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Terrestrial methylmercury bioaccumulation in a pine forest food chain revealed by live nest videography observations and nitrogen isotopes☆

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ABSTRACT

In comparison to the extensively documented mercury (Hg) biomagnification in food chains of aquatic/or aquatic-related systems, Hg biomagnification in food chains in strictly terrestrial systems is poorly explored. Here, we report Hg biomagnification through food webs in a monoculture subtropical pine forest in southwest China. A clear pine needle-caterpillar-tit nestling food chain was determined with the use of live nest videography observations combined with stable isotope analysis (SIA). Simultaneously, a potential pine needle-herbivorous/omnivorous insect-mantis/lacewing/spider food chain was identified by SIA. The results verify that dietary composition plays a pivotal role in terrestrial food chains. Distinct total Hg (THg) and methylmercury (MeHg) biomagnification through the determined and potential food chains was observed, with quite similar efficiency of Hg biomagnification based on the trophic magnification slope (*TMS*) (TMS_{THg} was 0.18 ± 0.03 and TMS_{MeHg} was 0.36 ± 0.05 for the determined food chain; and TMS_{THg} was 0.18 ± 0.04 and TMS_{MeHg} was 0.38 ± 0.07 for the potential food chain). The *TMS* values were significantly higher than those from freshwater studies in tropical regions (0.12 ± 0.12 for THg and 0.16 ± 0.07 for MeHg) and forest studies in temperate regions (0.20–0.28 for MeHg). We recommend live nest videography observations combined with nitrogen isotope analysis when specifically assessing the biomagnification of environmental pollutants through food webs involving nestlings of free-living birds.

1. Introduction

Mercury (Hg) is a global pollutant and a potent neurotoxin and endocrine disruptor, posing a significant risk to human and wildlife health (Lindberg et al., 2007; Tsui et al., 2018; Hsu-Kim et al., 2018; Eagles-Smith et al., 2018). In aquatic ecosystems, Hg released from industrial processes often finds its way into water systems (Evers et al., 2005), and anoxic conditions favor the bacterial transformation of inorganic Hg to methylmercury (MeHg) (Morel et al., 1998), meaning that the basal sources in aquatic ecosystems have been found to have much higher MeHg levels than their terrestrial counterparts (Tsui et al., 2019). Accordingly, information regarding the sources and trophic transfer of MeHg in aquatic ecosystems is extensively well documented (Kidd et al., 2012; Lavoie et al., 2013; Hsu-Kim et al., 2018; Eagles-Smith et al., 2018). It has also been documented that Hg can accumulate through food webs in strictly terrestrial ecosystems by recently several studies (Rimmer et al., 2010; Tsui et al., 2019; Yung et al., 2019).

Terrestrial songbirds that feed primarily on predatory invertebrates can bioaccumulate Hg in their tissues at concentrations similar to those in piscivorous birds due to the lengthen of food chain via riparian invertebrates (Cristol et al., 2008). Several laboratory studies (or dosing experiments) have been performed to

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understand the transfer of MeHg through the food chain (usually involving only 2 trophic levels from the diet to target species) associated with terrestrial birds (e.g., Scheuhammer, 1988; Jackson et al., 2015; Ma et al., 2018). Although their potential diets can be identified on the basis of the avian literature and associated collected samples, studies on MeHg flow in food webs involving free-living terrestrial birds are relatively limited (Abeysinghe et al., 2017; Rodenhouse et al., 2019). Currently, one of the greatest challenges in quantifying MeHg transfer through food webs involving free-living animals is the uncertainty of prey consumed by organisms at each trophic level (Hebert and Weseloh, 2006; Jardine et al., 2006; Dolgova et al., 2018). The environmental controlling factors and the extent of bioaccumulation and biomagnification of Hg in terrestrial ecosystems are still unclear and need more comprehensive studies in diverse terrestrial ecosystems.

Stable isotope analysis (SIA) is usually used for identifying dietary exposure to and biomagnification of contaminants in wild animal populations (Jardine et al., 2006). The stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) techniques have improved the knowledge of trophic ecology especially in estimating the trophic positions of and carbon flow to consumers in food webs (Post et al., 2000; Hyodo, 2015; Swan et al., 2019). Previous studies found that $\delta^{13}C$ of a consumer is similar to that of its diet, being considered as the proxy of dietary sources (Froehle et al., 2010; Song et al., 2018). Whereas, $\delta^{15}N$ of a consumer is higher than that of its diet and was considered as the proxy of trophic levels (Post, 2002; Jardine et al., 2006; Rodenhouse et al., 2019). Since the early 1990s, the slope of linear equation of logarithmic Hg concentration with $\delta^{15}N$ values (Trophic Magnification Slope, *TMS*) has been used as a quantitative indicator of biomagnifying potential of Hg in food webs. A positive slope (*TMS*>0) indicates Hg biomagnification in a food web (Lavoie et al., 2013).

The insectivorous great tits (*Parus major*) are good bioindicators of environmental pollutants (Dauwe et al., 2000; Costa et al., 2013; Lasters et al., 2019; Lopez-Antia et al., 2019) because they are ubiquitous, abundant and favor nesting in man-made nest boxes, hence their breeding populations can easily be manipulated and sampled. In the present study, we placed artificial nest boxes in a subtropical pine forest, southwest China for great tit breeding and randomly monitored parental provisioning behavior with video cameras, allowing us to identify the most provisioned prey items to nestlings (Sinkovics et al., 2018). We also performed the SIA, together with the videography observations as tools to reduce the uncertainty of prey consumed by tit nestlings (Pagani-Núñez et al., 2017). Samples of nestling feathers and potential prey items (invertebrates), pine needles as well as soils were collected from the subtropical pine forest. The aims of the study were to 1) elucidate the distribution of THg and MeHg in soils, pine needles, invertebrates, and tit nestlings; 2) identify trophic levels of the organisms according to video monitoring combined with SIA; and 3) quantify Hg and MeHg bioaccumulation and biomagnification in a strictly terrestrial ecosystem.

2. Materials and methods

2.1. Sampling and preprocessing

2.1.1. Ethical guidelines

In the present study, all field work was conducted with approval from the Forestry and Grassland Administration of Jingdong County, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (XTBG CAS), and the Institute of Geochemistry, Chinese Academy of Sciences (IG CAS).

2.1.2. Study area

Jingdong County is located in the southwestern part of Yunnan Province (Fig. 1a) and contains two large cordillera and national nature reserves (Mt. Wuliang and Mt. Ailao) and one drainage, the *Chuan River*, a tributary of the Red River (Fig. 1b). In this study, the artificial nest box plot is located at a peak in the monoculture subtropical pine forest on Mt. Wuliang (N24°15′59.12″, E100°55′17.94″; 1650 m above sea level), covering an approximately 15 ha area, with 100 nest boxes installed (Fig. S1). The nearest distance of the nest boxes to the *Chuan River* is more than 2 km, and each is over 0.2 km from any creeks, streams or ponds (Fig. 1c). We determined that nest box plot receives little aquatic input to consumer diets because the abundance of emerged aquatic insects decreases exponentially with distance from the stream edge (Baxter et al., 2005; Tsui et al., 2019). *Pinus kesiya* var. *langbianensis* is the dominant species of the monoculture subtropical pine forest (Fig. S1).

The climate of this region is influenced by the southwest monsoon throughout the year, and it has distinct dry (from November to May) and wet (June to September) seasons. The annual average atmospheric Hg concentration monitored at the ecological station was 1.6–2.0 ng m^{-3} (Zhang et al., 2016), consistent with the background atmospheric Hg concentration in the Northern Hemisphere (Sprovieri et al., 2016).

2.1.3. Sampling

The breeding peak of the great tit (the dominant species that occupied the nest boxes) in this area is from mid-March to late June (KL, pers. observ.). The feather development of tit nestlings was monitored every 1–3 days, and feathers were noninvasively collected continuously from April to June in 2018. Twelve-to fifteen-day-old nestling birds were sampled by pulling two secondaries from each individual nestling. Fifty-six nestlings were sampled and feathers were stored in clean polyvinyl chloride (PVC) bags and keep it in a fridge (+4 °C). For non-avian sample collection, in late June 2018, we established four areas (each area covers approximately 100 × 100 m, Fig. 1c) to conduct sampling. Newly emerged pine needles (<1 year old) of *Pinus kesiya* (from 20 individual trees) and paired forest surface soil samples (from 20 soil profiles with 0–10 cm depth) in 4 areas covering 10 × 10 m were collected. For invertebrates, at least 20 individuals of each taxa of *Dendrolimus kikuchii* and *D. houi* (caterpillars), *Orthoptera* spp. (grasshoppers), *Phasmatidea* spp. (stick insects), *Tettigoniidae* spp. (katydids), *Blattella bisignata* (cockroaches), *Mantodea* spp. (mantis), *Myrmeleontidae* spp. (lacewings), and *Araneae* spp. (spiders) were collected with insect nets. All invertebrates were stored in gauze-covered centrifuge tubes in coolers with ice bags, then transferred to laboratory.

In the laboratory, the nestling feathers were thoroughly washed in tap water, ultrasonically cleaned with detergent, acetone and deionized water, and then dried at room temperature. The feathers were cut into pieces of approximately 0.1–0.2 mm with ceramic scissors. For THg analysis, feathers from two individuals from a single nest were mixed together as one sample, while the remaining two samples were mixed together for MeHg analysis. The soil samples were air dried in a ventilated location, ground in a ceramic mortar and sieved with 200 mesh for analysis. Pine needle samples were cleaned three times in deionized water and then freeze-dried (−56 °C, LGJ-12, China), ground with a pulverizer (IKA A11, Germany) and sieved with 100 mesh for analysis. Invertebrates were placed in gauze-covered tubes and starved for approximately 24 h, lightly washed in deionized water, freeze-dried, and finally ground with a pulverizer prior to analysis.

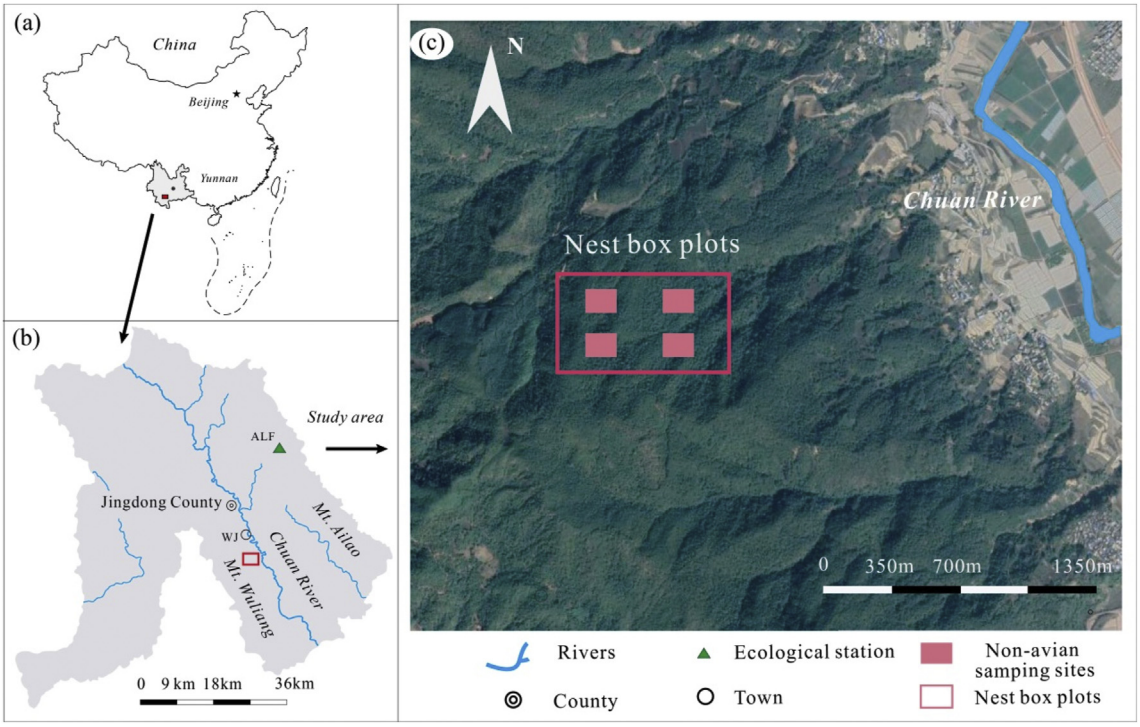

Fig. 1. The sampling sites in Jingdong, Southwest China (a). WJ represents the sampling site and ALF is the ecological station of the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (b). The empty red rectangle represents the artificial nest box installed site and the red rectangles represent the non-avian sampling sites (c).

2.2. Observation of nestling diet

We positioned micro charge coupled device (CCD) cameras (Yi Technology, 4K ACTION, China) inside the nest boxes to record parental provisioning behavior, especially to determine the mainly provided prey items. We randomly placed the cameras into nest boxes with nestlings of different ages for approximately 1 or 2 h and acquired approximately 40 h of recordings from 22 broods with high visibility that allowed us to check most of the prey items fed to nestlings. We identified the common and easily distinguished prey items to the suborder level, and most of the prey items were identified to the level of order or class.

2.3. Analytical methods

2.3.1. THg

For the biotic samples (feathers, invertebrates and pine needles), approximately 0.1 g of dry samples was weighed in a 25 ml glass tube and digested at 95 °C in a water bath with 5 ml of ultrapure HNO_3 for approximately 3 h. A suitable aliquot of the digestion was used for THg concentration determination and treated with BrCl oxidation, $NH_4OH{\cdot}HCl$ neutralized, $SnCl_2$ reduction, purging, and passing through a gold amalgam trap and measured using cold-vapor atomic absorption spectrometry (CVAFS, Brooks Rand Model III, Brooks Rand Laboratories, Seattle, USA) following USEPA method 1631e (US EPA, 2002). For non-biotic samples (soil), approximately 0.2 g of dry sample was weighed in a glass tube and digested at 95 °C in a water bath with fresh aqua regia (HNO_3:HCl = 1:3, v/v) for approximately 3 h and then treated as the biotic samples for measurement. Except for the data for nestling feathers, which are expressed according to fresh weight (f.w.), the concentrations for both biotic and non-biotic samples are presented according to dry weight (d.w.).

2.3.2. MeHg

For the biotic samples (invertebrates and feathers), approximately 0.01–0.02 g of dry samples was digested with 25% KOH-methanol solvent for 3 h in a water bath at 75 °C. A suitable aliquot of the digestion was taken for MeHg analysis via $NaBEt_4$ ethylation, purging, and passing through a Tenax trap and measured using gas chromatography coupled with cold-vapor atomic fluorescence spectroscopy (GC-CVAFS, Brook Rand Model III, Brooks Rand Laboratories, Seattle, USA) following USEPA method 1630 (US EPA, 2001) and Liang et al. (1994, 1996). For the soil samples, we followed the extraction method recommended by Liang et al. (2004). In brief, approximately 0.2–0.4 g of dry samples was digested with $CuSO_4$ (1 M) and HNO_3 (3 M). The MeHg was then extracted with CH_2Cl_2 after HNO_3 leaching, back extracted in a water bath at 75 °C, and measured with a GC-CVAFS as for the biotic samples. For the pine needle samples, approximately 0.2–0.3 g of dry samples was digested with 5 ml of KOH-methanol solvent, extracted with CH_2Cl_2, back extracted and measured as for the biotic samples.

2.3.3. Stable isotopes

Dry biotic samples (feathers, pine needles and invertebrates) were weighed for SIA. Carbon and nitrogen isotope ratios were determined with a continuous flow mass spectrometer (MAT 253, Thermo Finnigan Instrument, Germany) coupled to a flash analyzer (EA 2000, Thermo Scientific, Germany). The precision of the analytical measurements was <0.1‰, and the results are expressed in standard delta notation according to the following formula:

$$\delta X = (R_{sam} / R_{std} - 1) \times 1000 \quad (1)$$

where X refers to $^{15}N/^{14}N$ and $^{13}C/^{12}C$ and R refers to the abundance ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$.

Cellulose (IAEA-C3, $\delta^{13}C = -24.7‰$) and KNO_3 (IAEA-NO_3, $\delta^{15}N = 4.7‰$) were used to calibrate the $\delta^{13}C$ and $\delta^{15}N$ values,

respectively. All isotope values were converted on the basis of Vienna Pee Dee Belemnite (V-PDB) and standard atmospheric nitrogen.

2.3.4. QA/QC

For THg measurements, method blanks, duplicate samples (10%) and certificated reference materials (CRMs) were defined to ensure data quality. For CRMs, human hair (GBW09101b, Shanghai Institute of Applied Physics, China), citrus leaves (GBW10020, Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences (IGGE, CAGS)), and yellow-red soil (GBW07045, IGGE, CAGS) were used for feathers, pine needles, and soil samples, respectively. The recoveries of THg were 101.6 ± 4.5%, 102.8 ± 4.8%, and 101.7 ± 9.3%, respectively. All relative standard deviations (RSDs) were less than 10%.

Method blanks, duplicate samples (10%) and CRMs were also defined for the MeHg measurements. For the feather and invertebrate samples, the CRM was Tort 2 (lobster hepatopancreas, National Research Council Canada, Canada). For soil samples, the CRM was ERM CC580 (estuarine sediment). The recoveries of the CRMs were 93.1 ± 4.9% and 96.3 ± 2.5%, respectively, and all RSDs were less than 10%.

2.4. Data analysis

2.4.1. Trophic level (TL)

The nitrogen isotopic ratio ($\delta^{15}N$) was used to calculate the *TLs* of each trophic organism using the equation suggested by Post. (2002):

$$TL = \left(\delta^{15}N_c - \delta^{15}N_{base}\right) \Big/ \Delta_n + \lambda \quad (2)$$

where *TL* is the trophic level, $\delta^{15}N_c$ is the isotope ratio of the consumers, $\delta^{15}N_{base}$ is the isotope ratio of the organism at the base of the food chain, λ is the trophic position of the organism used to estimate $\delta^{15}N_{base}$ (for primary producers, λ is 1), and Δ_n is the mean trophic fractionation (3.4‰ for $\delta^{15}N$). In the present study, the pine needle was selected as the base to calculate *TLs*.

2.4.2. Biomagnification factors (BMF)

The biomagnification factor (*BMF*) between prey and related predators was calculated according to the following equations (adapted from Yung et al., 2019):

$$BMF_{(THg)predator} = [THg]_{predator} \Big/ [THg]_{prey} \quad (3)$$

$$BMF_{(MeHg)predator} = [MeHg]_{predator} \Big/ [MeHg]_{prey} \quad (4)$$

2.4.3. Trophic magnification slope (TMS)

The trophic magnification slope (*TMS*) was also used to quantify the Hg biomagnification in the food chain (Yoshinaga et al., 1992; Borgå et al., 2011; Lavoie et al., 2013). The THg and MeHg concentrations of all samples were log transformed, and the *TMS* (*b*, the slope of the regression line) was calculated according to equation (5).

$$\log_{10}[THg\ or\ MeHg] = b \times \delta^{15}N + \alpha \quad (5)$$

When *TMS* >0, it means significant biomagnification exists in a food chain, and the greater *TMS* values indicate stronger Hg biomagnification. The linear multiple regression analyses, and t-tests were performed with R software v.3.5.1 (R Development Core Team, 2013).

3. Results

3.1. Mercury concentrations in biotic and non-biotic samples

For non-biotic samples (soil), the THg concentrations (d.w.) ranged from 29.0 to 64.6 ng g^{-1}, with a mean of 43.3 ± 10.6 ng g^{-1} (mean ± SD, n = 17), while the MeHg concentrations ranged from 0.02 to 0.27 ng g^{-1}, with a mean of 0.11 ± 0.07 ng g^{-1} (n = 13). The mean percentage of MeHg to THg (%MeHg) in soils was 0.3 ± 0.2% (n = 13) with a range of 0.1–0.6%.

For the biotic samples, the THg and MeHg concentrations (d.w.) in the pine needles were 2.6–27.0 ng g^{-1}, with a mean of 11.1 ± 7.7 ng g^{-1} (n = 15), and 0.06–0.28 ng g^{-1}, with a mean of 0.13 ± 0.07 ng g^{-1} (n = 12), respectively. In terms of the THg concentrations in the consumers (herbivorous insects), the grasshoppers and caterpillars showed relatively low values of 6.3 ± 1.5 ng g^{-1} (n = 3) and 27.2 ± 13.2 ng g^{-1} (n = 25), respectively. The highest value was observed in spiders, up to 459.1 ng g^{-1}, with a mean of 404.3 ± 48.4 ng g^{-1} (n = 3), and the nestling feathers showed the greatest variation, ranging from 24.8 to 811.1 ng g^{-1}, with a mean of 175.1 ± 191.4 (n = 28) (Fig. 2a; Table 1). From the perspective of the MeHg concentration, herbivorous insects (caterpillars, stick insects, grasshoppers, and katydids) presented relatively low values (0.65–10.51 ng g^{-1}), while the highest value was observed in spiders (up to 187.63 ng g^{-1}), and the largest variation (3.36–43.62 ng g^{-1}) occurred in nestling feathers (Fig. 2b; Table 1).

For %MeHg, the highest values were observed in the mantis (48.2 ± 15.7%, n = 2), followed by grasshoppers (45.4 ± 2.9%, n = 3), spiders (39.5 ± 9.2%, n = 3), lacewings (37.3%, n = 1), nestling feathers (26.2 ± 12.0%, n = 6), katydids (20.9 ± 9.2%, n = 3), cockroaches (14.9 ± 8.1%, n = 3), caterpillars (3.1 ± 1.3%, n = 6), stick insects (2.5%, n = 1), and pine needles (2.2 ± 2.2%, n = 12), as shown in Fig. 2c and Table 1.

3.2. Observations of nestling diet based on video recordings

Across the 40 h of video recording, we observed 404 instances (10.1 times per hour) of parents feeding the nestlings. Approximately 82% of the total provisioned prey items were distinguished, and ~96% of the distinguishable items were invertebrates. In particular, the caterpillars (*Dendrolimus kikuchii* and *D. houi*) were the dominant food items provided to the nestlings with different ages (all>50%). Although approximately 18% of the provisioned invertebrates were not identified, it was clear that those identified invertebrates of katydids, cockroaches, and spiders accounted for less than 5% of the diet of great tit nestlings (Fig. 3).

The distinguishable dominant prey caterpillars (up to 67%), *D. kikuchii* and *D. houi* are the most destructive defoliators in *Pinus kesiya* var. *langbianensis* conifer forest by consuming pine needles during their whole larval stage (KL, pers. Observ.). Hence, a clear pine needle-caterpillar-tit nestling food chain was determined in the present study, and the simple food chain suggested that tit nestlings were mainly provisioned with caterpillars, which were developed on pine needles (Fig. S2 and S3).

3.3. Stable isotope values and the trophic structure of the food chain

The values of $\delta^{13}C$ for all samples could be categorized into three groups. The low-value group included pine needles and caterpillars. The pine needles showed little variation in $\delta^{13}C$ values, ranging between −30.4‰ and −27.7‰, with a mean value of −29.0 ± 1.1‰ (n = 11), and caterpillars showed a range of −29.3‰ to −27.7‰

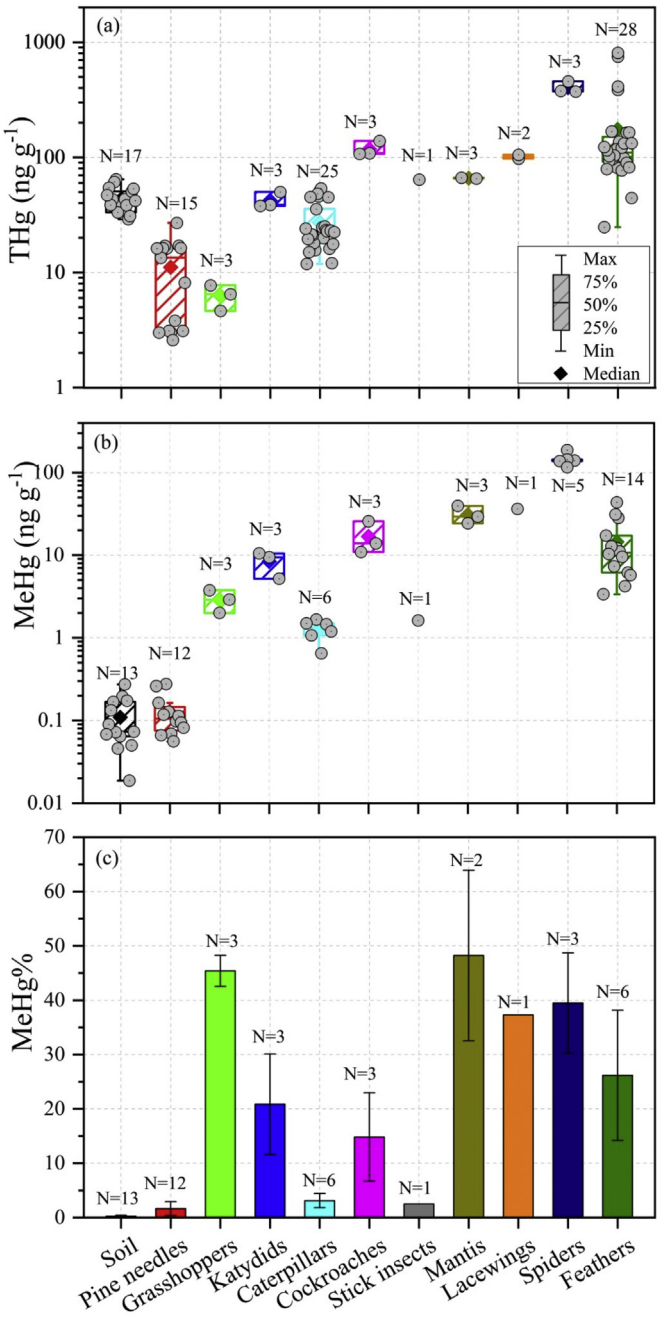

Fig. 2. Boxplots of (a) total mercury (THg) and (b) methylmercury (MeHg) and (c) histogram of the percentage of THg as MeHg (MeHg%) among all samples (on a dry weight basis) from a pine forest in Jingdong, southwest China. In Fig. 2a and b, N is the samples numbers.

(mean: -28.8 ± 0.5‰, n = 8). The high-value group included two herbivorous insects, stick insects and grasshoppers, presenting little variation, ranging between −13.8‰ and −12.6‰ (13.0 ± 0.4‰, n = 10) and between −14.6‰ and −14.1‰ (14.3 ± 0.2‰, n = 6), respectively. The remaining samples represented the intermediate-value group and showed relatively variation, except high variation occurred in katydids, ranging between −27.6‰ and −20.7‰ (25.3 ± 2.2‰, n = 12) (Fig. 4 and Table 1). In the present study, stick insects and grasshoppers were highly dependent on C4 plants and were different from the target species of pine trees (C3 plants) and contributed little to tit nestling diet based on the video results, hence, both of them were excluded in further analysis.

For $\delta^{15}N$, pine needles showed the lowest value on average but exhibited relatively high variation, ranging between −2.65‰ and 1.77‰ (−0.12 ± 1.63‰, n = 11), followed by one type of omnivorous insect (cockroaches) (0.64 ± 0.15‰, n = 6), four types of herbivorous insects (stick insects (0.52 ± 0.15‰, n = 10), caterpillars (0.68 ± 0.54‰, n = 8), grasshoppers (1.60 ± 0.29‰, n = 10), and katydids (1.68 ± 0.40‰, n = 12)), and one type of carnivorous insect (mantis) (3.64 ± 0.36‰, n = 10), and finally the tit nestling feathers (4.05 ± 0.27‰, n = 10). Unexpectedly, the highest values were detected in two carnivorous invertebrates (spiders (up to 5.8‰) and lacewings (up to 6.1‰)), as shown in Fig. 4.

The *TL* pattern was as follows: $TL_{(\text{spiders and lacewings})} > TL_{(\text{nestlings})} > TL_{(\text{mantis})} > TL_{(\text{herbivorous insects and omnivorous cockroaches})} > TL_{(\text{needles})}$. In the present study, despite the clear pine needle-caterpillar-tit nestling food chain, another potentially distinguishable pine needle-herbivorous/omnivorous insect-mantis/lacewing/spider food chain was determined based on the $\delta^{15}N$ signatures.

3.4. Trophic-level effects on Hg bioaccumulation

In the present food chains, the organisms at higher *TL*s tend to have higher $\delta^{13}C$ (excluding the grasshoppers and stick insects) (Fig. 4) and a significantly positive correlation ($p < 0.0001$) between $\delta^{13}C$ and THg and MeHg was observed (Fig. 5a, c). In the determined pine needle-caterpillar-tit nestling food chain, the $BMF_{(THg)}$ between the caterpillars and pine needles was 2.5, between the nestling feathers and caterpillars was 6.5, and between the nestling feathers and pine needles was 16.3. The $BMF_{(MeHg)}$ values were significantly greater than the $BMF_{(THg)}$ values, being 9.9, 11.5, and 113.9 times greater between caterpillars and pine needles, between nestling feathers and caterpillars, and between nestling feathers and pine needles, respectively. For the potential pine needle-herbivorous/omnivorous insect-mantis/lacewing/spider food chain, the $BMF_{(THg)}$ and $BMF_{(MeHg)}$ were 36.7 and 1115.4 between spiders and pine needles, respectively.

The *TMS* values of the determined pine needle-caterpillar-tit nestling food chain were 0.18 ± 0.03 for THg and 0.36 ± 0.05 for MeHg (Fig. 5b, d). Similar *TMS* values of the potential pine needle-herbivorous/omnivorous insect-mantis/lacewing/spider food chain were observed, averaging 0.18 ± 0.04 for THg and 0.38 ± 0.07 for MeHg (Fig. 5b, d).

The *TMS* values of both THg ($t = -2.7$, $p = 0.01$) and MeHg ($t = -8.0$, $p < 0.001$) were significantly higher than those in freshwater food webs for diverse temperate regions, which were 0.12 ± 0.12 and 0.16 ± 0.07, respectively (Lavoie et al., 2013). Similarly, the *TMS* values for MeHg (0.36–0.38) in the present study were significantly ($t = -6.2$, $p = 0.003$) higher than the reported *TMS* values (0.20–0.28) from temperate forest floor food chains (Tsui et al., 2019).

4. Discussion

4.1. Comparisons with earlier studies, including in aquatic ecosystems

In comparison to published data on THg in feathers of great tit nestlings, concentrations in the present study were at the median (Table S1). The investigated nestlings had mainly been provisioned with the most accessible and available food sources, caterpillars that contained low Hg concentrations (THg, 27.2 ± 13.2 ng g^{-1} and MeHg, 1.26 ± 0.37 ng g^{-1}). Hence, the low Hg concentrations in nestling feathers could be attributed to short trophic levels involved in the targeted food web structure (Bartrons et al., 2015;

Table 1
Stable carbon isotope composition ($\delta^{13}C$) and nitrogen isotope composition ($\delta^{15}N$) in the biotic samples and the mean concentrations of total mercury (THg), methylmercury (MeHg), and the percentage of methylmercury (%MeHg) for all samples from a pine forest in Jingdong, southwest China.

Sample	$\delta^{13}C$‰		$\delta^{15}N$‰		THg (ng g^{-1})			MeHg (ng g^{-1})			%MeHg	
	Mean ± Std	N	Mean ± Std	N	Range	Mean ± Std	N	Range	Mean ± Std	N	Mean ± Std	N
Soil	–	–	–	–	29.0–64.6	43.3 ± 10.6	17	0.02–0.27	0.11 ± 0.07	13	0.3 ± 0.2	13
Pine needle	−29.0 ± 1.1	11	−0.12 ± 1.61	11	2.6–27.0	11.1 ± 7.7	15	0.06–0.28	0.13 ± 0.07	12	2.2 ± 2.2	12
Grasshoppers	−14.3 ± 0.2	6	1.60 ± 0.29	10	4.6–7.7	6.3 ± 1.5	3	2.00–3.75	2.88 ± 0.88	3	45.4 ± 2.9	3
Cockroaches	−26.6 ± 0.2	6	0.64 ± 0.15	6	107.5–138.8	118.2 ± 17.8	3	10.92–25.84	16.91 ± 7.87	3	14.9 ± 8.1	3
Mantis	−25.4 ± 0.2	10	3.64 ± 0.36	10	65.6–66.5	66.1 ± 0.7	2	24.41–39.53	31.13 ± 7.72	3	48.2 ± 15.7	2
Stick insects	−13.0 ± 0.4	10	0.52 ± 0.15	10	64.2	64.2	1	1.62	1.62	1	2.5	1
Lacewings	−26.4 ± 0.1	10	5.80 ± 0.30	10	97.5–105.5	102.1 ± 5.7	2	36.42	36.42	1	37.3	1
Spiders	−25.6 ± 0.1	10	5.60 ± 0.11	10	373.4–459.1	404.3 ± 48.4	3	116.31–187.63	145.11 ± 26.34	6	39.5 ± 9.2	3
Katydids	−25.3 ± 2.2	12	1.68 ± 0.40	12	38.0–50.1	42.3 ± 6.8	3	5.20–10.51	8.40 ± 2.82	3	20.9 ± 9.2	3
Caterpillars	−28.8 ± 0.5	8	0.68 ± 0.54	8	11.9–53.8	27.2 ± 13.2	25	0.65–1.66	1.26 ± 0.37	6	3.1 ± 1.3	6
Feathers	−24.7 ± 0.2	10	4.05 ± 0.27	10	24.8–811.1	175.1 ± 191.4	28	3.36–43.62	14.54 ± 11.82	14	26.2 ± 12.0	6

Only for soil and pine needles, and soil were analyzed individuals, samples of invertebrates for each texa was mixed by at least 20 individuals. For feathers, THg is analyzed individuals while MeHg was measured by mixed two individuals.

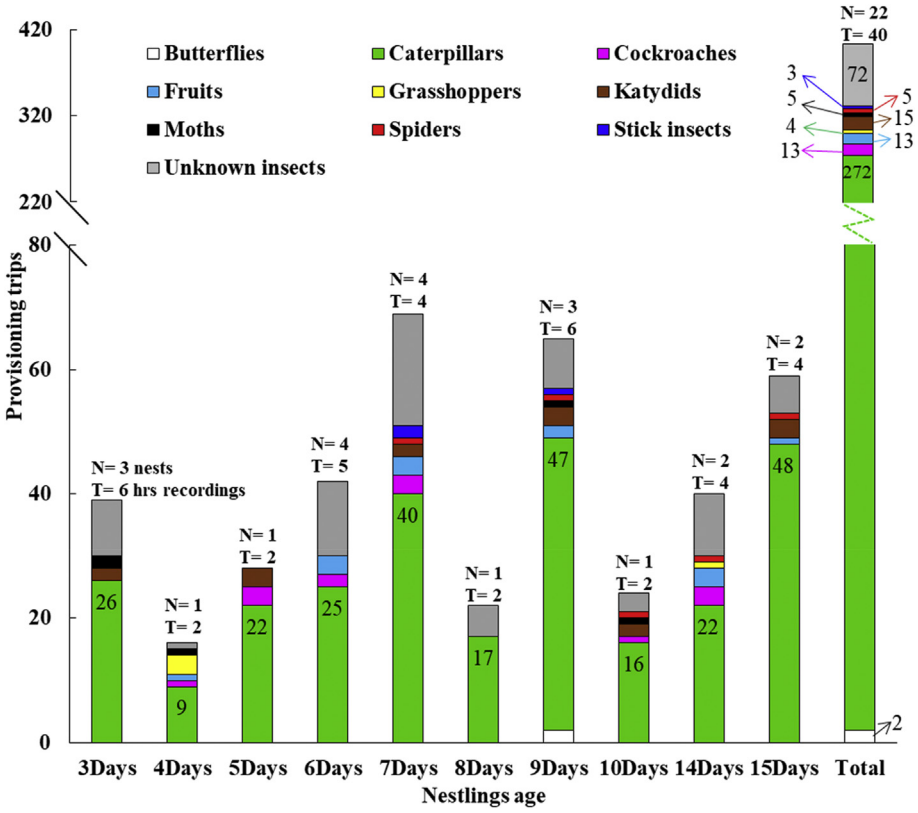

Fig. 3. The composition of the provisioned prey items composition for the great tit (*Parus major*) nestlings identified by video recordings from a pine forest in Jingdong, southwest China.

Dolgova et al., 2018) since there are no distinct Hg contamination source at the sampling site (Zhang et al., 2016).

In this study, we observed a distinct transfer of both THg and MeHg from lower to higher trophic levels (Fig. 5) with MeHg biomagnified more efficiently than THg as expected (Watras et al., 1998). Since *TMS* values are expected to decrease when basal inputs increase (DeForest et al., 2007; Jardine et al., 2013), the higher efficiency of both THg and MeHg biomagnification in our food chains was likely due to the low basal inputs in ecosystems (THg, 11.1 ± 7.7 ng g^{-1} and MeHg, 0.13 ± 0.07 ng g^{-1}).

In the present study, higher *TMS* values, particularly TMS_{MeHg} were observed for terrestrial food chains compared to those reported in diverse temperate freshwater systems (Lavoie et al., 2013). A recent study conducted in a terrestrial system also detected extremely high *TMS* values for THg (0.45) and MeHg (0.80) in a soil-nestle-insect food web (Yung et al., 2019). These results may highlight different efficiencies both with which THg and MeHg flow up the food chains in these contrasting ecosystem types.

Differences in taxa, regions or habitats might contribute to the large variations of *TMS* values observed in terrestrial food webs (including those with aquatic connections, Table S2) (Borgå et al., 2011; Tsui et al., 2019). Hence, methods (e.g., the SIA and live nest videography observations in this study) for detecting true predatory relationships in terrestrial food webs are recommended when assessing pollutant biomagnification. More research on similar taxa in different geographic locations would help to identify what

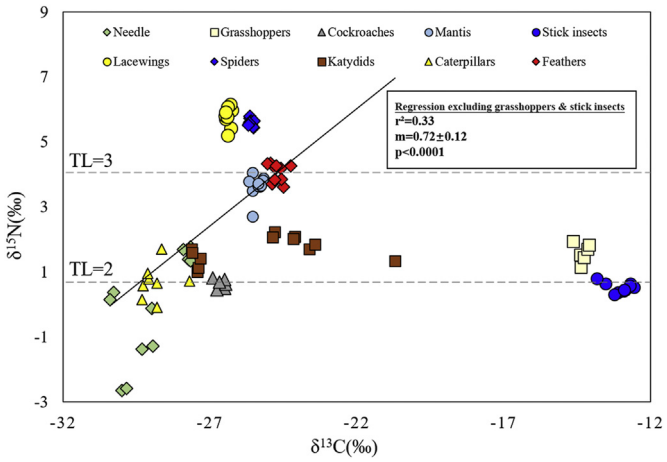

Fig. 4. Relationships between stable carbon isotope compositions ($\delta^{13}C$; as a proxy of dietary sources) and nitrogen isotope compositions ($\delta^{15}N$; as a proxy of trophic positions) of biotic samples from a pine forest in Jingdong, southwest China. The dashed lines indicate the trophic levels (*TLs*).

factors influence *TMS* values in terrestrial ecosystems.

4.2. Trophic level determination by nest videography versus $\delta^{13}C$-$\delta^{15}N$ analysis

In the present study, a clear pine needle-caterpillar-nestling food chain was determined by nest videography of the diet of tit nestlings (Fig. 3) as well as field observations on the diet of the caterpillars. The values of $\delta^{13}C$ of pine needles and caterpillars were much closed, suggesting pine needles was the main diet source of caterpillars (Zhang et al., 2014, Fig. S2). The fractionation values for $\delta^{15}N$ between caterpillars and nestling feathers was 3.4‰, which is consistent with the empirical value (3.4 ± 1.1‰, Post, 2002), suggesting the main potential food source of nestlings was caterpillars. Noted that the $\delta^{15}N$ fractionation value of 0.8‰ between caterpillars and pine needles did not match with the empirical fractionation value of 3.4‰ but was consistent with the suggested value between leaves and caterpillars ~1‰ (Hyodo, 2015), indicating a special processing of $\delta^{15}N$ enrichment from plants to caterpillars. However, the $\delta^{13}C$ fractionation value of 4.1‰ between nestling feathers and caterpillars was larger than the empirical fractionation value (0.8 ± 1.1‰, Post, 2002) and the recommended fractionation value (1.9 ± 0.3‰, Becker et al., 2007) from seabirds. Given the large difference of 4.1‰ between nestling feathers and caterpillars, $\delta^{13}C$ in the feathers of nestlings may in part reflect the diet of the parents, which could have included prey other than the caterpillars. In the present study, the videography helped to interpret the nestling diet results in light of the different $\delta^{13}C$ of the caterpillars and nestlings.

Contrary to our expectation, the highest average THg concentration was observed in spiders rather than in the insectivorous birds. The MeHg concentrations in nestling feathers were 3.7–55.8 times lower than those for spiders. Our results seem to contradict the general concept that the birds are the predators and the invertebrates (spiders) are the prey. With the help of video recordings, we confirmed that the nestlings were mainly provisioned

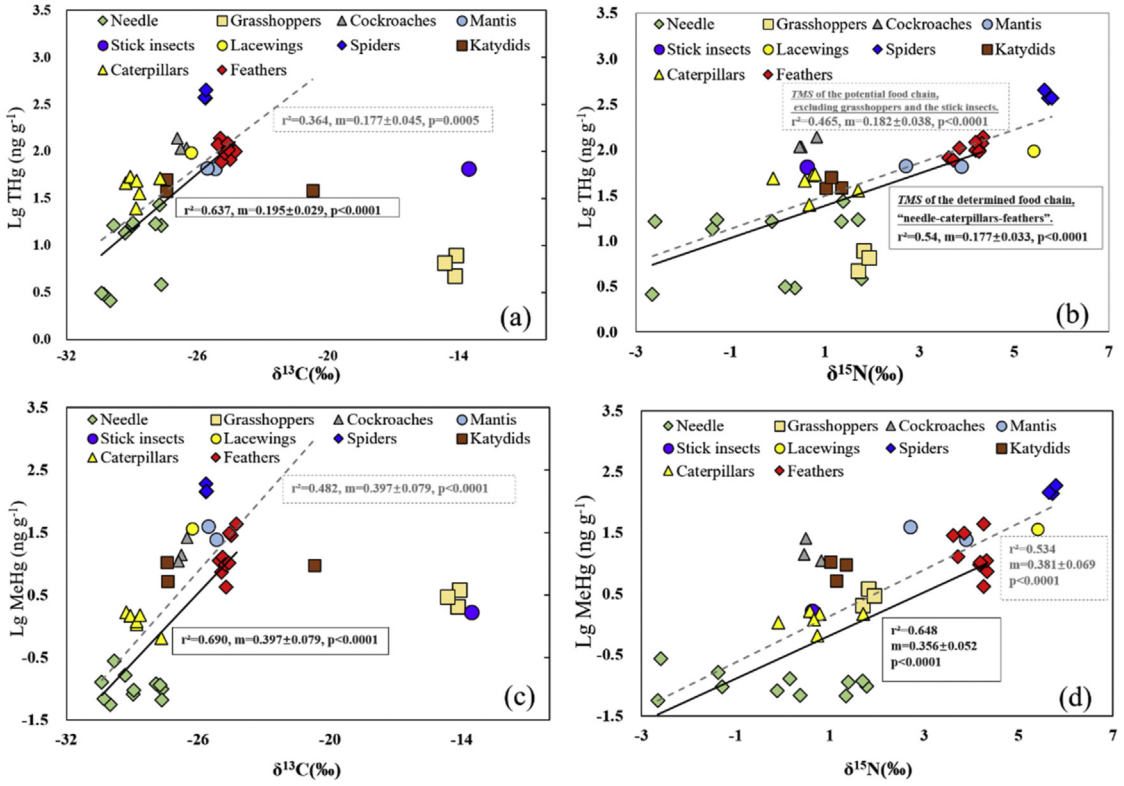

Fig. 5. Relationships between stable carbon isotope compositions ($\delta^{13}C$; as a proxy of dietary sources) and total mercury (THg) (a), methylmercury (MeHg) (c) tissue concentrations (on dry weight basis) of biotic samples in the pine forest. Relationships between stable nitrogen isotope compositions ($\delta^{15}N$; as a proxy of trophic positions) and total mercury (THg) (b), methylmercury (MeHg) (d) tissue concentrations (on dry weight basis) of biotic samples in the pine forest. The black lines indicate the regressions of the determined food chains and the statistical results shown in black boxes. While the dashed lines indicate the regression of potential food chain excluding the grasshoppers and the stick insects and the statistical results shown in dashed boxes.

with caterpillars instead of spiders, which is a quite different result than that found in a Mediterranean Iberian forest (Pagani-Núñez et al., 2011, 2017). Therefore, the diet is a critical factor in regulating exposure to, uptake of, and biomagnification of Hg contaminants (Hebert and Weseloh, 2006).

Notably, even in such a monoculture subtropical pine forest with low Hg concentrations in basal sources (pine needles), elevated Hg concentrations were observed in spiders (up to 459.1 ng g^{-1} for THg and 187.6 ng g^{-1} for MeHg) as reported in other studies (e.g., Cristol et al., 2008; Abeysinghe et al., 2017). Since our sampled spiders were mostly collected on the forest floor in the litter layer, their high trophic levels (according to our $\delta^{15}N$ signature, spiders were in a higher *TL* than the tit nestlings, Fig. 3) might be explained by the prevalence of detrital food webs (Steffan et al., 2017; Tsui et al., 2019). The presence of microbial components would lengthen the trophic food chain, as the Hg in detritus would be initially transformed into bacterial and fungal biomass (Steffan et al., 2017), which can be consumed by small predators, resulting in elevated Hg concentration levels through biomagnification (Lavoie et al., 2013). Although spiders contribute little to the diets of the target species in our case, it is still noteworthy that apex animal consumers can obtain large amounts of MeHg from terrestrial invertebrates, such as spiders (Tsui et al., 2019).

4.3. Nestling feathers as indicators of Hg transfer

Collection and usage of nestling feathers to monitor Hg is a simple, nondestructive, noninvasive, and convenient sampling procedure. The Hg in nestling feathers originated from both diet and maternal transfer (Ackerman et al., 2016, 2017, 2019). Researchers also argued that nestling feathers represent local Hg exposure since feathers in growing nestlings were formed almost from provisioned prey (Spalding et al., 2000, Herring et al., 2009, Rubio et al., 2016, Zabala et al., 2019a, 2019b). It is suggested that the nitrogen signature of nestling feathers was significantly dependent on the provisioned dietary (Higher *TL* dietary resulted in higher *TL* nestlings, Pagani-Núñez et al., 2017).

Due to the relative simple food items composition and caterpillars being the dominant dietary along the nestling ages (Fig. 3), our present study partially shed light on the Hg transfer through pine needle-caterpillar-tit nestling food chain. However, the transfer of Hg from female to their nestlings (Ackerman et al., 2016, 2017, 2019) would suggest that we might overestimated the *TMS*, suggesting the future work should take maternal transfer effects into consideration. In addition, a recent study revealed that the feather Hg concentration was not consistent with internal tissues (Low et al., 2019), indicating that future study of contrasted measurements among tissues and/or blood would contribute to a more comprehensive understanding of Hg transfer through dietary of nestlings.

5. Conclusions

The present investigation was effectively conducted with live nest videography observations in conjunction with modern SIA techniques to provide a better understanding of the links between physical and biological factors that govern Hg uptake and accumulation in a simple pine forest food chain. We revealed clear THg and MeHg biomagnification through food webs (including the determined and potential food chain) in a terrestrial ecosystem. The distinct transfer of both THg and MeHg from low to high *TL*s through the pine forest food chains with low Hg concentrations of basal resources was observed. The *TMS* values of THg and MeHg in this study are higher than those found in tropical freshwater and temperate forest food webs. Dietary composition plays a pivotal role in Hg toxin biomagnification in food webs. In addition to the traditional methods (e.g., stomach dissection, direct behavioral observations, neck collars, artificial nestling gape and fecal analysis) used for dietary composition analysis, we highly recommend live nest videography, especially when assessing the biomagnification of environmental pollutants in food webs related to free-living birds. Note that the effectiveness of nestling feathers as indicators of Hg exposure still need more case studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Kang Luo: Investigation, Methodology, Writing - original draft, Visualization, Data curation, Writing - review & editing. **Zhidong Xu:** Investigation, Methodology, Visualization, Data curation, Writing - review & editing. **Xun Wang:** Validation, Formal analysis. **Rui-Chang Quan:** Supervision, Project administration. **Zhiyun Lu:** Investigation, Funding acquisition. **Wenqi Bi:** Investigation. **Hai Zhao:** Project administration. **Guangle Qiu:** Supervision, Project administration, Funding acquisition.

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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.2020.114530.

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