



Distributions of Total Mercury and Methylmercury in Dragonflies from a Large, Abandoned Mercury Mining Region in China

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Abstract

Dragonflies (Order Odonata) often are considered to be biosentinels of environmental contamination, e.g., heavy metals and/or persistent organic pollutants (POPs). Dragonflies ($n=439$) belonging to 15 species of 8 genera were collected from an abandoned mercury (Hg) mining region in China to investigate the bioaccumulation of total Hg (THg) and methylmercury (MeHg). THg and MeHg concentrations in dragonflies varied widely within ranges of 0.06–19 mg/kg (average: 1.5 ± 2.2 mg/kg) and 0.02–5.7 mg/kg (average: 0.75 ± 0.65 mg/kg), respectively. THg and MeHg were positively correlated with body-weight (THg: $r^2=0.10$, $P=0.000$; MeHg: $r^2=0.09$, $P=0.000$). Significant variations were observed among species, with the highest MeHg value (in *Orthetrum triangulare*) was fivefold higher than the lowest (in *Pantala flavescens*). These variations were consistent with those of nitrogen isotope ($\delta^{15}\text{N}$) values, indicating that increased $\delta^{15}\text{N}$, i.e., trophic levels, may reflect increased exposure and uptake of biomagnifying MeHg in dragonflies. A toxicological risk assessment found hazard quotients for specialist dragonfly-consuming birds of up to 7.2, which is 2.4 times greater than the permissible limit of 3, suggesting a potential toxicological risk of exposure.

Mercury (Hg) is a globally distributed pollutant that can cause severe exposure risks to the biota of both aquatic and terrestrial ecosystems (Cristol et al. 2008; Walters et al. 2010). Apart from unintentional accidents, the dominant pathway of Hg poisoning is through the consumption of foodstuffs contaminated with Hg, particularly methylmercury (MeHg). As an organic form of Hg, MeHg has extremely neurotoxic effects and is readily accumulated in

biota due to its lipophilic and protein-binding properties (Ullrich et al. 2001). Compared with inorganic Hg (iHg), MeHg is more easily taken up and can be transported and biomagnified several million-fold from the water to top predators via aquatic food chains. Currently, the toxicology of Hg in vertebrates, especially mammals and humans, has been comprehensively studied since the Minamata disease of the 1950s (Harada 1995). Consumption of fish and rice is considered the major pathway for human exposure to MeHg (Clarkson and Magos 2006; Feng et al. 2008; Qiu et al. 2008; Kathryn et al. 2009).

In recent years, numerous ecological indicators have been identified that are sensitive to changes in aquatic, terrestrial, and atmospheric environments and can provide an early warning of pollution (Zheng et al. 2018; Eagles-Smith et al. 2020). Insects, spiders, and other vertebrates, usually those belonging to high trophic levels, have been verified as effective ecological indicators (Cristol et al. 2008; Jeremiason et al. 2016; Popova et al. 2016; Yang et al. 2016; Williams et al. 2017). Among them, amphibiotic insects play an important role in the transfer of heavy metals from aquatic to terrestrial environments due to their demands for high-carbohydrate diets (Martin-Creuzburg et al. 2017).

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The dragonfly (Odonata) is a typical metamorphosis developmental insect with aquatic and terrestrial life stages. Its larvae live in freshwater for one to several years then adults live in a terrestrial environment for several days or weeks (Córdoba-Aguilar 2008). Both adults and larvae are predacious, with larvae mainly preying on tadpoles, shrimp, and small fish (Johansson and Brodin 2003). Because dragonflies feed on small insects from both aquatic and terrestrial environments, they play an important role in pollutant transfer from aquatic to terrestrial ecosystems and are regarded as a biosentinel for environmental contamination (Eagles-Smith et al. 2020). Simultaneously, the dragonfly is preyed upon and consumed by frogs, spiders, and some insectivorous songbirds, suggesting that it may represent a vector of pollutant transfer to vertebrates through food chains (Sullivan and Rodewald 2012; Buckland-Nicks et al. 2014; Williams et al. 2017).

Intensive Hg mining activities have generated Hg-contaminated sites worldwide. Although Hg mines closed decades ago, significant quantities of mine-waste calcine produced during the retorting process continue to release Hg, posing threats to local ecosystems (Gray et al. 2002; Qiu et al. 2013). Most previous studies on Hg mining areas have focused on Hg contamination of water, soil, the atmosphere, and agricultural crops (Gray 2003; Horvat et al. 2003; Qiu et al. 2008), confirming that mining areas were heavily Hg contaminated. Recently, Abeyasinghe et al. (2017) reported elevated levels of Hg in the feathers of songbirds dwelling in Hg mining areas and found that the spiders played an important role in the transfer of Hg via food chains. Recently, Lesch and Bouwman (2018) and Buckland-Nicks et al. (2014) suggested that amphibiotic insects, especially dragonflies, which have both aquatic and terrestrial life stages, can become vectors for both Hg and MeHg biomagnification in aquatic or terrestrial food webs. However, few studies have focused on dragonflies that may play an important role in Hg transfer at those typical Hg-contaminated sites.

In the present study, total Hg (THg) and MeHg levels were determined in dragonflies and the waterbodies of their habitat at an abandoned large-scale Hg mine in Southwest China. The objectives were to (1) ascertain the characteristics of THg and MeHg in dragonflies impacted by historic Hg mining and retorting activities; (2) investigate the distribution of Hg and its influencing factors in different dragonfly species; and (3) assess the toxicological risk of Hg exposure on specialist dragonfly-eating birds.

Experimental and Methods

Study Area

The Wanshan Hg mining area (WMMA; 27° 24' 53"–27° 37' 46" N, 109° 8' 2"–109° 22' 43" E) lies in eastern

Guizhou Province, Southwest China. It was once the largest national production center of metallic Hg. It has a typical mountainous and karstic landform characterized by low terrain in the east, high terrain in the west, and an uplift in the middle, with elevations ranging between 270 and 1149 m above sea level. The WMMA has a subtropical humid climate with abundant precipitation (1378 mm per year) and mild temperatures (annual average temperature: 13.7 °C). The Aozhai and Xiayi Rivers are the main rivers flowing through the mining area and originate from the Hg mining sites of Wukeng and Shibakeng, respectively (Fig. 1).

Intensive retorting of cinnabar ores in the WMMA generated significant quantities of mine-waste calcines, which contain amounts of water-soluble secondary Hg compounds (Jasinski 1995; Xu et al. 2019). The water-soluble Hg is readily released from mine-waste calcines into the surroundings. As much as 12,000 ng/L Hg has been observed in surface water from nearby ponds receiving leachates of mine-waste calcines (Qiu et al. 2009). Under certain conditions, the released inorganic Hg can be converted into more toxic and bioavailable MeHg, causing elevated Hg levels and toxicological risks in biota.

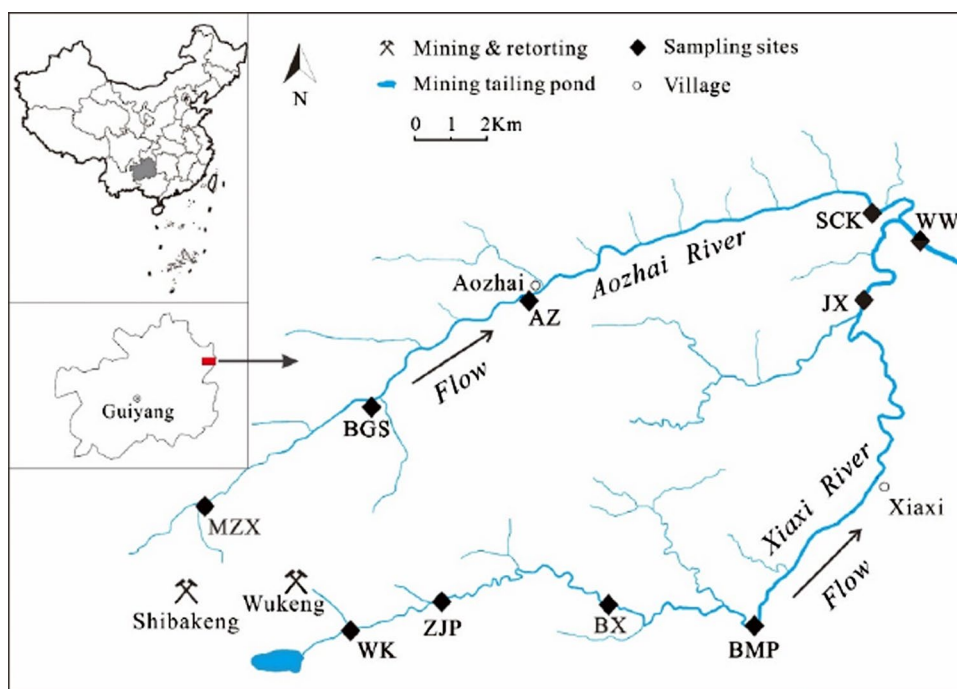
Sampling and Preparation

Dragonfly samples were collected from ten sites along the Xiayi River (XXR) and Aozhai River (AZR) using nylon dip nets in July 2018 and August 2019: six sites, Wukeng (WK), Zhongjiapo (ZJP), Boxi (BX), Baimuping (BMP), Jianxi (JX), and Wawu (WW), are located in XXR; and four sites, Meizixi (MZX), Baiguoshu (BGS), Aozhai (AZ), and Shenchongkou (SCK), in AZR (Fig. 1). According to the density of dragonflies, at least 40 individuals were sampled at each site. After collection, the species and sex of dragonflies were identified according to illustrated handbooks (Zhang 2019a, b).

A total of 439 individuals belonging to 15 species of 8 genera were collected. Species include *Acisoma panorpoides*, *Diplacodes trivialis*, *Orthetrum albistylum*, *Orthetrum japonicum*, *Orthetrum melanium*, *Orthetrum prunosum*, *Orthetrum sabina*, *Orthetrum triangulare*, *Pantala flavescens*, *Pseudothemis zonata*, *Rhyothemis fuliginosa*, *Sympetrum croceolum*, *Sympetrum kunckeli*, *Sympetrum speciosum*, and *Trithemis aurora*. The eight genera are *Acisoma*, *Diplacodes*, *Orthetrum*, *Pantala*, *Pseudothemis*, *Rhyothemis*, *Sympetrum*, and *Trithemis*. All the samples belong to the family Libellulidae (Order Odonata).

Among them, 198 are female, and 257 are male. 201 individuals (8 species of 4 genera) were collected from AZR and 238 individuals (11 species of 6 genera) from XXR. *Orthetrum*, *Pantala*, and *Sympetrum* were the dominant genera, accounting for 90.7% of all individuals. The four most common species were *O. albistylum*, *P. flavescens*, *S.*

Fig. 1 Sampling sites in the WMMA



croceolum, and *S.kunckeli*, with their population accounting for 78.6% of individuals. The dominant species were *S. kunckeli*, *P. flavescens*, and *O. albistylum* in AZR and *P. flavescens*, *O. triangulare*, and *S. kunckeli* in XXR.

In the laboratory, all samples were treated individually with starvation overnight, then anesthetized with alcohol and weighed. Samples were then thoroughly cleaned with deionized water to remove any contamination, freeze-dried (FDU-2110, EYELA, Japan) and their dry weight recorded. The dried samples were crushed individually into powder using a grinder (Pulverisette 23, Fritsch, Germany). The grinder was thoroughly cleaned with ethanol between operations to avoid cross-contamination.

Simultaneously, surface water samples ($n = 50$) for THg analysis were collected with 200 ml volume borosilicate glass bottles at each corresponding dragonfly sampling site. The borosilicate glass bottles were rigorously pre-cleaned in a muffle furnace at 500 °C for at least 1 h. After surface water collection, all samples were acidified in situ by adding 0.4% (v/v) of ultra-pure HCl, then stored in a refrigerator (4 °C) before analysis.

THg and MeHg Analyses

For THg, appropriately 20 mg of sample was weighed and digested at 95 °C for 3 h with 4 mL of a fresh acid mixture of $\text{H}_2\text{SO}_4:\text{HNO}_3$ (1:4, v/v). The digestion was then oxidized by BrCl overnight and filled with deionized water to a constant volume of 25 mL prior to analysis. THg concentration was detected by cold vapor atomic fluorescence spectrometry

(CVAFS, Model III, Brooks, USA) followed the reduction of SnCl_2 according to USEPA Method 1631e (USEPA 2002). For THg analysis in surface water, all samples were oxidized directly by BrCl with 24 h and then were determined by SnCl_2 reduction followed USEPA Method 1631e similar to THg analysis of dragonflies.

For MeHg, approximately 20 mg of sample was digested at 60 °C for 12 h with 4 mL 4.6 M HNO_3 according to Hammerschmidt and Fitzgerald (2005). MeHg was measured by gas chromatographic-cold vapor atomic fluorescence spectrometry (GC-CVAFS, Model III, Brooks, USA) following an ethylating process according to USEPA Method 1630 (USEPA 2001).

Quality Assurance and Quality Control

Quality assurance and quality control (QA/QC) for the THg and MeHg analyses were conducted using duplicates (~10%), method blanks, matrix spikes, and certified reference material (TORT-2, NRC Canada). The method blanks for THg and MeHg were <5.4 ng/g (~2% of the reference value of TORT-2) and <0.02 ng/g (detection limit value), respectively. The average THg concentration of TORT-2 was 268.6 ± 7.2 ng/g (2 SD, $n = 6$), which is comparable to the certified value of 270 ± 60 ng/g. The measured MeHg concentration in TORT-2 was 154.1 ± 4.8 ng/g (2 SD, $n = 6$) on average, which is comparable to the certified value of 152 ± 13 ng/g. The recoveries of THg and MeHg for the matrix spikes were 96.8–102.1% and 98.2–104.5%, respectively. Recoveries on matrix spikes of THg in water samples

were in the range of 90–110%. The relative standard deviation was < 8.5%.

Stable Nitrogen Isotope ($\delta^{15}\text{N}$) Analysis

In the present study, $\delta^{15}\text{N}$ was analyzed in two species of *O. triangulare* and *P. flavescens* collected from the WK site, which exhibited significant differences in THg and MeHg concentrations. Approximately 400–500 μg of sample were packaged into a small tin container, and then $\delta^{15}\text{N}$ was determined using an isotope ratio mass spectrometer (MAT 253, Thermo Fisher Scientific Inc., USA) and an elemental analyzer (Flash EA 2000 HT, Thermo Fisher Scientific Inc., USA).

$$\delta^{15}\text{N} = \frac{R_{\text{sa}} - R_{\text{st}}}{R_{\text{st}}} \times 1000(\text{‰})$$

where R_{sa} is the $^{15}\text{N}/^{14}\text{N}$ ratio of the samples and R_{st} is the $^{15}\text{N}/^{14}\text{N}$ ratio of the reference standard, which was atmospheric N_2 .

The mean percent recoveries of $\delta^{15}\text{N}$ were in the range of 99.2–100.4%. Each sample was measured in triplicate and the relative standard deviation was < 0.2%.

Hazard Quotient

High Hg concentrations in dragonflies may pose significantly high Hg exposure to their predators. The *Merops* (*M. viridis* and *M. philippinus*), whose diets depend heavily on dragonflies, were found in the study region. A hazards quotient (HQ) was applied to evaluate the exposure risk of the specialized dragonfly predator of *Merops* and was calculated as follows (Liu et al. 2015; Ai et al. 2019):

$$\text{HQ} = \sum \frac{\text{MDI}_{\text{IHg or MeHg}}/\text{BW}}{\text{TDI}_{\text{IHg or MeHg}}}$$

$$\text{MDI} = \text{DI} \times C \times \rho$$

$$\text{TDI} = \frac{\sqrt{\text{LOAEL} \times \text{NOAEL}}}{\text{UF}}$$

where MDI is the daily intake of IHg and MeHg via dragonflies (mg), DI is the daily intake of food (g), which is 3.84 g (d.w.) according to Krebs and Avery (1984), BW is the body weight (g), which is 35 g on average according to Zhao (2001), C is the Hg concentration in dragonflies (mg/kg), ρ is the proportion of dragonflies in food (%), ranging between 38.3 and 45.1% for Blue-throated bee-eater (Ke et al. 2017), between 57.9 and 63.0% for Blue-tailed bee-eater (Wu et al. 2009; Cheng et al. 2012), TDI is the tolerable daily intake, LOAEL is the lowest observed adverse

effect level of Hg: 0.9 mg/kg per day for IHg, and 0.025 mg/kg per day for MeHg (Sample et al. 1996), NOAEL is the no observed adverse effect level of Hg: 0.45 mg/kg per day for IHg, 0.015 mg/kg per day for MeHg, and UF is the uncertainty factor (a value of 10 was selected).

Statistical Analysis

Data processing was conducted using Microsoft Excel 2010 (Microsoft Corporation, USA). Statistical analysis was performed using one-way ANOVA with SPSS 22 software (IBM, USA). The map of sampling sites was drawn by CorelDRAW Graphics Suite X8 (Corel, Canada) and figures were plotted by Origin Pro 2016 (Origin Lab, USA).

Results

THg

The mean THg in all dragonflies was 1.5 ± 2.2 mg/kg ($n = 439$), ranging from 0.06 to 18.5 mg/kg. As expected, dragonflies from the WK site, which is close to the largest mining-waste calcine pile in the WMMA, exhibited the highest mean THg of 3.7 ± 3.9 mg/kg. This is almost tenfold higher (Mann–Whitney U test, $P = 0.017$) than the lowest value of 0.37 ± 0.21 mg/kg found at the JX site, which is approximately 20 km away from a pollutant source. THg in dragonflies from XXR (average: 2.31 ± 3.10 mg/kg; range: 0.14–18.5 mg/kg) was greater (Mann–Whitney U test, $P = 0.000$) than that of those from AZR (average: 1.0 ± 0.84 mg/kg; range: 0.06–4.8 mg/kg). Dragonflies collected from the mid-downstream to downstream areas of ZJP, BX, and BMP exhibited comparable mean levels of THg, which were 0.93 ± 0.60 mg/kg, 0.90 ± 0.34 mg/kg, and 1.0 ± 0.92 mg/kg, respectively. Overall, the THg in dragonflies gradually decreased with distance from upstream pollutant sources (Fig. 2a, d).

Variations in mean THg were observed among species, ranging between 0.63 ± 0.28 mg/kg in *S. croceolum* and 8.3 ± 4.3 mg/kg in *O. triangulare*. Species *O. japonicum* and *P. zonata* also exhibited high values of THg, reaching 7.1 ± 3.4 mg/kg and 5.3 ± 2.0 mg/kg on average, respectively. Of the four most common species, the highest and lowest THg values were found in *O. albistylum* and *S. croceolum*, averaging 1.8 ± 1.5 mg/kg and 0.63 ± 0.28 mg/kg, respectively. Interestingly, a significant difference in THg was observed between females and males, with the mean concentration in males (1.9 ± 2.7 mg/kg, $n = 244$) almost double that of females (1.0 ± 1.1 mg/kg, $n = 195$; Mann–Whitney U test, $P = 0.000$).

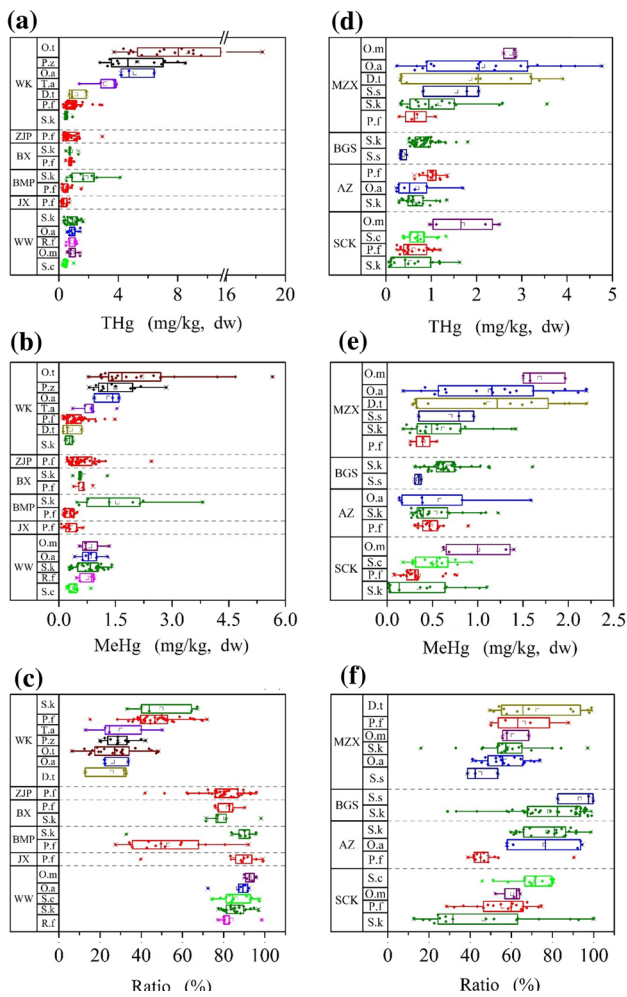


Fig. 2 Boxplots of THg (a, d), MeHg (b, e) and MeHg/THg ratio (c, f) in dragonflies collected from the Aozhai and Xiaxi River watersheds (*D. trivialis*-D.t, *O. albistylum*-O.a, *O. melanium*-O.m, *O. triangulare*-O.t, *P. flavescens*-P.f, *P. zonata*-P.z, *R. fuliginosa*-R.f, *S. croceolum*-S.c, *S. kunkeli*-S.k, *S. speciosum*-S.s, and *T. aurora*-T.a)

MeHg

The mean MeHg in all dragonflies was 0.75 ± 0.65 mg/kg ($n = 439$). The maximum and minimum values were 5.7 mg/kg and 0.02 mg/kg, which occurred at WK and JX, respectively. No significant difference in MeHg was observed between XXR (mean: 0.88 ± 0.86 mg/kg; range: 0.06–5.7 mg/kg) and AZR (mean: 0.64 ± 0.46 mg/kg; range: 0.02–2.2 mg/kg; Mann–Whitney U test, $P = 0.079$). Similar to the case for THg, both WK and MZX, which were adjacent to pollutant sources, recorded peak mean values of 1.1 ± 1.0 mg/kg and 0.93 ± 0.62 mg/kg, respectively. The lowest mean MeHg of 0.32 ± 0.19 mg/kg was observed in JX (Fig. 2b, e). Although dragonflies collected from mid-downstream to downstream of the ZJP, BX, and BMP sites exhibited comparable MeHg levels, there was a gradual decrease in MeHg with distance from upstream pollutant sources across the study region (Table 1).

Species also exhibited a wide range of MeHg values, ranging from 0.47 to 2.1 mg/kg, of which the highest and lowest values were recorded in *O. triangulare* and *P. flavescens*, respectively. In addition to *O. triangulare*, species *O. japonicum*, *O. melanium*, *O. pruinatum*, and *P. zonata* exhibited peak MeHg values > 1.0 mg/kg; interestingly, all of these four species belong to the genus *Orthetrum* (Table 2). In the common species, *O. albistylum*, *P. flavescens*, *S. croceolum*, and *S. kunkeli* showed comparable levels of MeHg, ranging between 0.47 ± 0.31 mg/kg and 0.95 ± 0.57 mg/kg. Similar to the case of THg, MeHg was greater in males than in females (Mann–Whitney U test, $P = 0.001$), with mean values of 0.85 ± 0.76 mg/kg ($n = 244$) and 0.61 ± 0.44 mg/kg ($n = 195$), respectively.

Table 1 Comparisons of THg and MeHg concentrations in dragonflies from the study area

Location	N	Body weight (mg, dw)				THg Conc. (mg/kg, dw)				MeHg Conc. (mg/kg, dw)				MeHg (%)				
		Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	
XXR	WK	90	94.5	32	46.8	173	3.69	3.85	0.309	18.5	1.061	1.02	0.114	5.66	37.38	15.1	6.40	71.9
	ZJP	40	69.8	25.1	30.1	146	0.930	0.600	0.302	2.93	0.744	0.521	0.220	2.45	79.6	11.1	41.8	95.9
	BX	14	68.8	26.7	39.1	122	0.900	0.340	0.458	1.58	0.747	0.320	0.371	1.28	82.6	7.11	71.5	98.1
	BMP	27	84.9	19	48.2	126	1.02	0.920	0.201	4.11	0.705	0.873	0.122	3.81	62.9	24.4	27.5	95.7
	JX	8	90.3	16.5	62.4	113	0.370	0.210	0.139	0.720	0.316	0.194	0.061	0.642	84.6	18.8	39.8	99.1
	WW	59	84.3	46.7	26.3	191	0.822	0.370	0.231	1.63	0.712	0.322	0.207	1.41	86.7	6.10	72.5	98.5
AZR	MZX	58	112	34.6	46.1	207	1.69	1.22	0.237	4.77	0.931	0.621	0.178	2.20	60.9	16.6	16.1	99.0
	BGS	44	73	20.8	42.5	129	0.840	0.300	0.309	1.80	0.654	0.258	0.309	1.61	79.4	17.8	29.0	99.7
	AZ	42	61.1	38.1	26.4	170	0.771	0.330	0.232	1.69	0.528	0.288	0.131	1.59	69.3	19.2	39.0	98.6
	SCK	57	92.5	33.2	41.2	183	0.690	0.49	0.063	2.51	0.427	0.334	0.019	1.39	55.7	21.6	12.7	99.9
	Total	-	439	86.1	36.0	26.3	207	1.52	2.20	0.063	18.5	0.751	0.652	0.019	5.66	64.5	23.6	6.40

Table 2 Inter-species and -genus comparisons of bodyweight, THg and MeHg concentrations, and MeHg ratio

Genus	Species	N	Body weight (mg, dw)					THg Conc. (mg/kg, dw)					MeHg Conc. (mg/kg, dw)					MeHg (%)		
			Mean	SD	Min	Max		Mean	SD	Min	Max		Mean	SD	Min	Max		Mean	SD	Min
<i>Acisoma</i>	<i>A. panorpoides</i>	3	39.4	8.80	29.3	44.9	0.951	0.040	0.931	1.00	0.882	0.041	0.839	0.912	89.6	0.501	89	89.9		
<i>Diplacodes</i>	<i>D. trivialis</i>	15	84.5	30.3	50.7	146	1.72	1.32	0.322	3.92	0.948	0.752	0.114	2.21	63.4	26.7	12.8	99.0		
<i>Orthetrum</i>	<i>O. albistylum</i>	37	128	34.0	46.8	175	1.83	1.54	0.231	6.41	0.947	0.568	0.131	2.23	62.9	21.3	22.2	94.3		
	<i>O. japonicum</i>	3	109	10.1	101	120	7.05	3.38	3.64	10.4	1.64	0.851	0.663	2.18	22.9	6.90	18.1	30.9		
	<i>O. melanium</i>	13	162	35.1	75.2	207	1.77	0.852	0.569	2.87	1.25	0.550	0.552	2.19	74.8	16.6	52.4	95.8		
	<i>O. pruinotum</i>	2	108	15.1	97.3	119	1.41	0.427	1.09	1.72	1.19	0.391	0.921	1.47	84.6	2.02	83.2	86.0		
	<i>O. sabina</i>	4	89.6	12.0	75.9	105	0.799	0.771	0.121	1.58	0.614	0.681	0.020	1.27	48.6	38.5	14.8	83.5		
	<i>O. triangulare</i>	21	136	19.5	87.7	173	8.27	4.28	0.351	18.5	2.12	1.35	0.285	5.66	29.8	17.1	6.40	82.7		
<i>Pantala</i>	<i>P. flavescens</i>	140	72.0	22.4	30.1	146	0.781	0.473	0.142	2.93	0.469	0.311	0.062	2.45	61.7	19.7	15.1	99.1		
<i>Pseudohemis</i>	<i>P. zonata</i>	12	78.1	13.3	61.2	101	5.28	2.03	2.77	8.52	1.52	0.611	0.804	2.85	29.1	7.01	19.9	42.1		
<i>Rhyothemis</i>	<i>R. fuliginosa</i>	5	68.9	6.70	59.6	76.0	0.908	0.310	0.479	1.21	0.751	0.221	0.469	0.942	83.8	8.40	77.3	98.5		
<i>Sympetrum</i>	<i>S. croceolum</i>	26	115	14.9	85.8	149	0.631	0.288	0.231	1.33	0.483	0.204	0.170	0.927	76.8	12.3	45.9	97.3		
	<i>S. kunckeli</i>	142	68.7	26.3	26.3	144	0.910	0.602	0.064	4.11	0.671	0.482	0.019	3.81	72.7	21.5	12.7	99.9		
	<i>S. spectosum</i>	10	120	21.3	79.3	149	0.821	0.603	0.312	2.04	0.542	0.221	0.311	0.954	78.4	23.9	38.7	99.7		
<i>Trithemis</i>	<i>T. aurora</i>	6	96.3	37.0	58.1	146	2.89	1.12	1.38	3.86	0.951	0.422	0.371	1.53	37.5	22.7	13.0	75.1		

MeHg Ratios

The ratios of MeHg to THg (MeHg%) in dragonflies varied widely, ranging from 6.45% to 99.95% with a mean of $64.5 \pm 23.6\%$. A maximum mean value of $86.7 \pm 6.1\%$ was recorded at WW, and a minimum of $37.4 \pm 15.1\%$ was observed at WK. A positive correlation (Spearman test, $r=0.891$, $P=0.000$) was observed between the MeHg and THg values at the 10 sites, with a logarithmically increasing pattern (Figure S1). This suggests that MeHg% values first increased and then decreased at THg concentrations above ~ 1.2 mg/kg. Overall, a significant negative correlation was observed between MeHg% and THg (Spearman test, $r=-0.287$, $P=0.000$).

A large variation in MeHg% was observed among species, ranging between 22.9 ± 6.9 and $89.6 \pm 0.5\%$ on average (Fig. 2c, f). Peak values of MeHg% mostly occurred in *A. panorpoides*, *O. pruinosum*, and *R. fuliginosa*, which were collected from the WW site. Of the common species, *O. albistylum*, *P. flavescens*, *S. croceolum*, and *S. kunckeli* showed comparable levels of MeHg% ranging between 61.7 ± 19.7 and $76.8 \pm 12.3\%$. In addition, species *O. japonicum*, *P. zonata*, and *O. triangulare*, which had the highest THg values, exhibited low mean MeHg% values that ranged from 22.9 ± 6.9 to $29.8 \pm 17.0\%$. A significant difference in MeHg% was observed between female and male *S. croceolum* (one-way ANOVA, $P=0.000$), of which the females ($85.9 \pm 7.0\%$) had a higher mean value than males ($70.1 \pm 11.0\%$).

Correlation Between Body Weight and Hg Concentration

Both THg and MeHg gradually increased with bodyweight in all dragonflies (THg: $r^2=0.10$, $P=0.000$; MeHg: $r^2=0.09$, $P=0.000$). Similar trends were observed at sites AZR (THg:

$r^2=0.15$, $P=0.000$; MeHg: $r^2=0.14$, $P=0.002$) and XXR (THg: $r^2=0.30$, $P=0.003$; MeHg: $r^2=0.20$, $P=0.000$; Fig. 3a, b). The result is consistent with a previous report of a positive relationship (THg: $r^2=0.38$, $P \leq 0.001$) between bodyweight and THg concentration in predatory insects such as *Nabidae* (Yung et al. 2019).

$\delta^{15}\text{N}$

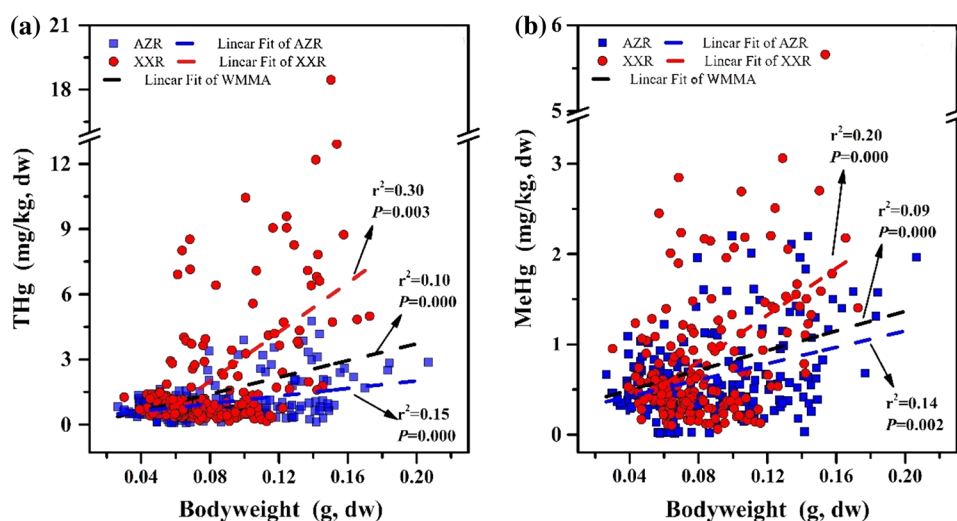
Species *O. triangulare* and *P. flavescens* from site WK exhibited significant differences in THg ($P < 0.001$) and MeHg ($P < 0.001$). The $\delta^{15}\text{N}$ value in *O. triangulare* (mean: $8.26 \pm 1.15\text{‰}$, range 6.63–10.3‰, $n=11$) was significantly higher than in *P. flavescens* (mean: $5.86 \pm 2.06\text{‰}$, range 2.64–8.42‰, $n=11$; $P=0.002$).

Discussion

Spatial Variation in THg and MeHg in Dragonflies

Dragonflies appear to provide a more stable and effective indication of the status of environmental pollutants than direct indicators such as water, soil, and the atmosphere (Lesch and Bouwman 2018; Eagles-Smith et al. 2020). As a typical carnivorous insect, adult dragonflies respond sensitively to Hg contamination in food chains. As expected, dragonflies in WK had extremely high THg levels, while dragonflies from other sites showed slightly decreasing or comparable levels of both THg and MeHg with distance from upstream Hg sources. This was coincident with the THg concentrations of surface water (Fig. 4a, b); both THg ($r=0.894$, $P=0.000$) and MeHg ($r=0.684$, $P=0.020$) in dragonflies were positively correlated to surface water THg, suggesting that they are good indicators of aquatic Hg concentrations.

Fig. 3 Correlations between dragonfly bodyweights and THg/MeHg concentrations



In the present study, Hg concentrations in all dragonflies were significantly elevated: fivefold and threefold higher than in dragonflies from the Eagle Mountain Fish Hatchery (MeHg: 0.15 mg/kg, dw) and Kejimikujik National Park (THg: 0.26 ± 0.071 mg/kg, dw; MeHg: 0.23 ± 0.074 mg/kg, dw) as reported by Williams et al. (2017) and Buckland-Nicks et al. (2014), respectively. However, they are slightly lower than those reported by Abeysinghe et al. (2017) in an Hg mining-affected rice paddy field (THg: 2.7 ± 0.84 mg/kg, MeHg: 1.8 mg/kg, dw). Also, an elevated THg value of 4.2 mg/kg (range: 0.23–12 mg/kg) was reported in Odonata from the area of zinc smelting and chlor-alkali areas (Zhang et al. 2009). High levels of Hg in dragonflies may imply heavy Hg contamination of the local environment (Zhang et al. 2012; Lesch and Bouwman 2018).

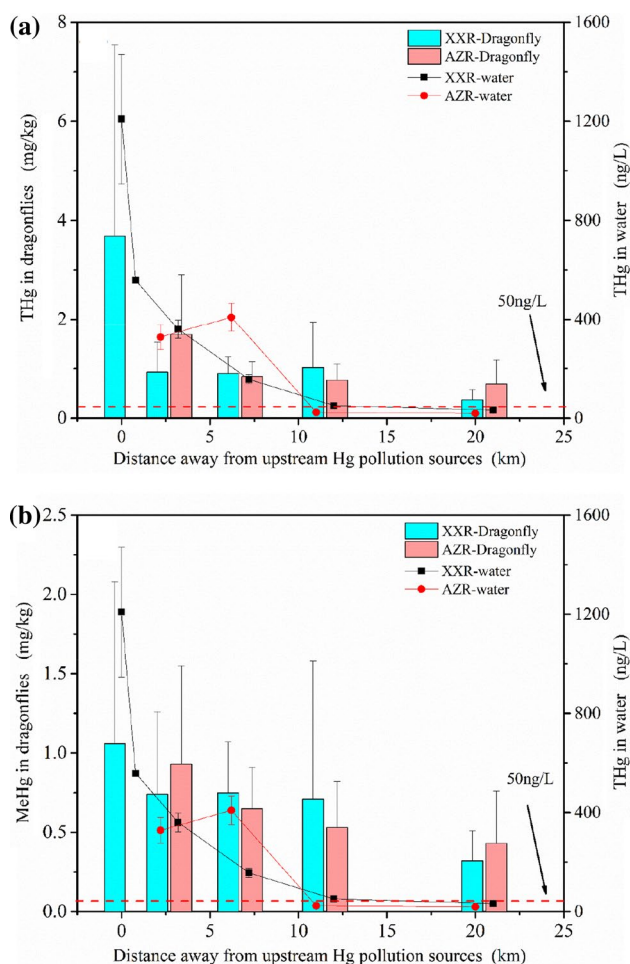


Fig. 4 Distributions of THg and MeHg in dragonflies and surface water with distance from upstream Hg pollution sources

Interspecies Variation in THg and MeHg Concentrations

Concentrations of THg and MeHg as well as MeHg-to-THg ratios (MeHg%) varied widely among dragonfly species. The lowest and highest THg concentrations were found in *S. croceolum* and *O. triangulare*, of which the latter was 13-fold higher than the former ($P=0.000$). Similarly, *O. triangulare* also exhibited the highest MeHg, which was approximately fivefold higher than the lowest value, which was found in *P. flavescens* ($P=0.000$). In the common genera, the highest THg and MeHg concentrations found in *Orthetrum* were 4.7-fold and 2.8-fold higher ($P<0.001$) than the lowest values found in *Pantala*. *Orthetrum* is usually two times higher in bodyweight than *Pantala*, allowing it to be a more effective predator and even prey on smaller dragonflies. This may explain why *Orthetrum* can accumulate more Hg than *Pantala*.

Significant differences in Hg concentration were found between species. In the present study, THg and MeHg in *O. triangulare* were found in higher concentrations than in *P. flavescens* ($P=0.002$), both of which were collected from the heavily Hg-contaminated WK site. The distribution of $\delta^{15}\text{N}$ found in the two species was coincident with THg and MeHg concentrations. *O. triangulare* had the higher values of $\delta^{15}\text{N}$, while *P. flavescens* showed lower values (Fig. 5). As higher $\delta^{15}\text{N}$ values indicate a higher trophic position (Hyodo 2015; Rodenhouse et al. 2019), elevated Hg, particularly the MeHg in *O. triangulare*, may be attributed to a higher trophic position in the food chain.

Interestingly, male dragonflies exhibited higher levels of both THg and MeHg than those of female dragonflies in the present study. Heckel and Keener (2007) and Zheng et al. (2010) reported similar phenomena in cicadae and suggested

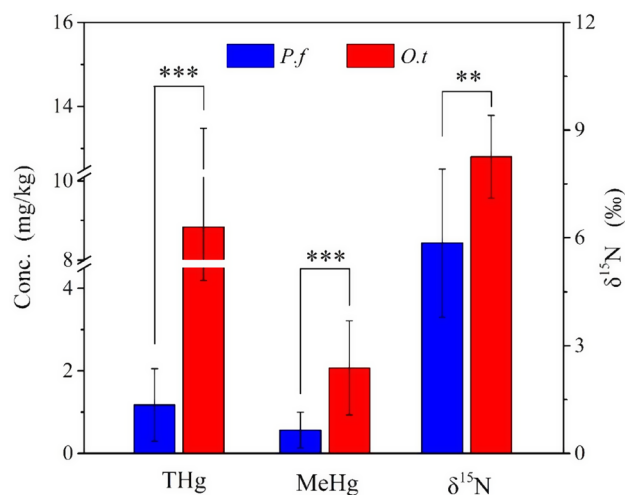


Fig. 5 $\delta^{15}\text{N}$ values (‰) in species *P. flavescens* and *O. triangulare* (***)Significant difference at $P<0.001$, ** $P<0.01$)

that egg-laying by adult females may cause Hg to excrete from the body. However, an alternative plausible explanation for the observed sexual differences in both THg and MeHg accumulation in dragonflies may be attributed to the shorter life-span of adult females than of adult males (Córdoba-Aguilar 2008), therefore, an apparent lower Hg concentration in females than males.

Influence of Bodyweight on Hg

Life traits such as feeding guilds, life cycle, and habitat are the main factors controlling the accumulation of Hg (Yung et al. 2019). In the present study, weakly positive correlations were observed between dragonfly bodyweights and THg ($r^2=0.10$, $P=0.000$) and MeHg ($r^2=0.09$, $P=0.000$), suggesting that bodyweight can lead to an increase of Hg in dragonflies, but does not make a significant contribution. Previous investigations have suggested that insects may demonstrate biomass dilution, causing a decrease in toxins with increasing bodyweight (Karimi et al. 2007; Clayden et al. 2013; Verburg 2014). Rapid growth could significantly reduce MeHg concentrations in organisms as biomass increases (Karimi et al. 2007; Zheng et al. 2008; Zhang et al. 2008).

Under certain condition, however, a diet once polluted by Hg may become the key factor in Hg bioaccumulation. As a heavily Hg-contaminated site in the present study, dragonfly predation of insects inhabiting the WMMA, which usually contained elevated Hg, may become the key pathway of their Hg bioaccumulation, leading to an anti-biomass dilution. Compared with many herbivorous insects observed in other regions, dragonflies in the present study exhibited higher Hg concentrations (Zheng et al. 2018), probably verifying that diet rather than bodyweight plays a dominant role in Hg accumulation.

Potential Risks to Consumers

Adverse effects on avian due to exposure to elevated Hg levels have been reported (Wada et al. 2009; Sauer et al. 2020). In a previous study, extremely high Hg concentrations of up to 123 ± 34 mg/kg were found in the feathers of songbirds in the study region (Abeyasinghe et al. 2017). However, the accumulation and transfer of elevated Hg levels in songbirds via food chains remain unclear. Several studies have reported that members of the *Merops*, such as the Blue-throated bee-eater (*M. viridis*) and Blue-tailed bee-eater (*M. philippinus*) depend heavily on dragonflies, which can account for 38.3–45.1% (Ke et al. 2017) and 57.9–63.0% (Wu et al. 2009; Cheng et al. 2012) of their diets, respectively. Such high rates of dragonfly consumption may contribute to heavy Hg burdens in songbirds inhabiting Hg mining regions.

Here, we evaluate the hazard quotient (HQ) of the two species *M. viridis* and *M. philippinus* according to their dietary ratios of Odonata. The results indicate that the HQs of Blue-throated bee-eater and Blue-tailed bee-eater are 4.39–5.17 and 6.63–7.22, respectively, both of which exceed the permissible limit of exposure risk (HQ=3; Liu et al. 2015; Ai et al. 2019). It is clear that the Blue-tailed bee-eater, which has the higher dragonfly dietary ratio, has a higher toxicological risk than the Blue-throated bee-eater at a particular site. Because both THg and MeHg at site XXR were higher than at AZR, the two species may experience greater exposure risk at XXR than at AZR. The emergent stage of the dragonfly coincides with the reproductive period of birds, which may greatly increase Hg exposure risk, especially to nestlings.

Conclusions

Dragonflies (Odonata) collected from large-scale Hg mining regions exhibited high concentrations of both THg and MeHg, which had ranges of 0.23–19 mg/kg and 0.06–5.7 mg/kg, respectively. Both correlated with the THg concentration of surface water. A positive correlation was observed between bodyweight and Hg concentration. Large variations in THg and MeHg occurred among different species, of which *O. triangulare* and *O. japonicum* showed extremely elevated THg and MeHg concentrations of 8.3 ± 4.3 mg/kg and 2.1 ± 1.4 mg/kg, respectively. Species with high Hg usually had high $\delta^{15}\text{N}$ values, indicating that they occupy high trophic levels. The MeHg-to-THg ratios also exhibited large variations, ranging between 6.4 and 99.9%. Our findings indicate that dragonflies are sensitive to Hg pollution and can be considered a sentinel for environmental Hg contamination. High Hg concentrations in dragonflies also may represent a vector through which Hg is passed to vertebrates. Future studies should focus on the mechanisms of Hg accumulation in dragonflies, particularly of MeHg, as well as Hg transfer in the process of metamorphosis from larva to adult. In addition, the exposure risk to specialist dragonfly consumers, such as *Merops* songbirds, warrants greater attention. Dragonflies are effective biosentinels of environmental Hg, particularly MeHg, and can be utilized to measure and predict Hg pollution. We strongly recommend future application of monitoring Hg in dragonflies for better understanding Hg bioaccumulation, predicting the potential risk to vertebrates, and evaluating the effectiveness of Hg reductions from locally employed environmental managements.

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Declarations

Conflict of interest The authors declare that they have no actual or potential conflicts of interest that could have influenced the work reported in the manuscript.

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