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RESEARCH ARTICLE

Ecotoxicology

Effects of dietary lead exposure on the call of Japanese quail (*Coturnix japonica*) hatchlings

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Abstract: Effects of experimental dietary exposure to the heavy metal Lead (Pb) on the calls of Japanese quail (*Coturnix japonica*) hatchlings were investigated in the present study. Trials with the heavy metal were conducted over three weeks (5 days of exposure, 3 days of non-exposure and 13 days of exposure) with two-week old hatchlings, following recommendations for exposure trials. A control trial was also included. The Pb concentrations in the prepared feed used in the exposure trials were 238, 389 and 532 µg kg^{-1}. The daily food consumption was quantified to calculate the ingested amounts of the heavy metal. Calls of the hatchlings were recorded prior to exposure, and on days 5 and 21 of the exposure trial, and the sonograms were assessed for five endpoints –frequency of notes, duration of syllables, notes per syllable, inter-syllable duration and inter-note duration. Exposure to all three tested levels of Pb resulted in calls with shorter syllables and fewer notes. Changes were evident only at day 21. Both directional and hormetic responses were noted for the different endpoints. These findings highlight that vocalization patterns, which are relatively easily detected, could serve as an important non-invasive tool for monitoring Pb pollution in the environment.

Keywords: Birds, calls, heavy metals, pollution, toxicity.

INTRODUCTION

Communication is a vital aspect of animal behaviour, and in birds the primary means of communication are visual and vocal (Kumar, 2003). Elaborate plumage, brightly coloured bare skin, wattles, and colouration in tail, beak and feet are physical features that play a role in communication (Torres & Velando, 2003). Acoustic signals may be produced only when required (Kumar, 2003) and can be used to transmit a high volume of information efficiently making it an ideal method for communication over long distances.

Two categories of bird sounds can be recognized, namely, songs and calls. Usually, songs are of longer duration and are more complex and contain more musical variations than calls. Songs mainly play a role in mate choice and territorial defence (Catchpole, 1987). Calls have less musical variation than songs and tend to be of shorter duration (Marler, 2004). Combinations of different calls or note types have been documented as forms of communication in birds (Kumar, 2003). Unlike songs, calls have a wider range of functions which include indicating a bird's location or the location of food sources, maintaining cohesiveness within a flock, raising an alarm in the presence of predators, and expressing hunger (Marler, 2004).

In their development, songs and calls have a learnt and/or a genetic component. Recently, evidence has come to light of the development of song and other secondary sexual signals being linked to physiological stress. For instance, food deprivation (Buchanan *et al*., 2004), parasite infestations (Buchanan *et al*., 1999) and challenges to the immune system (Garamszegi *et al*., 2004) have been shown to cause changes in song characteristics in birds. A few studies have shown that

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stress imposed by environmental pollution could also adversely affect vocal characteristics of birds (Wingfield & Sapolsky, 2003). Slabbekoorn and Peet (2003) have reported that birds sing at a higher pitch in areas with noise pollution. Gorissen *et al.* (2005) document that heavy metal exposure altered song patterns of the male Great tit (*Parus major*). Fields and Mitchell (2014) report that songs of wrens and sparrows along a methyl mercury contaminated river were simpler, shorter and lower-pitched than songs of birds in unpolluted sites. De Leon *et al.* (2013), studying the effect of polychlorinated biphenyls (PCBs) on the songs of two passerines, Black-capped chickadee (*Poecile atricapillus*) and Song sparrow (*Melospiza melodia*), document that there was a link between the pollutant load and features of the songs, *viz*, the species-specific identity signal in the Black-capped chickadee and the trill performance of the Song sparrow. These studies show that environmental pollution can have an impact on song attributes, but its effect on bird calls has not been investigated.

Lead (Pb) is a highly toxic non-essential trace metal which has been recorded in the environment at levels that exceed safety thresholds (Wijayawardhana *et al*., 2016; Merismon *et al*., 2017). Work on over 120 avian species has shown that Pb exposure leads to sublethal or lethal toxic responses, and that there was considerable interspecific variation in tolerance to this metal (Haig *et al*., 2014). Pb ingestion is reported to cause direct mortality in some bird species (Hoffman *et al*., 1981; Finkelstein *et al*., 2012). Among other impacts are the alteration of the structure and function of the kidney, bone, the central nervous system, and the haematopoietic system, leading to adverse biochemical, histopathological, neurological, and reproductive effects, with many of these effects being evident at a very young age as shown in studies with nestlings of the Western bluebird (*Sialia mexicana*) and Japanese quail (*Coturnix japonica*) (Fair & Myers, 2002; Fair & Ricklefs, 2002). Li *et al*. (2021) have shown that air pollutants including aluminium, arsenic, cadmium, iron, manganese and lead have caused changes in immunological, antioxidative and haematological parameters, and body condition of Eurasian tree sparrows (*Passer montanus*). Adverse effects of Pb have been observed at levels above 4 mg kg^{-1} (Burger & Gochfeld, 2000).

The objective of this study was to investigate the effects of dietary exposure of Pb on the calls of hatchlings of the Japanese quail (*Coturnix japonica*). To the best of our knowledge, this work is the first attempt to experimentally test the possible impacts of a heavy metal on bird calls. An extensive review by Richard *et al*. (2021) has outlined the impacts of the most common anthropogenic pollutants on birds, which included heavy metals and their impact on direct mortality and sublethal effects, although the impact of vocalization has not been documented.

MATERIALS AND METHODS

Collection and housing of the experimental animals for acclimatization

The experimental facility to conduct the trials was set-up in an isolated part of a residential building located in proximity to the poultry farm in a rural area in Sri Lanka (6.890489 N, 80.086396 E). This avoided the hatchlings from being unduly stressed owing to transport and allowed the experimental conditions (with respect to silence, physical space, ventilation, and lighting) to be satisfactorily provided. Twenty four one-week old hatchlings of the Japanese quail were collected from the nearby selected poultry farm. In doing so, it was ensured that the hatchlings were from different parental stocks and hence non-related. Selection for sexes was not attempted since males and females could not be distinguished with high accuracy before hatchlings are three weeks old (Rathert *et al*., 2017). The hatchings were then individually picked at random to form four lots of six hatchlings – the four lots being for the control and the three levels of Pb to be tested. These were housed in four specially constructed cages for acclimatization. The cages (each 75 cm × 24 cm × 25 cm) were constructed out of wood and wire-mesh following Richard *et al.* (2010). Noting that previous studies have reported that the development of quails of this species takes about 4 weeks (Sant'Ana *et al*., 2005), the acclimatization period was set as one week (OECD, 1984), and the hatchlings were exposed to the heavy metal when they were 14 days old. During acclimatisation, the hatchings were supplied with a basal diet of commercial starter feed (Chick starter, Prima, Sri Lanka) at the prescribed weights following Ahuja (1990). Water was provided *ad libitum.* Each cage was provided with heat from an incandescent bulb of 100 W (Rezende *et al*., 2017). The bulb was suspended above the centre of the cage and its height was adjusted as necessary to keep the temperature within the cage at 31.5° $\pm$ 0.1 °C (IR thermometer gun ±0.1 °C, Sper Scientific, Taiwan) following Randall and Bolla (2008). The one-week old hatchlings were provided with 24 hour lighting during the acclimatization period (following Ahuja, 1990).

Selection of test concentrations

Previous work on the Japanese quail has reported zero mortality for dietary exposure to 10^6 µg kg^{-1} of Pb acetate (Edens *et al*., 1976; Hamidipour *et al*., 2016). Hence any impact of much lower concentrations was expected to be sub-lethal. Therefore, 250, 400 and 550 µg of Pb per kilogram of dry commercial feed were used for preparing the test feed. The mean levels of Pb recorded by Diyabalanage *et al.* (2016) in rice (*Oryza sativa*) growing in the three climatic regions of Sri Lanka, which were 257 µg kg^{-1} (wet zone), 271 µg kg^{-1} (intermediate zone) and 322 µg kg^{-1} (dry zone) were also considered. The low and mid test levels of the metal selected for the study broadly covered this range and could hence be considered environmentally relevant on a local scale.

Preparation of the exposure diets

Commercial feed (Chick starter, Prima, Sri Lanka) was used as the basal diet for the hatchlings (Emadi *et al*., 2015). For preparing each test level feed the following procedure was adopted. Initially, 500 g of starter feed was taken. The starter feed, which is in the form of pellets, was ground in a blender and approximately 100 g of this was then transferred to a plastic mixing bowl and the appropriate quantity of Pb acetate was added. The rest of the ground feed was then gradually added, and the mixture was thoroughly homogenized using a hand-held mixer for 10 to 15 min, following Chowdhury *et al*. (2004) and Zhou *et al*. (2014). Hot distilled water was then gradually added until the consistency of the mix was appropriate for pelleting. The paste was then processed through a meat grinder with a 3 mm diameter outlet and the emerging mix pelleted, and the pellets were air dried (<50 °C) to a moisture content of 8 – 10%. After drying, a small quantity of each mixture was analysed for Pb, and the pellets were crumbled, packed in sealed plastic bags, and stored in a freezer until used (Zhou *et al*., 2014). This procedure was repeated as required for providing an adequate quantity of the feed. The measured concentrations (mean ± SD) of Pb in the test feed were 238.0 ± 3.7 µg kg^{-1}, 389.0 ± 5.2 µg kg^{-1} and 532.0 ± 6.6 µg kg^{-1}. The pellets for the controls were also prepared in this manner but without Pb. The measured Pb concentrations in the control (with no metal addition) were 1.2 ± 0.4 µg kg^{-1}.

Exposure procedure

For exposing the hatchlings to the heavy metal, they were kept in four cages especially constructed for this purpose. Each cage had a wooden circular base (radius 24 cm) and six compartments separated by thin wooden partitions arranged radially. The cage was enclosed on the outside with wire mesh, with provision to access each compartment. Each compartment had a floor area of approximately 300 cm^2 (as per OECD guidelines). The six hatchlings from each acclimatization cage were weighed and ringed with different coloured bands for identification, and transferred to one of the circular experimental cages, placing one hatchling in each of the six compartments. One set of six hatchlings would serve as the control and the other three sets would be exposed to the three levels of the heavy metal. They remained in their compartments during the trial period of 21 days. During the entire exposure trial of 21 days, they were maintained under natural light, 12 L: 12 D, (Calandreau *et al*., 2011; Simova-Curd *et al*., 2013), and at room temperature 28.82 ± 1.01°C (Edens *et al*., 1976; Gayathri *et al*., 2004). The four cages were set well apart; this would have reduced the possibility of hatchlings in a cage hearing the calls of those in other cages. The hatchlings used were 18 to 25 g in weight, with no significant differences among the treatments and controls (one-way Anova, $F_{3,20} = 0.93$, $p > 0.05$).

The trial was conducted over 21 days, which included 5 days of exposure (short term exposure), 3 days of non-exposure and 13 days of exposure (long term exposure), following OECD (1984) for dietary exposures. During the period of non-exposure, the hatchlings were provided with the basal feed without Pb. The amount of food provided per hatchling (which was increased twice during the trial) was as prescribed by Ahuja (1990) (days 1 to 5, 12 g per hatchling per day; days 6 to 8, 15 g per hatchling per day; days 9 to 21, 18 g per hatchling per day). The food provided to each hatchling was weighed and placed within its compartment daily at around 1100 h. The food which remained after 2 h was removed and weighed. These values were used for calculating the amount of food consumed and thereby the mean weight of the heavy metal ingested per hatchling (Table 1). Water was provided *ad libitum.* The weights of the quail hatchlings were recorded prior to exposure and at days 5 and 21. Ethical clearance was obtained from the Institute of Biology Sri Lanka for conducting the exposure trials (Reference No. ERC IOBSL 190 03 2019).

Recording calls

Prior to commencing the exposure trials, the methods used for attempting to record bird calls were reviewed. Two previously used methods, *viz*, (i) holding an individual within a cloth bag (Moore *et al*., 2008) and (ii)

Table 1: Mean (± SD) amounts of Pb ingested per day per hatchling for each exposure level. Values are means for six hatchlings per treatment and control.

Exposure period	Mean ± SD values of the amounts of metal consumed (µg g^{-1} body wt* of the hatchling)			
Pb exposure concentration (µg kg^{-1} of feed)	Control 1.20 ± 0.40	Low 238.00 ± 3.70	Mid 389.00 ± 5.20	High 532.00 ± 6.60
D 1 to D 5 (5 days of exposure)	0.00 ± 0.00	0.85 ± 0.14	1.38 ± 0.21	1.98 ± 0.39
D 6 – D 8 (3 days of non-exposure)	0.00 ± 0.00	0.61 ± 0.11	1.02 ±0.15	1.44 ± 0.30
D 9 – D 21 (13 days of exposure)	0.01 ± 0.00	2.97 ± 0.42	4.86 ± 0.67	7.08 ±1.34
Mean food provided per day per hatchling (g)	16.14 ± 2.59			
Mean food ingested per day per hatchling (g)	15.08 ± 0.29	14.94 ± 0.27	14.71 ± 0.36	15.14 ± 0.05
Total quantity of food ingested per hatchling over 21 days(g)	316.67 ± 6.09	313.67 ± 5.61	308.83 ± 7.49	317.83 ± 0.98
Total quantity of Pb ingested per hatchling over 21 days (µg g^{-1} body wt*)	0.02 ± 0.00	4.43 ± 0.67	7.26 ± 1.01	10.51 ± 2.03

*Body weight is the pre-treatment weight of the hatchlings of the exposure trial.

placing the hatchling individually in a meshed cage (Shafi *et al*., 1984) were also tested. Both methods failed as the hatchlings did not call. Hence, it was decided to record calls while the hatchlings were in their experimental cages. A sound recorder (TASCAM DR- 40 Linear PCM Recorder, USA) was placed directly above the centre of the cage with the microphone facing down. Recordings were done on three days – day 1 (prior to exposure), day 5 (after acute exposure), and day 21 (after chronic exposure). Three recordings were made, each of 2 min duration (following Simmons & Zuk, 1992), between 0800 – 1000 h for each treatment and the control. The individuals were distinguished by auditory and visual cues (the latter facilitated by the presence of coloured rings). When calls were emitted, the individual that was emitting the call was noted.

Analyses of sonograms

Syllables were used as the unit of assessment. A syllable is a group of notes separated by silent intervals (O'Reilly & Harte, 2017). The three 2-min sonograms recorded for each of the treatments/control on a given day were examined to pick six pairs of syllables for analysis. In doing so it was assured that each of the six pairs of syllables were those emitted by each of the six different hatchings. The first syllable of a pair was used to record the mean frequency of notes (Hz), duration of the syllable (in seconds), number of notes per syllable, and inter-note duration (in seconds), whereas both syllables of each pair was used to record the inter-syllable duration (in seconds) (n = 6 for each of the five endpoints). The sonograms were analysed using Raven Pro 1.5 software.

Statistical analysis

Body weights and song characteristics were tested for significant differences in means among the control and exposure concentrations using one-way analysis of variance (ANOVA) followed by Tukey's tests. The nature of the dose-responses was tested using linear and non-linear regression analyses for each of the endpoints. To approximate normal distribution, the endpoints used as variables were log-transformed prior to analyses. All statistical analyses were performed with SPSS 20.0 (IBM Corporation, 2011).

RESULTS AND DISCUSSION

The study showed that the body weights of the Japanese quail hatchlings at the end of the trial, *i.e.*, at day 21, were significantly different across the treatments and control ($F = 5.38$, $p < 0.01$, $R^2 = 44.65$). Those in the control (not exposed to heavy metals) were significantly heavier than those that were exposed, which was observed across all three concentrations (Table 2). No differences in weight were apparent between low, mid and high exposure levels.

Table 2: Mean weight (±SD) of quail hatchlings (n = 6 per treatment and control) at the end of the 21-day trial with exposure to three levels of Pb. Ingested amounts of the metal are given as µg g^{-1} initial body wt.

	Control	Low	Mid	High	One-way Anova and posthoc Tukey		
					$F_{3,20}$	P	R^2
Provided (µg kg^{-1} feed)	1.2 ± 0.4	238.0 ± 3.7	389.0 ± 5.2	532.0 ± 6.6			
Ingested (µg g^{1})	0.02 µg g^{-1}	4.43 µg g^{-1}	7.26 µg g^{-1}	10.51 µg g^{-1}			
Body weight (g)	84.2 ± 10.2^{a}	62.7 ± 10.3^{b}	60.0 ± 12.3^{b}	60.0 ± 10.8^{b}	5.3	0.01	44.65

Different superscripts indicate significant differences in body weights at $p < 0.05$

Table 3: Call parameters in quail hatchlings (n = 6 per treatment and control) prior to exposure, and at two stages of exposure (day 5 and day 21) to three Pb levels. The ingested metal levels at each exposure (low, mid and high) are given as µg g^{-1} initial body wt.

Parameter	Control	Low	Mid	High	One-way Anova and posthoc Tukey		
	0.02 µg g^{-1}	4.43 µg g^{-1}	7.26 µg g^{-1}	10.51 µg g^{-1}	$F_{3,20}$	p	R^2(%)
Day 0 (Prior to exposure)							
Frequency of notes (Hz)	4376.6 ± 206.3	4358.2 ± 151.9	4575.0 ± 257.0	4383.0±261.0	1.17	0.35	14.97
Duration of a syllable (s)	3.34 ± 0.69	3.98 ± 0.50	3.45 ± 1.07	3.61±1.71	0.48	0.70	6.75
Notes per syllable	5.50 ± 0.55	6.17 ± 0.98	5.50 ± 1.52	5.83±2.23	0.31	0.82	4.45
Inter-syllable duration (s)	1.77 ± 1.44	1.97 ± 1.07	1.28 ± 0.53	1.16±0.92	1.51	0.24	18.46
Inter-note duration (s)	0.48 ± 0.11	0.50 ± 0.12	0.54 ± 0.08	0.39±0.17	1.78	0.18	21.08
Day 5 (Short term exposure)							
Frequency of notes (Hz)	3987.0 ± 293.0	3956.9 ± 224.2	3863.0 ± 128.8	4057.0 ± 334.0	0.55	0.66	7.56
Duration of a syllable (s)	3.80 ± 0.87	2.83 ± 1.41	2.71 ± 0.87	3.99 ± 1.48	2.03	0.14	23.34
Notes per syllable	5.83 ± 0.75	5.33 ± 1.97	4.83 ± 1.72	6.33 ± 1.63	1.22	0.33	15.51
Inter-syllable duration (s)	1.31 ± 0.57	1.74 ± 1.26	1.42 ± 0.70	1.47 ± 1.23	0.06	0.98	0.96
Inter-note duration (s)	0.38 ± 0.06	0.37 ± 0.05	0.42 ± 0.05	0.44 ± 0.08	2.08	0.14	23.80
Day 21 (Long term exposure)							
Frequency of notes (Hz)	1867.0^{a} ± 544.0	2971.0^{b} ± 880.0	3204.6^{b} ±114.8	2400.0^{a,b,} ± 505.0	5.54	0.01*	45.38
Duration of a syllable (s)	1.24^{a} ± 0.59	0.32^{b} ± 0.11	0.13^{c} ± 0.11	0.12^{c} ± 0.08	20.29	0.001*	75.26
Notes per syllable	4.50^{a} ± 2.07	2.83^{a} ± 0.41	1.67^{b} ± 0.82	1.17^{b} ± 0.41	14.68	0.001*	68.77
Inter-syllable duration (s)	0.77 ± 0.22	0.55 ± 0.15	1.02 ± 0.95	0.87 ± 0.44	0.62	0.61	8.45
Inter-note duration (s)	0.39^{a} ± 0.32	0.13^{a,b} ± 0.03	0.04^{b} ± 0.05	0.05^{b} ± 0.07	5.81	0.01*	57.26

In each row, different superscript letters indicate significant differences between these values ($p < 0.05$)

The study showed that the exposure of Japanese quail hatchlings to Pb resulted in changes to the properties of their call (Tables 2 & 3). The nature and magnitude of these changes varied with the test level of the metal, and the duration of exposure. Prior to exposure, there were no significant differences in the five measured endpoints of toxicity across the control and the three groups that would be subsequently exposed to the heavy metal. Therefore, any significant changes could be attributed to the ingestion of Pb.

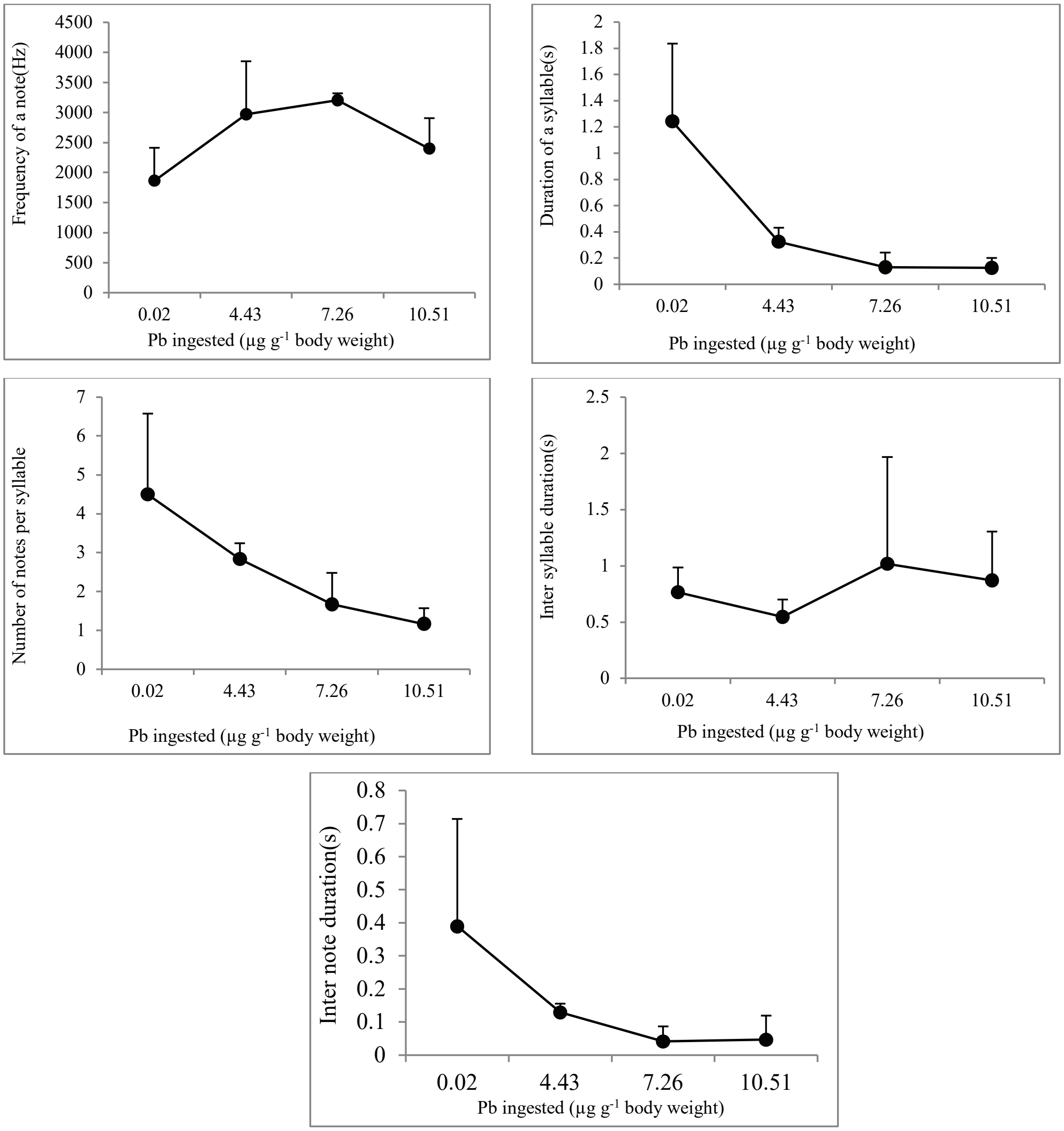

Figure 1: Five call parameters in the quail hatchlings exposed to three concentrations of Pb at the end of a 21-day trial (n = 6 sonograms per concentration). Amounts of metal ingested µg per g body weight were calculated using the pre-treatment body weights.

At day 5 there was no significant change in any of the five measured endpoints indicating that the nature of the calls remained unchanged following short-term exposure to Pb (Table 3). However, at day 21, except for the inter-syllable duration, significant changes were noted in the other four endpoints in the exposed birds compared to the control set (those that were not exposed). Significant directional trends were observed in three endpoints, *viz.*, duration of a syllable, number of notes per syllable, and inter-note duration (Figure I; Table 3). For example, by day 21, the duration of a syllable had declined by 74% in hatchlings exposed to the lowest dose of Pb (ingested levels were 4.43 µg g^{-1}) and by 90% in those exposed to the highest dose (ingested levels were 10.51 µg g^{-1}). The number of notes per syllable also declined progressively with the increasing level of exposure to Pb, decreasing

Table 4: Results of the linear and non-linear regression analyses conducted between the exposure levels and the five call parameters at day 21 of the trial.

Parameter	Equation	F	p	R^2 (%)
Frequency of notes *Non-linear*	Log Freq. = 3.536 - 0.01729(Log Pb) - $0.1071(\text{Log Pb})^2$	6.28	0.01[a]	37.4
Duration of a syllable *Linear*	Log Du. of a syll. = - 0.5411 - 0.3608(Log Pb)	39.51	0.001[a]	64.2
Notes per syllable *Linear*	Log Note. p. syll. = - 0.3572 – 0.1657(Log Pb)	20.02	0.001[a]	47.2
Inter-syllable duration *Non-linear*	Log Int.syl.Du. = - 0.4445 + 0.146(Log Pb) - $0.1953(\text{Log Pb})^2$	0.92	0.41	8.1
Inter-note duration *Linear*	Log Int.note.Du = 0.8074 – 0.1698(Log Pb)	15.66	0.001[a]	51.5

by 37% at the lowest exposure dose and by 74% at the highest dose. Thus, at day 21 the syllables were shorter and the notes were fewer than those in the control. The linear dose-dependent trends for duration of syllable, number of notes per syllable, and inter-note duration were highly significant, with R^2 being between 47–64% (Table 4). Hormetic or biphasic responses were observed for the other two endpoints. In the case of call frequency low and moderate concentrations induced significantly different responses from those of the control, whereas no significant difference was noted between the frequency of those exposed to the highest concentration and those of the control (Table 4 and Figure 1).

It has been reported that anthropogenic changes in the natural environment could lead to alterations in communication signals of wild birds (Slabbekoorn & Peet, 2003). The present study provides evidence for the potential of Pb to induce changes in the vocal characteristics of a bird species. Specifically, the study demonstrated that dietary exposure of Japanese quail hatchlings to environmentally relevant levels of Pb could bring about changes in the nature of their calls measured through five standard endpoints.

Some of the responses observed in the present study are consistent with those reported by others in field studies. Gorissen *et al.* (2005) reported that male Great tits inhabiting a heavily polluted site near a smelter sang significantly less frequently (almost 35% less) and their songs had a significantly lower repertoire size (almost 30% less) than those of males only 4 km away from the smelter. Fields and Mitchell (2014) document that wrens and sparrows exposed to methyl mercury sang simpler and shorter songs than birds that were not exposed. These observations could be taken as corresponding to the reduction in the number of syllables and the shortening of each syllable by incorporating fewer notes, in the calls of hatchlings that were exposed to Pb at day 21 of the trials in the present study. Dose-dependent trends were noted in the duration of a syllable, notes per syllable, and inter-note duration – the syllables were shorter and had fewer notes with increase in the exposure level. This is consistent with the findings of De Leon *et al.* (2013) that the song attributes of two passerines were linked to the load of PCBs to which they were exposed.

Fields and Mitchell (2014) also report that the songs of the birds exposed to methyl mercury had a lower pitch, but in the present study such a change was not seen. On frequency, which is responsible for pitch, a biphasic response (hormetic response) was observed. At low to moderate levels of exposure to Pb the calls were at a higher pitch, whereas at the highest levels of exposure calls were at a reduced pitch with the frequency not differing from those of the control hatchlings. Such biphasic responses were also seen in other endpoints. Several authors have reported such responses in parameters other than those of vocalization when birds are exposed to toxicants. A hormetic response for growth has been reported in chickens and ducks exposed to aflatoxins (Ostrowski-Meissner, 1984; Huff *et al*., 1986). Harding (2008) found that, in Red-winged blackbirds, at high levels of selenium uptake, egg production was greater and the eggs were heavier than when the birds were exposed to lower levels. Similar egg growth stimulatory responses were noted for two species of songbirds and for Mallard ducks exposed to selenium (Stanley *et al*., 1996; Ratti *et al*., 2006). Hormetic responses in breeding success have been shown in

European blackbirds exposed to Pb (Fritsch *et al*., 2019). Love *et al*. (2003) observed inverted U-shaped dose response curves for growth and adrenocortical hormones in captive American kestrels (*Falco sparverius*) after exposure to different PCBs.

Food deprivation has been shown to affect the song quality of the European starling (*Sturnus vulgaris*); when provided with a limited supply of food, songs were of shorter duration and less frequent (Buchanan *et al*., 2003). Such effects are reported to be more severe in nestlings (Spencer *et al*., 2003). In the present study, the Japanese quail hatchlings that were exposed to Pb, though showing no clear trend in the quantity of the food consumed, showed growth impairment, compared with the controls. This might suggest that the exposed birds experienced developmental stress. In studies on songbirds, Nowicki *et al.* (1998) have shown that in early life, developmental stress can have a deleterious effect on neural development, which may affect behaviour, life history strategies, and/or the development of secondary sexual signals. In males of the Great tit (*Parus major*) a reduced repertoire size at a heavy metal polluted site has been attributed to direct neurotoxic effects interfering with song system development or song learning (Gorissen *et al*., 2005). It has been shown that, since brain tissue is costly to produce and maintain, it is likely that only birds that are healthy could meet the neurological demands required for learning songs (Garamszegi & Eens, 2004). Hence, as costs are likely to be highest during development, exposure to a heavy metal such as Pb can cause severe learning and memory deficits (Finkelstein *et al*., 1998). It has been shown that Pb accumulates in bird brains and is reported to substitute for calcium even at minute concentrations, and that this affects protein kinase C resulting in the malfunctioning of neural excitation leading to memory loss in animals (Cid *et al*., 2009; Jaishankar *et al*., 2014). It is reported that another heavy metal, Cd, causes an abnormality in the size of neurons in the brain and the amounts of neurotransmitters, and that high doses lead to enlargement of brain cells (Gabol *et al*., 2014). The neurotoxic impacts arising from heavy metal exposure are likely to be linear, *i.e*., increased neurotoxicity at higher exposure levels. It is possible that such neurotoxic effects caused by Pb might have been responsible for some of the alterations in the nature of the call in the Japanese quail hatchlings exposed to Pb in the present study.

Systemic effects have also been implicated as a cause for observed changes in vocal characteristics of birds exposed to heavy metal contaminants. Devocalization in falcons (*Falco cherrug*) that suffered from acute Pb poisoning has been attributed to narrowing of the lumen of the syrinx (Molnar *et al*., 2008). The deposition of heavy metals in the syrinx and ventriculus in birds exposed to heavy metals has been linked to the flow of contaminated blood via branches of the carotid artery (Abdalla & King, 1976). The heavy metals Fe, Pb, Cd, and other inorganic and organic substances are said to be the probable cause of bronchi anthracosis associated with the narrowing of bronchi that would affect vocalization in ducks (Karadi & Al-Badri, 2018). Calabrese (2017) has documented that several physiological mechanisms have been cited as being responsible for different types of hormetic responses following exposure to a range of xenobiotics. Garamszegi and Eens (2004) report that intraspecific disparities observed in repertoire size, song length, and strophe length may be linked to brain space for a learned task. Hence, any or all of these factors combined might have led to the hormetic responses observed in some endpoints in our study.

The findings of the present study are important mainly because some of the noted changes in the nature of the calls were evident at environmentally relevant levels and over a limited period of exposure. The lowest levels of exposure corresponded to a level observed in rice grains in Sri Lanka. Through repeated ingestion of such material over a long period, birds would run the risk of acquiring quantities of Pb that are high enough to induce adverse impacts on vocalization. The present study is the first to experimentally test the relation between the level of heavy metal exposure and the magnitude of change in a range of call parameters. It was found that exposure to sublethal levels of Pb altered, to a considerable degree, the calls of Japanese quail hatchlings. Calls emitted by birds, as signals of different kinds, are linked to their survival. Therefore, changes induced by heavy metals, such as the shortening or dulling of calls, might, with time, lead to disruptions in bird communication, thereby affecting the long-term survival of the species. As suggested by Lackey (1994) and Suter *et al.* (1993), such alterations in vocalizations occurring in the wild could provide a snapshot of the integrity of an ecological system.

CONCLUSION

The present study has, for the first time, experimentally demonstrated the potential of the toxic heavy metal Pb to alter the nature of bird calls. In Japanese quail hatchlings subject to dietary exposure to Pb, the calls were shorter and contained fewer notes in comparison to those not exposed. Changes in calls were seen at the end of the

21-day trial period. Hormetic responses were noted for some of the endpoints. In the wild, such toxicity related endpoints could serve as an early warning of impending ecological change from chemical stress. In birds, vocalization patterns are relatively easily detected, and thus might function as a useful non-invasive tool for indicating heavy metal pollution in the environment. This in turn suggests that future studies must be more integrative and strive to document links between behavioural ecology and toxicology.

Conflict of interest

There are no financial or any other conflicts of interests.

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