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Total and methylmercury concentrations in nocturnal migratory birds passing through Mount Ailao, Southwest China

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ABSTRACT

Despite growing concerns over mercury (Hg) accumulation in birds in recent decades, little is known about Hg exposure in nocturnal migratory birds. Here, total mercury (THg) and methylmercury (MeHg) were detected in the feathers of nocturnal migratory birds (n = 286, belonging to 46 species) passing through Mount Ailao in Southwest China. The stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were also determined to clarify the effects of trophic position, foraging guild, and foraging behavior on Hg bioaccumulation. Our results show that the THg and MeHg concentrations varied by two orders of magnitude among all nocturnal migratory birds investigated, with the lowest values (THg: 0.056 mg kg^{-1}; MeHg: 0.038 mg kg^{-1}) in the Asian koel (*Eudynamys scolopaceus*) and the highest (THg: 12 mg kg^{-1}; MeHg: 7.8 mg kg^{-1}) in the hair-crested drongo (*Dicrurus hottentottus*). Waterbirds showed higher $\delta^{15}N$ values and higher THg and MeHg concentrations than songbirds, and the Hg concentrations in piscivorous species were significantly higher than those in herbivores, omnivores, and insectivores. Significant effects of foraging guilds (Kruskal–Wallis one-way ANOVA, $p < 0.001$) and foraging behaviors (Kruskal–Wallis one-way ANOVA, $p < 0.001$) on the Hg concentrations in migratory bird feathers were detected. A risk assessment indicated that approximately 7.0% of individuals were at moderate (2.4–5.0 mg kg^{-1}) to high (>5.0 mg kg^{-1}) risk of Hg exposure, and were therefore vulnerable to adverse physiological and behavioral effects. A long-term monitoring campaign during the migratory period is highly recommended to better understand the bioaccumulation of Hg in these nocturnal migratory bird populations over time.

1. Introduction

Mercury (Hg) is a well-known global pollutant because it is subject to long-distance atmospheric transportation in its elemental form (Tsui et al., 2012; Driscoll et al., 2013). Methylmercury (MeHg) is highly toxic, and it is readily accumulated in organisms and biomagnified along food chains (Lavoie et al., 2013; Abeysinghe et al., 2017; Li et al., 2021). Birds accumulate high concentrations of MeHg because they are sensitive to environmental changes and pollutants, particularly MeHg, and they occur at high trophic positions in food chains (Eagles-Smith et al., 2018; Hartman et al., 2019; Chételat et al., 2020). Recently, birds have shown increasing levels of MeHg exposure globally (Bond et al., 2015; Carravieri et al., 2016). Perkins et al. (2020) reported a significant increase in MeHg in the feathers of North American songbirds over the past 150 years, with the rusty blackbird (*Euphagus carolinus*) recording the greatest increase (17-fold), and this species has also shown an approximately 90% decline in its population since the 1960s (Greenberg and Droege, 1999). By contrast, the only species that showed no increase in MeHg was the red-eyed vireo (*Vireo olivaceus*), the populations of which displayed an increasing rather than decreasing trend (Rosenberg et al., 2019). This phenomenon implies a relationship between MeHg exposure in birds and their population growth.

It is widely accepted that birds' exposure to Hg depends strongly on their habitat, foraging guild, and trophic position. Many studies have

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characterized the differences in Hg exposure in water birds and songbirds (Keller et al., 2014; Mashroofeh et al., 2015; Burnham et al., 2018). In general, water birds, which obtain food from aquatic environments, show much higher Hg concentrations than songbirds from terrestrial habitats as a result of the different availability of MeHg in the distinct ecoregions (Peterson et al., 2017; Hall et al., 2020). Birds that feed on prey that occupy high trophic levels accumulate more MeHg than those that feed on prey at low trophic levels (Keller et al., 2014; Ackerman et al., 2016, 2019). Therefore, assessing the effects of ecological factors such as trophic position, foraging guild, and foraging behavior on Hg exposure in birds from distinct geographic sites can extend our understanding of the Hg burden in bird species across the landscape.

Avian migration is the regular seasonal movement of birds from their overwintering grounds to their breeding grounds (Webster et al., 2002). The two basic types of migratory birds are long-distance and short-distance migrants. Long-distance migrants travel great distances, covering thousands of kilometers across mountains, oceans, and deserts. In contrast, short-distance migrants rarely travel more than a few hundred kilometers (Newton, 2008). Migratory birds appear to be particularly vulnerable to Hg because they range widely across geographic locations and/or consume diverse diets during their long-distance migrations (Lavoie et al., 2014; Adams et al., 2020b). The migration of birds requires not only high energy, but also the complex interaction of neurological and physiological processes (Seewagen, 2020). Because MeHg potentially affects both neurological and physiological responses, the exposure of birds to MeHg may influence their migratory behavior (Seewagen et al., 2019). Several laboratory and field studies have shown that MeHg exposure reduces the takeoff ability, slows the initial flight speed, causes a reluctance to fly, lowers the homing efficiency, and reduces endurance flight performance of birds (Carlson et al., 2014; Moye et al., 2016; Ma et al., 2018a). Autumn migrant songbirds showed higher Hg levels than the same species passing through the same place in the following spring, suggesting that Hg exposure at breeding sites affects the migratory success of songbirds (Ma et al., 2018b; Seewagen et al., 2019).

To date, most studies of the effects of MeHg exposure have focused on fish-eating birds or aquatic birds. However, studies of Hg in songbirds that consume invertebrates, such as riparian spiders and aquatic insects, have also reported significant biomagnification of Hg (Cristol et al., 2008; Rimmer et al., 2010; Howie et al., 2018; Jackson et al., 2019). Songbirds living at Hg-contaminated sites accumulate high levels of 123.3 mg kg^{-1} Hg in feathers (Abeysinghe et al., 2017), which is even higher than the highest value of 91.6 mg kg^{-1} Hg reported in the feathers of fish-eating birds (Renedo et al., 2017). Recently, we found that songbirds from remote areas can also accumulate Hg to a level that presents a health risk (Li et al., 2021). This demonstrates that like aquatic birds, terrestrial birds can also be exposed to elevated levels of MeHg. Compared with the extensive studies that have been conducted on the ecological risks to aquatic birds caused by Hg exposure worldwide (Zamani-Ahmadmahmoodi et al., 2010; Watanuki et al., 2016; Su et al., 2020), little attention has been paid to the risk posed by MeHg to migratory terrestrial birds during their migration. Therefore, a better understanding of the MeHg burden in migratory terrestrial birds during their period of migration is required to clarify the exposure risk across species, and to advise regulators of their conservation.

Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are widely used to estimate the structures and energy flows of both aquatic and terrestrial food webs and can provide time-integrated information on the food sources and trophic positions of consumers (Binkowski et al., 2021; Souza et al., 2020., Ekblad et al., 2021). Typically, $\delta^{13}C$ reflects the major source of carbon for consumers (Peterson and Fry, 1987; Hebert et al., 2009), whereas $\delta^{15}N$ reflects the trophic position of individuals or species (Post, 2002; Bearhop et al., 2002; Keyel et al., 2020). Hg concentrations in consumers in the food chain generally increase with trophic position (Tsui et al., 2019; Li et al., 2021). Therefore, the integrated $\delta^{15}N$ and $\delta^{13}C$ values of consumers can be used to clarify the effects of ecological factors on potential variations in Hg in migratory birds (Brasso and Polito, 2013).

Mount Ailao in Yunnan Province, Southwest China is located at the intersection of two flyways: the Central Asia Flyway (CAF) and the East Asian–Australasian Flyway (EAAF). Massive numbers of nocturnal migratory birds fly over Mount Ailao during the autumn. From 1985 to 2017, a total of 150,561 individual birds of 310 species were recorded by banding projects that focused on nocturnal migratory birds (Wang, 2020) passing Mount Ailao. Studies of these birds have mainly addressed species composition, diversity, migration routes, and phototaxis (e.g., Yang et al., 2009; Luo et al., 2012; Zhao et al., 2020), but little is known about their exposure to Hg.

To address the knowledge gap described above, in the present study, we aimed to a) describe the variation in THg and MeHg across migratory populations of both aquatic and terrestrial birds; b) assess the influence of ecological factors affecting Hg and MeHg exposure using the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$); and c) evaluate the potential health risk posed by Hg exposure. We hypothesized that Hg levels in migratory birds were influenced by ecological factors such as foraging guild, foraging habitat, and trophic position.

2. Materials and methods

2.1. Sample collection site

Mount Ailao lies in the center of Yunnan Province, Southwest China. It forms the boundary between the Yunnan–Guizhou Plateau and the Cross Mountain Range and the watershed that divides the Yunjiang River and the Amo River. The Mount Ailao range extends from northwest to southeast, and it is administratively under the jurisdiction of the five counties of Shuangbai, Jingdong, Xinping, and Zhenyuan, all of which belong to Yunnan Province. The southwest monsoonal climate has a distinct rainy season extending from May to October and a dry season extending from November to March. The annual temperature and precipitation are 13.0 ± 5.0 °C and 1400 ± 700 mm, respectively (Wang et al., 2016). Jinshanyakou (JSYK, N23°94′, E101°49′, 2441 masl), at the junction of Xinping and Zhenyuan counties, is a narrow pass running from northwest to southeast in the middle of the Mount Ailao ranges (Fig. 1).

Eight migration routes are recognized worldwide, three of which pass through China: The Central Asia Flyway (CAF), the East Asian–Australasian Flyway (EAAF), and the West Asia–East Africa Flyway (WAEF). In Southwest China, the CAF and EAAF converge at Mount Ailao, and JSYK, a narrow pass and stopover site, is an important corridor for migrating birds. During the autumn migratory season, massive numbers of nocturnal migratory birds congregate at and migrate through the JSYK area, passing through the Mount Ailao range to their overwintering grounds.

Therefore, the JSYK site, at the juncture of the CAF and EAAF at Mount Ailao in Southwest China, provides an excellent opportunity to better understand the exposure of migratory birds to Hg. JSYK also has a national bird monitoring and banding station that has been banding birds since 2004 (Wang, 2020). This facility allowed us to monitor the exposure of nocturnal migratory birds to Hg. To date, no data for Hg or the exposure risk it poses to migratory birds have been reported. Therefore, in this study, we selected JSYK as the sampling site to conduct our investigation.

2.2. Sample collection and preparation

The chest feathers of 286 nocturnal migratory birds (belonging to 46 species and 18 families) were collected without harm to the birds in September and November 2018. Permission for avian sampling was obtained from the Yunnan Forestry and Grassland Bureau (Administrative License Decision of the Zhenyuan Management Bureau of Yunnan Mount Ailao National Nature Reserve to Hunt Wild Animals under

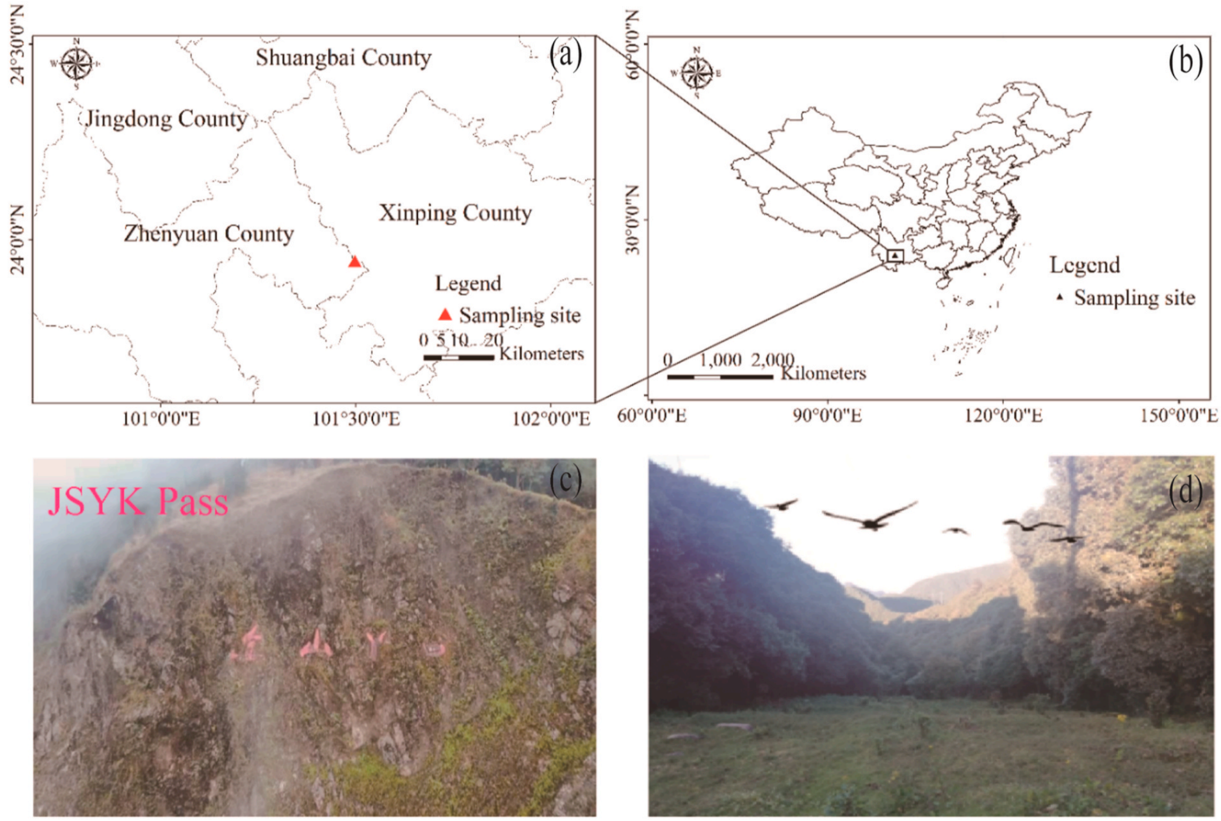

Fig. 1. Sampling site at Jinshanyakou (JSYK).

National Key Protection [ID 1584]).

The birds were captured with mist nets (mesh 36 mm, length 12 m, height 2.7 m) between 20:00 and 07:00. The bird species were identified online according to the Handbook of the Birds of China (https://www.cnniao.com) and the Handbook of the Birds of the World (https://www.hbw.com). The foraging guilds were categorized into herbivorous (including frugivores), omnivorous, insectivorous, and piscivorous guilds (Zhao, 2001). Detailed information on the migratory birds sampled is given in Table S1 and Table S2.

In the laboratory, chest feather samples were washed with tap water, detergent, and acetone to remove exogenous particles and organic pollutants, and then finally with deionized water. The cleaned feathers were air-dried and cut into approximately 0.1–0.5 mm pieces before analysis.

2.3. Analytical methods

2.3.1. THg and MeHg analyses

In this study, an acid digestion method was used to treated the feather samples before the THg and MeHg contents were determined according to Hintelmann and Nguyen (2005) and Tsui et al. (2019). Approximately 0.045–0.010 g of feather was accurately weighed into a centrifuge tube (50 mL) and then digested with 4 mL of HNO_3 (4 mol L^{-1}) in an oven at 55 °C for 30 h, with shaking every 30 min during digestion.

To determine the THg content, 2 mL of digestate was digested again with 5 mL concentrated HNO_3 (16 mol L^{-1}) in a water bath at 95 °C for 3 h and then oxidized with 25% (*m/v*) BrCl, neutralized with 25% (*m/v*) $NH_4OH{\cdot}HCl$, reduced with 20% (*m/v*) $SnCl_2$, and finally analyzed by cold-vapor atomic fluorescence spectrometry (CVAFS), using U.S. Environmental Protection Agency (USEPA) Method 1631E (USEPA, 2002).

To determine the MeHg content, 2 mL of digestate was neutralized with 2 mL of 20% KOH to adjust the pH to 4.9 and then treated with $NaBEt_4$ ethylation, purging, Tenax trapping, and gas chromatography (GC)–CVAFS, using USEPA Method 1630 (USEPA, 2001).

Quality assurance and quality control (QA/QC) were achieved with method blanks, duplicate samples, standard curves, and certified reference materials (for THg: GBW09101b, human hair, Shanghai Institute of Applied Physics, China; for MeHg: Tort 2, lobster hepatopancreas, National Research Council Canada, Canada). The value determined for THg in GBW0910b was 1.0 ± 0.28 mg kg^{-1} (with 96 ± 2.5% recovery), which was similar to the certified value of 1.1 ± 0.28 mg kg^{-1}. The value determined for MeHg in Tort 2 was 0.14 ± 0.053 mg kg^{-1} (with 91% ± 3.5% recovery), which was similar to the certified value of 0.15 ± 0.13 mg kg^{-1}. All relative standard deviations (RSDs) in the duplicate samples were <10%.

2.4. Stable isotope analyses

To determine the $\delta^{13}C$ and $\delta^{15}N$ values, approximately 0.50 mg of each feather sample was weighed into a tin cup and analyzed with an elemental analyzer (EA 2000; Thermo Scientific, Germany) coupled to a continuous-flow mass spectrometer (MAT 253, Thermo Finnigan Instrument, Germany). Cellulose (reference IAEA-C3, $\delta^{13}C$ = 24.7‰) and KNO_3 (reference IAEA-NO3, $\delta^{15}N$ = 4.70‰) were used to calibrate the $\delta^{13}C$ and $\delta^{15}N$ values, respectively, and the precision of the analytical measurements was <0.100‰. The values for $\delta^{13}C$ and $\delta^{15}N$ were converted based on Vienna Pee Dee Belemnite (V-PDB) and standard atmospheric nitrogen (AIR), respectively.

The isotopic ratios were expressed with delta notation (δ, ‰), relative to the international standard, using equation (1):

$$\delta X = \left(R_{sample} / R_{standard} - 1\right) \times 1000 \quad (1)$$

where X represents the isotope ^{13}C or ^{15}N, R_{sample} represents the isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the sample, and $R_{standard}$ represents the isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the international standard.

2.5. Statistical analysis

All statistical analyses were performed with Excel (Microsoft Corp., USA) and SPSS 24 (IBM, New York, USA). The raw data were checked for normality of distribution and homogeneity of variance using the Shapiro–Wilk and Kolmogorov–Smirnov tests, respectively. Nonparametric tests (Kruskal–Wallis one-way ANOVA) were performed to determine the significance of differences ($p < 0.05$) in feather Hg between different trophic levels, ecological types, and taxonomic affiliations because the datasets were not normally distributed or homoscedastic. Pearson's correlation and a linear regression analysis were used for the correlation analysis of THg, MeHg, $\delta^{13}C$, and $\delta^{15}N$. Figures were drawn using ArcMap10.2 (ESRI, USA) and Origin 9.0 (Origin Lab Corporation, Northampton, USA).

3. Results

3.1. THg and MeHg

3.1.1. THg

The THg concentrations in migratory bird feathers ranged from 0.056 to 12 mg kg^{-1}, with a mean of 1.1 ± 1.1 mg kg^{-1} (n = 286; Table 1, Fig. S1). Among the songbirds, the blue-and-white flycatcher (*Cyanoptila cyanomelana*, 2.8 ± 1.6 mg kg^{-1}) and brown-breasted flycatcher (*Muscicapa muttui*, 2.8 ± 1.1 mg kg^{-1}) had the greatest mean feather THg, whereas the wedge-tailed green pigeon (*Treron sphenurus*, 0.19 ± 0.057 mg kg^{-1}) had the lowest mean feather THg. The highest individual THg value of 12 mg kg^{-1} was observed in the hair-crested drongo (*Dicrurus hottentottus*), and was approximately two orders of magnitude higher than the lowest value, observed in the Asian koel (*Eudynamys scolopaceus*). THg thus displayed a wide and variable range. This large variation may also be attributable to the small sample sizes of the Asian koel in the present study.

Among the waterbirds, the striated heron (*Butorides striatus*), ruddy-breasted crake (*Porzana fusca*), and black-capped kingfisher (*Halcyon pileata*) had the highest mean THg concentrations, with values of 3.5 ± 1.0, 2.4 ± 1.1, and 2.4 ± 1.0 mg kg^{-1}, respectively. The highest individual THg values of 7.0 and 6.8 mg kg^{-1} were recorded in von Schrenck's bittern (*Ixobrychus eurhythmus*) and the yellow bittern (*Ixobrychus sinensis*), respectively. The lowest mean THg concentrations occurred in the rufous-backed crake (*Porzana bicolor*, 1.6 ± 0.22 mg kg^{-1}) and Eurasian woodcock (*Scolopax rusticola*, 1.0 ± 0.44 mg kg^{-1}).

3.1.2. MeHg

The MeHg concentrations in migratory bird feathers ranged from 0.027 to 7.8 mg kg^{-1}, with a mean of 0.70 ± 0.86 mg kg^{-1} (n = 286; Table 1, Fig. S1). Among the songbirds, similar to THg, the highest mean MeHg concentration was observed in the brown-breasted flycatcher (2.3 ± 1.1 mg kg^{-1}), followed by the blue-and-white flycatcher (1.7 ± 0.54 mg kg^{-1}) and the Eurasian wryneck (*Jynx torquilla*, 1.5 ± 0.99 mg kg^{-1}), and the lowest mean MeHg concentration was also observed in the wedge-tailed green pigeon (0.045 ± 0.020 mg kg^{-1}). Also similar to THg, the hair-crested drongo displayed the highest individual MeHg value of 7.8 mg kg^{-1}.

Among the waterbirds, the highest mean MeHg concentration was recorded in the striated heron (2.2 ± 0.90 mg kg^{-1}), followed by the ruddy-breasted crake (1.8 ± 1.4 mg kg^{-1}) and the grey-headed lapwing (*Vanellus cinereus*, 1.3 ± 0.65 mg kg^{-1}). The lowest mean MeHg concentration occurred in the Eurasian woodcock (0.59 ± 0.080 mg kg^{-1}). The highest individual MeHg values of 5.8 and 5.1 mg kg^{-1} were recorded in von Schrenck's bittern and the yellow bittern, respectively.

3.1.3. Ratio of MeHg to THg (MeHg%)

The ratio of MeHg to THg (MeHg%) in the analyzed feathers ranged between 10% and 99%, with an average value of 56% ± 23% (Table 1, Fig. S1). Among the songbirds, the highest mean MeHg% ratios in feathers were in the brown-breasted flycatcher (82% ± 7.9%), Asian stubtail (*Urosphena squameiceps,* 81% ± 18%), dusky warbler (*Phylloscopus fuscatus*, 81% ± 15%), and russet bush warbler (*Locustella mandelli*, 81% ± 11%). The lowest mean MeHg% ratios in the feathers analyzed were in the wedge-tailed green pigeon (26% ± 20%) and Asian emerald cuckoo (*Chrysococcyx maculatus*, 26% ± 10%).

Among the waterbirds, the rufous-backed crake (69% ± 19%), ruddy-breasted crake (67% ± 25%), and grey-headed lapwing (65% ± 21%) had the greatest mean feather MeHg% ratios, whereas the black-capped kingfisher (43% ± 19%) had the lowest mean feather MeHg% ratio. The highest individual MeHg% value of 99% was observed in the grey-headed lapwing.

3.2. Stable isotopes

3.2.1. $\delta^{13}N$

The values of $\delta^{15}N$ wide ranged widely from 1.27‰ to 14.0‰, with a mean value of 7.2‰ ± 2.6‰ (Table 1, Fig. S1). Among the songbirds, the chestnut-tailed starling (*chestnut-tailed starling*) showed the highest $\delta^{15}N$ value (14‰ ± 0.33‰), whereas the lowest value was observed in the oriental turtle dove (*Streptopelia orientalis*, 1.3‰). Among the waterbirds, the ruddy-breasted crake showed the highest $\delta^{15}N$ value (11‰ ± 1.4‰), and the lowest value was observed in the black-capped kingfisher (7.1‰ ± 2.5‰). Overall, the waterbirds (6.4‰ ± 2.5‰) had higher $\delta^{15}N$ values than the songbirds (9.4‰ ± 1.7‰, $p < 0.001$; Fig. 2b).

3.2.2. $\delta^{13}C$

The values of $\delta^{13}C$ ranged widely from −11.9‰ to −27.4‰, with a mean value of −23.3‰ ± 2.2‰ (Table 1). Among the songbirds, the largest $\delta^{13}C$ value was recorded in the chestnut bunting (*Emberiza rutile*, −11.9‰), and the lowest in Pallas's grasshopper warbler (*Helopsaltes certhiola*, −25.5‰ ± 0.83‰). Among waterbirds, the largest $\delta^{13}C$ value was recorded in the grey-headed lapwing (−21.7‰ ± 1.6‰) and the lowest in the yellow bittern (−27.4‰). Carbon isotopes are strongly fractionated by photosynthesis, and they are distinctly different in C_3 and C_4 plants. Therefore, C_4 plants have higher $\delta^{13}C$ values (−10‰ to −20‰) than C_3 plants (−22‰ to −34‰; O'Leary, 1988). In the present study, the $\delta^{13}C$ values for most migratory bird feathers fell in the range of −26.0‰ to −23.0‰, suggesting that the birds' diets were derived from C_3 sources (Fig. 2a, c).

3.3. Relationships among THg, MeHg, $\delta^{13}C$, and $\delta^{15}N$

No significantly positive relationships were observed between the $\delta^{15}N$ value and the THg concentration (songbirds: r = 0.22, $p > 0.05$; waterbirds: r = 0.32, $p > 0.05$) or the MeHg concentration (songbirds: r = 0.24, $p > 0.05$; waterbirds: r = 0.34, $p > 0.05$) in either songbirds or waterbirds. However, there was a tendency for the THg and MeHg concentrations to increase as the δ15N value increased (Fig. 3a, b).

Similarly, there was no significantly positive relationship between $\delta^{13}C$ and the THg concentration (songbirds: r = 0.25, $p > 0.05$; waterbirds: r = −0.49, $p > 0.05$) or the MeHg concentration (songbirds: r = 0.18, $p > 0.05$; waterbirds: r = −0.38, $p > 0.05$) in either songbirds or waterbirds. However, in waterbirds, there was a tendency for $\delta^{13}C$ values to decrease as THg and MeHg concentrations increased (Fig. 3c, d).

4. Discussion

In the present study, the feather THg and MeHg concentrations in different species varied by more than two orders of magnitude. Within the same species, individuals also showed very wide ranges of variation in their THg and MeHg concentrations. These marked variations may be

Table 1
Concentrations of total mercury (THg) and methylmercury (MeHg) and the compositions of stable carbon ($\delta^{13}C$) and nitrogen isotopes ($\delta^{15}N$) in the feathers of nocturnal migratory birds.

Species	THg ($mg \cdot kg^{-1}$)		MeHg ($mg \cdot kg^{-1}$)		MeHg%		$\delta^{13}C$‰		$\delta^{15}N$‰	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Red collared dove *Streptopelia tranquebarica*	0.27 ± 0.015	0.26–0.28	0.13 ± 0.067	0.17–0.080	47 ± 22	31–63	−20.7 ± 0.41	−21.0–20.4	7.9 ± 7.9	5.0–7.9
Oriental turtle dove *Streptopelia orientalis*	0.17	–	0.060	–	35	–	−23.5	–	1.3	–
Common emerald dove *Chalcophaps indica*	0.27	–	0.080	–	30	–	−23.8	–	4.1	–
Wedge-tailed green pigeon *Treron sphenurus*	0.19 ± 0.057	0.10–0.28	0.045 ± 0.020	0.027–0.098	26 ± 20	11–96	−24.5 ± 0.43	−24.1–24.9	4.3 ± 1.3	2.9–5.4
Chestnut-winged cuckoo *Clamator coromandus*	0.38 ± 0.030	0.36–0.40	0.15 ± 0.027	0.13–0.17	39 ± 4.0	37–42	–	–	–	–
Asian emerald cuckoo *Chrysococcyx maculatus*	0.43 ± 0.20	0.29–0.57	0.10 ± 0.0069	0.097–0.11	26 ± 10	19–33	–	–	–	–
Lesser cuckoo *Cuculus poliocephalus*	0.55 ± 0.24	0.29–1.3	0.23 ± 0.18	0.083–0.88	41 ± 20	20–97	−24.3 ± 0.40	−24.7–23.9	4.5 ± 1.6	3.5–6.3
Common cuckoo *Cuculus canorus*	0.40 ± 0.11	0.29–0.58	0.16 ± 0.069	0.080–0.27	39 ± 8.7	28–49	−29.3 ± 0.48	−24.5–23.5	4.7 ± 1.8	2.7–5.9
Large hawk-cuckoo *Hierococcyx sparverioides*	0.29 ± 0.12	0.13–0.53	0.10 ± 0.070	0.038–0.31	35 ± 12	21–63	−24.7 ± 0.69	−24.2–25.5	3.8 ± 1.6	2.5–5.5
Himalayan cuckoo *Cuculus saturatus*	0.37 ± 0.21	0.10–1.3	0.19 ± 0.17	0.027–0.88	36 ± 0.17	11–97	−24.8 ± 1.5	−25.8–23.1	4.6 ± 3.1	2.2–8.1
Asian koel *Eudynamys scolopaceus*	0.056	–	0.038	–	68	–	–	–	–	–
Eurasian wryneck *Jynx torquilla*	2.2 ± 1.3	1.3–3.1	1.5 ± 0.99	0.82–2.2	69 ± 4.6	65–72	–	–	–	–
Fork-tailed swift *Apus pacificus*	2.4 ± 0.056	2.1–2.4	0.86 ± 0.22	0.71–1.0	37 ± 8.3	31–42	–	–	–	–
Chestnut bunting *Emberiza rutila*	1.5	–	0.28	–	19	–	−11.9	–	5.0	–
Yellow-breasted bunting *Emberiza aureola*	1.1	–	0.17	–	16	–	−14.0	–	7.7	–
Chestnut-tailed starling *Sturnia malabarica*	0.88 ± 0.22	0.72–1.0	0.47 ± 0.35	0.22–0.72	50 ± 28	31–70	−23.0 ± 0.079	−23.0–22.9	14 ± 0.33	14–14
Paddyfield pipit *Anthus rufulus*	1.5 ± 0.97	0.92–2.6	0.79 ± 0.76	0.29–1.7	45 ± 16	32–63	−22.5 ± 0.90	−23.4–21.6	9.3 ± 1.2	8.5–11
Olive-backed pipit *Anthus hodgsoni*	1.1 ± 0.19	0.97–1.2	0.38 ± 0.22	0.22–0.53	33 ± 14	23–43	–	–	–	–
Lanceolated warbler *Locustella lanceolata*	1.4 ± 0.34	1.0–1.7	1.1 ± 0.55	0.58–1.9	79 ± 21	58–80	−24.3 ± 1.1	−25.1–23.5	9.1 ± 0.80	8.6–9.7
Pallas's grasshopper-warbler *Helopsaltes certhiola*	0.80 ± 0.70	0.38–1.7	0.71 ± 0.76	0.23–1.6	79 ± 18	61–96	−25.5 ± 0.83	−26.0–24.9	8.8 ± 0.19	8.6–8.9
Russet bush-warbler *Locustella mandelli*	0.40 ± 0.18	0.29–0.62	0.32 ± 0.12	0.21–0.45	81 ± 11	74–94	−24.5 ± 1.5	−25.6–23.4	7.5 ± 0.14	7.4–7.6
Spotted bush warbler *Locustella thoracica*	0.62 ± 0.34	0.37–1.3	0.45 ± 0.22	0.23–0.86	75 ± 14	58–93	−25.2 ± 0.20	−25.3–25.0	7.3 ± 1.7	5.8–9.1
Asian stubtail *Urosphena squameiceps*	1.5 ± 1.1	0.78–3.2	1.4 ± 1.2	0.51–3.1	81 ± 18	62–97	−25.1 ± 0.26	−25.3–24.9	3.9 ± 0.26	3.7–4.1
Thick-billed warbler *Arundinax aedon*	0.56 ± 0.16	0.38–0.95	0.25 ± 0.96	0.92–0.38	45 ± 16	34–60	−24.0 ± 0.74	−24.8–23.4	8.5 ± 2.8	5.5–11
Yellow-browed warbler *Phylloscopus inornatus*	1.1 ± 0.49	0.64–1.7	0.73 ± 0.44	0.38–1.3	62 ± 13	47–79	−24.4 ± 0.66	−24.8–23.9	6.0 ± 1.4	5.0–7.1
Pallas's leaf warbler *Phylloscopus proregulus*	1.0 ± 0.25	0.65–1.4	0.81 ± 0.26	0.38–1.3	78 ± 14	56–92	−25.2 ± 0.87	−26.0–24.3	5.0 ± 2.4	2.2–6.7
Dusky warbler *Phylloscopus fuscatus*	0.47 ± 0.029	0.45–0.49	0.38 ± 0.044	0.34–0.41	81 ± 15	71–91	–	–	–	–
Blue rock thrush *Monticola solitarius*	1.5 ± 0.63	0.91–2.7	0.97 ± 0.80	0.19–2.4	57 ± 25	21–92	−23.2 ± 0.43	−23.6–22.7	6.3 ± 1.8	4.4–7.9
Red-breasted flycatcher *Ficedula parva*	1.2 ± 0.52	0.49–2.2	0.92 ± 0.52	0.27–1.9	72 ± 16	38–92	−23.3 ± 1.3	−24.1–21.8	7.6 ± 0.58	6.9–8.0
Blue-and-white flycatcher *Cyanoptila cyanomelana*	2.8 ± 1.6	1.6–3.9	1.7 ± 0.54	1.3–2.1	67 ± 19	53–81	–	–	–	–
Brown-breasted flycatcher *Muscicapa muttui*	2.8 ± 1.1	2.0–3.6	2.3 ± 1.1	1.5–3.1	82 ± 7.9	76–87	–	–	–	–
Hill blue-flycatcher *Cyornis whitei*	1.7 ± 0.91	0.87–3.2	1.3 ± 0.87	0.36–2.5	71 ± 23	40–97	−22.3 ± 1.3	−23.6–21.0	4.9 ± 1.6	3.1–5.9
Grey bushchat *Saxicola ferrea*	1.4 ± 0.51	0.82–2.7	0.96 ± 0.64	0.41–2.6	65 ± 20	31–97	−23.5 ± 1.3	−24.4–22.1	6.3 ± 0.66	5.5–6.7
Siberian blue robin *Larvivora cyane*	0.92 ± 0.49	0.45–2.9	0.55 ± 0.25	0.15–1.3	63 ± 19	27–96	−23.9 ± 1.6	−25.0–22.1	9.0 ± 1.3	7.7–10
Black-naped oriole *Oriolus chinensis*	0.84 ± 0.35	0.34–1.3	0.49 ± 0.37	0.13–1.2	52 ± 21	28–93	−24.0 ± 0.55	−24.4–23.4	6.2 ± 1.2	5.3–7.4
Maroon oriole *Oriolus traillii*	0.71	–	0.21	–	30	–	–	–	–	–
Hair-crested drongo *Dicrurus hottentottus*	1.9 ± 3.4	0.37–12	1.3 ± 2.3	0.20–7.8	61 ± 15	37–87	−24.1 ± 1.3	−25.2–22.7	5.4 ± 1.2	4.8–6.8
Eurasian woodcock *Scolopax rusticola*	1.0 ± 0.44	0.59–1.5	0.59 ± 0.080	0.50–0.65	63 ± 22	45–87	−22.8 ± 1.7	−24.6–21.3	8.7 ± 0.50	8.3–9.3

(*continued on next page*)

Table 1 (*continued*)

Species	THg (mg·kg^{-1})		MeHg (mg·kg^{-1})		MeHg%		δ^{13}C‰		δ^{15}N‰	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Striated heron *Butorides striata*	3.5 ± 1.0	2.8–4.2	2.2 ± 0.90	1.6–2.8	62 ± 7.4	56–67	−23.4 ± 3.1	−25.6–21.2	9.9 ± 1.2	9.1–11
Von Schrenck's bittern *Ixobrychus eurhythmus*	7.3	–	5.8	–	79	–	−26.0	10.7	–	–
Yellow bittern *Ixobrychus sinensis*	6.8	–	5.1	–	74	–	−27.4	9.65	–	–
Grey-headed lapwing *Vanellus cinereus*	1.9 ± 0.54	1.0–3.0	1.3 ± 0.65	0.26–2.7	65 ± 21	21–99	−21.7 ± 1.6	−23.1–20.0	9.7 ± 0.70	9.0–10
Ruddy-breasted crake *Zapornia fusca*	2.4 ± 1.1	1.5–4.0	1.8 ± 1.4	0.60–3.8	67 ± 25	40–97	−22.3 ± 4.9	−25.4–16.7	11 ± 1.4	9.5–12
Common moorhen *Gallinula chloropus*	1.8	–	1.2	–	65	–	−25.0	–	11	–
Rufous-backed crake *Porzana bicolor*	1.6 ± 0.22	1.5–1.8	1.2 ± 0.46	0.83–1.5	69 ± 19	56–83	–	–	–	–
Black-capped kingfisher *Halcyon pileata*	2.4 ± 1.0	1.2–3.4	1.1 ± 0.85	0.34–2.6	43 ± 19	29–75	−23.6 ± 0.42	−23.2–24.0	7.1 ± 2.5	5.1–10

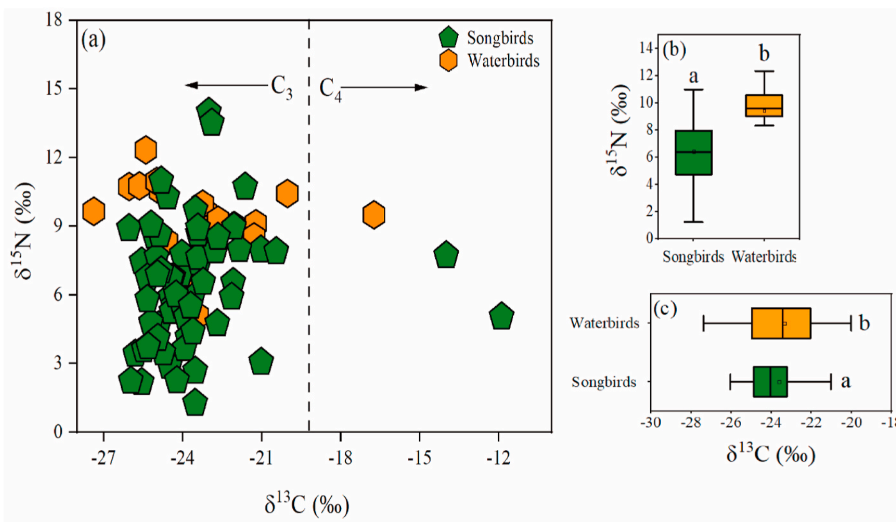

Fig. 2. Distribution of stable carbon (δ^{13}C) and nitrogen isotopes (δ^{15}N) in all feather samples.

related to their feeding guilds, trophic positions, and/or habitats.

4.1. Foraging guilds

The foraging guild is an important factor driving THg and MeHg concentrations and the variations in them among species (Mashroofeh et al., 2015; Knutsen and Varian-Ramos, 2020). Significant differences (Kruskal–Wallis one-way ANOVA, $p < 0.001$) in THg and MeHg concentrations were observed among birds belonging to distinct foraging guilds (Fig. 4a). The THg and MeHg concentrations in piscivorous species were significantly higher than those in herbivores, omnivores, and insectivores. These results indicate that diet is an important pathway via which birds accumulate Hg (Ma et al., 2021; Liu et al., 2020), and that Hg levels in birds are closely associated with their prey (Mashroofeh et al., 2015; Jackson et al., 2021).

In this study, flycatchers showed high concentrations of both THg and MeHg, similar to those reported in California (Ackerman et al., 2019) and on Mount Ailao (Li et al., 2021). The high Hg concentrations in flycatching species may be related to their diets of >90% invertebrates (Gray, 1993; Cooper et al., 2017). The hair-crested drongo also showed elevated levels of both THg and MeHg, attributable to its diet of predominantly vertebrates and insects (Gardner and Jasper, 2014). By contrast, the wedge-tailed green pigeon, oriental turtle dove, common emerald dove (*Chalcophaps indica*), and red collared dove (*Streptopelia tranquebarica*), all of which belong to the family Columbidae, showed the lowest Hg concentrations, probably because their diets are based on fruits, seeds, grasses, fresh leaves, and sprouts of plants in terrestrial ecosystems (Zhao, 2001; Table S1). These low Hg concentrations are consistent with those observed in the mourning dove (*Zenaida macroura*, 0.020 mg kg^{-1}) in California (Ackerman et al., 2019). Waterbird species of the family Ardeidae recorded peak values for both THg and MeHg in the present study, which may be attributable to their diets derived from aquatic sources, which generally contain high levels of Hg.

Similarly, the THg concentrations and MeHg% showed large variations among species (Table 1, Fig. S2), which is consistent with those reported for songbird feathers (10%–96%; Li et al., 2021) and tit nestling feathers (13%–44%) in another region of Mount Ailao (Luo et al., 2020). Dias dos Santos et al. (2021) reported low MeHg% (40%) in aquatic and scavenger bird feathers in the western Amazon. This significant difference in MeHg% may be attributable to the birds' diets, which are derived from terrestrial ecosystems and characterized by large variations in MeHg. Marine birds feed mainly on fish, and the high MeHg% in fish plays a key role in the high MeHg% in marine birds. Conversely, songbirds feed primarily on terrestrial invertebrates, which are always associated with low MeHg%, resulting in low MeHg% in terrestrial birds. As mentioned above, the small sample sizes of different species in this study may also be a factor contributing to the large variations in and wide range of MeHg levels within species.

Additionally, birds' sex, weight, age, physical condition, etc., are important factors, which might impact the bioaccumulation of Hg in birds (Tavares et al., 2013; Jackson et al., 2011; Ackerman et al., 2019;

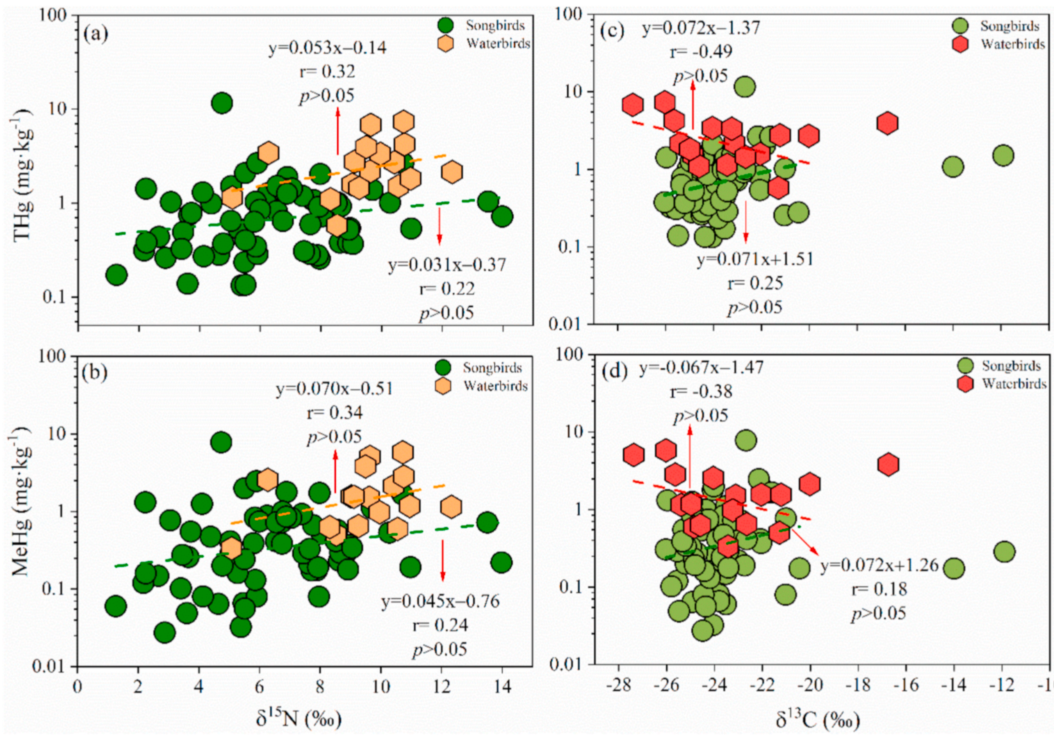

Fig. 3. Relationships between the δ^{15}N and THg or MeHg concentration in (**a**) songbirds and (**b**) waterbirds, and between δ^{13}C and THg or MeHg concentrations in (**c**) songbirds and (**d**) waterbirds.

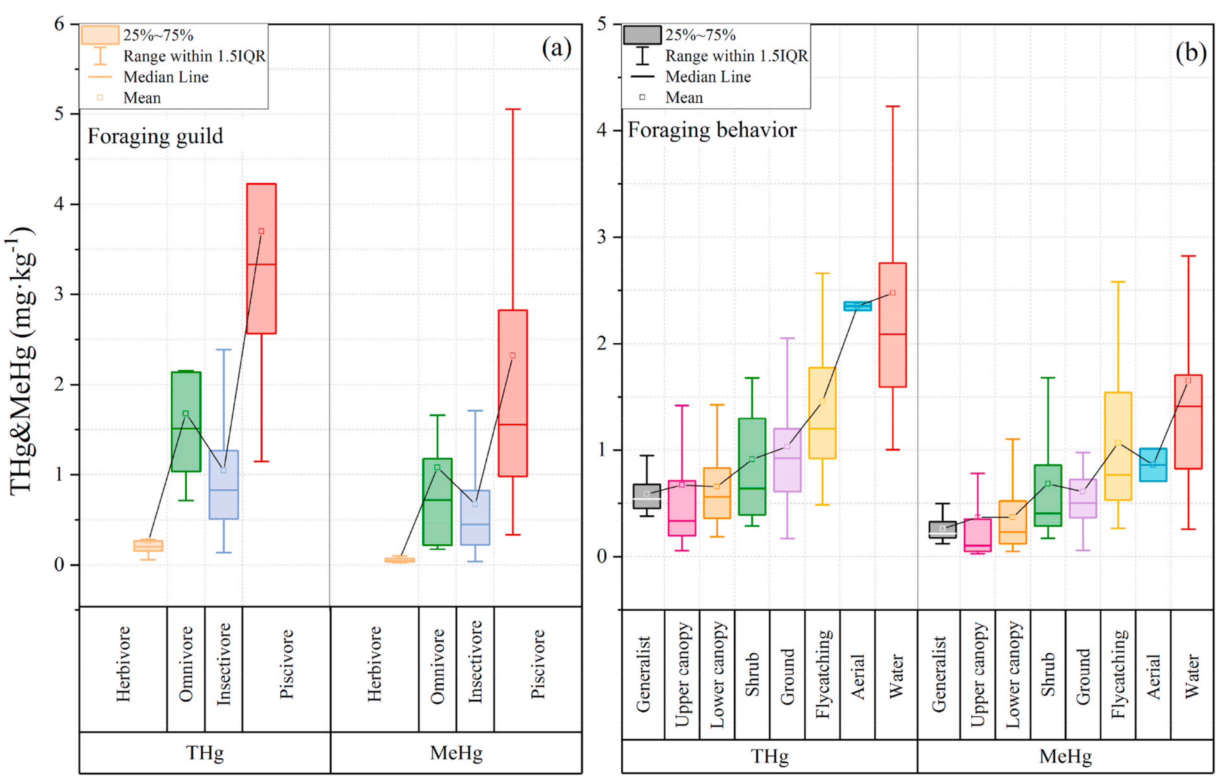

Fig. 4. Concentrations of THg and MeHg in bird feathers according to (**a**) foraging guild and (**b**) foraging behavior.

Adams et al., 2020b). Those data on birds' weight, sex, age and physical condition were not available in this study. Future work on the effects of these factors will help understanding Hg accumulation in birds.

4.2. Foraging behaviors

Foraging behaviors can also affect the different amounts of Hg in birds (Knutsen and Varian-Ramos, 2020). Significant differences (Kruskal–Wallis one-way ANOVA, $p < 0.001$) in THg and MeHg concentrations were also observed among birds with different foraging behaviors (Fig. 4b).

The mean THg concentrations were in the order: water foragers (2.7 ± 1.5 mg kg^{-1}, n = 31) > aerial foragers (2.4 ± 0.56 mg kg^{-1}, n = 2) > flycatching foragers (1.5 ± 0.74 mg kg^{-1}, n = 51) > ground foragers (1.0 ± 0.64 mg kg^{-1}, n = 51) > shrub foragers (0.91 ± 0.68 mg kg^{-1}, n = 23) > lower canopy foragers (0.66 ± 0.0.36 mg kg^{-1}, n = 46) > upper canopy foragers (0.67 ± 1.5 mg kg^{-1}, n = 55) > generalists (0.59 ± 0.18 mg kg^{-1}, n = 27).

Water foragers tend to feed on aquatic animals with markedly high Hg levels, so elevated levels of Hg in their bodies can be expected. In the present study, two generalist species, the chestnut-tailed starling and thick-billed warbler, had the lowest Hg concentrations of all the birds. A possible explanation is that these species feed mainly on terrestrial herbivorous insects, in which Hg concentrations are always low (Rimmer et al., 2010; Luo et al., 2020; Zhang et al., 2022).

4.3. Trophic position

The trophic level is another important factor driving Hg bioaccumulation and biomagnification in food webs (Ackerman et al., 2019; Binkowski et al., 2021). Generally, $\delta^{15}N$ is used to determine the trophic dynamics of individuals (Hobson et al., 1999), and high $\delta^{15}N$ values in consumers in a food chain often correlate with elevated Hg concentrations (Tsui et al., 2019; Seco et al., 2021). In the present study, $\delta^{15}N$ values did not correlate significantly positively with THg (songbirds: r = 0.22, $p > 0.05$; waterbirds: r = 0.32, $p > 0.05$) or MeHg (songbirds: r = 0.24, $p > 0.05$; waterbirds: r = 0.34, $p > 0.05$) in either songbirds or waterbirds, but weak positive correlations between $\delta^{15}N$ values and both THg and MeHg were observed (Fig. 3a and b), implying the occurrence of efficient Hg biomagnification processes in their food webs.

Waterbirds had higher $\delta^{15}N$ values and Hg concentrations than songbirds in this study (Fig. 2b, Fig. S3), which suggests that the biomagnification of Hg is related to trophic level. The $\delta^{15}N$ values for waterbird feathers ranged from 5.1% (black-capped kingfisher) to 12.3% (ruddy-breasted crake). These results correspond to about two trophic levels within the waterbirds when the trophic discrimination factor for $\delta^{15}N$ that is frequently applied to aquatic food webs for waterbirds or seabirds (3.4‰) is taken into consideration (Post, 2002; Borgå et al., 2012). The $\delta^{15}N$ values for songbird feathers ranged from 1.3% (oriental turtle dove) to 14% (chestnut-tailed starling). These results correspond to about three trophic levels within songbirds when the trophic enrichment factor for songbirds (3.7‰) is taken into consideration (Becker et al., 2007).

Previous studies have demonstrated that Hg concentrations and $\delta^{15}N$ values in bird assemblages increase along the food chain or web (Blévin et al., 2013: Li et al., 2021). A species of cuckoo (Himalayan cuckoo, *Cuculus saturatus*), the spotted bush warbler, and the black-capped kingfisher had a wide range of δ15N values and Hg concentrations, which may be related to the different composition of their prey (Bezerra et al., 2021).

4.4. Feeding habitats

Feeding habitats can also affect the Hg burden in the body of organisms due to their distinct differences of the biogeochemical processes of Hg and food webs (Adams et al., 2020a; Kramar et al., 2019). The stable isotope of carbon, $\delta^{13}C$, is a proxy for feeding habitats and can be used to determine ecosystem connectivity (Kelly, 2000). A wide range of $\delta^{13}C$ values in bird feathers usually suggests different food resources and feeding habitats (Bryan et al., 2012; Bezerra et al., 2021).

In the present study, the chestnut bunting and yellow-breasted bunting (*Emberiza aureola*) had the highest $\delta^{13}C$ values and low Hg concentrations (Table 1), which may be attributable to habitat availability and specific foraging behaviors because these species mainly inhabit relatively open and sparse forests and feed primarily on chestnuts, sorghum, and other grains of C_4 plants (Zhao, 2001). However, the yellow bittern and von Schrenck's bittern, which are piscivorous waterbirds, showed the lowest $\delta^{13}C$ values and high Hg concentrations, which is consistent with the differences observed between terrestrial and aquatic diets.

Water-associated habitats provide ideal conditions for Hg methylation, so elevated MeHg production is usually observed in these habitats (Hall et al., 2008; Marvin-DiPasquale et al., 2014), causing elevated Hg concentrations in piscivorous birds (Peterson et al., 2017; Chiang et al., 2021; Gerstle et al., 2019). Furthermore, the Hg risk can extend beyond the point source in contaminated aquatic areas to riparian songbirds because the surrounding habitats are also conducive to the methylation of Hg (Jackson et al., 2019).

4.5. Potential risk of Hg exposure to migratory birds passing Mount Ailao

The risk benchmark for Hg concentrations in feathers is reported to be 5.0 mg kg^{-1} (Burger and Gochfeld, 2000), and high Hg concentrations can significantly affect a bird's reproductive capacity. A previous study reported that as THg concentrations in the body feather of songbirds increased from 2.4 to 12.8 mg kg^{-1}, the reduction in nest success increased from 10% to 99%, suggesting that elevated Hg reduces a bird's nest success (Jackson et al., 2011). Here, we defined three risk categories for feather Hg in migratory birds: a) < 2.4 mg kg^{-1}, low risk; b) 2.4–5.0 mg kg^{-1}, moderate risk; and c) > 5.0 mg kg^{-1}, high risk. We then used these risk categories to evaluate the health risk that Hg exposure poses to these birds, based on our analytical data.

Overall, approximately 93% of individual birds were at low risk, 6% of individuals were at moderate risk, and 1% of individuals were at high risk of adverse physiological and/or behavioral effects (Fig. 5).

Significant effects of MeHg have been reported by researchers, including low nesting rates, fewer eggs and chicks, and thinner eggshells (Jackson et al., 2011; Hallinger et al., 2011; Tartu et al., 2013; Olivero-Verbel et al., 2013). The increasingly unsuccessful reproduction of birds may eventually lead to population decline. A recent study reported that since 2007, the population of breeding birds in North America has declined steeply by 2.9 billion, and that nocturnal migratory birds have decreased by 13.6% ± 9.1% (Rosenberg et al., 2019). Among these birds, the rusty blackbird (*Euphagus carolinus*) has shown a decline in population of ~90% since the 1960s (Greenberg and Droege, 1999), and its feather MeHg concentrations have increased significantly (17-fold) over the past 150 years (Perkins et al., 2020). Therefore, the elevated MeHg levels in the rusty blackbird and the dramatic decline in its population may well be linked, although no direct data are currently available.

5. Conclusions

The feathers of migratory birds are cost-effective bioindicators that can be used to monitor their THg and MeHg exposure at breeding or nonbreeding sites. Here, we have reported the THg and MeHg concentrations in nocturnal migratory birds in the Asia–Pacific region for the first time. These are important data for assessing the Hg burden in migratory birds in two global flyways (the CAF and EAAF). Significant variations in the THg and MeHg concentrations were observed among these nocturnal migratory birds, and the variations in Hg are attributed to differences in habitat, trophic position, and foraging guild. Our results suggest that approximately 93% of individual birds were at low risk of adverse physiological and/or behavioral effects, 6% of individual birds were at moderate risk, and 1% of individual birds were at high risk. Because the exposure of migratory birds to MeHg can affect their survival during migration through carry-over effects (Ma et al., 2018b), future long-term studies of the MeHg concentrations in nocturnal migratory birds and their association with breeding and nonbreeding areas are required.

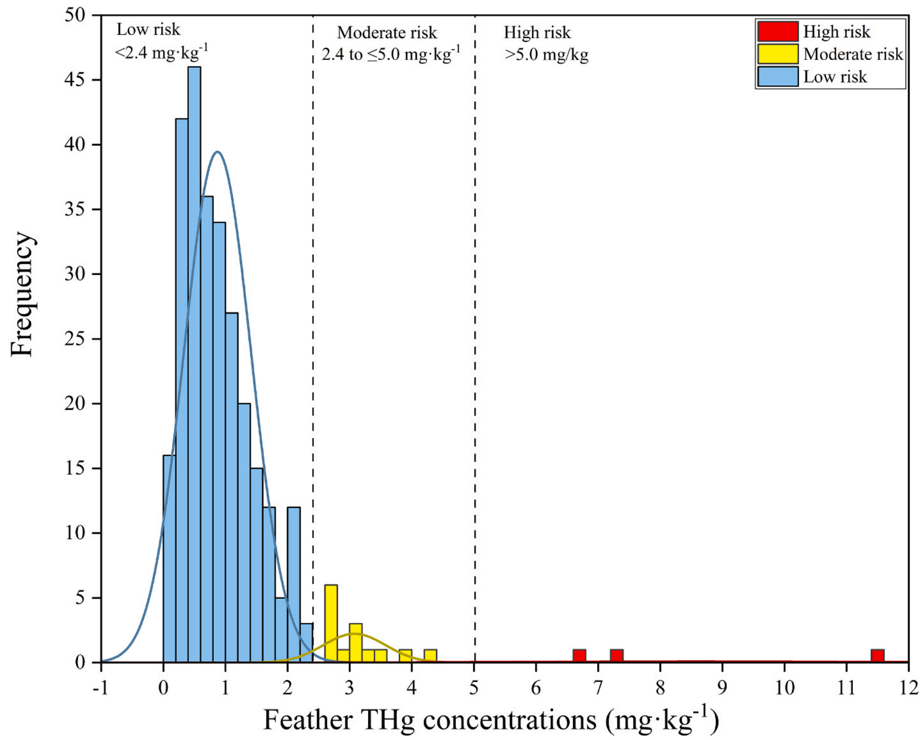

Fig. 5. Frequency histogram of feather THg concentrations in all individuals by risk category.

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CRediT authorship contribution statement

Chan Li: Investigation, Formal analysis, Methodology, Visualization, Data curation, Writing – original draft, Visualization, Data curation. **Kang Luo:** Investigation, Visionary, Methodology, Writing – review & editing. **Yuxiao Shao:** Investigation, Methodology, Resources, Formal analysis. **Xiaohang Xu:** Investigation, Methodology, Writing – review & editing, Funding acquisition. **Zhuo Chen:** Supervision, Validation, Funding acquisition. **Tao Liang:** Investigation, Data curation. **Zhidong Xu:** Validation, Writing – review & editing. **Xian Dong:** Investigation, Data curation. **Hongdong Wang:** Methodology, Investigation, Resources. **Guangle Qiu:** Supervision, Conceptualization, Project administration, Writing – review & editing, Funding acquisition.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.114373.

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