metabolism in the striatum of the lesion side. Furthermore, the existence of a vestibulo-thalamo-striatal pathway has been proven (Lai, et al. 2000) and it has been suggested that vestibular input from the medial vestibular nucleus may be modulating motor integration in the striatum by regulating dopamine receptor activity of striatal
neurons (Shima 1983). Thus, vestibular damage is able to generate striatal deficiencies and exacerbate dopaminergic stereotypies, such as circling.

Vestibular damage can be induced by chronic noise exposure. This has been extensively studied in human exposure to occupational noise. Subjects with a history of work-related noise exposure of non-specified sources showed vestibular injury positively correlated with the severity of the acoustic trauma when evaluated by electronystagmography (ENG), a test designed to assess deficiencies in the vestibular system through the vestibule-ocular-reflex (Man, et al. 1980). Likewise, a study evaluating human males that have been exposed to loud noises during their military service (impulse and impact noises from firearms, helicopters, tanks and other military machinery) demonstrated that asymmetrical hearing loss (but not the severity of it) was related to vestibular deficiencies (Golz, et al. 2001). Studies have shown as well that the human vestibular saccule (the structure containing the macular cells) can be stimulated at or above 100 dB SPL (Akin, et al. 2003, Welgampola & Colebatch 2005), and furthermore, that vestibular structures are able to withstand much less pressure than the cochlea, which implies that the probability of the vestibular damage is greater by intense noise exposure when compared to the cochlea (Raghunath, et al. 2012)

In rodents, vestibular damage in the macula has been induced in guinea pigs exposed to continuous 6 hours noise at 120 dB when compared with animals exposed to intermittent noise (1 hour of silence/ 1 hour of noise) (Akdogan, et al. 2009). In mice when wild type females were exposed to noise below 0.5 kHz at 70 dB SPL for one month continuously, animals showed impairments in balance compared with controls and high frequency exposure (16 kHz, 70 dB SPL). They also had fewer vestibular hair cells and an increased level of oxidative stress in the vestibule (Tamura, et al. 2012). Thus, it is possible that noises that are greatly energetic at low frequencies, as mining noise could be, could generate vestibular impairment and subsequent changes in the vestibulo-thalamo-striatal pathway. Whether vestibular damage potentiated the effects of mining noise stress on circling during high amplitude and/or impaired the abilities of animals to circle during low frequency exposure is a possibility that should not be overruled. Further research assessing long-term exposure to anthropogenic noise needs to consider possible vestibular damage in connection with dopamine release and stereotypy expression.
7.6. **Nonlinearity: an acoustic characteristic with possible consequences for stress**

It is also important to state that even when amplitude and frequency are the most important characteristics of sound, there are other characteristics that could determine the physiological effects of noise. For instance, nonlinearity is described as highly complex sounds that include frequency modulations, frequency jumps warbles, subharmonics and biphonation and is a characteristic of distress calls that could be easily included in anthropogenic noise (Blesdoe & Blumstein 2014). For example, it has been established that animals respond with stress-related reactions to distress calls from conspecifics in the same way they do to synthetically-constructed nonlinear sounds (Blesdoe & Blumstein 2014). When a normal distress call is mixed with a nonlinear synthetic sound, animals decrease the time spent foraging, which was interpreted as a corroboration of nonlinearity as a characteristic that increases stress arousal and also, as a way to add variability to calls and avoid habituation (Blumstein & Récapet 2009). In our study, it is possible that the presence of discordant mixtures of frequencies was greater in the amplitude experiment conducted with unfiltered mining noise compared to the filtered tracks used to evaluate separate frequency ranges. This difference could decrease the amount of nonlinearity contained in filtered tracks and affect the necessity to generate behavioural coping strategies, as observed in this part of our research. Further experiments should include the assessment of these sound characteristics.

7.7. **Mining noise and wildlife: possible measures to improve wildlife welfare**

Finally, although it was largely concluded that mining machinery noise is a dangerous environmental input for animal and that its effects were widely determined by high amplitudes and both low and high frequencies, noise regulations in mining sites do not include the use of high quality noise barriers to absorb the energetic input of mining noise and contain both high and low frequencies. For example in Queensland, the Environmental Protection Act (EPA) (Queensland 1994) and the Environmental Protection (Noise) Policy (EPNP) (Queensland 2008) do not have specific requirements for noise barriers. Therefore, mining projects such as the South Galilee Coal Project (SGCP) do not consider the establishment of permanent noise barriers when modelling the effects of noise pollution by mining, except for the presence of casual waste rock emplacement (SGCP 2012). Therefore, this research is crucial to enforce the use of specific materials for noise barriers, such as wave trapping barriers that have been tested in mines in Western Australia and have shown around 10dB (A) of noise reduction (Pan, et al. 2004).
Similarly, as it was proven that sex-hormones are deeply related to noise sensitivity and considering the fact that these hormones are crucial for reproduction, the establishments of yearly working schedules in which the reproductive seasons of affected species with seasonal reproduction are considered is a possibility that should be considered and supplied. Such working schedules should be also considered on daily basis, establishing periods of the day where noise is high and other periods where it is absent, as it has been proven that animals can adapt to predictable noise by allocating communication to certain hours of the day (Laiolo 2010, Warren, et al. 2006, Wright, et al. 2007). This strategy could help alleviate mining noise exposure in existing mining sites while better strategies are developed.

7.8. Conclusions
1. The methodology designed to observe EBT lizards stress-related behaviours contributes to the assessment of the effects that noise and other environmental stimuli have on the well-being of lizards, as well as to the establishment of welfare standards during transport, as it was found that cold is the greatest contributor to stress during transport.
2. The sound processing technique and exposure methodology generated to evaluate the effects of mining noise exposure is a positive contribution to the study of anthropogenic noise exposure under laboratory conditions, since it allows the control of amplitude and frequency, as well as the influence of other factors that could not be accounted in the field.
3. For EBT lizards, behavioural lateralization became an important tool to assess noise-related stress and it was observed that mining noise of high frequencies at high amplitude was the most noxious experience, as it decreased right-side of the body behaviours, presumed to be processed in the left hemisphere.
4. In wild mice, the increase of behavioural coping was intensity-dependent, as high amplitude mining noise increased the amount of circling behaviour while tending to decrease levels of fecal corticosterone.
5. Behavioural lateralization of stereotypical circling was a useful tool to infer the degree of annoyance and physiological inhibition required by different amplitude levels, since only high amplitudes increased the amount of clockwise circling, which indicates interhemispheric inhibition due severe stress.
6. In addition to organ weight, a new methodology was utilised to assess changes on organ morphology, providing a good indicator of the physiological effects of noise exposure. Through this
assessment, it was observed that females were more affected by high amplitude exposure, due the decrease in adrenal cortex thickness accompanied with stereotypic behaviour.

7. It was proven that mining noise at different frequency ranges has noxious effects for wild mice that are highly sex-related. Females were more affected by high frequency mining noise, having the highest fecal cortisol levels and spleen atrophy, possibly due to oestrogen-dependent sensitivity, whereas low frequencies increased fecal cortisol in males, perhaps due the slightly increased thresholds below 1 to 2 kHz that males have.

8. My research suggests that the current legislation to regulate noise pollution in the mining industry is insufficient, as there are no specifications for the characteristics that noise control systems, such as barriers and work schedules, which would diminish the effects on wildlife welfare. Impact assessment would determine the feasibility of such research.

9. This type of research is recommended as a tool for the implementation of environmental noise control techniques in areas of high interest for animal conservation.

7.9. **Further research**

Through this research, several related areas of research interest have been identified:

A. The assessment of the behavioural effects that other types of environmental stimuli have on reptiles in the pet industry or in the wild utilizing the methodology I have proposed and validated for noise exposure and some road transport stressors.

B. The development of techniques (such as the use of collars to record lateral head movements) to evaluate head lateralization in wild or captive lizards to investigate the degree of stress that is experienced in different kinds of habitats.

C. The evaluation of the effects on welfare and behaviour of other meaningful characteristics of anthropogenic noise, such as nonlinearity, since sound components other than energy intensity and perceived pitch could play an important role in the effects of stress generated by anthropogenic noise.

D. The assessment of anthropogenic noise as a sound mimicking experience for wild animals which should comprise the examination of different hearing ranges, as well as the analysis of different types of animals calls and their patterns, to establish resemblance with noises generated by man-made activities.

E. The evaluation of the relationship between dopamine secretion and noise exposure, as there is evidence to suggest that the stress generated by noise is linked with dopaminergic stereotypies and the subsequent inhibition of stress arousal.
F. The relation between noise exposure and motivational approach, as dopamine is not only related to locomotion, but also to aggression, anxiety and other emotions that could relate noise annoyance with other cognitive processes utilizing physiological evidence.

G. The evaluation of the detrimental effects of mining noise and other anthropogenic noises on the hearing system and its relation with vestibular damage, as dopaminergic circling can be also related to the direct action of noise intensity on hearing structures.

H. Research and subsequent implementation of specific types of noise barriers that contribute to decrease the effects of noise exposure, leading to the establishment of specific regulations that reduce the noise pollution generated by mining facilities.

I. The assessment of the efficacy of hearing schedules for mining noise exposure to establish possible avenues of mitigation in areas where mining without noise control strategies for wildlife is taking place.

7.10. References


Davidson RJ 1995 *Cerebral asymmetry, emotion, and affective style.* In: Davidson RJ and Hugdahl K (Eds) *Brain Assymetry* pp 361-387. MIT Press: Masachussetts, USA.


Schust M 2004 Effects of low frequency noise up to 100 Hz. Noise and Health 6: 73-85.


