

Mercury accumulation in bats near hydroelectric reservoirs in Peninsular Malaysia

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Abstract In large man-made reservoirs such as those resulting from hydroelectric dam construction, bacteria transform the relatively harmless inorganic mercury naturally present in soil and the submerged plant matter into toxic methylmercury. Methylmercury then enters food webs and can accumulate in organisms at higher trophic levels. Bats feeding on insects emerging from aquatic systems can show accumulation of mercury consumed through their insect prey. In this study, we investigated whether the concentration of mercury in the fur of insectivorous bat species was significantly higher than that in the fur of frugivorous bat species, sampled near hydroelectric reservoirs in Peninsular Malaysia. Bats were sampled at Temenggor Lake and Kenyir Lake and fur samples from the most abundant genera of the two feeding guilds—insectivorous (*Hipposideros* and *Rhinolophus*) and frugivorous (*Cynopterus* and *Megaerops*) were collected for mercury analysis. We found significantly higher concentrations of total mercury in the fur of

insectivorous bats. Mercury concentrations also differed significantly between insectivorous bats sampled at the two sites, with bats from Kenyir Lake, the younger reservoir, showing higher mercury concentrations, and between the insectivorous genera, with *Hipposideros* bats showing higher mercury concentrations. Ten bats (*H. cf. larvatus*) sampled at Kenyir Lake had mercury concentrations approaching or exceeding 10 mg/kg, which is the threshold at which detrimental effects occur in humans, bats and mice.

Keywords Mercury · *Hipposideros* · *Rhinolophus* · *Megaerops* · *Cynopterus* · Hydroelectric reservoirs

Introduction

Mercury (Hg) contamination has become a well-known global issue (Pacyna et al. 2006; Selin et al. 2007) as the burning of coal, creation of hydroelectric dams, metal mining and municipal waste incineration have increased and augmented the amount of inorganic mercury entering the atmosphere and water sources (Chan et al. 2003). Extensive deforestation and agricultural land use also release mercury from soils creating point sources of local, acute contamination (Barbosa et al. 2003). Lake-sediment records suggest locations distant from point source contamination can also receive significant inputs of anthropogenically released mercury due to transcontinental and global distribution of highly volatile, atmospheric mercury (Fitzgerald et al. 1998; Chan et al. 2003).

In aquatic systems, relatively harmless inorganic mercuric (Hg^{2+}) or mercurous (Hg^+) forms of mercury are naturally present in the substrate, but can be transformed by sulphate-reducing and iron-reducing bacteria to methylmercury

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(MeHg) (Chan et al. 2003; Poulain and Barkay 2013). Significant amounts of mercury can be introduced into aquatic food webs during the flooding of forests (Barbosa et al. 2003), such as during the construction of hydroelectric dams (Bodaly et al. 1984; Stokes and Wren 1987; Ikingura and Akagi 2003). When a reservoir is created, submerged vegetation and organic material start to slowly decompose (Rodgers et al. 1995), leading to a rise in the dissolution rate of organic carbon, increased release of mercury bound to organic material and higher net mercury methylation rates (Chan et al. 2003). A deeper water column and increased decomposition creates anoxic conditions which are ideal for mercury methylation (Hylander et al. 2006). A study of reservoirs up to 67 years old suggested that it may take 20–30 years before mercury concentrations return to pre-dam levels (Hylander et al. 2006).

Methylmercury has been shown to be a potent neurotoxin in humans (Mergler et al. 2007) and other mammals including bats and otters (Basu et al. 2005; Nam et al. 2012). Central nervous system damage caused by methylmercury toxicity in mammals includes motor and sensory deficits and behavioral impairment (Wolfe et al. 1998). Increased levels of methylmercury in vertebrates have been shown to impair reproductive system function (Wada et al. 2010; Nam et al. 2012). Methylmercury is readily transferred across the placenta and can concentrate selectively in the fetal brain, causing developmental alterations leading to fetal death (Wolfe et al. 1998). Infants can also be exposed to methylmercury during lactation (Mergler et al. 2007).

Mercury biomagnifies as it moves up the food chain, with high trophic level species, such as top predators showing higher concentrations of mercury in their tissues than primary consumers, which absorb mercury (Barbosa et al. 2003; Stewart et al. 2008). Insects that have aquatic larval stages could act as biovectors, exporting methylmercury from aquatic systems upon emergence (Benoit et al. 2013; Mogren et al. 2013). The biomass of aquatic insects can reach 190 kg/ha per day in productive lake systems (Mogren et al. 2013).

Most studies of environmental mercury contamination have been conducted in temperate regions (e.g. Baxter 1977; Tweedy et al. 2013), have measured total mercury in fish (e.g. Barbosa et al. 2003), aquatic insects (e.g. Hall et al. 1998; Benoit et al. 2013); or fish-eating birds and mammals (see Chan et al. 2003 and references therein). In Malaysia, studies have examined mercury levels in fish and seafood (e.g. Bloom 1992; Agusa et al. 2005; Hajeb et al. 2009) and in humans living in coastal communities, or in fishing communities near lakes (e.g. Sivalingam and Sani 1980; Hajeb et al. 2008).

Sixty reservoirs have been created as the result of hydroelectric damming over the past 80 years in Malaysia (ICOLD 2014). Twelve more dams, slated for construction

by 2020, have been planned for Malaysian Borneo alone (Herbertson 2013; Thin 2013). Despite the increasing concern regarding mercury contamination in this global biodiversity hotspot, no studies currently exist of methylmercury accumulation in non-human mammals.

One mammalian group showing potential as a model for the study of mercury contamination and bioaccumulation through trophic levels is bats (Chiroptera) (Nam et al. 2012; Yates et al. 2014). Bat assemblages occupy high and low trophic levels, are species rich and abundant, and represent several distinct feeding guilds including frugivorous and insectivorous species (Rojas et al. 2013). Insectivorous bats eat 20–50 % of their pre-feeding body mass in insects every night (Brunet-Rossinni and Austad 2004) including insects with an aquatic larval life stage (e.g. Megaloptera, Trichoptera, certain Diptera, certain Coleoptera, Neuroptera, Ephemeroptera, Odonata) and/or insects without an aquatic larval life stage (e.g. most Lepidoptera, certain Coleoptera) (Bogdanowicz et al. 1999; Fukui et al. 2006). The limited studies of the diet of insectivorous bat species found in Malaysia (*Hipposideros*, *Rhinolophus*) suggest 1–4 % of the insects consumed have an aquatic larval stage (Thabah et al. 2006; Jiang et al. 2008). If mercury is present in aquatic insect prey, there should be accumulation of mercury in the tissues of insectivorous bats. Hair and blood mercury concentration are closely correlated (Yates et al. 2014) and both are accepted as valid biomarkers of methylmercury exposure (US EPA 2001). Hair generally has a 250–300-fold higher mercury concentration than blood (Mergler et al. 2007; Wada et al. 2010) and mercury fixed in the hair at the time of collection is stable and can give a longitudinal history of blood mercury levels (US EPA 2001). To our knowledge no studies have compared mercury concentration in insectivorous and frugivorous bat species and there is only a single unpublished report of Hg concentrations in bats from Malaysia (Yates et al. 2011).

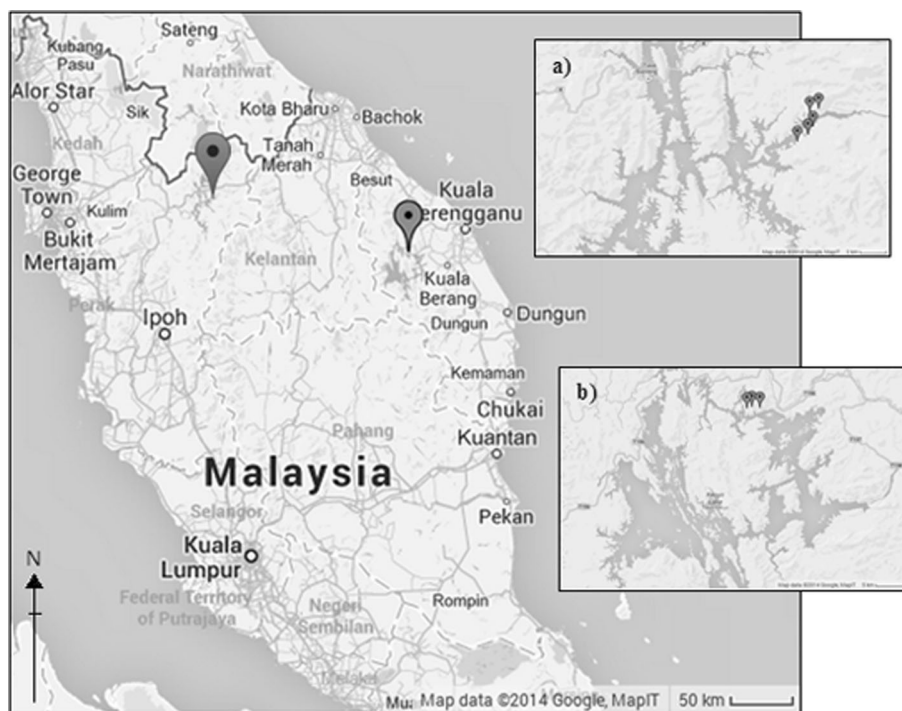
The overall objective of this study was to investigate whether the concentration of total mercury in the fur of insectivorous bat species was significantly higher than that in the fur of frugivorous bat species sampled near reservoirs created by hydroelectric damming in Peninsular Malaysia.

Materials and methods

Study area

Bats were sampled on the shores of two major hydroelectric reservoirs in Peninsular Malaysia: Temenggor Lake, Perak (N05°31', E101°26') and Kenyir Lake, Terengganu (N05°08', E102°46') (Fig. 1) between 16 and 25 July 2013.

Fig. 1 Study sites in Peninsular Malaysia where bat fur was sampled for mercury analysis (2013): **a** Temenggor Lake and **b** Kenyir Lake



Temenggor Lake was created in 1979 and is the second largest man-made lake in Malaysia, covering 15,200 ha (Lin 2006) with an average depth of 127 m and average width of 537 m (Davidson et al. 1995). The reservoir is filled by two major river systems in the north, two in the east and one in the west (Norizam and Ali 2000). The lake is used as a supply for domestic water consumption (Khalik and Abdullah 2012) and is also fished by the local aboriginal community. We sampled bats along the eastern edge of Temenggor Lake (Fig. 1a). Kenyir Lake was created in 1986 and is the largest man-made lake in Malaysia covering 36,900 ha with an average depth of 37 m and a maximum depth of 145 m (Kamaruddin et al. 2011). The lake receives water from two main rivers (Rouf et al. 2010). We sampled bats along the northeastern edge of Kenyir Lake (Fig. 1b).

Capture, sample collection and handling

Bats were captured using 4 four-bank harp traps positioned across flight paths (trails, logging skids or streams) and ten mist nets set near the lake edge. Traps were set at 19:00 until 07:30 and were checked at 30 min intervals with sampling continuing until morning unless it rained. A small wing punch was collected from each captured bat into a 1.5 ml microcentrifuge tube following AMNH (2013). Hair samples were taken from each captured bat by snipping a small amount of hair (0.02 g) from the upper part of the body using stainless steel scissors. Hair was stored in a

1.5 ml microcentrifuge tube. Scissors and forceps were cleaned with alcohol and sterile tissues between bats to avoid cross-contamination. If a bat with a wing punch was captured it was treated as a re-capture (Faure et al. 2009) and not subjected to another wing punch or further hair sampling. Sex and lifestage of the captured bats were recorded. Bats were identified in the field using morphological guides (Kingston et al. 2006; Francis 2008), but given the prevalence of cryptic bat species in Malaysia (Sing et al. 2013; Wilson et al. 2014) species identification was confirmed using DNA barcoding (Francis et al. 2010), following standard methods used in previous studies (see Sing et al. 2013; Wilson et al. 2014).

Mercury analysis

Hair samples from adults of the most abundant genera of the two feeding guilds—insectivorous (*Hipposideros*, *Rhinolophus*) and frugivorous (*Cynopterus*, *Megaerops*) were selected for analysis of total mercury. We measured total mercury concentration in fur which is a standard approach, and is directly proportional to the concentration of methylmercury in the fur (Yates et al. 2014). Total mercury concentration was measured using a Milestone Direct Mercury Analyzer (DMA-80) following US EPA Method 7473 (US EPA 2007). The total mercury detection limit is 0.001 mg/kg.

Quality control included the use of standard reference materials DORM-3 (percentage recovery = 89.2–101.1 %)

and DOLT-4 (percentage recovery = 96.1–106.2 %), running method blanks, sample blanks, and sample duplicates (percentage recovery = 83.5–103.6 %), initially and then every 20 samples. The percentage recovery of spiked material was 92.7 %.

Statistical analysis

Mercury concentration was compared between bats of different genera and species (with singleton species omitted from the test) using one-way ANOVA with a post hoc Tukey HSD test; and between feeding guilds (frugivorous vs. insectivorous), sites (Temenggor Lake vs. Kenyir Lake) and sexes using two-way ANOVA with a post hoc Tukey HSD test. All statistical analyses were performed using JMP 11.1.1 (SAS Institute Inc.).

Results

Forty-one samples (nine frugivorous bats and 32 insectivorous bats) from Temenggor Lake and 87 samples (22 frugivorous bats and 65 insectivorous bats) from Kenyir Lake were analysed for mercury concentration, comprising 12 species (two genera) of insectivorous bats and three species (two genera) of frugivorous bats. Note that bat species in Malaysia are often “dark taxa”, species which have been recognized and recorded previously but which have not yet been formally described (Sing et al. 2013; Wilson et al. 2014), so a few of the species are referred to using non-Linnaean species names.

Insectivorous genera (5.13 ± 3.10 SD mg/kg) had significantly higher concentrations of mercury than frugivorous genera (0.02 ± 0.01 SD mg/kg) ($F(3,124) = 48.64$, $p < 0.0001$). The post hoc Tukey HSD test indicated that the genera *Hipposideros* (6.26 ± 2.98 SD mg/kg) and *Rhinolophus* (3.14 ± 2.22 SD mg/kg) had significantly higher concentrations of mercury than the two genera, *Megaerops* (0.023 ± 0.009 SD mg/kg) and *Cynopterus* (0.013 ± 0.006 SD mg/kg) (Fig. 2).

Hipposideros cf. larvatus (7.136 ± 2.546 SD mg/kg) and *Rhinolophus chiewkweeae* (7.393 ± 1.793 SD mg/kg) both had significantly higher mercury concentrations than the other insectivorous species (with singleton species omitted). There were no significant differences in mercury concentration among the frugivorous bat species ($(F(7,113) = 40.29$, $p < 0.0001$); Table 1).

Mercury concentrations in insectivorous bats at Kenyir were significantly higher than insectivorous bats at Temenggor ($F(1,124) = 10.41$, $p = 0.0016$). Grouped separately by site, mercury concentrations in insectivorous bats were significantly higher than frugivorous bats at both

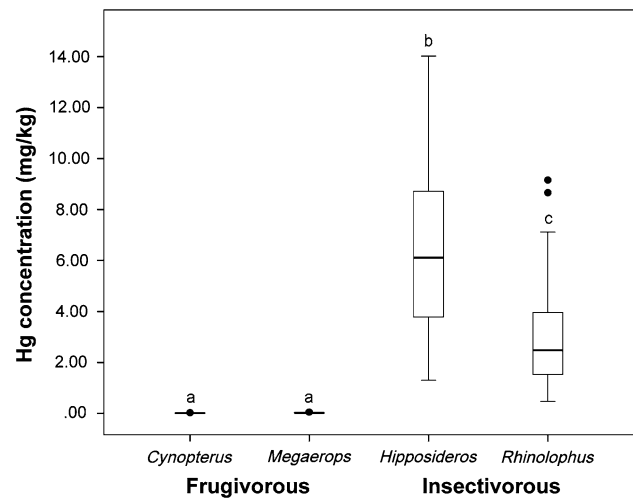


Fig. 2 Mean mercury concentration (mg/kg) in fur from bats of different feeding guilds (Frugivorous or Insectivorous) grouped by genus with standard deviation bars. Different letters above the bars indicate significant differences between the means based on post hoc Tukey HSD test. Black circles are outliers

sites. The interaction between guild and site was significant ($F(1,124) = 10.50$, $p = 0.0015$) (Fig. 3).

Comparison of mercury concentrations between sex was not significant ($F(1,124) = 0.0006$, $p = 0.9810$). On average, females exhibited slightly lower mercury concentrations (3.412 ± 3.669 SD mg/kg) than males (4.347 ± 3.261 SD mg/kg) (Fig. 4).

Discussion

We found that mercury concentration was significantly higher in the hair of insectivorous bats than mercury concentration in the hair of frugivorous bats sampled at two hydroelectric lakes in Peninsular Malaysia. This suggests that insectivorous bats could be accumulating methylmercury through their diet. The diet of insectivorous bats includes emergent aquatic insects (Fukui et al. 2006) that are, plausibly, contaminated with mercury from the lakes (Tremblay and Lucotte 1997; Tweedy et al. 2013). Other studies have demonstrated that aquatic insects can act as biovectors transferring sedimentary mercury from lakes into terrestrial predators on the shoreline (Haro et al. 2013; Tweedy et al. 2013). Interestingly, Reidinger (1972) argued that mercury contamination in bats probably occurred from their free water drinking source rather than through their insect prey, however, this idea has largely been abandoned (Yates et al. 2014).

Of the two insectivorous genera analyzed for mercury concentration, *Hipposideros* made up the largest proportion

Table 1 Total mercury concentrations in fur (mg/kg) for bat species sampled near Temenggor Lake and Kenyir Lake, Peninsular Malaysia

| Guild | Genus | Species | <i>n</i> | Mean/ value | Standard deviation | Statistical significance ^a | |
|------------------|----------------------|------------------------|-------------------|----------------|-----------------------|--|---|
| Frugivorous | <i>Cynopterus</i> | <i>C. horsfieldii</i> | 10 | 0.012 | 0.004 | a | |
| | | <i>C. JLE sp. A</i> | 7 | 0.015 | 0.007 | a | |
| | <i>Megaerops</i> | <i>M. ecaudatus</i> | 14 | 0.023 | 0.009 | a | |
| Insectivorous | <i>Hipposideros</i> | <i>H. cf. bicolor</i> | 11 | 2.293 | 0.856 | a, b | |
| | | <i>H. cf. larvatus</i> | 47 | 7.136 | 2.546 | d | |
| | | <i>H. cervinus</i> | 1 | 8.988 | | | |
| | | <i>H. diadema</i> | 1 | 3.789 | | | |
| | | <i>H. doriae</i> | 1 | 5.135 | | | |
| | | <i>H. dyacorum</i> | 1 | 9.525 | | | |
| | | <i>Rhinolophus</i> | <i>R. affinis</i> | 23 | 2.686 | 1.985 | b |
| | | <i>R. chiewkweeae</i> | 2 | 7.393 | 1.793 | c, d | |
| | <i>R. trifolius</i> | 7 | 3.969 | 1.987 | b, c | | |
| | <i>R. acuminatus</i> | 1 | 0.627 | | | | |
| | <i>R. lepidus</i> | 1 | 1.760 | | | | |
| <i>R. luctus</i> | 1 | 3.132 | | | | | |

^a Species that share common letters do not differ significantly. Singleton species were omitted from the post hoc Tukey HSD test

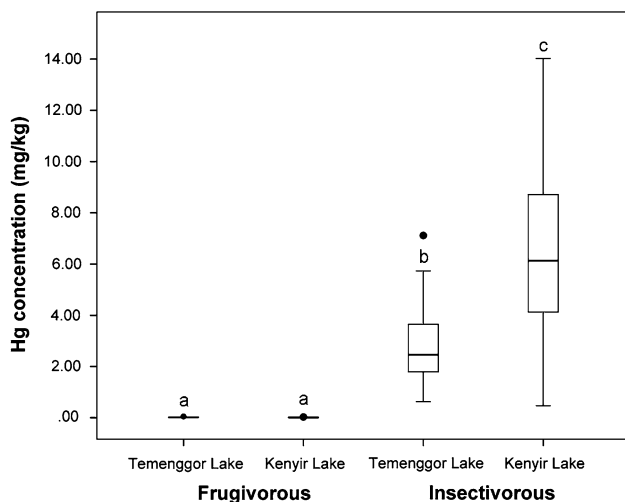


Fig. 3 Mean mercury concentration (mg/kg) in fur from bats of different feeding guilds (Frugivorous or Insectivorous) grouped by study site (Temenggor Lake or Kenyir Lake) with standard deviation bars. Different letters above the bars indicate significant differences between the means based on post hoc Tukey HSD test. Black circles are outliers

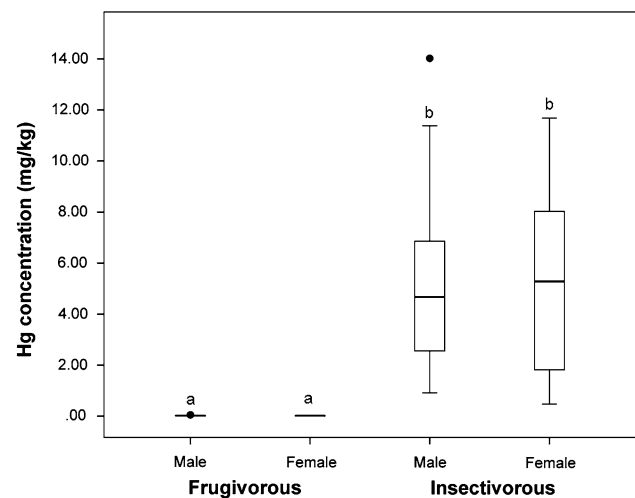


Fig. 4 Mean mercury concentration (mg/kg) in fur from bats of different feeding guilds (Frugivorous or Insectivorous) grouped by sex with standard deviation bars. Different letters above the bars indicate significant differences between the means based on post hoc Tukey HSD test. Black circles are outliers

of analyzed samples (37 %) and showed significantly higher mercury concentrations than the *Rhinolophus* bats. This could be due to a larger proportion of aquatic insects in the *Hipposideros* diet; however, records on the diet of bat species in Malaysia are limited. One study conducted in various secondary forests in Malaysia reported that 17 % of the diet of *H. larvatus s.l.* consisted of Coleoptera with the rest comprising unidentifiable insect fragments (Muda

1991). Thabah et al. (2006) reported that the diet of *H. larvatus s.l.* collected from 11 sites in the Indo-Malayan region (India, China, Myanmar, Malaysia) comprised more than 80 % coleopterans. Alternatively, the diet of *Rhinolophus affinis* from a cave in Jiangxi Province, China contained more than 50 % of Lepidoptera (Jiang et al. 2008). While the diet of insectivorous bats is likely to be opportunistic, relying on the presence and density of prey species in the bats particular foraging area, this could suggest a

larger proportion of aquatic insect species in the diet of *Hipposideros* compared to *Rhinolophus* bats, and may explain the increased exposure to mercury contamination of *Hipposideros* bats in our study areas. It is also a possibility that *Hipposideros* bats could be foraging more frequently and cover a larger area including over water bodies, but no studies have documented this yet. The use of next generation DNA sequencing (NGS) to establish the taxonomic identity of prey fragments—“DNA metabarcoding”—in feces could help resolve this question (Razgour et al. 2011). Both the frugivorous bat genera sampled, *Cynopterus* and *Megaerops*, showed significantly lower concentrations of mercury (~99 % lower than the insectivorous genera). We would expect the frugivorous bats to exhibit low concentrations of mercury because their diet likely contains little mercury. Plant roots absorb small amounts of mercury from soils and the mercury is not directly translocated from root tissues to the tissues at the top of plants (Patra and Sharma 2000) where the bats are feeding.

The comparison between the mercury concentrations in insectivorous bats collected at the two lakes showed a significantly higher concentration of mercury in bats sampled at Kenyir Lake. There was no known point source or intense agricultural activities near our study area at the lake. Kenyir Lake is shallower than Temenggor Lake, based on average depth, allowing rapid erosion of soils which increases bioavailability of mercury-rich particles to filter feeding invertebrates (Lucotte et al. 1999). Limitations on methylmercury production in Temenggor Lake might include low total mercury concentrations in the flooded soils and sediments and rapid oxidation and decay of organic matter leading to low total organic carbon in the reservoir (Ikingura and Akagi 2003). Our study area in Temenggor Lake can be considered pristine without human encroachment except for small-scale fishing and collecting of forest resources by aborigines. Ikingura and Akagi (2003) reported it was a common phenomenon for fish mercury concentration to be negatively correlated with age of reservoirs even with a difference of only 5 years. Therefore, the age of the reservoirs could potentially be a partial explanation for the differences in mercury concentration in bats from the two lakes in our study, as Kenyir Lake is 7 years younger than Temenggor Lake.

We found no significant variation in mercury concentration among sexes within each feeding guild. Similarly, no significant difference in mercury concentration between sexes was observed for both adult and juvenile bats from Oneida Lake, New York, USA (Yates et al. 2014) and Southwest England (Walker et al. 2007). Mercury contamination would not be expected to vary significantly between males and females of the same species as they live in colonies and most likely have a very similar diet.

Comparing mercury concentration in bats on a global scale, mean mercury concentrations in the fur of *Myotis lucifugus*, *M. septentrionalis*, *M. leibii* and *M. grisescens* from non point source sites in Quebec exceeded the threshold for mercury concentration in hair (10 mg/kg) (Hickey et al. 2001) at which detrimental effects occur in humans (Murata et al. 1999) and neurobehavioral disorders occurred in rodents (Burton et al. 1977). Mercury concentrations in fur from bats at point source sites in North America have been reported as 28–132 mg/kg (Wada et al. 2010; Nam et al. 2012; Yates et al. 2014) 5–30 times higher than the values for insectivorous bats in the present study. However our values are similar to the mean mercury concentration in fur from bats at 69 non-point source sites in North America (6.44 mg/kg) (Yates et al. 2014).

This is the first study comparing mercury concentrations in frugivorous and insectivorous bats at hydroelectric reservoirs. Fur from ten bats (*H. cf. larvatus*) sampled at Kenyir Lake had mercury concentrations approaching or exceeding 10 mg/kg which is the threshold at which harmful effects occur in mammals (Murata et al. 1999; Burton et al. 1977). Insectivorous bats consuming large numbers of prey emerging from new reservoirs could be exposed to increased, and potentially harmful, levels of mercury as has been shown previously in insectivorous songbirds (Gerrard and St. Louis 2001). A reduction in bat populations due to neurological problems as a result of mercury toxicity could have serious consequences for the local ecosystem: insectivorous bats are important for controlling insect populations and for nutrient recycling (Jones et al. 2009). Malaysia has created 60 reservoirs as a consequence of hydroelectric damming since 1920; however, the ecological consequences of hydroelectric damming have never received serious consideration. Likewise, many other countries have embraced hydroelectricity as a renewable energy resource resulting in the creation of thousands of reservoirs around the world (Barros et al. 2011).

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Conflict of interest The authors declare that they have no conflict of interest.

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