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The effects of simulated transport on the behaviour of eastern blue tongued lizards (Tiliqua scincoides)

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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 analysed 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 min, after which each lizard was removed. Lizards exposed to Cold spent less time in the test chamber (330 vs 434 s) and more time inactive in the escape chamber (148 vs 40 s). They also spent longer walking towards the hiding chamber (18.0 vs 10.5 s) and walking in the hiding chamber away from the stimulus (3.6 vs 2.3 s). 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 tongued lizard, noise, temperature, transport, wildlife trade*

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 30,000 individuals), with a further 2–3% mortality within two days of importation (or approximately 30,000–45,000 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 *Scinicidae*, *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).

Reptiles are highly represented in the illegal trade worldwide; it has been estimated from the bulletins issued by a wildlife monitoring network, TRAFFIC (www.traffic.org) 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 $US6 million (Tapley *et al* 2011). 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 5,000 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 over-exploitation; 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 are 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 7,700 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 recognised 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 the 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, ie species inhabiting limited ecological niches with specific characteristics required for the lifecycle 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). Some of the noise generated by vehicles is within the lizards' hearing range (Saunders *et al* 2000; Christensen-Dalsgaard 2005). 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 Chinese soft-shelled 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 (ie, 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 apoptosis, 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 co-ordination 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 both for 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 1988).

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. We hypothesised that transport stimuli can be negative experiences for the lizards, which would generate aversion.

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).

Study 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 60 × 39 × 40 cm; length × width × height and three 95 × 52 × 53 cm). 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).

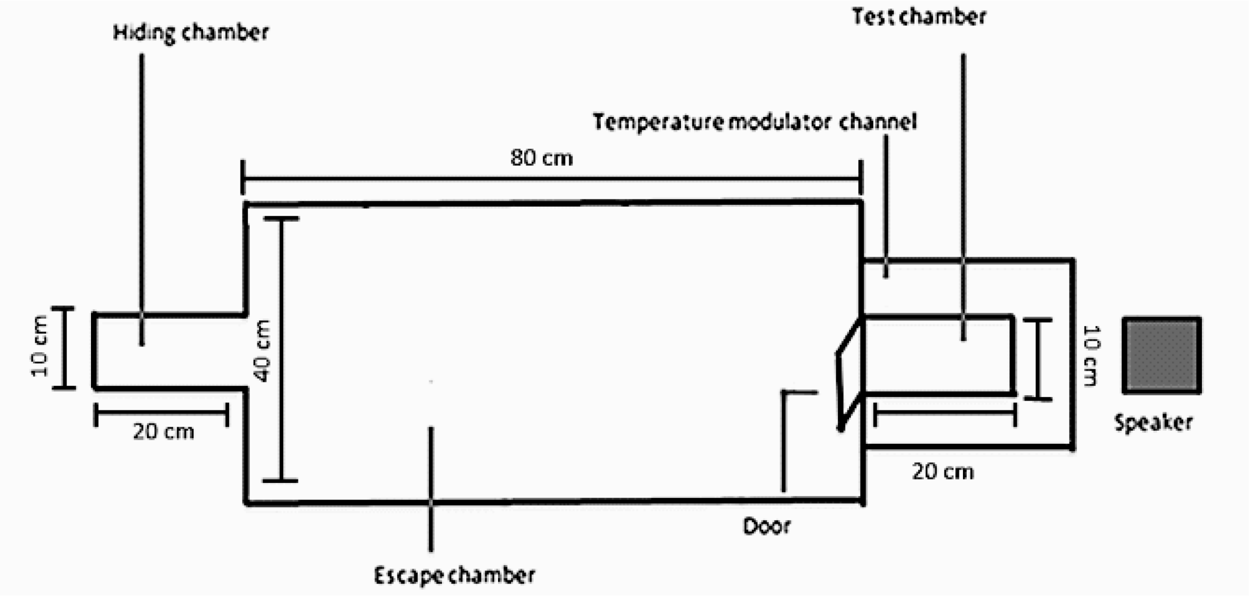
Diet

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 fruit 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).

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

Figure 1



Stimulus aversion experimental apparatus with test, escape and hiding chambers, scale 1:10 cm.

chamber that was 20 × 10 cm (length × width), 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 × 40 cm), 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 × 10 cm), 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 optimum level of detail possible in the experimental recordings.

The testing apparatus was inside a room at ambient temperature, recorded daily (mean [± SEM] temperature: 27.7 [± 0.05]°C). 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 min 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 fruit (mangos and strawberries) for the final test.

Generation of stimuli

Aversion to four different stimuli was tested during 15-min periods: Cold (C), Heat (H), High Frequency Noise (HFN) and Low Frequency Noise (LFN), with a Control (CT) treatment for comparison. Taking into account the recommended temperature zone for EBT lizards (20/25 to 30/35°C) (Turner & Valentic 2001), treatment C 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 C) or two heating blankets (treatment H), placed inside the channel for 10 to 20 min 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 (27.7 [+ 0.05]°C). Background noise levels were verified to be below 50 (± 0.1) dB (A) using a Digital Sound Level Meter (model Q1362, Dick Smith Electronics, Australia).

To broadcast noise and generate vibrations, two speakers (Multimedia Computer Speakers ACS5, Altec Lansing, CA, USA) 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 90 dB (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 (Hendricks 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 [LFN] ≤ 500 and High Frequency Noise [HFN] > 500 Hz) using a sound editing software programme (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 (90 dB [A]) to the escape chamber (83.3 dB [A]) to the hiding chamber (82.2 dB [A]).

The vibrations produced by treatments HFN and LFN 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, USA). The sensor was coupled to a signal conductor via a USB port (National Instruments NI9233 Compact Daq Signal conducter, 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 32,525 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 in a Latin Square design with four replicates of each treatment for each lizard and five days per week used to present the five treatments to each lizard. Thus, each lizard was tested once daily and experienced all five treatments within a week. Lizards were placed individually in the test chamber, facing the escape chamber with the door closed. For a preliminary period of 5 s 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 h 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 were 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.

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 were transformed using square root or logarithm_{10} as required. Four specific contrasts were tested in the model: Control (CT) vs all stimuli, temperature (C and H) vs noise (LFN and HFN), C vs H and LFN vs HFN. 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 $P \leq 0.05$. All calculations were performed with the programme Minitab Statistical Software, version 16.

Table 1 Behaviour of lizards exposed to simulated transport stimuli.

Behaviour	Treatment means					SED	*P*-value		
	H	C	LFN	HFN	CT		C vs all stimuli	Temperature vs noise	H vs C
Inactive in TC									
s 15 min^{-1}	426	330	482	423	406	122.3	0.0007	0.09	0.13
Inactive in EC									
Log$_{10}$ s 15 min^{-1}	1.6	2.2	1.4	1.6	1.8				
s 15 min^{-1}	38.9	148.0	24.0	40.7	56.2	0.40	0.03	0.01	0.006
Inactive in HC									
Log$_{10}$ s 15 min^{-1}	0.70	1.01	0.61	0.77	0.53				
s 15 min^{-1}	5.01	10.3	4.1	5.9	3.4	0.40	0.03	0.28	0.14
Walk EC to HC away from wall									
Log$_{10}$ s 15 min^{-1}	0.57	0.84	0.58	0.73	0.57				
s 15 min^{-1}	3.8	7.0	3.8	5.5	3.8	0.26	0.04	0.59	0.05
Walk EC to HC by wall									
Log$_{10}$ s 15 min^{-1}	0.84	1.07	0.69	0.66	0.93				
s 15 min^{-1}	7.0	11.9	5.0	4.6	8.6	0.34	0.02	0.02	0.18
Walk HC away from stimulus									
Log$_{10}$ s 15 min^{-1}	0.35	0.55	0.34	0.43	0.31				
s 15 min^{-1}	2.3	3.6	2.2	2.7	2.0	0.20	0.04	0.38	0.06
Tongue flick									
Square root s 15 min^{-1}	4.1	4.7	4.7	4.0	4.6				
s 15 min^{-1}	2.0	2.2	2.2	2.0	2.1	1.04	0.47	0.96	0.24

H: Heat; C: Cold; LFN: Low Frequency Noise; HFN: High Frequency Noise; CT: Control treatment; TC: Test chamber; EC: Escape chamber; HC: Hiding chamber. SED: Standard error of the difference.

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 1). 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 LFN was compared with HFN there were no significant differences in any of the behaviours analysed. Also, there were no treatment effects in any of the chambers on the time that lizards spent with their head up or down, analysed with the Kruskal-Wallis test ($P = 0.45$ and $P = 0.40$, respectively), or other behaviours analysed as binary variables. The means (± SEM) across all treatments 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.

Discussion

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 × 140 cm) than small (70 × 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-min 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, the western shingleback (*Tiliqua 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 *et al* 2004). When C and H were compared, lizards were more inactive in the escape chamber when C 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 the bobtail lizard (*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 the western fence lizard (*Sceloporus occidentali*), this rhythm is not only determined by the environment but also by an endogenously generated behaviour pattern, because the lizards maintain their nychthermal (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 C should be further studied, taking into account the evidence discussed above regarding the nychthermal 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, well-being 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 inactive in the test chamber when heat was applied, which could be linked with their natural heat-seeking behaviour.

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 (Guillette *et al* 1995) which can induce, amongst other effects, infections in the respiratory system and a reduction in digestion rate, which may even 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

We expected animals to experience LFN as a vibrational stimulus and HFN as an auditory stimulus since the hearing range of lizards is 1–3 kHz (Saunders *et al* 2000; Christensen-Dalsgaard 2005). In one study most of the spectral noise energy experienced inside the cabin of a simple transport vehicle was between 20 and 200 Hz (Soltani & Demneh 2011). LFN 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, such as the leopard lizard (*Gambelia w. wislizenii*), good auditory perception of LFN (300–700 Hz) has been acknowledged, as well as perception of vibrations below their hearing range (Wever *et al* 1966). For the chameleon (*Chamaeleo lyptrat*), 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 LFN 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 related purely 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 LFN generated by equipment such as air conditioning and lights, which may lead to habituation. This also applies for HFN, which did not have any measurable effects on lizards' behaviour. Furthermore, because of the lizards' hearing range (in which the best hearing sensitivity lies in low frequencies) and the greatest amount of energy in a vehicle cabin noise lying between 20–200 Hz (as mentioned above), reactions to HFN were not observed.

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 transport 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 emphasised 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 C (15°C), thus making avoidance an expected consequence for treatment C (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 the 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 carried out addressing different sets of frequencies at different amplitudes, as well as different sources of vibration apart from sound. Also, long- 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 does not 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.

Conclusion

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 mean (± SEM) 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.

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