

## The effects of acute exposure to mining machinery noise on the behaviour of eastern blue-tongued lizards (*Tiliqua scincoides*)

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### Abstract

The mining industry is an important source of noise for wildlife, and the eastern blue-tongued (EBT) lizard (*Tiliqua scincoides*) is an Australian animal that may be impacted. We analysed the behaviour of nine EBT lizards during and after exposure for 5 s to one of five combinations of mining machinery noise frequency and amplitude (frequency < or > 2 kHz, low [60–65 dB (A)] and high [70–75 dB (A)] amplitude, or a control treatment). Following exposure, lizards could leave the test chamber and enter an escape chamber, which led into a small hiding chamber. Chambers were monitored for 15 min after initial exposure. In the test chamber, lizards exposed to high frequency, high amplitude noise spent more time freezing, a typical stress response in reptiles, when compared with animals in all the other treatments. This was especially the case for lizards exposed to high frequency noise. In the hiding chamber, high frequency noise at high amplitudes decreased durations of head positioning to the right and downwards, suggesting a lateralised fear reaction, but decreased standing and freezing behaviours. We hypothesise that lizards have lateralised behaviour reactions to mining noise, with high frequency, high amplitude noise being the most detrimental. Our results demonstrate that acute exposure to mining noise had negative effects on EBT lizards' behaviour and welfare, which may suggest a threat for lizards experiencing chronic mining noise in the wild, making the study of mining machinery noise *in situ* a research priority.

**Keywords:** animal welfare, anthropogenic noise, eastern blue-tongued lizard, lateralisation, mining noise, sound mimicking

### Introduction

Anthropogenic activities are an important source of acoustic noise for wildlife (Pijanowski *et al* 2011). Such noise has been acknowledged as a major stressor with the capability to mask and alter calls between conspecifics, hamper the detection of predators, change the hearing thresholds of individuals and alter animals' distribution (Blickley & 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 immunosuppression (Kight & Swaddle 2011).

In Australia, mining is a widespread industry that has a potential impact on wildlife. Open-cast mining is commonly used as a method of mineral extraction (Britt *et al* 2014), creating environmental noise pollution (Haigh 1993; Tripathy 1999). There have been few analyses of mining noise frequency spectrums and amplitudes at a distance to test their impact on wildlife, despite a possible influence on animal populations and their welfare being recognised (Armstrong 2010; Saha & Padhy 2011). Studies that have analysed mining

noise as a human workplace hazard have revealed that low frequencies, usually below 2 kHz, are dominant and energetic, as they exist in many other anthropogenic noises (Slabbekoorn & Peet 2003; Barber *et al* 2011). Machinery used in open-cast mining and rock crushing emits most energy at low frequencies, with dumper trucks having the lowest frequency (0.25–0.5 kHz) and cooling fans from bulldozers the highest (0.3–3.5 kHz) (Vardhan *et al* 2005). However, even though frequencies below 2 kHz predominate, mining noise does have a broad frequency range. Some special machines, such as diamond-cutters, generate dominant frequencies between 2 and 4 kHz (Pal *et al* 2006), with a dominant frequency range of 3.16 to 8 kHz (Peng *et al* 2010).

The amplitude of mining noise *in situ* can reach 90 to 110 dB (A) (Mohapatra & 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 & Goswami 2012). These levels are similar to measurements near a rock-crushing facility, which is operationally similar to open-cast mining, with measurements of 86 ( $\pm$  0.42) dB (A) in a forest between 0 and 500 m from the noise source and

lower values ( $64.4 [\pm 0.25]$  dB [A]) at 500–1,000 m from the source (Saha & Padhy 2011).

In addition to its pitch and volume, mining noise is unusual in relation to other anthropogenic noises, since it originates from a wide range of sources (eg industrial machinery, railway transport and construction and vehicle traffic). Such a combination can enhance noise properties that increase annoyance and aversion, in particular sound complexity (the degree of mixture of different sounds) and an excessive complexity (discordant mixtures of frequencies) (Cone & Hayes 1984), both of which are components of non-linear 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 characterised by non-linear equations and a degree of distortion as they travel. Non-linear acoustic phenomena are composed of desynchronised 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 (Blesdoe & Blumstein 2014).

Mining machinery has only been evaluated as a noxious stimulus in relation to its impacts on human health in the workplace (Roy & Adhikari 2007; Peng *et al* 2010). The impacts of mining noise on the behaviour, physiology and welfare of free-ranging animals have been widely overlooked, although there is evidence that mining and related industries can affect the migration patterns and selection of breeding and feeding grounds in elks (*Cervus canadensis*) (Kuck *et al* 1985), elephants (*Loxodonta africana*) (Rabanal *et al* 2010) and bird populations (Read 2000; Saha & 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 in the field. Therefore, investigations into the effects of mining noise on captive terrestrial animals may suggest 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, the Environmental Protection Act of Queensland 1994).

It is important that research into the effects of noise pollution on animals includes an evaluation of the effects 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 birds, because of the potential for their vocalisations to be masked by the noise; there is little information on less vocal species, including reptiles (Shannon *et al* 2015).

The eastern blue-tongued (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 & 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 these areas is probable due to its widespread distribution across north- and south-eastern Australia (Anon 2012) and its capacity to adapt to environments with a strong anthropogenic influence, including 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 (Cottle & Keys 2014). Since noise can decrease immunocompetence (Kight & 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 *Tiliqua rugosa*, have similar hearing ranges (Köppl & Manley 1992). Therefore, mining noises are 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 species or subspecies that are listed as vulnerable, eg *T. adelaidensis* is endangered, and the subspecies *T. 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 help to generate conservation strategies for others.

Behaviour is an important tool to evaluate animal welfare in a non-invasive manner. Locomotion is frequently reduced among captive reptiles (Warwick 1995), but exploratory behaviours and stress-related inactivity are important tools to assess discomfort and stress in these taxa (Warwick *et al* 2013; Mancera *et al* 2014). In order to determine whether mining noise is deleterious to captive EBT lizards' welfare, they were exposed to mining machinery noise in a modified open-field facility designed to assess avoidance patterns. We hypothesised that mining noise in an acute, non-continuous exposure would have negative impacts on their welfare, as assessed by their behaviour, and that such effects would be frequency- and amplitude-dependent.

## 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).

## Study 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). They were permanently housed in nine individual enclosures, consisting of a tubular plastic frame supporting plastic mesh walls (six were  $60 \times 39 \times 40$  cm; length  $\times$  width  $\times$  height and three were  $95 \times 52 \times 53$  cm). 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 the area where the testing took place, had its greatest energy component between 0 and 400 Hz and was always below 55 dB (A) when measured with a Sound Level Meter (Model QM-1589, Digitech, California, USA).

### Diet

Animals were fed twice weekly with 7 g of fruit and vegetables. For protein, lizards were given either a live giant mealworm (*Zophobas morio*) or a steamed chicken egg chopped into pieces, mixed with the fruit and vegetables and sprinkled with a reptile supplement (Repti-vite, Aristopet, Australia).

### Test enclosure design, habituation and training

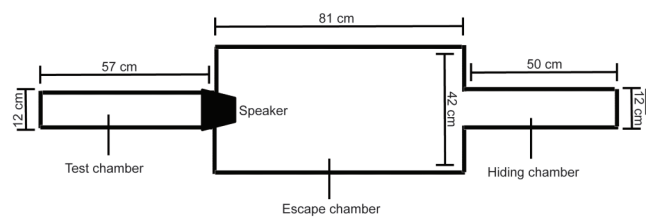
We designed a test of aversion to mining noise which assumed that the lizards would move away from an aversive auditory stimulus to seek a hiding place. A three-chamber facility (Figure 1), that had been previously developed to test aversion to various stressors that included road traffic noise in lizards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

The 20-cm high walls surrounding the chamber system were made of plywood and lined with insulating material (Reflecta, GID Double Layer Insulation, 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 onto a table in an isolated room away from any vehicle or human activity. The room was kept at ambient temperature, which was recorded daily (mean temperature  $26.4 \pm 2.02^\circ\text{C}$ , range  $24.4\text{--}28.4^\circ\text{C}$ ). Lizards were first habituated to the chamber system by placing them individually into the TC facing the HC for 15 min on three occasions over three days prior to the experiment.

### Creation of mining noise and sound processing

Based on the characteristics of mining noise described in the literature (Read 2000; Roy & Adhikari 2007; Saha & Padhy 2011) and in consultation with a mining geologist, a mining machinery noise soundtrack was created. Online sources, specialised in the creation of sound effects were used (<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>) 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 min. From this recording, a 15-min section containing the simultaneous

**Figure 1**



Auditory stimuli aversion experimental apparatus. Consisting, firstly, of a test chamber (TC) ( $57 \times 12$  cm; length  $\times$  width), in which lizards were exposed to the noise. This comfortably accommodated a single lizard from head to tail, while preventing excessive movement or visual stimulation. Then, an escape chamber (EC) ( $81 \times 42$  cm), in which there was sufficient room to perform activities, creating an open-field area for behavioural responses after exposure to the noise stimuli. And, finally, a hiding chamber (HC) ( $50 \times 12$  cm). 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 are used for hiding in the wild and provided in their normal enclosures.

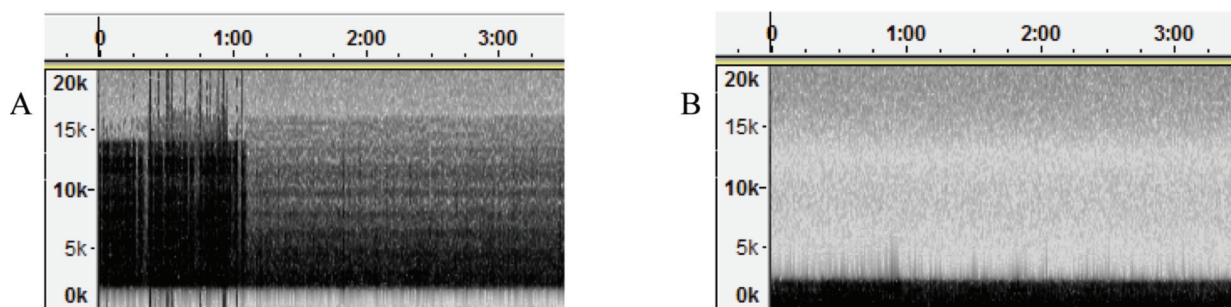
noise of all three pieces of equipment was selected at random and further processed using the high- and low-pass filter functions of the Audacity® software to create two tracks of low frequency (LF)  $\leq 2$  kHz, and high frequency noise (HF)  $> 2$  kHz, respectively (Figure 2). This recognised that anthropogenic noise has most of its energy output below 2 kHz (Slabbekoorn & Peet 2003; 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 (Young 2003), we hypothesised that 2 kHz would be the most appropriate division in relation to animal perception and, therefore, behaviour.

### Further experimental treatments and procedures

Auditory treatments were further established at two different levels of amplitude, recorded in the TC: high amplitude (HA), mean  $73.90 (\pm 0.83)$  dB (A), range 70–75 dB (A), and low amplitude (LA), mean  $62.94 (\pm 0.91)$  dB (A), range 60–65 dB (A). Amplitude ranges were calculated from recordings of the high and low frequency noises at both amplitudes. Decibel values were extracted from successive samples using the function ‘Sample Data Export’ in the software Audacity®. An increase of ten decibels is an increase in (noise) power by a factor of ten (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). This use of dB (A) 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 is not widely

Figure 2



Frequency spectrums of (A) high and (B) low frequency noise obtained with the programme Audacity®. The vertical axis corresponds to frequencies in kHz while the horizontal axis corresponds to time (min). The grey scale represents the amount of acoustical energy contained in the correspondent frequency; the darker the area, the greater energy component in that area of the spectrum.

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

Treatments	Sound components
HF HA	HF = high frequency noise ( $\geq 2$ kHz) HA = high amplitude noise (70–75 dB [A])
HF LA	HF = high frequency noise ( $\geq 2$ kHz) LA = low amplitude noise (60–65 dB [A])
LF HA	LF = low frequency noise ( $< 2$ kHz) HA = high amplitude noise (70–75 dB [A])
LF LA	LF = low frequency noise ( $< 2$ kHz) 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 ( $\pm 0.1$ ) dB (A)

available. The study assessing noise levels in a forest close to a rock-crushing facility (Saha & 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: i) HF HA; ii) HF LA; iii) LF HA; and iv) LF LA. A speaker (output power: 2.5 W  $\times$  2; response 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 TC (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). This position also prevented the generation of substrate vibrations. 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 s of the sound recording, which was considered to be a representative fragment due to 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) ( $\pm 2$ ) dB (A) from the TC to the mid-point of the EC, and by a gradient of 15 dB (A) ( $\pm 2$ ) dB (A)

from the TC to the end-point of the HC. 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.

Lizards were exposed to the noise treatments in a Latin Square design with three replicates of each treatment for each lizard, achieved by one treatment being presented to each lizard each day over a five-day week. Lizards experienced all five treatments each week, for three weeks, providing three replicates of each treatment. At the beginning of the trials, animals were placed individually inside the TC facing the EC. Immediately after the animal was positioned, a transparent Perspex lid was used to cover the entire chamber system to prevent animals escaping as well as the intrusion of exterior sounds. Then, for a preliminary period of 5 s after initiation of the mining noise, the lizard was held in the TC by a wooden removable door (Figure 1), thus experiencing the noise without any opportunity to escape. After these 5 s, the door was opened and behaviour recording commenced, whilst the lizards responded to the continued stimulus. Each test lasted for a 15-min period, with tests conducted daily between 0900 and 1200h.

### 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). Two experimenters 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-related effect on lizard behaviour. They recorded the rates and durations of behaviours performed in each chamber using behaviour analysis software (Cowlog; Hänninen & Pastell 2009) while tests were taking place. 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 a knowledge of behavioural measurement criteria for bout determination (Martin & Bateson 1993).

An ethogram was defined which focused upon the type of movement and where it occurred, as well as behaviours that had been observed in other lizard behaviour studies (Langkilde 2006; Mancera *et al* 2014). The behaviours recorded were categorised into whole body movements (recording of the position of the centre of gravity) and accessory movements (change in head position, sneezing and tongue-flicking). Whole body movements were walking, climbing, standing (remaining in the same position while exploring the environment visually by head motions and/or by tongue-flicking) and freezing (remaining in the same position without any other movement than those related to breathing). The accessory movements recorded were 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).

Within the two behavioural categories created, behaviours were designed to be mutually exclusive, that is, behaviours under the same category could not occur simultaneously. At the same time, behaviours from different categories could be observed together, since the existence of whole body movement could not prevent the animal from engaging in accessory activities (for instance, head movements), except in the case of freezing, where all motions are restricted.

### Statistical analysis

A General Linear Model was constructed which included the following factors: lizard, 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 were transformed using square root or  $\log_{10}$  — whichever most effectively returned residuals to a normal distribution. After preliminary exploration of the data using the aforementioned model, five specific contrasts were tested: i) comparison of all treatments; ii) control versus noise treatments; iii) HF HA treatment versus all other treatments; iv) HF treatments versus LF treatments; and v) HA treatments versus LA treatments. 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 were 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  $P \leq 0.05$  and are presented as means (+ SED). All calculations were performed with Minitab Statistical Software, version 16.

## Results

### Occupation of the chambers

Following release of the restraining door, animals spent most time (358.5 [ $\pm$  20.7] s) in the EC, with similar amounts of time spent in the TC (266.7 [ $\pm$  20.5] s) and HC (291.1 [ $\pm$  26.4] s). Both high frequency and high amplitude treatments extended the mean time spent in TC (HF = 260.2 s per

900 s, LF = 160.8 s per 900 s;  $P < 0.01$ ; HA = 245.9 s per 900 s, LA = 172.4 s per 900 s;  $P = 0.05$ ). In EC and HC the time animals spent in the chamber was not affected by treatment (Table A; see supplementary material to papers published in *Animal Welfare* on the UFAW website: <http://www.ufaw.org.uk/t-ufaw-journal/supplementary-material>).

### Lizard behaviour in the three chambers

As a proportion of time spent in the chamber, lizards spent much more time with their head down and to the left when they were in the TC, compared with when they were in EC or HC (Table 2). Animals in EC spent most of their time with their heads down to the front, and also more time standing (Table 2). Lizards in the HC spent more time with their heads up (left or right) (Table 2). Animals froze in both the TC and EC, but rarely in the HC (Table 2). Figure 3 is a graphic representation of these results. Full statistical analyses and other behaviours analysed in the three chambers are contained in Table B (see supplementary material to papers published in *Animal Welfare* on the UFAW website: <http://www.ufaw.org.uk/t-ufaw-journal/supplementary-material>).

### Behaviour in the test chamber

Lizards tended to spend more time moving their heads in the high compared with the low frequency treatments ( $P = 0.07$ ), particularly in the HF HA treatment ( $P = 0.03$ ) (Table 3). They also spent more time and froze more regularly in high frequency treatments ( $P < 0.001$ ), again particularly in the HF HA treatment (Table 3). Figure 4 is a graphic summary of these results.

### 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 3).

### Behaviour in the hiding chamber

Overall, lizards in the HC 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 3, Figure 5). 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 (Table 3). Lizards in the HF HA treatment spent less time turning their heads down and right when compared with all other treatments combined ( $P = 0.01$ ) (Table 3, Figure 5). They also spent less time standing than those in the other treatments ( $P = 0.02$ ) (Table 3, Figure 5). They also froze less frequently compared with the other treatments ( $P = 0.05$ ) (Table 3).

### Behaviour analysed across all chambers

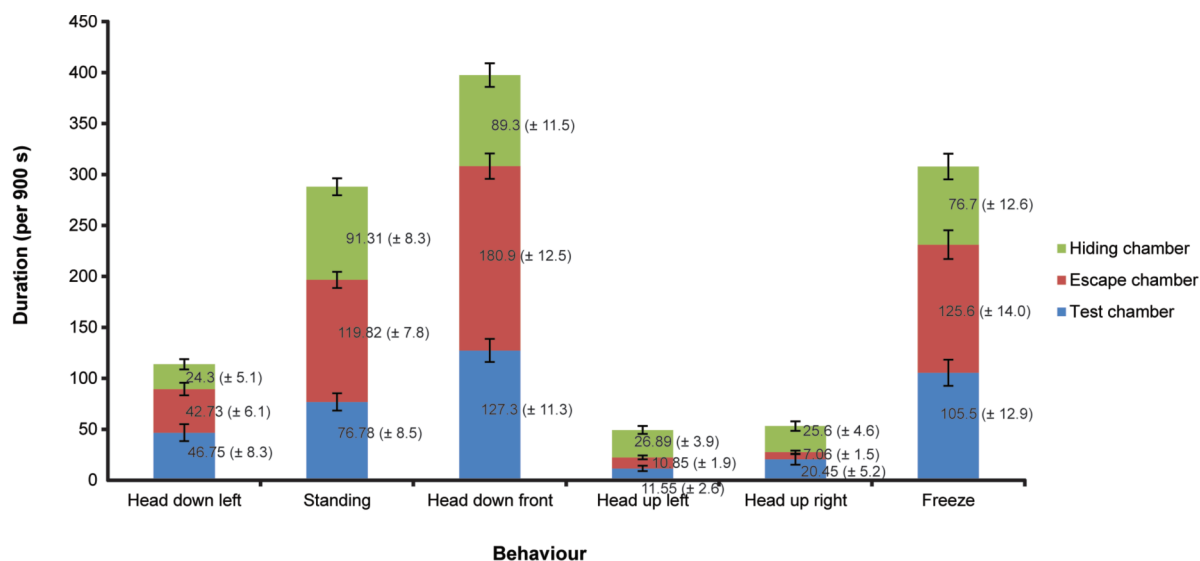
Lizards spent more time with their head oriented to the right and downwards when exposed to low amplitude treatments compared with high amplitude treatments (HA = 43.41 s per 900 s, LA = 81.92 s per 900 s;  $P = 0.02$ ), an effect which tended to be greatest in HF HA ( $P = 0.07$ ) (Table 3).

**Table 2 Mean ( $\pm$  SEM) proportion (%) of time in each chamber spent in the different behaviours.**

Behaviour (% total time/total time per chamber)	Test chamber	Escape chamber	Hiding chamber	SED	F-value	P-value
Head up left (median % total time per chamber)*	0	0	1.77	–	–	< 0.001
Head up left (mean % total time per chamber)	2.95 ( $\pm$ 0.54)	2.94 ( $\pm$ 0.46)	9.91 ( $\pm$ 1.33)	–	–	–
Head up right (median % total time per chamber)*	0	0	0	–	–	< 0.01
Head up right (mean % total time per chamber)	5.17 ( $\pm$ 0.89)	1.86 ( $\pm$ 0.36)	8.36 ( $\pm$ 1.22)	–	–	–
Head down left (median % total time per chamber)*	0.73	4.93	0.33	–	–	< 0.01
Head down left (mean % total time per chamber)	93.9 ( $\pm$ 73.2)	11.6 ( $\pm$ 1.48)	6.7 ( $\pm$ 1.24)	–	–	–
Head down front ( $\sqrt{\%}$ total time per chamber)	5.84 <sup>a</sup>	6.37 <sup>a</sup>	4.30 <sup>b</sup>	1.447	16.59	< 0.001
Head down front (% total time per chamber)	34.1	40.6	18.5	–	–	–
Standing ( $\log_{10}$ % total time per chamber)	0.29 <sup>b</sup>	0.42 <sup>a</sup>	0.31 <sup>b</sup>	0.095	13.92	< 0.001
Standing (% total time per chamber)	1.95	2.63	2.04	–	–	–
Freezing (median % total time per chamber)*	0.41	0.59	0	–	–	< 0.001
Freezing (mean % total time per chamber)	1.03 ( $\pm$ 0.45)	0.75 ( $\pm$ 0.06)	0.31 ( $\pm$ 0.03)	–	–	–

SED = Standard error of the difference between two treatments. Means that do not share a superscript are statistically different.

\* Behaviours compared using Kruskal-Wallis test. For the complete analysis of all behaviours refer to Table C (see supplementary material to papers published in *Animal Welfare* on the UFAW website: <http://www.ufaw.org.uk/t-ufaw-journal/supplementary-material>).

**Figure 3**

Comparative analysis of duration of lizards' behaviours in the three chambers. Graph shows untransformed means ( $\pm$  SEM).

## Discussion

The aim of this study was to evaluate EBT lizards' behavioural reactions to acute exposure to mining noise, which is of great significance in Australia, where the EBT lizard is indigenous and the mining industry is of major importance (Connolly & 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 we had better control of acoustic variables: the position, processing and direction of auditory stimuli and the use of acoustic materials to line the walls of the chamber system. This is likely to have improved sound quality, potentially making the animals more responsive, even though the amplitude used in this experiment was lower than in our previous study (60–75 dB [A] vs 90 dB [A]).

Overall, the lizards' pattern of activities in each chamber demonstrated that when lizards were in TC, they spent much of their time freezing and with their heads orientated left and downwards; conversely, in HC, they spent more time with their heads up and less time freezing. Freezing is characterised by tension and immobility and considered a reaction to inappropriate and restrictive environments, and hence a sign of stress and fear (Warwick *et al* 2013). It was also considered a sign of aversion in EBT lizards when observed in response to different transport stimuli (Mancera *et al* 2014). The increased freezing in the test chamber and subsequent decrease in the hiding chamber suggests that this behaviour is an indicator of stress that is alleviated as the animal moves away from the noise source.

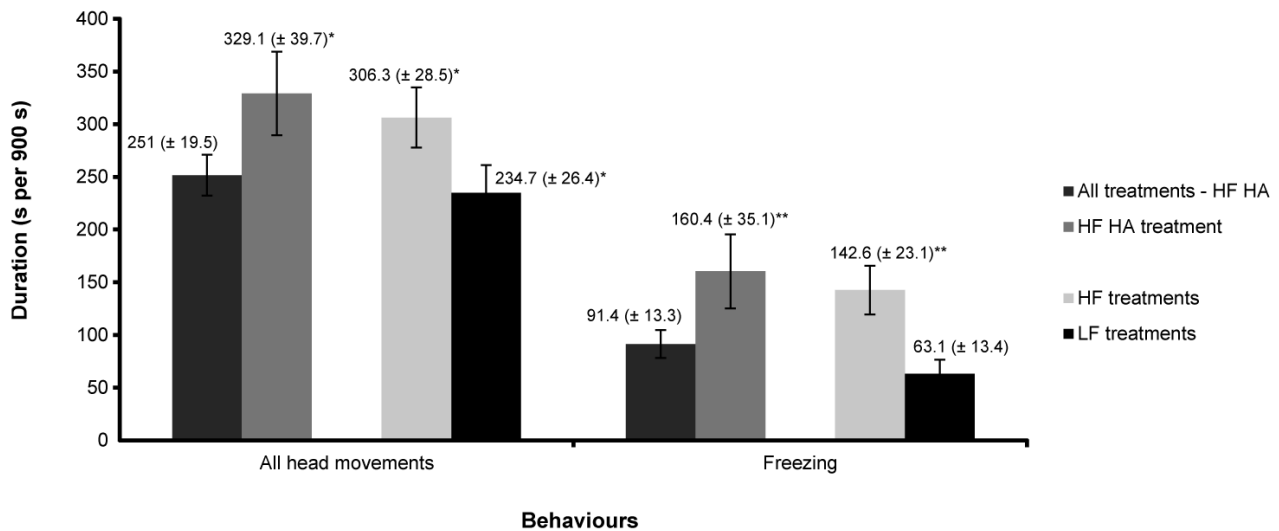
The EBT lizards moved freely after exposure, allowing them to move forward, towards the EC, but also to turn back into the TC. Any change in direction was a potential diffi-

**Table 3** Behaviour of lizards exposed to mining machinery noise in the test chamber, escape chamber, hiding chamber and the whole chamber system.

Behaviour	Mean					SED		P-value						
	HF	HA	HF	LA	LF	HA	LF	LA	C	All treatments	Noise vs CT	HF vs LF	HA vs LA	HF HA vs all other treatments
<i>Test chamber</i>														
Duration all head movements ( $\sqrt{s}$ /900 s)	17.1	14.9	14.2	13.01	13.8	3.01	0.702			0.48	0.07	0.18	0.03	
Duration all head movements (s per 900 s)	291.4	221.1	202.5	169.3	189.9	–	–			–	–	–	–	
Duration freezing ( $\sqrt{s}$ per 900 s)	10.99	8.8	6.1	5.1	8.2	2.73	0.63			0.76	< 0.001	0.17	0.004	
Duration freezing (s per 900 s)	120.1	77.4	37.8	25.8	66.6	–	–			–	–	–	–	
Rate freezing ( $\sqrt{\text{times}}$ per 900 s)	1.26	1.04	0.74	0.84	0.94	0.287	0.18			0.82	0.004	0.62	0.007	
Rate freezing (times per 900 s)	1.60	1.08	0.55	0.71	0.88	–	–			–	–	–	–	
<i>Escape chamber</i>														
Duration combined <sup>†</sup> head right ( $\sqrt{s}$ per 900 s)	4.7	6.5	4.8	7.3	5.5	2.17	0.67			0.77	0.62	0.02	0.21	
Duration combined <sup>†</sup> head right (s per 900 s)	22.5	41.9	23.1	53.4	30.6	–	–			–	–	–	–	
<i>Hiding chamber</i>														
Duration all head left/all head right ( $\log_{10}$ [(all head left (s per 900 s))/[(all head right (s per 900 s))])	0.203	0.31	0.18	0.27	–0.13	0.370	0.96			0.04	0.84	0.54	0.67	
Duration all head left/all head right ([all head left (s per 900 s)]/[all head right [s per 900 s]])	1.59	2.04	1.51	1.86	0.74	–	–			–	–	–	–	
Duration head down right ( $\log_{10}$ s per 900 s)	0.29	0.65	0.82	0.56	0.57	0.966	0.02			0.94	0.09	0.68	0.01	
Duration head down right (s per 900 s)	0.95	3.47	5.61	2.63	2.71	–	–			–	–	–	–	
Duration standing ( $\sqrt{s}$ per 900 s)	6.4	9.3	8.9	7.4	9.4	2.10	0.01			0.16	0.70	0.47	0.02	
Duration standing (s per 900 s)	40.9	86.1	80.5	54.5	89.1	–	–			–	–	–	–	
Rate head up front ( $\sqrt{\text{bouts}}$ per 900 s)	0.62	0.58	0.64	0.84	1.03	0.274	0.32			0.007	0.24	0.51	0.27	
Rate head up front (bouts per 900 s)	0.38	0.34	0.41	0.71	1.06	–	–			–	–	–	–	
Rate freezing ( $\sqrt{\text{bouts}}$ per 900 s)	0.49	0.79	0.94	0.69	0.71	0.318	0.046*			0.91	0.20	0.87	0.05	
Rate freezing (bouts per 900 s)	0.24	0.62	0.88	0.48	0.50	–	–			–	–	–	–	
<i>Chamber system</i>														
Duration head down right ( $\sqrt{s}$ per 900 s)	6.1	9.7	7.1	8.3	7.7	2.48	0.27			0.97	0.85	0.02	0.07	
Duration head down right (s per 900 s)	37.2	94.9	50.1	69.5	59.3	–	–			–	–	–	–	

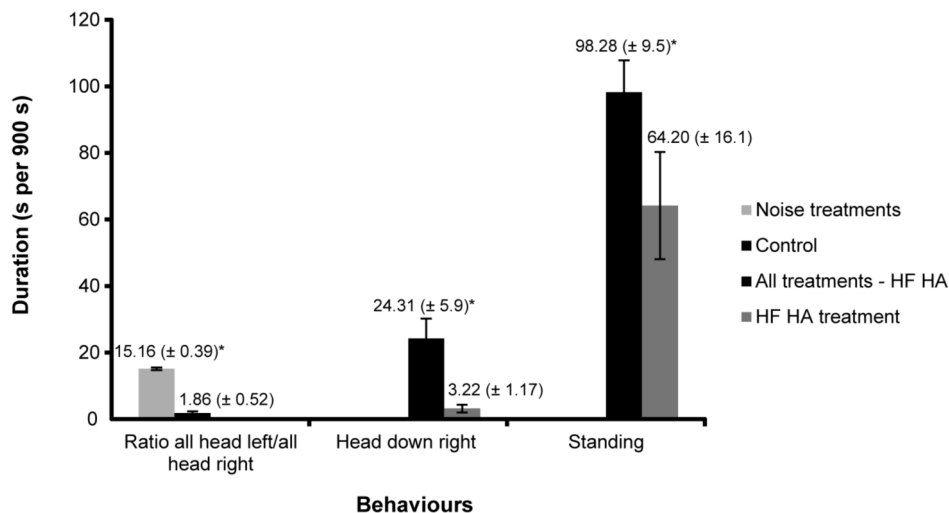
HF = high frequency, LF = low frequency, HA = high amplitude, LA = low amplitude C = control treatment.  
 SED = Standard error of the difference.

Figure 4



Duration of behaviours of lizards in the test chamber analysed with General Linear Model (GLM). Graph shows untransformed means (± SEM). \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

Figure 5



Duration of behaviours of lizards in the hiding chamber analysed with General Linear Model (GLM). Graph shows untransformed means (± SEM). \*  $P < 0.05$ .

culty for recording left and right head movements *in situ*, as the observers had left and right references based on the left and right of the chamber. If the animals turned back and faced the TC, the spatial reference would change and observers could potentially make mistakes when assigning right or left movements. Nonetheless, during the tests, lizards walked forward more than 90% of the time, which allowed us to record head lateralisation in a consistent manner. Therefore, it was possible to make deductions about the lizards' head directionality as they almost always kept their heads facing the EC. Furthermore, the observations were related to the particular traits of the mining noise, such as its broad frequency range when compared to other anthropogenic noises (Peng *et al* 2010), and any inaccuracies would have been independent of treatment.

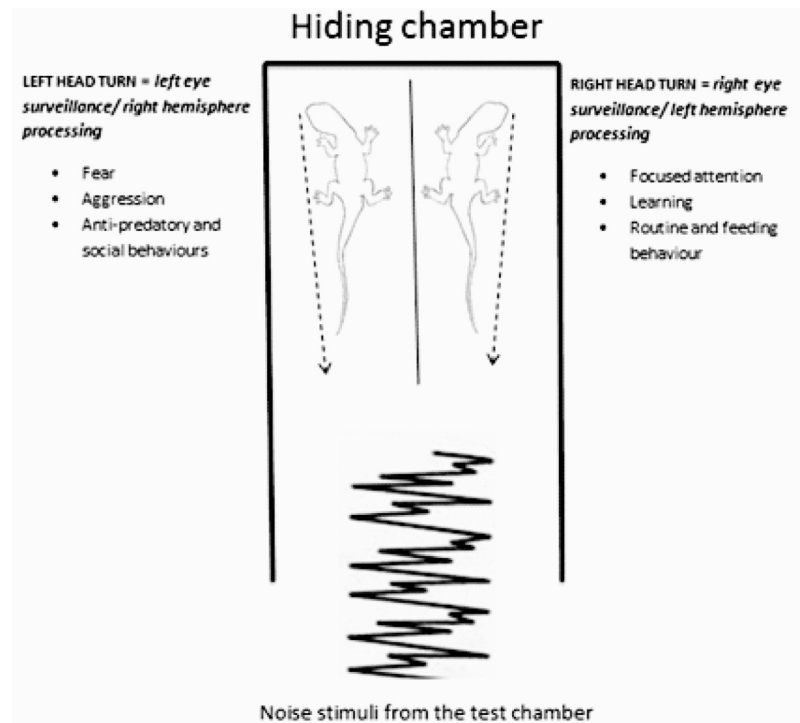
### Head positions as indicators of visual lateralisation and fear

In the hiding chamber, for each second the lizards held their heads to the right, they spent 1.75 s 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. Brain asymmetry is a central tenet in neuroscience, indicating the specialisation of the two hemispheres (which are also related to differences in size and anatomical structure) to control different tasks (Csermely & Regolin 2012). Lateralised behaviour in response to emotional input is regarded as a direct consequence of brain asymmetry; the



Figure 6

Conceptualised lateralisation and information processing in right and left brain hemispheres in response to mining noise from a posterior source (based on Bonati *et al* 2010).



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 involve fear and aggression as well as escape responses (Ocklenburg *et al* 2013).

Lateralisation has been well established in several species of lizards, for example, *Podarcis muralis* (Bonati & 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). Several studies have assessed visual lateralisation in lizards when exposed to both predatory and non-predatory stimuli. For example, the common wall lizard (*P. muralis*) shows a left eye/right hemisphere preference when inspecting predators, which is regarded as lateralised fear processing (Bonati *et al* 2010), whilst it exhibits a right eye/left hemisphere inclination when exposed to prey (Bonati *et al* 2008). This feeding-related response is also observed in the ornate dragon (*C. ornatus*), which increases its right eye preference as prey becomes more familiar (Robins *et al* 2005).

Along with the left eye/right hemisphere response to fear, there is a similar response in behaviours related to aggressiveness. For example, the Carolina anole (*A. carolinensis*) prefers to bite, threaten and perform aggressive movements to a conspecific using its left eye as guidance (Deckel 1995). Likewise, striped plateau lizards (*S. virgatus*) present the same lateralised response when gravid females are

exposed to males placed in different fields of vision, with the left visual field being the one that results in 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 the lizards, exercising a preference for left head turns allowed left side of the head exposure (right brain hemisphere) to the stimuli source, possibly indicating a fear-related reaction (Figure 6).

Animals with laterally positioned eyes, such as lizards, initiate visual monitoring of the environment before they decide to flee when threatened by possible predators (Cooper 2008). Likewise, lizards prefer to initiate surveillance with the left eye when first emerging from the refuge selected after they have fled (Martin *et al* 2010). Therefore, the results obtained in our study confirm the existence of visual lateralised responses to identify possible threats. Nevertheless, the present experiment was based solely on auditory stimulation. This suggests that even when animals sought to use their vision to identify possible threats, the responses observed were triggered primarily by noise identification and processing.

Even though there is no previous research studying lateralised hearing responses in reptiles, it has been proven that, despite the positioning of the ear canal behind the head, lizards have one of the best ear directionalities amongst vertebrates of the same size (Christensen-Dalsgaard 2005). This phenomenon typically occurs within a 1.8–2.4 kHz frequency band around the best frequency of hearing, due

the interference of ipsi- and contra-lateral inputs. Therefore, it is very likely that lizards can respond with lateralised behaviours to auditory stimuli, as these animals are capable of distinguishing the direction and angle of sound due to tympanic coupling, despite the size and characteristics of their ear canal (Christensen-Dalsgaard 2005). This makes our study the first account of noise causing lateralised behaviour responses in lizards.

In a previous study by Bonati *et al* (2010), lateralised escape reactions in the common wall lizard were stimulated by beating a brush against a transparent tube containing the animal. Afterwards, they were allowed to decide the direction of their flight in an open-field test. Animals performed frequent pauses during their locomotion that allowed them to visually survey the environment, as reported previously in other studies (Brana 2003), and there was a significant preference to turn their heads to the left (Bonati *et al* 2010). Although it was concluded that visual lateralisation was the main factor responsible for the lateralised head movements, it is worth noting that the stimulus used (brush-beating) also generates auditory stimulation that could have contributed to the lateralised behaviour observed. Therefore, it is possible that visual lateralisation is related to the auditory characteristics of the stimulus presented.

In addition to increases in movements of the head to the left in the hiding chamber, when exposed to low amplitudes lizards spent more time with their heads facing to the right and downwards 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 & Hayes 1984). Further research is required to fully understand the importance of the acoustic environment for EBT lizards' behavioural reactions.

#### Effects of high frequency mining noise on EBT lizards' acoustic processing

In addition to the lateralised behavioural responses to noise in general, EBT lizards also presented specific lateralised 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 behavioural 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 behaviour and have been previously categorised as posture changes, ie adjustments in posture that are not associated with locomotion and are predominantly head movements for visual surveillance (Greenberg 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 behaviour of the Carolina anole (*A. carolinensis*), an iguanid lizard, posture changes increased with perturbations of the environment (eg foliage- and air-induced movements) and were

positively correlated to handling or exposure to new surroundings (Greenberg 1993). Thus, our observations in the TC, where animals were initially exposed to noise, point to a stress-related reaction in high frequency, high amplitude noise.

In addition, in the hiding chamber, EBT lizards decreased the time spent with their heads facing 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, but it was particularly associated with high frequency, high amplitude sound.

In wildlife, anthropogenic noise distorts and masks auditory cues (Blickley & Patricelli 2010). If a noise overlaps with the 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 & Maderson 1973). Many lizards produce this vocalisation when afraid, being handled or during escape attempts, accompanied by aggression and the deliberate inflation of the body (Warwick *et al* 2013). EBT lizards produce hissing sounds when fearful or when displaying agonistic behaviour (Turner 2010). Hissing has been recorded when emitted by the lizard *Pristidactylus volcanensis*, and it comprises a frequency spectrum between 2.3 and 3.6 kHz (Labra *et al* 2007), ie above 2 kHz.

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 of 3–13 kHz (Young 1991). In addition to hissing, rattling or tail vibrations are recurrent sounds during aggression by snakes, with a frequency range of 2–20 kHz (Young 2003).

These acoustical signals that are 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 to the similarity of frequencies from both acoustic stimuli. Such deception could explain the lateralisation 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 behaviour in the hiding chamber in HF HA may have a similar explanation. Standing was defined in this experiment as maintaining the same body position (keeping the chosen centre of gravity unchanged), while exploring the environment visually (by head

movements) and/or engaging in tongue-flicking. As both tongue-flicking and head motions can constitute exploratory behaviours for lizards (Greenberg 1993, 2002), we can attribute an exploratory function to standing. Exploration activities may be used by reptiles to evaluate mild stressors (Greenberg 2002), but may also be considered a sign of good welfare when present with unhurried locomotion (Warwick *et al* 2013). Exploratory behaviours may be a sign of pronounced stress when completely absent, severely decreased or increased (Warwick *et al* 2013). Decreased freezing in the EC and HC suggests an overall reduction of stress levels, even though there are still remains of the suspected stress-related reaction from the left eye/right hemisphere head responses discussed above.

High amplitude noise apparently elicited stress, which is possibly related to the acoustic features that mining noise and other sounds with threatening characteristics share. This acoustic overlapping results in mining noise mimicking environmental signals, an erroneous interpretation of the surroundings and the consequential generation of certain behavioural responses by the EBT lizards. The possibility that noise pollution might mimic animals' signals has been little explored, although there is evidence that some animals do increase their vigilance patterns with anthropogenic noise (Quinn *et al* 2006), which could be related to acoustic deception. Nonetheless, it has been recently reported that an endangered species, Stephens' kangaroo rat (*Dipodomys stephensi*), responds with alertness and foot-drumming (a behaviour used in territorial and mating contexts) to traffic noise playback. This playback is an anthropogenic noise that can mimic specific auditory signals for some animals, thereby deceiving them into engaging in false responses that can be energetically costly and decrease their survival (Shier *et al* 2012).

Whether this phenomenon occurs in reptiles and other taxa should be the subject of further research, including the study of the frequency spectrum and composition of different noises, the analyses of distress and aggression calls and acoustic predatory cues of several species, as well as obtaining an in-depth knowledge of animals' hearing patterns.

### 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 and shelter. Such mitigation is exemplified by the fact that freezing in the HC was reduced when compared with TC. While the exposure designed for this experiment was variable and acute, in a natural environment exposure to noise may be constant and prolonged; further research is needed to test the responses over longer periods of time.

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 indi-

vidual, and the overall investment that an animal has made in a specific habitat (such as gaining territory, establishing dominance amongst peers) (Wright *et al* 2007).

The effects observed in our study could be greatly enhanced in the field with long-term exposure which, in turn, could generate long-term stress related to immunosuppression and reproductive malfunction, diminished body condition and accelerated ageing (Romero & Butler 2007). Moreover, noise is not the only stressor wild animals face and, when many negative stimuli are combined, a stronger physiological response is generated which proliferates inflammatory factors and increases sickness (Deak 2007). Nevertheless, animals that are subjected to long-term noise stress can habituate and learn that certain stimuli are neutral (Bejder *et al* 2009; Samson *et al* 2014). Most studies on anthropogenic noise and wildlife have assessed instantaneous reactions due to the difficulties of evaluating responses over long periods of time (Nisbet 2000). Habituation cannot be assumed to be a consequence for long-term noise exposure because this has rarely been tested (Bejder *et al* 2009). Likewise, exposure to anthropogenic noise may become neutral if animals lose their hearing or if they are too energetically challenged to respond (Wright *et al* 2007).

Due to the difficulties surrounding the measurement of habituation, tolerance (the stimulus intensity that an individual is able to endure without responding in a defined way) is often tested (Nisbet 2000). In this study, lizards' avoidance patterns and lateralised behaviour suggested that high frequency, high amplitude mining noise was the least tolerated. Since the process of habituation to chronic stressors may be influenced by a number of factors, further research is necessary to evaluate whether the results obtained in this experiment would be exacerbated or diminished by chronic continuous, exposure, which would resemble the regime that animals endure in the wild.

We have also hypothesised that the responsiveness observed with high frequencies associated with high amplitudes 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. Likewise, further research is needed to identify whether sound mimicking is occurring on mining noise exposure in the wild and if habituation to this phenomenon is possible.

### Conclusion

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 s with their head positioned left for every second that they positioned it to the right, and they decreased the amount of head orientation to the right with high amplitude exposure in the whole chamber system, suggesting a lateralised fear-response. Also, in the test chamber, they increased the time and number of occasions they spent freezing when exposed to

high frequency, high amplitude noise, 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.

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