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Passerine bird reproduction does not decline in a highly-contaminated mercury mining district of China☆

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A B S T R A C T

Mercury (Hg) is a neurotoxic element with severe effects on humans and wildlife. Widely distributed by atmospheric deposition, it can also be localized near point sources such as mines. Mercury has been shown to reduce the reproduction of bird populations in field observations in North America and Europe, but studies are needed in Asia, where the majority of emissions now occur. We investigated the reproduction of two passerines, Japanese Tit (*Parus minor*) and Russet Sparrow (*Passer rutilans*), in a large-scale Hg mining district, and a non-mining district, both in Guizhou, southwest China. Concentrations of Hg were elevated in the mining district (blood levels of 2.54 ± 2.21 [SD] and 0.71 ± 0.40 μg/g, in adult tits and sparrows, respectively). However, we saw no evidence of decreased breeding there: metrics such as egg volume, nestling weight, hatching and fledgling success, were all similar between the different districts across two breeding seasons. Nor were there correlations at the mining district between Hg levels of adults or juveniles, and hatching or fledgling success, or nestling weight. Nest success was high even in the mining district (tit, 64.0%; sparrow: 83.1%). This lack of reproductive decline may be related to lower blood levels in nestlings (means $<$ 0.15 μg/g for both species). Concentrations of selenium (Se), and Se-to-Hg molar ratio, were also not correlated to breeding success. Although blood levels of 3.0 μg/g have been considered as a threshold of adverse effects in birds, even leading to severe effects, we detected no population-level reproductive effects, despite ~25% of the adult tits being above this level. Future work should investigate different locations in the mining district, different life-stages of the birds, and a wider variety of species. The hypothesis that bird populations can evolve resistance to Hg in contaminated areas should also be examined further.

1. Introduction

Mercury (Hg) is a globally distributed pollutant that can be transmitted through atmospheric deposition to both aquatic and terrestrial ecosystems (Driscoll et al., 2013). It can be widely distributed by deposition even to the most remote regions of the world (Ariya et al., 2004); at the same time, it can be a particular threat near point source locations of pollution (Kocman et al., 2013). Mercury is a neurotoxic element that has been shown to have adverse effects on both humans (Mergler et al., 2007) and wildlife (Scheuhammer et al., 2007), to the degree that an international convention has been put in place to control its use and emissions (UNE, 2013). It is most biologically available, and toxic, in the form of methylmercury (MeHg), which is formed by bacteria in anoxic conditions (Ullrich et al., 2001). Because MeHg is difficult for animals to excrete, it biomagnifies across trophic levels (Boening, 2000; Lavoie et al., 2013). Therefore, MeHg has long been considered a particular problem for aquatic organisms. Recently, however, it has been shown to that MeHg can become elevated at high trophic levels even in terrestrial organisms, particularly in contaminated areas close to water sources (Brasso and Cristol, 2008; Mahbub et al., 2017).

Birds have become model organisms for the biomonitoring of Hg, because they are abundant and distributed across many trophic levels, and feathers and blood can be sampled non-destructively (Abdullah et al., 2015; Pilastro et al., 1993). Mercury can be toxic in many ways to

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birds, disrupting processes such as flight (Ma et al., 2018b), migration (Ma et al., 2018a), and cognition and decision-making (Kobiela et al., 2015). Recently, Ackerman et al. (2019) measured the total mercury (THg) concentration of nearly 1200 song birds of 53 species, and found a significant negative correlation with body condition – their model predicted that as the score of standard body mass and fat increased from 0 (worst) to 5 (best), the blood THg level declined an average of 44% and 34%, respectively. Further, numerous studies have demonstrated negative effects of Hg exposure on different aspects of reproduction (Whitney and Cristol, 2017). While some response variables (e.g., clutch or egg size) show inconsistent results, most studies have shown a reduction in the number of fledged young (Burgess and Meyer, 2008; Evers et al., 2008; Hallinger and Cristol, 2011; Jackson et al., 2011) or in the amount of breeding generally, including pre-egg-laying processes (Zabala et al., 2020).

In comparison to lab studies, field studies of bird reproduction may better indicate the real threat of Hg to the conservation of birds, given that birds under natural conditions may be further stressed by the requirements to forage, and by natural and anthropogenic threats other than Hg. Most such field studies, however, have been conducted in North America and Europe (e.g., of the 49 field studies reviewed in Whitney & Cristol, 40 were conducted in North America and six were conducted in Europe), where Hg emissions are trending downwards. In contrast, the majority of Hg emissions over the past two decades have been in Asia, and in some parts of Asia emissions are continuing to rise (AMAP/UN Environment, 2019; Pacyna et al., 2016; Pacyna et al., 2010). Hence, studies of the relationship of Hg levels to bird reproduction are particularly needed in Asia. Another gap-in-knowledge is how bird reproduction is affected near heavily contaminated sites, and particularly mines and their effluents (but see Hill et al., 2008). In such sites, birds might be challenged by the presence of multiple heavy metals (e.g., Ding et al., 2020). In this study conducted in mining and non-mining sites of China, we looked at both Hg and selenium (Se) concentrations in breeding birds. While Se can be toxic at very high levels, it can also be an antagonist to Hg (Ohlendorf and Heinz, 2011), and hence might play a protective role (Yang et al., 2008).

China is the country with the highest emissions globally (Pacyna et al., 2010), and it is also an important country for the mining of Hg (Jiang et al., 2006). We worked at the Wanshan Hg Mining District, known as the “capital of mercury”, since it was the largest Hg mine in Asia during the 1950s until the 1990s (Jiang et al., 2006), as well as at a reference district, Leishan, without a history of mining. We studied two cavity-nesting species that accept artificial nest boxes and are at different trophic levels: Japanese Tit (*Parus minor*), an insectivore during the breeding season, and Russet Sparrow (*Passer rutilans*), a granivore. We hypothesized that: 1) the Hg concentration in blood and feathers of birds would be higher in Wanshan than in Leishan; 2) the hatching success, fledgling success and nesting success of both species would be higher in Leishan than in Wanshan; 3) At Wanshan, reproductive success would be inversely correlated with the Hg concentration of the nestling or parent birds; 4) reproductive success at Wanshan would be positively correlated with the Se:Hg molar ratio of birds, due to Se’s potential protective effect, and its presence in the ores at Wanshan (Qiu et al., 2019).

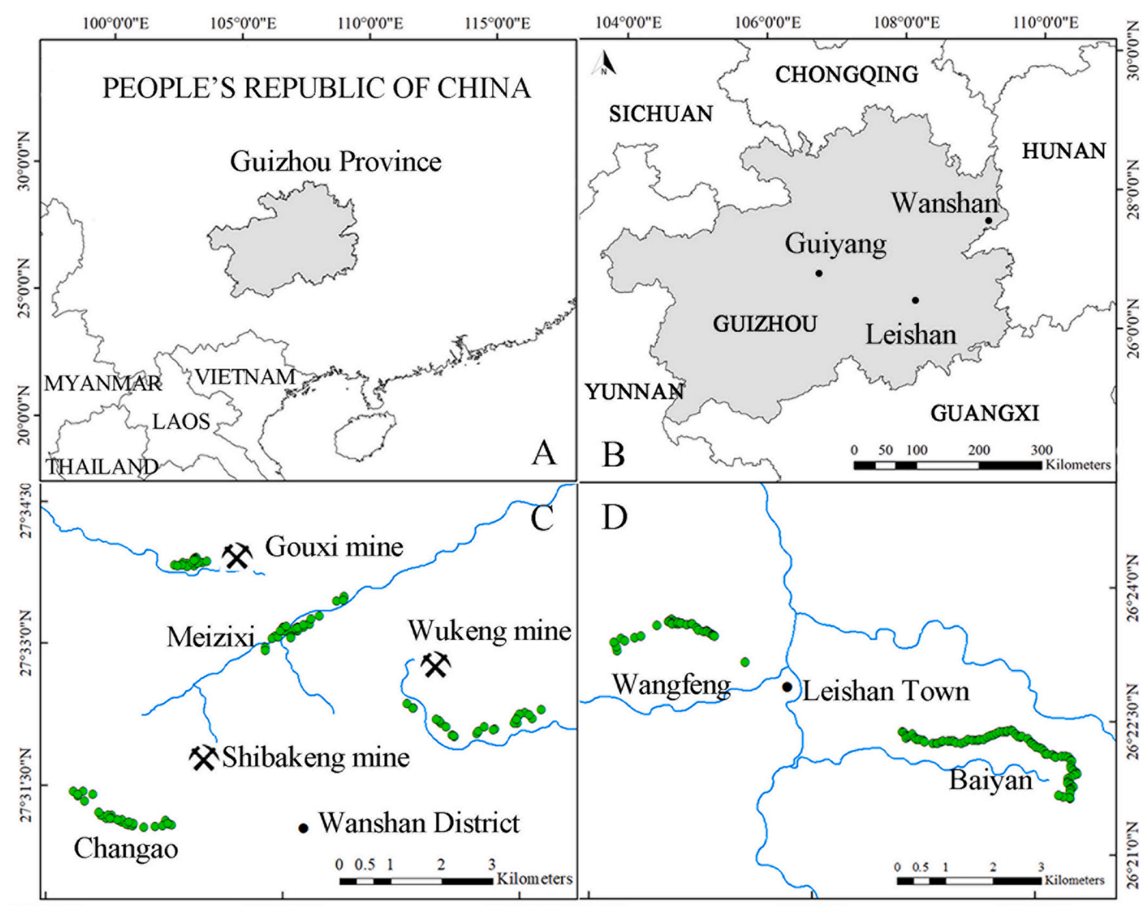

Fig. 1. Sites of the nest boxes that were sampled in this study. A: The location of Guizhou Province in China. B: The location of Wanshan Hg Mining District and Leishan (reference district) in Guizhou. C and D: The sites of the nest boxes (green points) in Wanshan and Leishan, respectively, which were attached to telephone phone along roads in agricultural areas, bordering open forests. Hammer and shovel icons indicate large mines or tailing areas. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2. Materials and methods

2.1. Study districts and sampling design

As the contaminated study site, we selected the Wanshan Hg Mining District, located in Tongren County in northeastern Guizhou Province, China (Fig. 1). Wanshan Hg Mining District has a 3000-yr long history of mining (Dai et al., 2012). Large-scale mining operations lasted between 1949 and the 1990s until the mines were officially closed in 2004, although it is reported that illegal artisanal mines continue to operate. Some of the largest tailings from the underground mines have been capped in concrete, but mine wastes continue to produce fluxes of Hg into the air (Dai et al., 2012), and into rivers (Zhang et al., 2010), which then produce soil contamination (Dai et al., 2013). This contamination leads to Hg bioaccumulation by rice, as well as poultry (Yin et al., 2017), and Hg exposure to humans (Qiu et al., 2008). Wildlife is also exposed, with MeHg levels rising across the food web (Abeysinghe et al., 2017). Although invertivorous animals usually have higher Hg levels than granivores (Ackerman et al., 2019), in Wanshan granivores can have high concentrations due to the contamination of the rice ecosystem (Abeysinghe et al., 2017). The reference district was near the town of Leishan, Leishan County, located in southeastern Guizhou, around 180 km distance from Wanshan District, where the general environment (elevation, precipitation, human disturbance level and crops) is similar, but there is no history of mining (Abeysinghe et al., 2017).

We placed artificial nest boxes in both districts beginning in the winter of 2018. Nest boxes were constructed of wood, with total dimensions of 10 cm (w) × 10 cm (l) × 20 cm (d), with an entrance hole of 4 cm in diameter in the front. The nest boxes were constructed so that the cover could be lifted up for checking on the nest progress and capturing the nestlings. All the nest boxes were set 2.5 m high on telephone poles, a position that ensures low nest predation, along roads in agricultural areas bordering open forests. In Wanshan Hg Mining District, the nest boxes were placed 200–2000 m from mine tailings in 2018 in three areas (Changao, Gouxi and Meizixi; see Fig. 1). In 2019, attempting to sample birds with higher Hg concentrations, we placed most boxes at another area, Wukeng, 50–300 m from the tailings, while continuing with some boxes at Meizixi. In Leishan, we placed the boxes in two areas in 2018, Baiyan and Wangfeng. In 2019, attempting to increase the numbers of tits, we placed all boxes in Baiyan, which has more of the open forest preferred by that species. In total, we placed 193 and 118 nest boxes in Wanshan and Leishan, respectively.

We selected two bird species, the Russet Sparrow (*Passer rutilans*) and the Japanese Tit (*Parus minor*), because they were the most common species to use nest boxes in the region, and differed in their diets. Both species are resident in Guizhou and do not migrate (MacKinnon et al., 2000). Adult sparrows are granivorous, whereas tits are generally insectivorous (with some seed-eating in the non-breeding season). Both species feed their young with terrestrial insect prey. In Guizhou, the breeding season of Japanese Tit is slightly earlier than Russet Sparrow, beginning from early April and lasting to the end of July in both study districts. Russet Sparrows, in contrast, begin their breeding in mid-May and continue to the end of August.

2.2. Monitoring of breeding ecology

We observed bird reproduction simultaneously in both districts to monitor breeding and take Hg samples. TS worked in both years in Wanshan. CH worked in Leishan in 2018, and then a local assistant (Yuanxi Yao) did so in 2019. However, all three observers trained and worked together during some periods to standardize methods. Starting in April and continuing through August, we checked all the nest boxes each week to determine if the box was being used by the target species. If nest material or a bird was seen, we then cut down the interval between inspections to three days. To estimate the exact day when eggs were hatched, we used the flotation method of Westerskov (1950). We then sequentially took information on the (1) egg weight and (2) volume (upon first observation of the eggs), (3) clutch size (when complete), (4) percent of eggs that hatched (hatching success), (5) nestling weight (at day 8 after hatching) and (6) percent of nestlings that fledged (fledgling success). By measuring these metrics, we covered the most common aspects of reproduction that have been investigated in Hg studies (clutch size, egg characteristics, hatching and fledgling; Whitney and Cristol, 2017). As a final cumulative response variable, we (7) classified a nest as successful if at least one nestling fledged; nesting success was thus assessed across the incubation and nestling periods. In total, we monitored 105 nests of sparrows (55 in Leishan, 50 in Wanshan) and 51 nests of tits (19 in Leishan, 32 in Wanshan). In 2018, there was some question as to whether the nestling weights were measured consistently on the eighth day, especially in Leishan, and we did not analyze these data.

2.3. Feather and blood sampling and sample storage

We captured the adult parents in the nest box when the nestlings were 10 days old, by inserting a transparent plastic door on the inside of the front of the nest box, so that the parent pushed it in when returning to the nest, and then got trapped inside. We primarily sampled female individuals, sexing the species by distinctive plumage (Russet Sparrows) or by brood patches (Japanese Tits). Approximately 20 μl of blood was sampled from 8-day old nestlings or adults from the vein at the elbow joint. We also sampled the second secondary flight feather from adults and ~10 breast feathers from nestlings (again on the 8th day). For Russet Sparrows, we took samples from 10 adult males and 12 females at 22 nests in Leishan, and from 15 males and 21 females at 36 nests in Wanshan. For nestling birds of this species, we took samples from 189 nestlings at 57 nests in Leishan and from 173 nestlings at 50 nests in Wanshan. For Japanese Tits, we took samples from one male and two females in Leishan (a small sample related to the field assistant not being able to conduct this work in 2019) and from 9 adult males and 19 females at 28 nests in Wanshan. For nestling tits, we took samples from 94 nestlings at 20 nests in Leishan, and from 163 nestlings at 30 nests in Wanshan.

Blood was saved in centrifuge tubes that contained anticoagulant to prevent clotting, and feathers were sealed in plastic bags; both were then stored at −14 °C until processing. Before testing, the feather samples were washed with acetone to clean off impurities on their surface, and then washed with ultrapure water. Finally, the feather samples were cut into small pieces, and both feather and blood samples were weighed with 0.001 g accuracy.

2.4. Laboratory analysis

The percentage of Hg that is MeHg in bird blood and feathers is generally high, usually greater than 90% (e.g., Rimmer et al., 2005 for blood, and Abeysinghe et al., 2017 for feathers), and therefore we tested only total Hg (THg). The Direct Mercury Analyzer (DMA-80, Milestone, Italy) was used to test the THg content of all blood ($n = 681$) and feather samples ($n = 709$). With DMA-80, both feather and blood samples can be analyzed without the need for acid digestion or other sample preparation; the DMA-80 has a detection limit of 0.005 ng. For these analyses, we used 0.5–10 mg of feather samples and 5–30 mg of blood samples. A calibration curve of 0–20 ng was used for testing the samples from Leishan, and a calibration curve of 20–1500 ng was used for samples from Wanshan. We used both blank samples and reference material of human hair (IAEA-086, International Atomic Energy Agency), to ensure quality control. The reference material was tested after each batch of 15 samples to make sure the error of testing was within the acceptable range (80–120%). The recovery rate was 100.77 ± 9.79 (standard deviation [SD]%, $n = 83$).

Before testing for Se, the feather samples ($n = 290$) were acid digested with nitric acid (HNO_3; Guarantee reagent) in a graphite oven at 120 °C, and ultrapure water was injected at a constant volume, followed by an injection of ferricyanide (FeCy; Hu et al., 2002). For these analyses, we used 5–10 mg of feather samples. Tests for Se concentrations used an atomic fluorescence morphological analyzer (SA-20, Beijing Jitian Instrumentals Co. Ltd., China). We built a standard curve using Se standard solution GSB 04-1751-2004, and the detection limit of the equipment was 0.002 μg/g. The reference material was tested after each batch of 15 samples to make sure the error of testing was within the acceptable range (80–120%), and the recovery rate was 96.50 ± 2.37% (n = 15).

2.5. Statistical analysis

For all analyses we used R statistical software (version 3.6.1), and we report mean values ± SD. Egg volume was calculated following Hoyt (1979): Egg volume = 0.51 × LW^2, where L = length, W = width. All data were analyzed at the nest level. This means that if there were multiple measurements per nest (e.g., egg measurements, nestling weights, nestling Hg or Se measurements), we averaged among them. We found no differences between the adult sexes in their Hg or Se concentrations. In the analysis, we used female measurements because we had more of them (52 nests), but if a female measurement was missing, we used the male measurement (in 26 nests).

Preliminary analyses found that results were generally similar among different areas within a district, and across years in the same area (see Supplemental Analysis, Tables S1-S4). Therefore, we pooled all years and all areas within the districts and then compared the two districts in their metal concentrations and breeding metrics. To compare the districts in egg weight, egg volume, nestling weight, we used Welch's T-tests, as variances were sometimes dissimilar. We also used Welch's T-tests to compare the districts in metal concentrations (Hg in blood, Hg in feathers, Se in feathers, Se:Hg molar ratio), log transforming the response variables to improve their fit to parametric assumptions, as visualized in residual plots. To compare the districts in clutch size, the percentage of eggs that hatched, and the percentage of nestlings that fledged, we used Mann-Whitney U-tests, as the data were not normally distributed. To compare the districts in their nesting success, a binomially distributed response variable, we used Fisher's Exact Tests. For the dataset in Wanshan, in which nests varied widely in metal concentrations, we investigated relationships between metal concentrations and breeding metrics with Pearson (relationships between Hg and nestling weight, or between Se:Hg and nestling weight) or Spearman (relationships between Hg and hatching or fledgling success) correlations.

To check how a lack of differences in breeding metrics between the districts was influenced by the sample sizes of the analyses, we performed a post-hoc power analysis. Specifically, we calculated the power we had to detect a 10% difference in means (10% greater than the mean at the reference district). Following Cohen (1988), we calculated d as $(u_1 – u_2)/\sigma$, where u_1-u_2 was 10% of the Leishan mean, and σ was the common error variance. Using the R package "pwr" (Champely et al., 2020), we then determined the power for our sample size (separate sample sizes for the two districts) at an alpha level of 0.05..

3. Results

3.1. Metal concentrations

Wanshan birds clearly and consistently had higher Hg concentrations in blood and feathers compared with those in Leishan: all comparisons had significance at the $P < 0.0001$ level, except for adult Japanese Tit blood and feathers, for which sample size in Leishan was only three individuals (Table 1, Fig. 2). Summarizing feather concentrations (as they had better sample size), Russet Sparrows adults in Wanshan had 4.5 times the concentrations of those in Leishan; nestling sparrows had 4.0 times the concentrations. For tits, adults in Wanshan had 26.2 times the concentrations of those in Leishan, and nestlings had 9.9 times the concentrations. Differences between the districts in blood concentrations showed similar patterns (see Table 1).

Adult Japanese Tits had higher Hg concentrations than Russet Sparrows, both in Wanshan and in Leishan, and in both blood and in feathers (the least significant t-value was $t_{7.12} = 4.80$, $P = 0.02$). Nestling Japanese Tits also had higher Hg levels in feathers and blood than Russet Sparrows in Leishan (least significant t-value was $t_{73.3} = 5.53$, $P = 0.0006$), but there was no difference between the nestlings Hg concentrations in Wanshan in either tissue (feathers, $t_{78} = 1.49$, $P = 0.19$ and blood $t_{70.55} = 0.26$, $P = 0.81$).

Selenium was elevated in Wanshan, but not by as much as Hg. Russet Sparrow adults had 1.8 times the feather concentrations of Se than those in Leishan, and nestlings of this species had 3.0 times the concentrations (Table 2). For tits, adults in Wanshan had 2.1 times the concentrations, and nestlings had 2.8 times the concentrations of those in Leishan. Hence, the molar ratios of Se:Hg were higher in Leishan than Wanshan: 2.1 times higher for Russet Sparrow adults, 1.4 times for Russet Sparrow nestling, 5.8 times for Japanese Tit adults, and 3.3 times for Japanese Tit nestlings (see Table 2).

3.2. Breeding metrics

Despite the differences in concentrations, the only statistically significant difference we found in the breeding metrics of either species between the two districts when pooling across years was that Russet Sparrows had larger clutch sizes in Wanshan (4.47 ± 0.71; $n = 81$) than in Leishan (3.99 ± 0.60; U = 5274.50, $P < 0.0001$; $n = 97$; Table 3). All other comparisons (seven breeding metrics for each of the two species) were non-significant (Fig. 2), despite sample sizes of $n > 20$ and as high as 80, in each of the two areas, for all but one comparison. In general, nest success was high (Japanese Tit: 64.0%, Russet Sparrow: 83.1%). When looking at the two years separately (see Table S5), 2018 fledgling success for Japanese Tits was lower in Wanshan (70%, n = 9) than for Leishan (96%, n = 10; U = 24, $P = 0.04$). For Russet Sparrows in 2019, there was the opposite pattern: fledgling success in Wanshan was higher (96%, n = 25) than Leishan (78%, n = 26; U = 410.0, $P = 0.02$).

Table 1
Comparison between the contaminated (Wanshan) and reference (Leishan) districts in Hg concentrations in blood and feathers of adults and nestlings of the two species over both years. Conclusions about Japanese Tit adults are limited by a sample size of three in Leishan. All statistics were Welch's t-tests on log-transformed response variables, and positive statistics indicate higher values in Leishan.

Species	Adult or Nestling	Tissue Type	Mean ± SD (N) (μg/g)		Statistic	*P*-value
			Wanshan	Leishan		
Russet Sparrow	Adult	Blood	0.71 ± 0.40 (31)	0.11 ± 0.06 (22)	$t_{25.18} = -11.40$	<0.0001
Russet Sparrow	Adult	Feather	2.38 ± 0.96 (36)	0.53 ± 0.17 (22)	$t_{49.56} = -15.08$	<0.0001
Russet Sparrow	Nestling	Blood	0.13 ± 0.13 (46)	0.04 ± 0.05 (48)	$t_{91.93} = -6.65$	<0.0001
Russet Sparrow	Nestling	Feather	3.60 ± 0.25 (50)	0.91 ± 0.76 (57)	$t_{84.89} = -5.55$	<0.0001
Japanese Tit	Adult	Blood	2.54 ± 2.21 (28)	0.37 ± 0.25 (3)	$t_{2.45} = -0.60$	0.50
Japanese Tit	Adult	Feather	7.08 ± 8.31 (28)	0.27 ± 0.06 (3)	$t_{15.49} = -1.08$	0.65
Japanese Tit	Nestling	Blood	0.14 ± 0.09 (28)	0.01 ± 0.009 (16)	$t_{27.52} = -11.51$	<0.0001
Japanese Tit	Nestling	Feather	2.96 ± 1.44 (30)	0.30 ± 0.22 (20)	$t_{36.21} = -15.45$	<0.0001

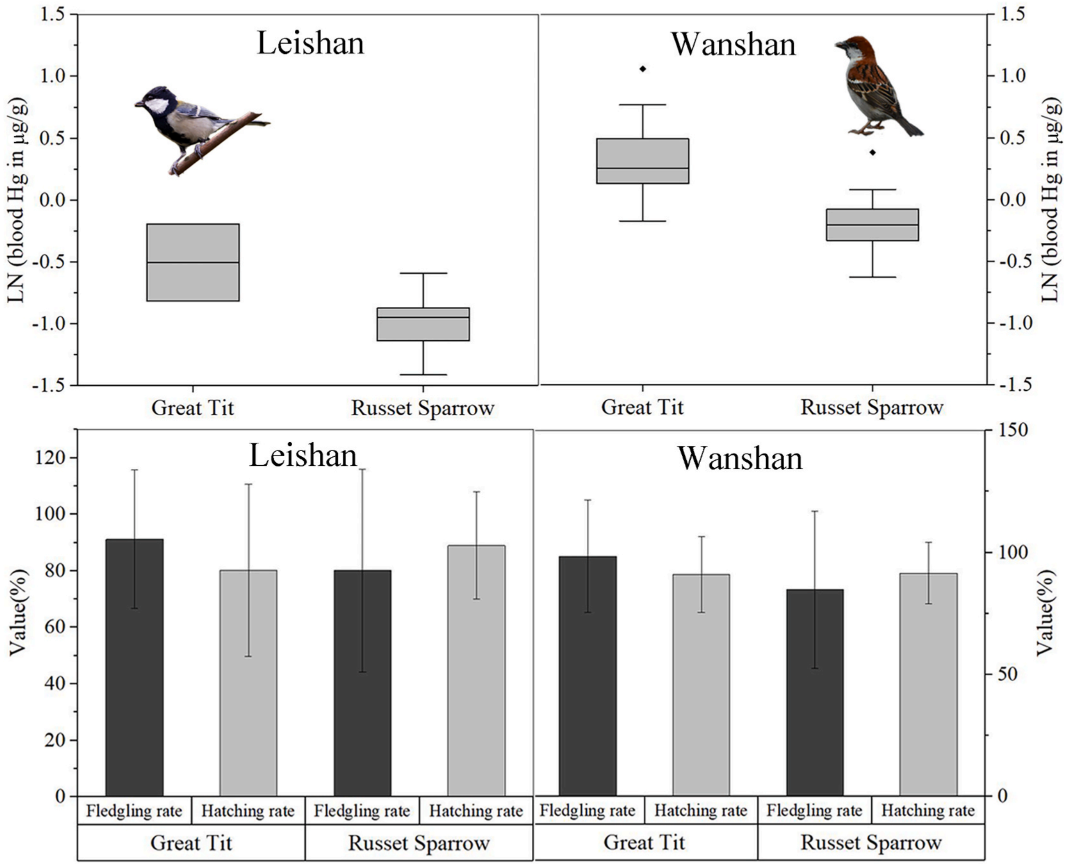

Fig. 2. The comparison of blood Hg level of adults, fledgling success and hatching success of Japanese Tit and Russet Sparrow between the Leishan and Wanshan districts. Photos of Japanese Tit by Lichun Huang and of Russet Sparrow by Donglian Huang.

Table 2
Comparison between the contaminated (Wanshan) and reference (Leishan) districts in Se concentrations and Se:Hg molar ratio in feathers of adults and nestlings of the two species over both years. All statistics were Welch's t-tests on log-tranformed response variables, and positive statistics indicate higher values in Leishan.

Comparison	Species	Adult or Nestling	Mean ± SD (N) (μg/g)		Statistic	*P*-value
			Wanshan	Leishan		
Se	Russet Sparrow	Adult	1.72 ± 1.243 (35)	0.94 ± 0.39 (21)	$t_{42.82} = -3.65$	0.003
	Russet Sparrow	Nestling	2.77 ± 3.82 (50)	0.94 ± 0.40 (43)	$t_{71.58} = -4.17$	0.0001
	Japanese Tit	Adult	2.41 ± 1.66 (28)	1.15 ± 0.43 (3)	$t_{20.31} = -3.45$	0.18
	Japanese Tit	Nestling	2.13 ± 3.80 (30)	0.75 ± 0.15 (17)	$t_{34.74} = -2.67$	0.04
			Ratio (unitless)			
Se:Hg molar ratio	Russet Sparrow	Adult	2.29 ± 3.15 (34)	4.91 ± 2.74 (21)	$t_{40.05} = 5.70$	<0.0001
	Russet Sparrow	Nestling	2.84 ± 4.22 (48)	3.87 ± 2.75 (40)	$t_{82.47} = 2.25$	0.03
	Japanese Tit	Adult	1.97 ± 1.85 (27)	11.32 ± 6.11 (3)	$t_{2.90} = 3.93$	0.003
	Japanese Tit	Nestling	2.76 ± 6.28 (28)	9.20 ± 5.16 (16)	$t_{40.01} = 4.79$	<0.0001

The post-hoc power analysis showed that power to see a 10% difference in means was greater than 0.96 for 7/8 comparisons that used continuous measurements (egg weight and volume, nestling weight) and clutch size, as these variables had low variance (the exception was clutch size for Japanese Tits, Supplemental Table S6). However, power was less than 0.80 for 3/4 analyses of hatching and fledgling success.

3.3. Correlations between breeding metrics and metal concentrations

At the Wanshan Hg Mining District, there were no relationships between the Hg concentrations in nestling or adults (either measured in blood or feathers) and nestling weight, or between Hg concentrations and fledgling or hatching success (Table S7), with sample sizes ranging between 21 and 44 nests for each analysis. There were also no relationships between Se concentrations and the same breeding characteristics (Table S8, analyses' samples sizes between 17 and 46 nests), or between Se:Hg molar ratios and them (Table S9, samples sizes between 11 and 30 nests). Interestingly, of the six correlations analyzed for Se:Hg molar ratios, five of them were negative, although all were non-significant (see Table S9).

4. Discussion

4.1. Hg concentrations

The Hg concentrations we report from the mining district, especially in the adult and invertivorous Japanese Tits (mean 2.54 ± 2.21 μg/g of blood THg, with a maximum of 11.53 μg/g; these levels were ~ 7X those of tits in the reference district, and 3.5 X those of granivorous Russet Sparrows in the mining district), are clearly above the background Hg

Table 3
Comparison between the contaminated (Wanshan) and reference (Leishan) districts in their breeding metrics for the two species over the two years. Samples sizes represent nests; positive statistics indicate higher values in Leishan. For nestling weight, we only used the 2018 data because there were some questions about the reliability of the 2019 data. Significant tests are highlighted in bold. Frequency tables were tested with Fisher Exact Tests.

Breeding Metric	Species	Year	Test	Mean ± SD (N)		Statistic	P-value
				Wanshan	Leishan		
Egg volume (cm^3)	Russet Sparrow	2018 and 2019	Welch's T-test	1.83 ± 0.15 (66)	1.86 ± 0.14(80)	$t_{135.62} = 1.27$	0.21
Egg weight (g)	Russet Sparrow	2018 and 2019	Welch's T-test	1.88 ± 0.16 (66)	1.89 ± 0.15 (80)	$t_{135.48} = 0.41$	0.68
Nestling weight (g)	Russet Sparrow	2018	Welch's T-test	15.67 ± 1.08 (25)	16.22 ± 1.52 (26)	$t_{45.23} = 1.48$	0.15
Clutch size	**Russet Sparrow**	**2018 and 2019**	**Mann-Whitney Test**	**4.47 ± 0.71 (81)**	**3.99 ± 0.60 (97)**	**U = -5274.50**	**< 0.0001**
Hatching %	Russet Sparrow	2018 and 2019	Mann-Whitney Test	0.92 ± 0.12 (64)	0.89 ± 0.19 (78)	U = −2577.00	0.69
Fledgling %	Russet Sparrow	2018 and 2019	Mann-Whitney Test	0.85 ± 0.32 (60)	0.80 ± 0.36 (63)	U = −1990.00	0.53
Nesting Success	Russet Sparrow	2018 and 2019	Fisher's Exact Test	54/65 (83%) (65)	55/72 (76%) (72)		0.40
Egg volume (cm^3)	Japanese Tit	2018 and 2019	Welch's T-test	1.43 ± 0.09 (27)	1.46 ± 0.16 (23)	$t_{34.42} = 0.94$	0.36
Egg weight (g)	Japanese Tit	2018 and 2019	Welch's T-test	1.42 ± 0.12 (27)	1.45 ± 0.15 (23)	$t_{42.73} = 0.82$	0.41
Nestling weight (g)	Japanese Tit	2018	Welch's T-test	13.13 ± 0.51 (6)	13.85 ± 0.77 (6)	$t_{8.73} = 1.91$	0.09
Clutch size	Japanese Tit	2018 and 2019	Mann-Whitney Test	6.56 ± 1.07 (41)	6.86 ± 1.30 (28)	U = 503.50	0.38
Hatching %	Japanese Tit	2018 and 2019	Mann-Whitney Test	0.91 ± 0.15 (34)	0.80 ± 0.30 (24)	U = −485.00	0.17
Fledgling %	Japanese Tit	2018 and 2019	Mann-Whitney Test	0.89 ± 0.23 (32)	0.91 ± 0.25 (20)	U = 285.00	0.38
Nesting Success	Japanese Tit	2018 and 2019	Fisher's Exact Test	32/50 (64%) (50)	19/25 (76%) (25)		0.43

concentrations of birds, when many individuals are sampled over large regions. For example, the highest mean values for any passerine species in any habitat of eastern North America was not above 1.0 μg/g of blood THg (Jackson et al., 2015). In another study on all kinds of birds from western North America, only five of 106 species had mean values greater than 1.0 μg/g of blood THg (three of these were piscivores; all had sample sizes < 5 despite the sample size of the total dataset being 4639; Ackerman et al., 2016).

These Hg concentrations are, however, similar to those found in birds in contaminated areas or especially sensitive ecosystems. For example, in the well-studied South River system of the United States, contaminated by the chemical industry in the 1950s, adult invertivorous birds (specifically Tree Swallow *Tachycineta bicolor* and Carolina Wren *Thryothorus ludovicianus*) averaged between 1.74 (Jackson et al., 2011) and 3.56 μg/g of blood THg (Brasso and Cristol, 2008). Kopec et al. (2018) recently reported four species of passerine that averaged between 0.95 and 4.97 μg/g of blood THg from another contaminated river in the United States. Birds in some sensitive ecosystems without specific point-source locations also have been shown to have high Hg exposure. For example, 25 of 44 samples of whole blood from seabirds in the arctic were above 1.0 μg/g of blood THg (Albert et al., 2019).

Our measurements of Hg concentrations for adults in this study were nonetheless quite substantially below those measured by Abeysinghe et al. (2017) also in the Wanshan Hg Mining District. Their feather measurements, of 40.27 ± 29.62 μg/g in tits and 19.34 ± 13.08 μg/g in "Tree Sparrows" (a similar species to Russet Sparrows, probably misidentified), were much above ours (7.08 ± 8.31 feather μg/g for tits, 2.38 ± 0.96 for sparrows). Differences between the studies in the exact capture sites and distances to the mines may account for some of these disparities. Our project required the presence of telephone poles to which we affixed the nest-boxes and thus we did not have the flexibility of Abeysinghe et al. (2017) to sample in a wide variety of areas, including very close to mine tailings.

4.2. Breeding metrics

Despite these Hg concentrations, we saw no significant decline in breeding metrics between Wanshan and the reference district when pooling the data across years. There was a hint of lowered fledgling success in Wanshan for Japanese Tits in 2018 ($P = 0.04$), but for Russet Sparrows in 2019, the pattern went in the opposite direction, with higher fledgling success in Wanshan. Russet Sparrows also had larger clutch sizes in Wanshan across both years. We suspect that the high fitness of Russet Sparrows in Wanshan may be connected to greater food availability with more rice paddies in the area that this granivore may feed on. Also, the town of Wanshan is larger than the towns in Leishan, and this species is often associated with human settlements. Further, Wanshan is at a higher latitude than Leishan and clutch size in general increases with latitude (Jetz et al., 2008).

The lack of differences between the contaminated and reference districts came despite samples of more than 20 nests in each district. For continuous measurements (egg weight and volume, nestling weight), and clutch size, the power to detect a 10% difference in means was high (usually greater than 0.96, Table S6). Power for success rates (hatching and fledgling) was lower, but here the observed effect sizes (d, the biggest mean divided by the smallest one) were small, too (1.02–1.13 for four measurements of the two species). Our findings of a lack of correlations between metal concentrations and breeding metrics in the Wanshan population may be related to low power due to sample size issues. For example, one would need a sample size of 24 in each district to detect a r-value of −0.5 with 0.80 power at 0.05 significance, and a sample size of 67 to detect a r-value of −0.3 (Cohen, 1988). But, again, we note that the relationships found between metal concentrations and breeding metrics were not strong. For example, the most negative correlation between Hg concentrations and breeding metrics that we observed had a r-value of −0.12. To demonstrate that such low effects sizes were significant, we would need sample sizes that were not logistically possible.

4.3. Adverse effects of Hg on bird reproduction

The levels of Hg in the Wanshan birds, and particularly in the tits, would lead one to expect reproductive declines. For example, Ackerman et al. (2016) identified reproductive effects starting below 1.0 μg/g of blood THg, 'substantial' above 2.0 μg/g, 'severe' above 3.0 μg/g, and often complete failure above 4.0 μg/g. Fuchsman et al. (2017) argued for a higher level of adverse effects, between 2.1 and 4.2 μg/g of blood THg, for small to medium sized birds. Their analysis could be considered conservative, as they cast doubt on some studies that have suggested lower thresholds of adverse effects, such as 10% reduction in reproduction at 0.7 μg/g of blood THg for a passerine species in a field study (Jackson et al., 2011), and reduced reproduction in a low-dosed treatment in an aviary study, where birds averaged 0.73 μg/g of blood THg (Frederick and Jayasena, 2011). Nor did Fuchsman et al. (2017) consider a recent paper (Rowse et al., 2015) that showed potential adverse effects at very low levels (mean 0.21 μg/g of blood THg). Ackerman et al. (2016) point out that the blood equivalent of adverse thresholds in livers from the study of Shore et al. (2011) is about 1.0 μg/g, giving more weight to the idea that 1.0 μg/g of blood THg could be a general threshold of adverse reproductive effects.

In our study, 85.7% of 28 adult Japanese Tits were above 1.0 μg/g of blood THg, 35.7% above 2.0 μg/g, 25.0% above 3.0 μg/g, and 17.9%

above 4.0 μg/g. For Russet Sparrows, 9.7% of 31 adults were above 1.0 μg/g of blood THg and 3.2% of adults were above 2.0 μg/g, with none above 3.0 μg/g. Earlier studies suggested a threshold of 5 μg/g in feathers for reproductive decline (Burger and Gochfeld, 2000; Eisler, 1987); 35.7% of adult Japanese Tits in our study had such levels, although no adult Russet Sparrows did.

Our study is in the minority of research projects that have found Hg to not be correlated with reproductive success in birds (Whitney and Cristol, 2017). Research on the effect of Hg on some particular aspects of breeding success, such as clutch size or egg volume, has led to mixed results (Whitney and Cristol, 2017). However, the majority of research shows some degree of reduction in the number of young birds that fledge due to Hg (similar to our overall measurement of nest success). This result has been found in experimental dosing studies (e.g., Albers et al., 2007; Heinz et al., 2009; Varian-Ramos et al., 2014). Lower breeding success has also been shown in free living birds, including loons (Burgess and Meyer, 2008; Evers et al., 2008), waterbirds (Frederick and Jayasena, 2011; Zabala et al., 2019), seabirds (Goutte et al., 2014a; Goutte et al., 2014b), and passerines (Brasso and Cristol, 2008; Hallinger and Cristol, 2011; Jackson et al., 2011; McCullagh et al., 2015; Rowse et al., 2014). The few field studies that have not shown negative effects of Hg on reproduction have been mostly in seabirds eating prey at non-point source locations (Bustamante et al., 2016; Carravieri et al., 2018; Pollet et al., 2017; Thompson et al., 1991). One study that looked at a passerine species (the Great Tit, *Parus major*, closely related to Japanese Tit studied here) in a contaminated area did not find reproductive effects of Hg (Costa et al., 2014). However, in that study the blood levels of Hg did not vary between contaminated and reference sites (Costa et al., 2014).

4.4. Lack of detection of adverse effects: confounding factors and limitations

There are a number of possible adverse effects that our study may not have been able to detect, and future research is needed in different locations, life-stages and species. First, the Leishan-Wanshan comparison might be influenced by factors other than Hg concentrations: for example, as mentioned above, we suspect that clutch size/nesting success for Russet Sparrows was higher in Wanshan because of the abundance of rice paddies there. Perhaps other parts of reproduction are affected by such factors and thus conceal a negative impact of Hg. Second, we did not sample birds with the extreme Hg concentrations of Abeysinghe et al. (2017), indicating there may be other locations in Wanshan where effects might be found. Third, we should point out that our analyses were limited to birds in the nest; once out of the nest birds will have less opportunity to shed Hg in feathers, and it is possible their survival is affected (Condon and Cristol, 2009). Also, recent studies have emphasized that pre-egg-laying (e.g., mating) processes can be affected by Hg (Zabala et al., 2020). Finally, species differ widely in their sensitivity to Hg. Heinz et al. (2009) and Fuchsman et al. (2017) showed wide variation among bird species in their sensitivities to Hg, and Goutte et al. (2014b) showed variation in Hg sensitivity even between closely related species. Hence, results might be different in other species.

4.5. Possible factors that could provide resilience to Hg in these populations

At a mechanistic level, it is important to note that the blood levels in the nestling birds that we detected were consistently low (no measurements over 0.7 μg/g). The concentrations in nestlings might be more important than those in parental birds, as far as a predictor of effects (Zabala et al., 2019). Interestingly, for nestlings, the levels in feathers were higher than in blood – for example, 11 Russet Sparrow nests (21.5%) had nestling feathers with greater than 5 μg/g THg (the level considered to be the threshold of adverse effects for adult birds by Eisler, 1987). This observation supports the hypothesis of Condon and Cristol (2009) that the sequestration of Hg in feathers, and subsequent feather molting, is a very important mechanism of excretion for young birds.

Another reason that could explain the resilience of the birds in this study has to do with the presence of Se. Selenium is naturally a part of the same ores as Hg, and also shows high levels in Wanshan (Qiu et al., 2019). An antagonism between Hg and Se has been confirmed in aquatic organisms, humans, and other mammals (Belzile et al., 2006; Peterson et al., 2009; Sørmo et al., 2011). While the exact mechanism of the antagonism is somewhat controversial, Se is known to bind to Hg in organisms, forming a biologically inactive compound, HgSe (although this compound is usually studied in tissues other than feathers; Yang et al., 2008). In Wanshan, when Hg concentrations are not too elevated, there is a positive correlation in bird feathers between Hg and Se (Qiu et al., 2019), supporting the idea that Se is inactivating Hg's effect.

Our selenium data had smaller sample sizes and a greater reliance on feathers compared to the data on Hg, as our blood samples were used up in the Hg testing. However, these analyses show no correlation between Se level and Se:Hg molar ratio and the breeding metrics. In fact, the non-significant trends between Se:Hg ratio and breeding (most significant P-value = 0.07) were in the opposite direction than expected if Se is having a protective effect: higher Se:Hg ratios tended to be associated with less success. Indeed, very high levels of Se (>5 μg/g in feathers) have been associated with reproductive problems for birds (Ohlendorf and Heinz, 2011). Yet our population at Wanshan had only a few individuals with such high levels (3/28 tit adults, 2/30 tit nestlings, 3/35 sparrow adults, and 6/50 sparrow nestlings; there were no birds in Leishan above 5 μg/g). Se does not biomagnify like Hg, so if it does have a protective effect, that would be weakened in higher trophic organisms like the Japanese Tit. Although our data suggest Se protection is not the mechanism of tolerance to Hg for these populations, future work using blood samples should confirm these results.

Another potential reason for tolerance to Hg in Wanshan, is that birds may have been selected to be resistant to the effects of Hg living over many generations in this contaminated area. Whitney and Cristol (2017) have argued that there may be an inherent bias towards negative results when studying free living birds in Hg contaminated areas due to such adaptation, as only resistant individuals continue to breed. This situation might apply specifically to Wanshan, where mining has been occurring for 3000 years (Dai et al., 2012). Also, both the Japanese Tit and the Russet Sparrow are well-adapted to human-dominated habitats, and some of this adaptation may include resistance to heavy metals common in such habitats. If bird populations are able to evolve resistance to elevated Hg levels, this has great significance to predicting their responses to increased Hg emissions in Asia in the future. More multi-generational laboratory tests are required in laboratory-controlled conditions to investigate this possibility (see Varian-Ramos et al., 2014).

4.6. Conclusions

In conclusion, the results of this study are surprising in that they show no statistically significant negative effects of Hg on bird reproduction across two years in a contaminated mining district, despite high levels of Hg being present in some birds (specifically > 3.0 μg/g of blood THg in 25% of adult tits). Mechanistically, we argue that this result may be related to low Hg blood levels in the nestling birds (no measurements above 0.7 μg/g), likely linked to the nestlings excreting Hg into their feathers (which reached greater than 5 μg/g in 22% of sparrow nests; also see Condon and Cristol, 2009). Future work should also investigate different locations in the mining district, different life-stages of the birds, and a wider variety of species. Finally, we hope future researchers will also examine the hypothesis that bird populations in contaminated areas can evolve resistance to Hg.

Ethical statement and permissions

This study did not involve endangered or protected species of the

People's Republic of China. The sampling protocol of our study was reviewed and approved by the Leishan Forestry Bureau and the Tongren Forestry Bureau.

Data accessibility

The full dataset is included in the supplemental information.

Credit author statement

Tongping Su was responsible for the sample collection, data analyses and writing the manuscript; Chao He and Zhidong Xu for coordination of sample collection and preparation. Aiwu Jiang and Guangle Qiu were responsible for advising on the project design. Eben Goodale was responsible for conceiving the research goals, data analyses, and writing the manuscript. All authors provided comments on the manuscript and approved its submission.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.117440.

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