Bulletin of Environmental Contamination and Toxicology (2021) 107:801–808
https://doi.org/10.1007/s00128-021-03274-9

FOCUSED REVIEW

A Review of Freshwater Invertebrates as Biomonitors of Methylmercury: the Importance of More Complete Physical and Chemical Reporting

Beatriz Malcata Martins[1] · **Nelson J. O'Driscoll**[2] · **Mark L. Mallory**[3] · **João Canário**[1]

Received: 17 February 2021 / Accepted: 20 May 2021 / Published online: 3 June 2021

Abstract

Methylmercury (MeHg) is a toxic and bioaccumulative organo-metallic compound that is naturally produced in many ecosystems. Organisms that occupy the lower trophic positions in food webs may be key factors in the assessment of MeHg biomagnification between ecosystems. Here we present a review of the peer-reviewed literature examining MeHg bioaccumulation in freshwater invertebrates, focused principally on insects. This review aims to characterize the invertebrates that bioaccumulate higher MeHg concentrations and therefore pose a higher risk to upper trophic levels and to clarify which ecosystems are more susceptible to bioaccumulation in lower trophic levels. However, we found that few studies provided robust environmental data (notably water chemistry) as part of their papers, dramatically limiting our ability to test for factors that might contribute to different concentrations of MeHg in invertebrates. We highlight the importance of providing physical and chemical characteristics of study sites in publications examining MeHg bioaccumulation and biomagnification. Adopting the proposed recommendations will improve the available information for future mercury risk assessment analyses.

Keywords Mercury · Methylmercury · Bioaccumulation · Biomagnification · Invertebrates · Risk assessment

Methylmercury (MeHg) is a toxic pollutant that is produced naturally in aquatic and terrestrial ecosystems such that it can be found in remote areas and at concentrations above background due to the atmospheric global dispersion of elemental mercury (Hg(0)) (Driscoll et al. 2013), which concentration is estimated to be 15 times higher than pre-industrial values (Amos et al. 2015). The methylation of divalent mercury (Hg(II)) is primarily a microbial process that produces MeHg, a neurotoxin with the capacity to bioaccumulate and biomagnify (Sakamoto et al. 2011; Lavoie et al. 2013). As a result, it can reach high concentrations in upper trophic levels. Concentrations of MeHg in a food web are affected by abiotic conditions that influence Hg(II) methylation and bioavailability, such as nutrients, dissolved organic matter (DOM) and pH (Paranjape and Hall 2017).

The input of nitrogen (N) and phosphorous (P) nutrients into an aquatic system may affect mercury (Hg) methylation (MacMillan et al. 2015; Kickbush et al. 2018). Nutrient sources to ecosystems may be anthropogenic (e.g. sewage and fertilizer) or natural sources such as biovectors (e.g. bird colonies nesting in an area) which may deposit guano containing previously accumulated contaminants such as N, P and Hg (Blais et al. 2007; Mallory et al. 2015). Alternatively, the over-enrichment of N and P in aquatic ecosystems can cause algal blooms that reduce the Hg concentration at the base of the food web and result in lower Hg bioaccumulation in consumers (Pickhardt et al. 2002). The role of DOM in Hg speciation is multifaceted and includes Hg complexation and sensitization of Hg photochemical processes (Klapstein and O'Driscoll 2018). Its effects are also influenced by the pH of the aquatic systems which may reduce the binding of Hg to functional groups on DOM with acidic conditions and in turn may influence Hg(II) methylation (Ravichandran 2004). The apparent favoring of methylation in ecosystems with low pH (Ullrich et al. 2001) may partially explain the inverse

✉ Beatriz Malcata Martins
beatriz.martins@tecnico.ulisboa.pt

[1] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisboa, Portugal

[2] Department of Earth and Environmental Science, K.C. Irving Environmental Science Center, Acadia University, Wolfville, NS B4P 2R6, Canada

[3] Department of Biology, Acadia University, Wolfville, NS B4P 2R6, Canada

correlation between pH and MeHg in biota observed in some studies (Allen et al. 2005; Chételat et al. 2011).

Methylmercury bioaccumulation and biomagnification are also affected by the ecological characteristics of the organisms and their relationships in the food web (Kidd et al. 2011). Studies show that MeHg concentration is influenced by life stage (higher concentrations in adults; Chételat et al. 2008), and diet or trophic position, with MeHg concentrations increasing with higher trophic level (Tremblay et al. 1996a). However, the ecology can be more relevant to the MeHg concentration of the organisms than its trophic position. An example is polychaete worms that, although considered a primary consumer, have high levels of MeHg because they create oxygenated burrows walls in anoxic sediments that accumulate DOM, leading to higher MeHg bioaccumulation (Sizmur et al. 2013).

Stable isotopes of δ^{15}N and δ^{13}C are commonly used by researchers to link organism diet and trophic position with its MeHg concentration (e.g. Cremona et al. 2009; Chételat et al. 2020). Due to the preferential retention of ^{15}N and excretion of ^{14}N in most organisms, as trophic position increases δ^{15}N values also tend to increase (Fry 2006). Since MeHg can biomagnify, the linear relationship between δ^{15}N and MeHg is used as a quantitative indicator of average food web biomagnification (Lavoie et al. 2013). At the same time, δ^{13}C can potentially assess the source of dietary carbon and its evolution through the food web (Fry 2006).

Recently, Chételat et al. (2020) conducted a thorough review of factors influencing MeHg exposure and magnification in wildlife, which builds on earlier reviews of MeHg accumulation in food webs (Peakall and Burger 2003; Wiener et al. 2003; Evers 2018). Most of these reviews noted ecological or physiological processes that influenced MeHg, rather than environmental characteristics. However, our focused review addresses a specific research gap related to Hg bioaccumulation in lower trophic levels of the food web, more precisely, freshwater invertebrates. These organisms are important food sources for higher trophic level organisms such as birds and fish and may be important biovectors of contaminants, such as MeHg, from aquatic to terrestrial ecosystems (Paterson et al. 2006; Sullivan and Rodewald 2012). This review focuses on what type of invertebrates are key vectors of MeHg in food webs, which aquatic ecosystems are susceptible to bioaccumulation in invertebrates, and which physical and chemical descriptors most influence bioaccumulation in invertebrates. We hypothesized that, across studies, MeHg would be higher in invertebrates occupying higher trophic positions, and from waterbodies with higher acidity.

Methodology

We performed a search of key databases (e.g. Google Scholar and Web of Science) for relevant articles using keywords such as “mercury”, “methylmercury”, “bioaccumulation”, “biomagnification, “invertebrate” and “freshwater”. The selection criteria included only studies that provided the values of MeHg in freshwater invertebrates. Collectively, we extracted data from 23 papers that were found to provide information on mean values of MeHg, total mercury (THg), percentage of total mercury in the form of MeHg (%MeHg), stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) in invertebrates, as well as characteristics of waterbodies where the invertebrates were collected (pH, total organic carbon [TOC], total nitrogen [TN]) (publications selected are listed in SI Table S1). However, across the 23 studies, the availability of supporting physical/chemical information on waterbodies (i.e., environmental factors that could influence Hg exposure in invertebrate biota such as pH, TOC, DOC and P and N concentration) varied dramatically from single to multiple variables. To maximize the number of studies used, we selected studies with waterbody pH and organism δ^{15}N, as these have been shown previously to be important parameters influencing MeHg content (Clayden et al. 2014) and six articles provided this information. Almost all the research gathered was from North America, mainly from Canada.

Using data extracted from the papers, we examined the general pattern of MeHg bioaccumulation in the dry tissue of freshwater invertebrates. We compared MeHg in similar taxa of invertebrates from lakes and wetlands using Mann–Whitney *U* tests. These two ecosystems were selected based on the data available of the invertebrates chosen for the analysis, that were well-represented across studies. We log-transformed MeHg concentrations from invertebrates, and then ran a stepwise multiple regression to examine the potential influence of trophic position (as assessed by δ^{15}N) and pH on MeHg concentrations in invertebrates, with MeHg as the response variable and δ^{15}N and pH as independent factors. All analyses were conducted with SPSS Statistics, version 26.

Results and Discussion

Patterns of MeHg Concentration in Invertebrates

Across studies, palaemonids (Palaemonidae, grass shrimps), nepids (Nepidae, water scorpions) and crayfish (Decapoda, crayfish) had the highest reported MeHg

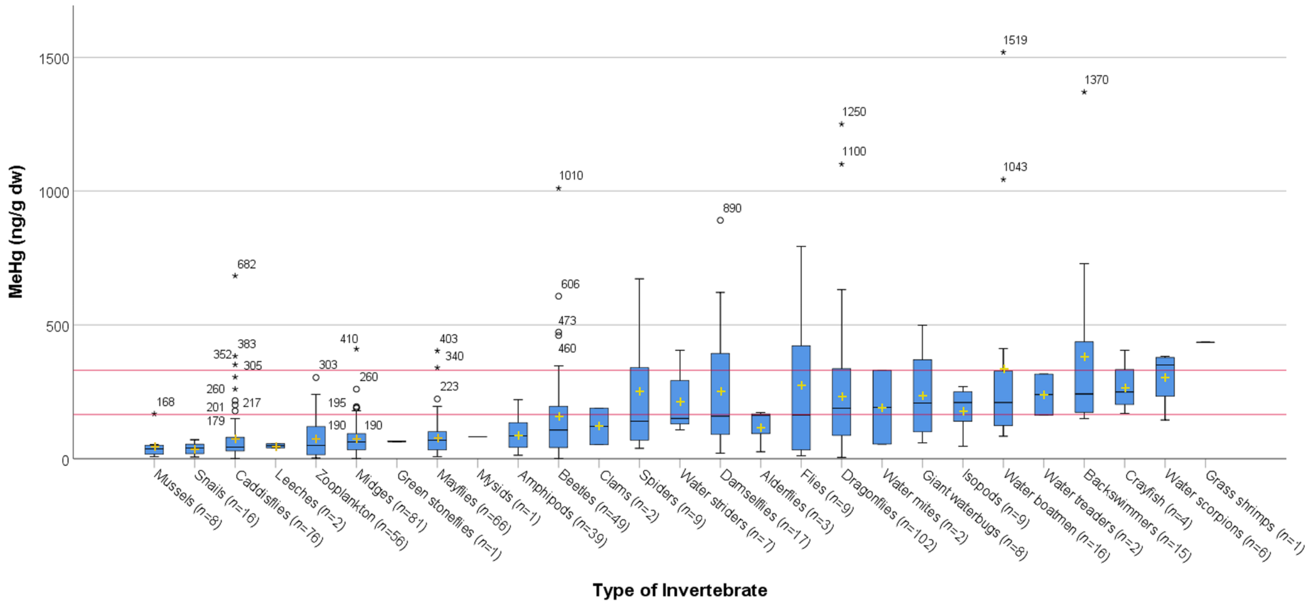

Fig. 1 Boxplots of MeHg concentration in the dry tissue of invertebrates separated by common name. The boxplots are ordered from lowest to highest median value. In each boxplot the median is represented with a black bar, the mean with a yellow plus sign, the outliers marked with circles and extreme outliers marked with asterisks. The former includes the values outside the range of the third/first quartile plus/minus 1.5 times the interquartile range (IQR) and the latter the ones outside the range of the third/first quartile plus/minus 3 times the IQR. The red lines represent the Canadian methylmercury tissue residue guideline for the protection of wildlife consumers of aquatic biota (CCME 2000), which is 33 µg/kg in wet weight (converted to dry weight values for 80% and 90% water content of invertebrates (Hall et al. 1998; Lavoie et al. 2010), which leads to an interval between 165 ng/g dw and 330 ng/g dw). The number of individual data points used for each boxplot (*n*) is also displayed. The data plotted is from publications listed in SI Table S1

(Fig. 1; range of medians and means 249–435 ng/g dw), although the number of individual data points of each type of invertebrate (referred to as "*n*") varied considerably among groups of invertebrates. Most of these taxa are predatory (Thorp and Rogers 2011), so their high MeHg concentration is expected due to MeHg capacity for biomagnification in food webs (Lavoie et al. 2013). Corixids (Corixidae, water boatmen) had unexpectedly high MeHg (median 210 ng/g dw, mean 340 ng/g dw), given that many are non-predatory, or feed on low trophic level organisms (Thorp and Rogers 2011) and all other invertebrates in high positions in Fig. 1 are commonly predators. Additionally, corixids also showed high variation in mean values of MeHg, with a coefficient of variation (CV) of 115% (n = 16). This is likely attributable to the variability in ecologies and diet of species within this group, including predation and scavenging (Hilsenhoff 2001; Hädicke et al. 2017).

Predatory invertebrates also showed the highest percentage of THg that was presented as MeHg, as expected (Riva-Murray et al. 2020). Specifically, notonectids (Notonectidae, backswimmers), mesoveliids (Mesoveliidae, water treaders) and nepids (median and mean ≥ 93%; Fig. 2) presented the highest values of %MeHg, although the number of studies that reported both THg and MeHg was low (n ≤ 5 for any group). Notonectids not only showed a high mean and median value of %MeHg but also a low variation between the values collected (CV = 4.1%). This could potentially indicate that the MeHg concentration could be estimated by measuring THg, which is a simpler and less expensive laboratory analysis.

In contrast, the greatest variation in mean values of MeHg was registered in a primary consumer, the trichopterans (Trichoptera, caddisflies). These invertebrates showed a CV of 134% (n = 76) that could be related to the susceptibility to the concentration of MeHg in the sediments and variability in species ecology. Clarke (2018) found that MeHg was related to Hg in sediments, which could vary greatly for trichopterans because some families build their protective cases from materials often on the waterbody benthos. Also, biosynthesized silk is used in this process which can be important to remove Hg(II) from their body (Clarke 2018).

Additionally, consumers showed high variation in %MeHg, specifically mussels (Unionidae, mussels; CV = 92%, n = 3), chironomids (Chironomidae, midges; 80%, n = 21) and ephemeropterans (Ephemeroptera, mayflies; 58%, n = 31). Riva-Murray et al. (2020) suggested that the %MeHg of aquatic primary consumers is correlated with the aqueous MeHg concentration, that is significantly influenced by the environmental conditions (Paranjape and Hall

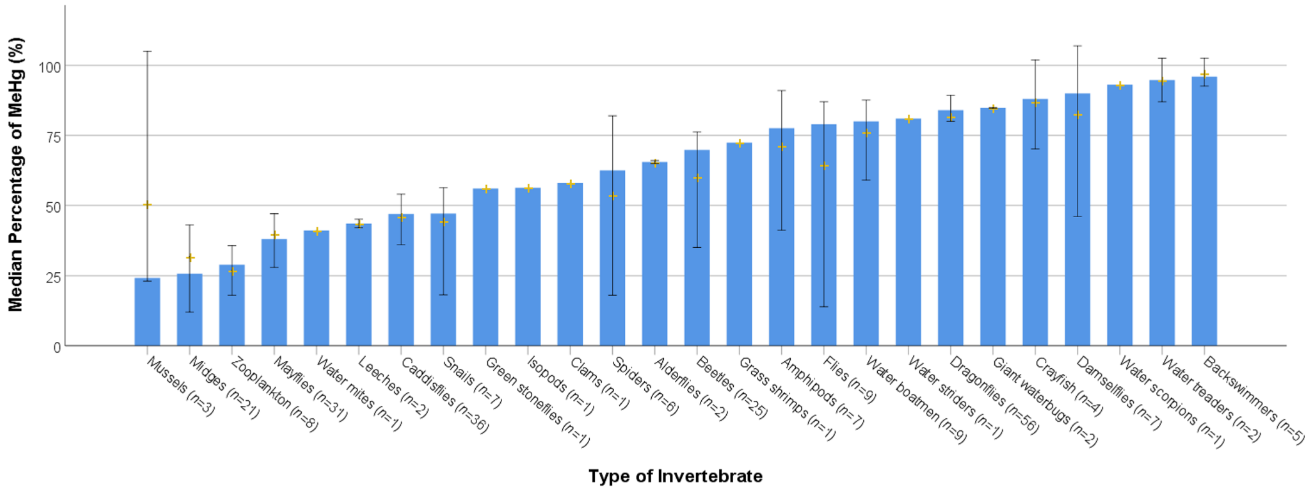

Fig. 2 Barplots of median percentage MeHg relative to total mercury concentration in the dry tissues of the invertebrates. The invertebrates are separated by common name and ordered from lowest to highest median value. The mean values are represented with a yellow plus sign and the error bars with confidence level of 95% are also plotted when applicable. The number of individual data points used for each boxplot (*n*) is also displayed. The data plotted is from publications listed in SI Table S1

2017). Thus, the high susceptibility of primary consumers to bioavailable MeHg concentration in the surrounding environment may explain their high variability.

From the 23 studies that reported the MeHg concentration in freshwater invertebrates, it was clear that many individual invertebrates had MeHg concentrations that exceeded the Canadian guidelines for the protection of wildlife consumers of aquatic biotas (CCME 2000; 33 ng/g ww or 330 ng/g dw assuming 90% of water content). The maximum values reported were 1519 ng/g MeHg in a corixid (Tremblay et al. 1996b), and 1370 ng/g MeHg in a notonectid (Sinclair et al. 2012), approximately the same as the maximum of 1411 ng/g reported in anisopterans (Anisoptera, dragonflies) by Eagles-Smith et al. (2020). Clearly in some systems, certain invertebrates are capable of acquiring quite high MeHg concentrations, although mean values are ~ 60% lower (Fig. 1). We noted that the majority of high values for individual invertebrates in Fig. 1 were from studies in wetlands and reservoirs, which could potently suggest a greater risk to top predators in these habitats. Jackson et al. (2015) assessed THg concentrations in songbirds inhabiting eastern North America and concluded that invertebrate-eating species had significantly higher concentrations when compared to omnivores, especially the ones that inhabited wetland habitats.

We note that the high variability in MeHg concentration and %MeHg we report in some of the invertebrates could be a consequence of the method by which we grouped organisms. Some groups may be at a higher taxonomic level and inherently include greater diversity of ecologies and life stages. But even the groups of invertebrates that only include one family showed high variability, which is the case of the corixids. Analysing patterns at lower taxonomic levels may be preferable in some experimental designs to reduce the masking of species-specific values, but the identification of invertebrate species requires substantial time and expertise that may not be available in many contaminant studies. Gerwing et al. (2020) showed that in ecological studies there was no significant difference in the conclusions when identifying organisms by species or by family in coastal ecosystems. Nevertheless, our review suggests that, for contamination studies, certain individual species' behaviors may create outliers in the bioaccumulation data. In these cases, the identification by species could provide useful information.

Comparison Between Wetlands and Lakes

To compare MeHg bioaccumulation and biomagnification in wetlands and lakes, we chose a smaller set of data containing information on only trichopterans, ephemeropterans, anisopterans and zygopterans (Zygoptera, damselflies; Fig. 3). These groups were chosen because they provided the most data across the greatest number of studies, are easy to collect, and anisopterans and zygopterans have recently been identified in particular as important biomonitor species in aquatic and terrestrial systems (Buckland-Nicks et al. 2014; Eagles-Smith et al. 2020).

Ideally, biomonitor species should cover a wide range of mean MeHg concentrations and show a wide variation in MeHg between ecosystems in response to physical and chemical differences. In lakes, trichopterans had the lowest median value of MeHg (40.0 ng/g dw, mean 54.3 ng/g dw) but exhibited the highest variation among mean values (CV = 94%). In contrast, anisopterans had the highest median MeHg (122.0 ng/g dw, mean 177.5 ng/g dw) yet

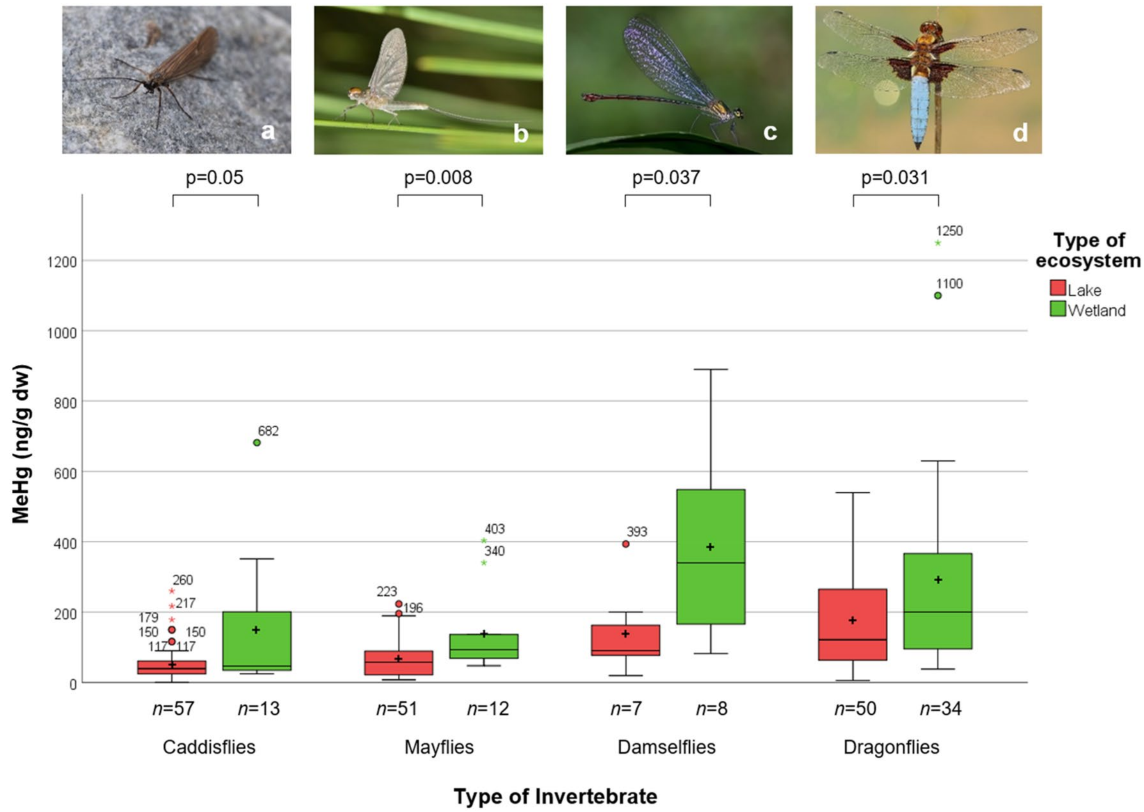

Fig. 3 Boxplots of mean values of MeHg extracted from the literature of caddisflies, mayflies, dragonflies, and damselflies separated by type of invertebrate in lakes and wetlands. In each boxplot the median is represented with a black bar, the outliers marked with circles and extreme outliers marked with asterisks. The former includes the values outside the range of the third/first quartile plus/minus 1.5 times the interquartile range (IQR) and the latter the ones outside the range of the third/first quartile plus/minus 3 times the IQR. The mean value is marked with a plus sign in each boxplot. The invertebrates are sorted from lowest to highest median value in each type of ecosystem. The number of individual data points used for each boxplot (*n*) is also displayed. The p value from Mann–Whitney *U* tests is provided. On top, pictures of the invertebrates used in the analysis: **a** caddisfly **b** mayfly **c** damselfly **d** dragonfly (all photographs from pixabay.com)

lowest variation (76%) among values. In wetlands, trichopterans had the lowest median value, 47.7 ng/g dw (mean 150 ng/g dw) and also the greatest variation in values (129%), as with lake studies. Wetland zygopterans had the highest median MeHg (340.0 ng/g dw, mean 385.3 ng/g dw) and lowest variation between values (71%).

For all four groups of invertebrates, MeHg concentrations were significantly higher in wetlands than in lakes (Fig. 3; Mann–Whitney *U* tests, all $p \leq 0.05$). Several studies have concluded that wetlands are hotspots for Hg methylation due to their high content of dissolved organic matter, anoxia, and low pH (Galloway and Branfireun 2004; Hall et al. 2008). For example, Eagles-Smith et al. (2020) found that THg averaged 35% higher in anisopterans from waterbodies with abundant wetland borders than those without wetlands. The study by Edmonds et al (2012), used in the analysis, also shows a negative correlation between the concentration of dissolved oxygen and pH in wetland ecosystems with the concentration of MeHg in biota, specifically in rusty blackbird (*Euphagus carolinus*) and its invertebrate prey.

While these species appear to be important Hg bioindicators, we observed that there is an underrepresentation of wetland invertebrates in studies when compared to lakes (14 studies in lakes and only 3 in wetlands in this analysis). Even with these limited data, they suggest that wetlands pose a higher risk to upper trophic level organisms due to foraging on invertebrates with relatively elevated content of MeHg.

Combined Influence of Physical and Chemical Variables Predicting MeHg Bioaccumulation

Bioaccumulation and biomagnification of MeHg is significantly affected by the physical and chemical characteristics of the ecosystem (Eagles-Smith et al. 2018). Using data from all invertebrates, log-MeHg was predicted by waterbody pH and $\delta^{15}N$ of the organism ($F_{2,169} = 29.84$, $p < 0.001$) but explained only 25% of the variation in MeHg. Consistent with expectations, MeHg was higher in invertebrates from acidic (lower pH) waterbodies ($\beta = -0.541$, $p < 0.001$) and higher for organisms at higher trophic levels

($\beta = 0.238$, $p = 0.001$). We followed this analysis with a second regression restricted to the four invertebrate groups in Fig. 3, which allowed us to include aqueous total organic carbon (TOC) as a potential predictor. However, TOC was not retained in the stepwise regression analysis, which predicted log-MeHg ($F_{2,62} = 51.0$, $p < 0.001$) from pH ($\beta = -0.691$, $p < 0.001$) and $\delta^{15}N$ ($\beta = 0.770$, $p < 0.001$) and explained 62% of the variation. Collectively, these results across studies align with other publications concluding that MeHg is higher in organisms from more acidic systems (Yu et al. 2011; Edmonds et al. 2012) and organisms in higher trophic positions (Clayden et al. 2014). Curiously, TOC was removed as a significant predictor of MeHg, even though studies have demonstrated that MeHg is higher in waterbodies with greater organic carbon content (Hall et al. 2005; Braaten et al. 2014). Dissolved organic matter can have several effects on Hg speciation and bioavailability that depend not only on its concentration but also its composition (Ravichandran 2004; Bravo et al. 2017) and research shows some contradictory results regarding its effects (Jiang et al. 2018). Additionally, the selection of TOC as the parameter to account for changes in organic matter, and not dissolved organic carbon (DOC; which was reported in too few studies), may also have influenced our results. The TOC content includes dissolved and particulate organic carbon, and high TOC concentration usually, but not always, relates to higher DOC. With relatively few studies in our sample, using TOC may have masked a correlation between DOC and MeHg concentration in invertebrates. Specifically, only six studies that provided MeHg concentrations in the invertebrates also provided $\delta^{15}N$ and the pH of the collection site which decreased to three studies when adding TOC to the analysis.

Recommendations for Reporting and Application to Risk Assessment

In 2017, the Minamata Convention entered into force and the Parties agreed to address the anthropogenic emissions of Hg, notably atmospheric emissions (You 2015) which has a separate article in the Convention report (UNEP 2019). In comparison to atmospheric Hg, direct Hg releases in aquatic systems have been understudied (Kocman et al. 2017), which may explain the discrepancy in Hg trends in environmental compartments. Although there has been a decline in atmospheric Hg over the past years (Zhang et al. 2016), this trend is not mirrored in aquatic biota, due to processes that affect Hg speciation, bioavailability and uptake that occur specifically in these ecosystems (Wang et al. 2019).

Consequently, Hg risk assessments may benefit from using monitoring data of Hg concentrations in aquatic invertebrates from field studies. Certain families of invertebrates are distributed globally and inhabit several types of ecosystems. Furthermore, the sampling of these organisms may be simpler than sampling upper trophic level organisms like fish or birds; fewer permits and simple equipment may be all that is required, and it can be facilitated through citizen science collections (e.g. Eagles-Smith et al. 2020). However, additional data are required to refine what invertebrates may be most suitable (e.g. anisopterans; Eagles-Smith et al. 2020), and how their monitoring may or may not be more suitable than other biota. For example, examining the bioconcentration of MeHg from the water column to plankton can be a good predictor of the vulnerability of the ecosystems to MeHg biomagnification (Wu et al. 2019), and certainly upper trophic level organisms may be a better indicator of Hg in certain ecosystems (Bodaly et al. 1993; Evers et al. 1998; Harris et al. 2007). Nevertheless, with their diversity of habitat use and ecologies, invertebrates are a key linkage in food webs and could help identify specific pathways important for biomagnification in ecosystems.

Consequently, using invertebrates as biomonitors could be a good way to track Hg in aquatic environments. However, as we have shown, we lack a more fulsome set of the physical and chemical parameters of the study sites to maximize their utility. For example, for our comparisons above, we found data from 6 studies that provided data on MeHg in invertebrates in addition to pH and $\delta^{15}N$ which decreased to 3 studies when adding TOC to the analysis (SI Table S1). We suspect that this limited sample precluded our ability to robustly test for the effects of TOC, and we could not find sufficient data in studies to test for other factors like nutrients (e.g. phosphorus), recently shown to influence MeHg concentrations in wetland water (Kickbush et al. 2018).

Based on this review, we recommend that researchers follow some best practices for the analysis of bioaccumulation and biomagnification of Hg in invertebrate food webs. In particular, for presenting data on aquatic invertebrate Hg in manuscripts, we recommend that researchers also provide robust water chemistry parameters (pH, total or dissolved organic carbon, dissolved oxygen, phosphorus, waterbody area, and other cations and anions). The publication of these datasets with the paper text would be very helpful for future focused reviews. Furthermore, we also recommend that researchers identify invertebrates to the lowest taxonomic level possible, preferably genus or species, which would better allow for discrimination between different organismal ecologies. Following these recommendations will provide the data needed for future insights of physical and chemical relationships with invertebrate Hg concentrations, and our ability to maximize their use as risk indicators, to provide stronger and more robust information to initiatives like the UN Global Mercury Assessment, the European Union Regulation 2017/852, the Canadian Mercury Science Assessment or the Minamata Convention on Mercury.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00128-021-03274-9.

Acknowledgements We gratefully acknowledge funding by the Portuguese Foundation for Science and Technology (FCT) through the project UIDB/00100/2020 to CQE and the Natural Science and Engineering Research Council (NSERC) Discovery grants, for financial support of this research project. We would also like to note that the photographs used in the paper were selected from Pixabay, an open-source image database. We also thank Molly Bradford and anonymous referees for reviewing the manuscript draft.

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