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Estimating mercury exposure in amphibians using non-lethal tissue sampling

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Abstract
Amphibian populations worldwide are declining in response to environmental threats, including contaminants, yet our ability to study sensitive populations in their exposure and response to environmental contaminants is limited by the reliability and accuracy of non-lethal sampling methods. Mercury (Hg)—a widespread contaminant—can be transformed in freshwater systems to methylmercury (MeHg), a more bioavailable and toxic form, threatening amphibians using these habitats. We refined methodology for non-lethal sampling and Hg determination across 13 amphibian species, where we demonstrate the efficacy of toe and tail clip tissues to estimate whole-body Hg burdens by examining whether (1) the primary form of Hg (i.e., MeHg or total mercury [THg]) is consistent across tissues and taxonomic groups; (2) clipped tissues correlate with whole-body MeHg and THg concentrations, and (3) all-species or species-specific calculations are appropriate to estimate whole-body concentrations from clips. Across all taxa, MeHg was the primary form of Hg across tissues (averaging 72–78%); toe/tail clip tissues were positively correlated with whole-body Hg, regardless of Hg speciation (i.e., THg or MeHg); measuring MeHg in toe/tail clips was the best analysis to estimate whole-body THg and MeHg (e.g., MeHg relationships were 1.4x and 1.06x less variable than THg relationships for toe and tail clip to whole-body estimates, respectively); and there were no significant species × toe/tail clip to whole body interactions, indicating the all-species model estimates are appropriate to use across taxa. These non-lethal sampling techniques allow for estimation of population-level effects of Hg, especially for rare or imperiled species.

Keywords Contaminant · Frog · Monitoring · Risk · Salamander · Tissue correlation

Introduction

Mercury is a contaminant of concern for human and wildlife populations worldwide due to its prevalence, global distribution, and toxicity. It enters the environment via direct point sources (e.g., mining) or indirect sources; for example, inorganic mercury (Hg) is released into the atmosphere via human activity (e.g., coal combustion) and natural geologic sources, such as volcanic activity, and can travel in the atmosphere for up to 1 year until deposition through precipitation (Schroeder and Munthe 1998; Driscoll et al. 2013). While proximity to Hg sources can be associated with elevated Hg in aquatic wildlife (Bartz et al. 2023), even remote environments are susceptible to Hg contamination (Fitzgerald et al. 1998; AMAP 2011; Eagles Smith et al. 2016; Drummond et al. 2022; Han et al. 2023). This is because in aquatic systems, localized biogeochemical processes can transform inorganic Hg into a more toxic and bioavailable form, methylmercury (MeHg), (Rice et al. 2014; Cariccio et al. 2019) that readily bioaccumulates and biomagnifies within food webs (Chasar et al. 2009; Gentès et al. 2021). Methylmercury production and concentrations in wildlife may be mediated via biogeochemical and ecological factors (Ullrich et al. 2001; Benoit et al. 2002; Eagles-Smith et al. 2016, 2016a), commonly

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resulting in a decoupling between abiotic and biological Hg concentrations. As a result, there is a variable landscape of exposure risk to wildlife that necessitates localized data collection to understand and manage mercury exposure and demographic effects (Eagles-Smith et al. 2016, 2016a; Tornabene et al. 2023).

Amphibian populations worldwide are declining in response to multiple stressors, including environmental contaminants (Stuart et al. 2004; Egea-Serrano et al. 2012; Sievers et al. 2019). Amphibians are susceptible to Hg exposure and experience individual-level effects such as behavioral changes (Burke et al. 2010; Bergeron et al. 2011), malformation (Unrine et al. 2004; Todd et al. 2011), disrupted thyroid function, liver oxidative stress, lipid metabolism disorder (Shi et al. 2018), and increased larval mortality (Unrine et al. 2004; Bergeron et al. 2011), all which can combine to have population-level effects (Willson and Hopkins 2013). Given many species of amphibian rely on aquatic habitats for reproduction and larval development, and these same habitats facilitate MeHg production, wild amphibian populations may be at risk of MeHg exposure, primarily through dietary pathways. However, limited information exists regarding Hg concentrations in amphibian tissues compared to other taxa like fish, piscivorous birds, and mammals due, in large part, to the contribution of fish consumption to human and wildlife health risk and the associated research attention this affords (Chan et al. 2003; Chételat et al. 2020). Because of the spatial variability in Hg concentrations across wild amphibian populations (Smalling et al. 2021; Drummond et al. 2022; Tornabene et al. 2023) and the lack of concurrence between abiotic Hg contamination and biological Hg concentrations (Eagles-Smith et al. 2016, 2016a), it can be difficult to estimate potential risk to amphibian populations without directly sampling individuals. However, many amphibian species and populations are declining or are of conservation concern (Stuart et al. 2004), precluding lethal sampling approaches. As a result, non-lethal sampling methods promise to serve as a valuable tool for estimating amphibian exposure to MeHg.

Non-lethal sampling methods (i.e., toe or tail clips) to estimate whole-body total mercury (THg) concentrations in multiple anuran and salamander species are established (Bergeron et al. 2010; Todd et al. 2012; Pfleeger et al. 2016; Haskins et al. 2019). However, there can be substantial variability in the proportion of Hg in the MeHg form in non-lethal clips (Rowland et al. 2022), which can add uncertainty to the use of THg as a proxy for MeHg concentrations. Moreover, Pfleeger et al. (2016) found species-specific differences in the relationships between tissue and whole-body THg concentrations that suggest taxonomically distinct equations may be more effective in estimating whole-body THg concentrations. Further refinement of Hg speciation and taxonomic variability may provide more accurate estimates of whole-body Hg concentrations when using non-lethal tissue samples.

In this study, we sampled 231 individuals representing 9 anuran and 4 salamander species with two general life histories (pond-breeding species that use both aquatic and terrestrial habitats and obligate neotenic species that only use aquatic habitats) from 14 states/provinces across the United States and Canada to assess THg and MeHg concentrations in non-lethal tissue clip subsamples and how they relate with paired whole-body samples (Table S1). We examine (1) the effectiveness of toe or tail clips to estimate whole-body MeHg and THg concentrations; (2) the proportion of THg in each tissue type that is comprised of MeHg; and (3) how the relationships between toe/tail clip and whole-body Hg concentrations vary across amphibian taxa.

Methods

Field collection

Individual adult amphibians were collected by hand, dip nets, or unbaited traps from 53 unique locations across the US (Table S1). Descriptions and details of sampling sites can be found in Tornabene et al. (2023). Upon capture, each individual was euthanized with MS-222 or benzocaine in accordance with U.S. Geological Survey or the University-specific Institutional Animal Care and Use Committee procedures and immediately placed in a sealed, labeled polyethylene bag. Individuals were frozen within a few hours of collection and stored at -20 °C until processing in the laboratory. Frozen samples were shipped to the USGS Contaminant Ecology Laboratory in Corvallis, Oregon, for processing and Hg determination.

Sample processing and tissue preparation

In the laboratory, snout-vent length was measured to the nearest mm with a ruler, and wet weight was determined to the nearest 0.0001 g on a digital balance. Sex was recorded when it could be determined and was otherwise recorded as "unknown". Toe and tail clip collection followed methodology outlined in Pfleeger et al. (2016). Briefly, for anurans, the posterior left and right toes in position 4 (see Pfleeger et al. 2016; Fig. 2) were removed and processed separately, such that for each anuran one toe was analyzed for THg and the other for MeHg. For salamanders, the distal 2 cm of tail was removed from each individual. All remaining whole bodies and tail clips were oven dried (50 °C), and toe clips were lyophilized (-40 °C) to constant mass, and dry weight of respective tissues was determined to the nearest

0.0001 g on a digital balance. Whole bodies and tail clips were homogenized via porcelain mortar with pestle and stainless steel scissors, respectively, whereas toe clips were analyzed whole.

Mercury determination

Whole bodies and tail clips were analyzed for THg and MeHg from the same homogenate, whereas left and right toe clips from each individual were randomly assigned for either THg or MeHg analysis because there was not enough mass to run both analyses on a single toe. Total Hg determination was conducted on either a Milestone tri-cell DMA 80 Direct Mercury Analyzer (Milestone Inc, Monroe, Connecticut, USA) or Nippon MA-3000 (Nippon Instruments Corporation, Tokyo, Japan) following US EPA Method 7473. Quality-assurance measures included certified reference materials (lobster hepatopancreas [TORT-3; National Research Council of Canada, Ottawa, Canada] and dogfish muscle [DORM-4; National Research Council of Canada, Ottawa, Canada]), continuing calibration checks, blanks, and duplicates. Percent recoveries for certified reference materials and calibration checks averaged 100.5 ± 0.4 SE (n = 66) and 101.6 ± 1.2 SE (n = 66), respectively; the relative percent difference for duplicates averaged 4.0 ± 0.7 SE (n = 25).

Methylmercury determination was conducted using a MERX-M automated MeHg analyzer (Brooks Rand Inc, Seattle, Washington, USA) following US EPA Method 1630. Homogenized whole bodies were weighed to a target mass of 7–9 mg; tail clips > 12 mg were homogenized and weighed to a target mass of 5–11 mg, while those < 12 mg were analyzed whole; all toe clips were analyzed whole due to limited mass of samples. Tail and toe clips < 1 mg were analyzed in triplicate to ensure accuracy. Dried whole or homogenized samples were digested in 30% nitric acid at 60 °C for 14 h and ethylated with 1% sodium tetraethylborate for analysis. Quality-assurance measures included certified reference materials (scallop [IAEA-452; International Atomic Energy Agency, Vienna, Austria] and fish homogenate [IAEA-407; International Atomic Energy Agency, Vienna, Austria]), continuing calibration checks, blanks, matrix spikes, and duplicates. Percent recoveries for certified reference materials and calibration checks averaged 100.5 ± 0.5 SE (n = 160) and 100.1 ± 0.8 SE (n = 67), respectively; the relative percent difference for duplicates averaged 3.8 ± 0.4 SE (n = 65).

Statistical analyses

We used linear regression to describe the relationships across all taxa between toe or tail clip Hg concentrations (both MeHg and THg) and their paired whole-body THg and MeHg concentrations. We also examined whether the %MeHg in each tissue type varied with THg concentrations (log-transformed) using logarithmic regression with a rise to a maximum, due to the fact that %MeHg is constrained to 100%. Additionally, we used analysis of covariance (ANCOVA) to test whether relationships between toe/tail clip and whole-body concentrations differed among taxa. We ran individual models for all combinations of toe/tail clip THg or MeHg (as independent covariates) and whole-body THg or MeHg (as dependent variables) to evaluate the relative strength of the relationships between different Hg species and tissue matrices. Species was included as a categorical factor in the model to test for differences in slopes and intercepts among taxa. All THg and MeHg concentration data were natural-log transformed prior to statistical analyses to normalize residuals and meet the assumptions of the statistical tests. All statistical analyses were conducted using JMP V16.0 (SAS Institute, Cary, NC).

Results

Descriptive summary results

Whole-body THg concentrations in anurans (n = 76) and salamanders (n = 155) ranged from 31 to 924 and 25–2148 ng/g dry weight (dw), respectively. MeHg concentrations (ng/g dw) ranged from 15 to 868 in anurans and 20–1790 in salamanders. In general, THg and MeHg concentrations were lower in toe clips (27–591 and 12–580 ng/g dw, respectively), and tail clips (89–317 and 26–221 ng/g dw, respectively) than in whole bodies. The proportion of THg present as MeHg (mean %MeHg ± standard deviation) was 77 ± 16% and 77 ± 12% in anuran and salamander whole bodies, respectively (Fig. 1), whereas %MeHg in toe clips averaged 78 ± 20% and in tail clips %MeHg averaged 72 ± 14% (Fig. 1). At THg concentrations below 100 ng/g dw, %MeHg in whole bodies and tail clips increased with THg, but both reached an asymptote of 81% and 79% MeHg, respectively, at higher THg concentrations (i.e. >100 ng/g) (Fig. 2a and c). In contrast, there was no relationship between %MeHg and THg concentrations in toe clips, regardless of tissue concentration (Fig. 2b).

Tissue vs. whole-body hg relationships

Across taxa

Across all species, THg concentrations in toe clips were positively correlated with their paired whole-body THg ($F_{1,80}$ = 137.9, P < 0.0001; R^2 = 0.63) and MeHg ($F_{1,80}$ = 145.7,

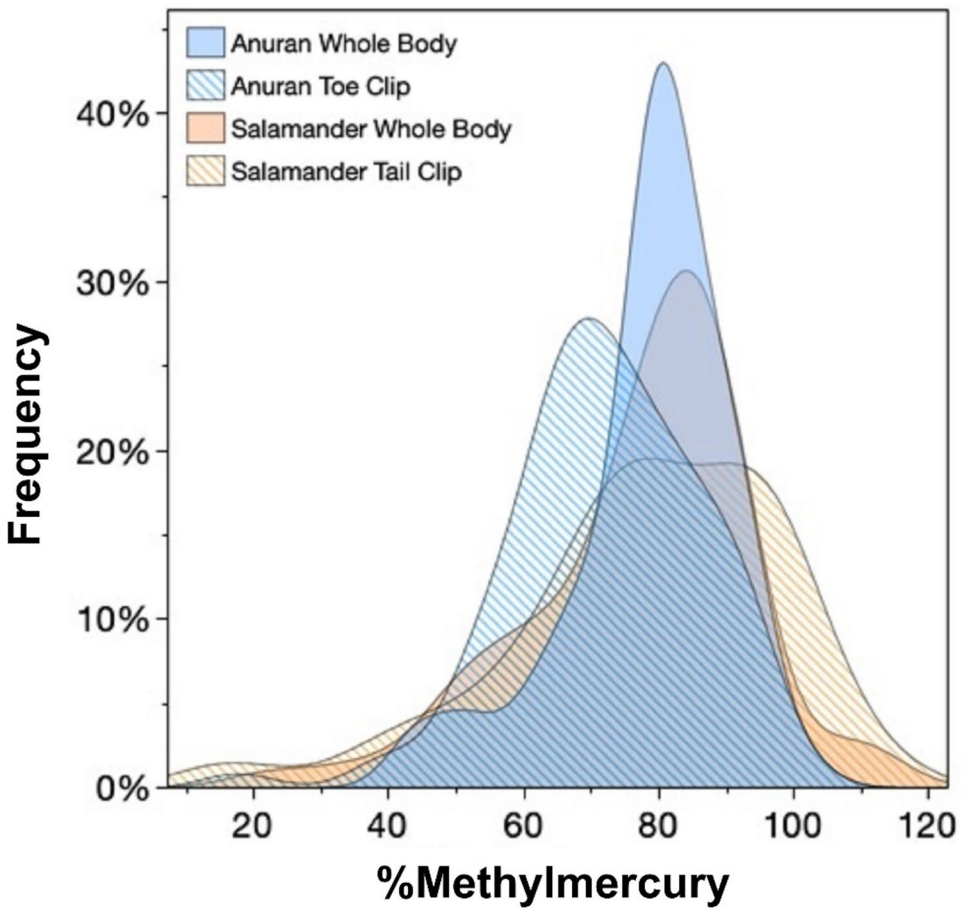

Fig. 1 Density histogram of proportion of total Hg (THg) in methylmercury (MeHg) form (%MeHg) in whole bodies (solid fill, $n=231$), and tail or toe clips (hatched fill) of adult anurans (blue, $n=76$) and salamanders (orange $n=155$)

$P<0.0001$; $R^2=0.65$) concentrations (Eqs. 1, 2; Fig. 3). The relationships between whole bodies and toe clips were substantially stronger for toe clip MeHg concentrations (whole-body THg: $F_{1,84}=537.7$, $P<0.0001$; $R^2=0.87$; whole-body MeHg: $F_{1,84}=923.5$, $P<0.0001$; $R^2=0.92$; Eqs. 3, 4; Fig. 3). Similarly, tail clip THg concentrations were positively correlated with their paired whole-body THg ($F_{1,93}=771.1$, $P<0.0001$; $R^2=0.89$) and MeHg ($F_{1,94}=576.0$, $P<0.0001$; $R^2=0.86$) concentrations (Eqs. 5, 6; Fig. 3). The correlations between whole bodies and tail clips were slightly stronger for tail clip MeHg concentrations (whole-body THg: $F_{1,140}=1562$, $P<0.0001$; $R^2=0.92$; whole-body MeHg: $F_{1,141}=1453$, $P<0.0001$; $R^2=0.91$; Eqs. 7, 8; Fig. 3), but the difference was less pronounced than with toe clips. See all-species equations below and full suite of equations with uncertainty estimates included in Table 1.

Whole-body THg vs. toe THg;
$$\ln THg_{wb}\left(ng \bullet g^{-1}dw\right) = 1.33 + 0.82(\ln THg_{toe}) \quad (1)$$

Whole-body MeHg vs. toe THg;
$$\ln MeHg_{wb}\left(ng \bullet g^{-1}dw\right) = 0.25 + 0.99(\ln THg_{toe}) \quad (2)$$

Whole-body THg vs. toe MeHg;
$$\ln THg_{wb}\left(ng \bullet g^{-1}dw\right) = 1.81 + 0.77(\ln MeHg_{toe}) \quad (3)$$

Whole-body MeHg vs. toe MeHg;
$$\ln MeHg_{wb}\left(ng \bullet g^{-1}dw\right) = 0.75 + 0.95(\ln MeHg_{toe}) \quad (4)$$

Whole-body THg vs. tail THg;
$$\ln THg_{wb}\left(ng \bullet g^{-1}dw\right) = 0.13 + 1.06(\ln THg_{tail}) \quad (5)$$

Whole-body MeHg vs. tail THg;
$$\ln MeHg_{wb}\left(ng \bullet g^{-1}dw\right) = -0.74 + 1.18(\ln THg_{tail}) \quad (6)$$

Whole-body THg vs. tail MeHg;
$$\ln THg_{wb}\left(ng \bullet g^{-1}dw\right) = 1.21 + 0.89(\ln MeHg_{tail}) \quad (7)$$

Whole-body MeHg vs. tail MeHg;
$$\ln THg_{wb}\left(ng \bullet g^{-1}dw\right) = 0.48 + 0.99(\ln MeHg_{tail}) \quad (8)$$

Taxonomic variability

We used Analysis of Covariance (ANCOVA) to test whether species affected the relationships between Hg concentrations in tissue clips and whole-body values (excluding Pacific Tree Frogs [*Pseudacris regilla; n = 3*] and Oregon Spotted Frogs [*Rana pretiosa; n = 2*] due to limited sample sizes). Both whole-body THg and MeHg concentrations were positively correlated with toe clip THg concentrations (whole-body THg: $F_{1,62}=6.24$, $p=0.01$; whole-body MeHg: $F_{1,62}$

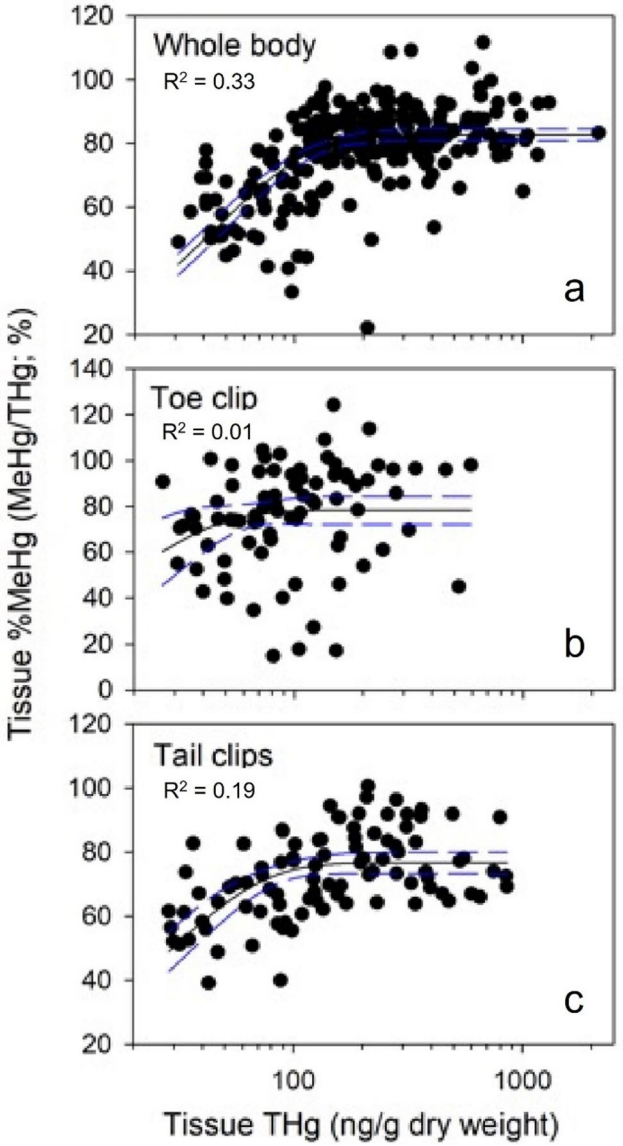

Fig. 2 Percent methylmercury (MeHg) in relation to tissue total mercury (THg) concentrations (ng/g dry weight) in **a** whole bodies, **b** toe clips, and **c** tail clips of adult anurans and salamanders. Fitted curves represent logarithmic regression with a rise to maximum, and dashed lines are 95% confidence bands

= 6.16, $p=0.02$) and tail clip concentrations (whole-body THg: $F_{1,87} = 158$, $p<0.0001$; whole-body MeHg: $F_{1,88} = 136$, $p<0.0001$; Figure S1). However, neither the species × toe clip Hg (whole-body THg: $F_{9,62} = 0.85$, $p=0.58$; whole-body MeHg: $F_{9,62} = 0.72$, $p=0.69$), nor species × tail clip Hg (whole-body THg: $F_{3,87} = 0.03$, $p=0.99$; whole-body MeHg: $F_{3,88} = 0.44$, $p=0.72$) interactions were significant (Figure S1). This indicates that the slopes of relationships between THg concentrations in toe clips or tail clips and either THg or MeHg concentrations in corresponding whole bodies did not differ among taxa.

Similarly, whole-body THg and MeHg concentrations were positively correlated with MeHg concentrations in toe clips (whole-body THg: $F_{1,66} = 92.6$, $p<0.0001$; whole-body MeHg: $F_{1,66} = 140.3$, $p<0.0001$; ; Figure S1) and tail clips (whole-body THg: $F_{1,134} = 2186$, $p<0.0001$; whole-body MeHg: $F_{1,135} = 276$, $p<0.0001$; Figure S1). Neither the species × toe clip MeHg (whole-body THg: $F_{9,66} = 0.84$, $p=0.58$; whole-body MeHg: $F_{9,66} = 1.13$, $p=0.35$) nor species × tail clip MeHg interactions were significant (whole-body THg: $F_{3,134} = 1.17$, $p=0.32$; whole-body MeHg: $F_{3,135} = 1.47$, $p=0.22$; Figure S1).

Intercept coefficients did not differ among taxa for any of the toe clip-whole-body relationships except for that between whole-body MeHg and toe clip THg concentrations ($F_{9,62} = 2.33$, $p=0.02$; Figure S1). In contrast, intercept coefficients did differ among taxa for all whole-body THg and MeHg relationships with tail clip THg or MeHg. Specifically, intercepts were substantially higher in Common Mudpuppy (*Necturus maculosus*) and Gulf Coast Waterdog (*N. beyeri*), than in Northern Two-lined Salamander (*Eurycea bislineata*) or Eastern Newt (*Notophthalmus viridescens*) (Figure S1). Complete regression equations for each taxon and whole-body vs. tissue Hg relationship can be found in Table 1.

Discussion

In addressing the objectives of this study we found that: (1) MeHg is the primary form of Hg (averaging 72–78%) across tissues and taxonomic groups—but it was highly variable—indicating that THg is not a consistent proxy for MeHg in amphibian tissues; (2) toe/tail clip and whole-body Hg concentrations were correlated regardless of Hg speciation, but MeHg relationships were, on average, 1.4x and 1.06x less variable than THg relationships in toe and tail clips, respectively, and therefore more precise than THg relationships; (3) there was limited among-species variability in the relationships between toe/tail clips and whole body across both anurans and salamanders.

Our results show that measuring MeHg in non-lethal tissue clip samples yields more precise and reliable estimates of whole-body Hg concentrations than measuring tissue clip THg concentrations. The %MeHg in clips closely matched whole-body values (e.g., 78 ± 20% versus 77 ± 16% in anuran toe clips and whole-body, respectively; 72 ± 14% versus 77 ± 12% in salamander tail clips and whole-body, respectively). Additionally, clip MeHg predicted whole-body THg and MeHg with high accuracy ($R^2 = 0.86–0.92$) outperforming clip THg (R^2 range = 0.63–0.89). Toe clip MeHg improved precision by 34% for whole-body MeHg and 31% for THg compared to toe clip THg, while tail clip

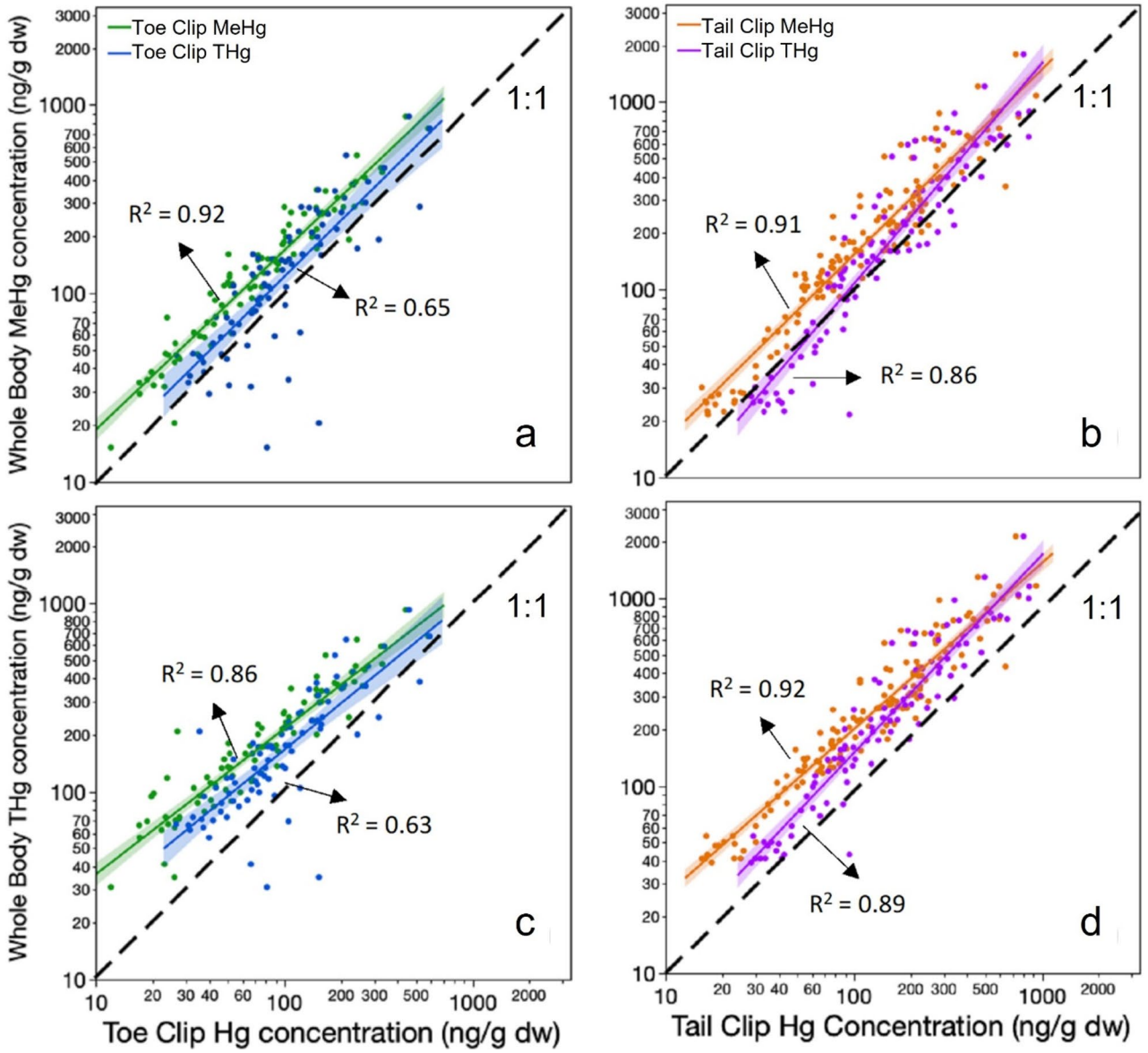

Fig. 3 Relationships of mercury concentrations (ng/g dry weight (dw)) between whole bodies and non-lethal tissue clips in adult anurans and salamanders. Shaded areas represent 95% confidence intervals. **a** Whole-body methylmercury (MeHg) concentrations versus toe clip MeHg (green) and toe clip total Hg (THg; blue); **b** Whole-body MeHg concentrations versus tail clip MeHg (orange) and tail clip THg (purple); **c** Whole-body THg concentrations versus toe clip MeHg (green) and toe clip THg (blue); **d** Whole-body THg concentrations versus tail clip MeHg (orange) and tail clip THg (purple)

MeHg was 6% and 3% better, respectively. The strongest correlations occurred between clip MeHg and whole-body MeHg ($R^2 = 0.92$ for toes clips; $R^2 = 0.91$ for tail clips), and whole-body MeHg consistently exceeded clip MeHg across exposure gradients.

The benefit of using clip MeHg to estimate whole body Hg concentrations is further highlighted by the variability in %MeHg across tissue concentrations. Specifically, tissue concentrations (both whole bodies and clips) had lower and more variable %MeHg below ~100 ng/g dw. As a result, estimates of whole body MeHg would be biased low if determined using THg in clip samples that had low concentrations.The mechanism behind this difference across the exposure gradient is unclear but may reflect dietary shifts, less biomagnification at low Hg exposure, or simply greater sensitivity of %MeHg to small errors in THg when concentrations are low. Toe clips exhibited greater %MeHg variability than tail clips, while whole-body concentrations were the most stable. This may be due to the relative differences in tissue Hg burden between these tissue types (e.g.,

Table 1 Tissue correlation equations for estimating whole-body total mercury (THg) or methylmercury (MeHg) from toe or tail clip MeHg and THg concentrations (±SE)

Species	Tissue Metric	Whole Body THg equation	R2	P	N	Whole Body MeHg equation	R2	P	N
All taxa	**Toe THg**	**$\ln THg_{wb}$= 1.33 (± 0.32) + 0.82 (± 0.07) *$\ln THg_{toe}$**	**0.63**	**<0.0001**	**82**	**$\ln MeHg_{wb}$= 0.25 (± 0.38) + 0.99 (± 0.08) *$\ln THg_{toe}$**	**0.65**	**<0.0001**	**82**
	Toe MeHg	**$\ln THg_{wb}$= 1.81 (± 0.14) + 0.77 (± 0.03) *$\ln MeHg_{toe}$**	**0.86**	**<0.0001**	**84**	**$\ln MeHg_{wb}$= 0.75 (± 0.14) + 0.95 (± 0.03) *$\ln MeHg_{toe}$**	**0.92**	**<0.0001**	**84**
	Tail THg	**$\ln THg_{wb}$= 0.13 (± 0.19) + 1.06 (± 0.05) *$\ln THg_{tail}$**	**0.89**	**<0.0001**	**94**	**$\ln MeHg_{wb}$= -0.75 (± 0.25) + 1.18 (± 0.05) *$\ln THg_{tail}$**	**0.86**	**<0.0001**	**95**
	Tail MeHg	**$\ln THg_{wb}$= 1.21 (± 0.11) + 0.89 (± 0.02) *$\ln MeHg_{tail}$**	**0.92**	**<0.0001**	**142**	**$\ln MeHg_{wb}$= 0.48 (± 0.13) + 0.99 (± 0.03) *$\ln MeHg_{tail}$**	**0.92**	**<0.0001**	**142**
American Bullfrog	Toe THg	$\ln THg_{wb}$ = 1.002 (± 1.31) + 0.94 (± 0.28) *$\ln THg_{toe}$	0.74	0.03	6	$\ln THg_{wb}$ = 1.72 (± 0.87) + 0.82 (± 0.19) *$\ln MeHg_{toe}$	0.82	0.01	6
	Toe MeHg	$\ln MeHg_{wb}$ = -0.15 (± 1.24) + 1.14 (± 0.26) *$\ln THg_{toe}$	0.83	0.01	6	$\ln MeHg_{wb}$ = 0.75 (± 0.75) + 0.96 (± 0.16) *$\ln MeHg_{toe}$	0.9	0.004	6
Boreal Chorus Frog	Toe THg	$\ln THg_{wb}$ = 1.91 (± 0.99) + 0.72 (± 0.18) *$\ln THg_{toe}$	0.73	0.007	8	$\ln THg_{wb}$ = 1.70 (± 0.59) + 0.79 (± 0.11) *$\ln MeHg_{toe}$	0.89	0.0004	8
	Toe MeHg	$\ln MeHg_{wb}$ = 1.31 (± 1.25) + 0.81 (± 0.23) *$\ln THg_{toe}$	0.68	0.01	8	$\ln MeHg_{wb}$ = 0.88 (± 0.65) + 0.92 (± 0.12) *$\ln MeHg_{toe}$	0.9	0.0003	8
Cascades Frog	Toe THg	$\ln THg_{wb}$ = 3.57 (± 2.26) + 0.22 (± 0.56) *$\ln THg_{toe}$	0.02	0.7	8	$\ln THg_{wb}$ = 1.24 (± 0.35) + 0.92 (± 0.10) *$\ln MeHg_{toe}$	0.93	<0.0001	8
	Toe MeHg	$\ln MeHg_{wb}$ = 2.85 (± 2.88) + 0.29 (± 0.71) *$\ln THg_{toe}$	0.03	0.7	8	$\ln MeHg_{wb}$ = -0.08 (± 0.45) + 1.17 (± 0.13) *$\ln MeHg_{toe}$	0.93	<0.0001	8
Columbia Spotted Frog	Toe THg	$\ln THg_{wb}$ = 1.91 (± 1.40) + 0.71 (± 0.30) *$\ln THg_{toe}$	0.58	0.08	6	$\ln THg_{wb}$ = 2.14 (± 0.40) + 0.70 (± 0.08) *$\ln MeHg_{toe}$	0.89	<0.0001	10
	Toe MeHg	$\ln MeHg_{wb}$ = 0.67 (± 1.84) + 0.96 (± 0.40) *$\ln THg_{toe}$	0.58	0.07	6	$\ln MeHg_{wb}$ = 1.80 (± 0.54) + 0.75 (± 0.11) *$\ln MeHg_{toe}$	0.84	0.0002	10
Green Frog	Toe THg	$\ln THg_{wb}$ = 3.16 (± 1.28) +0.36 (± 0.22) *$\ln THg_{toe}$	0.13	0.31	10	$\ln THg_{wb}$ = 2.45 (± 0.72) + 0.63 (± 0.22) *$\ln MeHg_{toe}$	0.51	0.02	10
	Toe MeHg	$\ln MeHg_{wb}$ = 1.76 (± 1.38) + 0.55 (± 0.36) *$\ln THg_{toe}$	0.22	0.17	10	$\ln MeHg_{wb}$ = 0.58 (± 0.16) + 1.00 (± 0.05) *$\ln MeHg_{toe}$	0.98	<0.0001	10
Western Toad	Toe THg	$\ln THg_{wb}$ = 0.86 (± 0.60) + 0.94 (± 0.14) *$\ln THg_{toe}$	0.7	<0.0001	20	$\ln THg_{wb}$ = 2.38 (± 0.54) + 0.61 (± 0.14) *$\ln MeHg_{toe}$	0.52	0.0004	20
	Toe MeHg	$\ln MeHg_{wb}$ = -0.09 (± 0.72) + 1.10 (± 0.17) *$\ln THg_{toe}$	0.69	<0.0001	20	$\ln MeHg_{wb}$ = 1.56 (± 0.61) + 0.75 (± 0.16) *$\ln MeHg_{toe}$	0.56	<0.0001	20
Wood Frog	Toe THg	$\ln THg_{wb}$ = 1.67 (± 0.92) + 0.81 (± 0.17) *$\ln THg_{toe}$	0.75	0.002	9	$\ln THg_{wb}$ = 1.63 (± 0.78) + 0.82 (± 0.15) *$\ln MeHg_{toe}$	0.81	0.0009	9
	Toe MeHg	$\ln MeHg_{wb}$ = 1.94 (± 1.02) + 0.73 (± 0.19) *$\ln THg_{toe}$	0.67	0.007	9	$\ln MeHg_{wb}$ = 1.83 (± 0.87) + 0.75 (± 0.17) *$\ln MeHg_{toe}$	0.74	0.003	9
Common Mudpuppy	Tail THg	$\ln THg_{wb}$ = 1.25 (± 1.58) + 0.94 (± 0.30) *$\ln THg_{tail}$	0.71	0.03	6	$\ln THg_{wb}$ = 1.86 (± 1.43) + 0.85 (± 0.28) *$\ln MeHg_{tail}$	0.7	0.04	6
	Tail MeHg	$\ln MeHg_{wb}$ = 0.19 (± 1.61) + 1.12 (± 0.30) *$\ln THg_{tail}$	0.77	0.02	6	$\ln MeHg_{wb}$ = 0.85 (± 1.41) + 1.02 (± 0.27) *$\ln MeHg_{tail}$	0.78	0.02	6
Eastern Newt	Tail THg	$\ln THg_{wb}$ = 0.47 (± 0.48) + 0.95 (± 0.12) *$\ln THg_{tail}$	0.75	<0.0001	22	$\ln THg_{wb}$ = 1.55 (± 0.15) + 0.77 (± 0.04) *$\ln MeHg_{tail}$	0.94	<0.0001	25
	Tail MeHg	$\ln MeHg_{wb}$ = -0.49 (± 0.53) + 1.05 (± 0.13) *$\ln THg_{tail}$	0.75	<0.0001	23	$\ln MeHg_{wb}$ = 0.45 (± 0.11) + 0.93 (± 0.03) *$\ln MeHg_{tail}$	0.98	<0.0001	26
	Toe THg	$\ln THg_{wb}$ = -0.49 (± 3.59) +1.14 (± 0.71) *$\ln THg_{toe}$	0.24	0.15	10	$\ln THg_{wb}$ = 1.12 (± 0.75) + 0.94 (± 0.17) *$\ln MeHg_{toe}$	0.8	0.0005	10
	Toe MeHg	$\ln MeHg_{wb}$ = -1.65 (± 3.75) + 1.29 (± 0.74) *$\ln THg_{toe}$	0.27	0.12	10	$\ln MeHg_{wb}$ = 0.35 (± 0.75) + 1.02 (± 0.17) *$\ln MeHg_{toe}$	0.82	0.0003	10
Gulf Coast Waterdog	Tail THg	$\ln THg_{wb}$ = 1.05 (± 0.45) + 0.98 (± 0.08) *$\ln THg_{tail}$	0.95	<0.0001	10	$\ln THg_{wb}$ = 1.37 (± 0.40) + 0.94 (± 0.07) *$\ln MeHg_{tail}$	0.95	<0.0001	10
	Tail MeHg	$\ln MeHg_{wb}$ = 1.15 (± 0.42) + 0.95 (± 0.07) *$\ln THg_{tail}$	0.95	<0.0001	10	$\ln MeHg_{wb}$ = 1.45 (± 0.37) + 0.91 (± 0.07) *$\ln MeHg_{tail}$	0.96	<0.0001	10
Northern Two-lined Salamander	Tail THg	$\ln THg_{wb}$ = 0.62 (± 0.19) + 0.98 (± 0.08) *$\ln THg_{tail}$	0.92	<0.0001	57	$\ln THg_{wb}$ = 1.42 (± 0.12) + 0.84 (± 0.02) *$\ln MeHg_{tail}$	0.92	<0.0001	101
	Tail MeHg	$\ln MeHg_{wb}$ = 0.43 (± 0.22) + 0.94 (± 0.04) *$\ln THg_{tail}$	0.9	<0.0001	57	$\ln MeHg_{wb}$ = 1.16 (± 0.13) + 0.84 (± 0.02) *$\ln MeHg_{tail}$	0.92	<0.0001	101

greater bone-to-soft tissue in toes; more muscle in tails; and inclusion of organs such as liver and brain in whole bodies that incorporate MeHg more readily than skin or bone). As evident by the slope of the tail clip THg to whole-body MeHg relationship (Fig. 3c), below 100 ng/g THg the tail clip concentration exceeds that of the whole-body MeHg concentration but, above 100 ng/g, the whole-body MeHg concentrations exceed that of their paired tail clip THg concentrations. This is consistent with our findings of %MeHg in tail clips decreasing below 100 ng/g (Fig. 2c), suggesting that surficial inorganic Hg may be affecting estimated whole-body values in clips with low Hg content.

To further illustrate this with the all-species models estimating whole-body MeHg (Table 1), a toe/tail clip MeHg or THg concentration of 100 ng/g would bias the estimated whole-body MeHg concentration low when using toe/tail clip THg rather than the toe/tail clip MeHg. In fact, estimated whole-body MeHg concentrations would be 122.6 ng/g and 109.3 ng/g based on toe and tail clip THg concentrations, respectively, whereas whole-body MeHg estimates derived from toe and tail clip MeHg would be 168.2 ng/g and 154.3 ng/g, respectively. In this example, whole-body MeHg estimates based on THg in clips are 31.4% and 34.1% lower than estimates from MeHg in clips for toes and tails, respectively. Underestimates of this magnitude could substantially alter estimates of risk of benchmark exceedances. Thus, our results suggest that measurements of THg are less precise and include more uncertainty than measurements of MeHg. In the worst case, THg estimates may greatly bias estimates of Hg exposure risk.

We found that taxon was not an important source of variation in the relationship between toe/tail clips and whole-body Hg concentrations, as indicated by the lack of a species × toe or tail clip interactions for both THg and MeHg (Figure S1). The consistency of estimates from toe/tail clips is important for ease of use as an all-species monitoring tool for amphibians, such that techniques need not differ across anurans or salamanders when using toe or tail clips, respectively. Comparing our all-species models to those from Pfleeger et al. (2016)—another study whose tissue subsampling methodology we use for the current study (i.e., rear toe in position 4 for anurans or 0–2 cm distal tail for salamanders)—we find very similar toe/tail clip THg to whole-body THg relationships. Despite Pfleeger et al. only examining Cascades Frogs, and even though our all-species toe clip models use both left and right rear toe clips, we still find that our all-species model had a similar toe clip THg to whole-body THg relationship as Pfleeger et al.'s rear right toe clip model (model slopes = 0.98 and 0.82 for Pfleeger et al. and our models, respectively), with relatively similar whole-body THg concentrations for any given toe clip THg value (i.e., ±35%). Compared to the Pfleeger rear left toe clip model, the toe clip THg to whole-body THg relationship is even more similar (i.e., model slopes = 0.9 and 0.82 for Pfleeger et al. and our models, respectively), but whole-body THg is consistently 43–67% greater for any given toe clip value in our model. Similarly, both studies' all-species tail clip THg to whole-body THg models have similar slopes (0.9 and 1.058 for Pfleeger et al. (2016) and our models, respectively); however, our model predicts roughly 67–116% greater whole-body THg for any given tail clip THg value, due to the substantially higher intercepts of Mudpuppy and Waterdog species integrated into our all-species model. The difference in salamander species comprising our respective all-species models highlights the potential importance of life history and exposure risk for certain species. In examining our salamander tail clip to whole body-models, there were no slope differences in the relationships between tail clips and whole body among taxa, but there were notable species-specific differences in intercept coefficients. To illustrate the model estimate differences by using an arbitrary 100 ng/g tail clip MeHg value, the all-species salamander model (Table 1) fits within the species-specific model error range for Northern Two-lined Salamander whole-body MeHg and narrowly overestimates Eastern Newt whole-body MeHg by 4.4 ng/g (3.8%) beyond the species-specific model's error range; however, the all-species salamander model underestimates Mudpuppy and Waterdog whole-body MeHg by 54.7 ng/g and 79.9 ng/g, respectively (or 23.9% and 33.1% less than the species-specific models' estimate range, respectively). The substantially higher intercept coefficients for Common Mudpuppy and Gulf Coast Waterdog models (Figure S1) indicate greater overall Hg burden in these species, and that whole-body concentrations are higher than tail clip concentrations relative to other salamanders. This could be due to habitat use, life histories, and differences in Hg exposure, as Mudpuppies and Waterdogs are fully aquatic and obligate paedomorphs, as opposed to the semiaquatic salamanders and newts with terrestrial life stages (Duellman and Trueb 1994; Wells 2007). However, it is also important to note that the Mudpuppy and Waterdog concentration ranges were limited, as all individuals had whole-body MeHg concentrations greater than 180 ng/g. Without a broader representation of Hg concentrations, these species-specific estimates could be inconsistent or skewed for concentrations not represented in our sampled populations. However, returning to the benefit of using the all-species model, this issue could be alleviated because that model incorporates a greater range of Hg concentrations. Thus, when selecting a salamander model, it may be important to weigh considerations for a species-specific model when examining taxa with substantially differing life histories and routes of Hg exposure or,

conversely, the benefit of the broad range of Hg concentrations accounted for in an all-species model.

Our study supports the use of these non-lethal sampling techniques across a wide array of amphibian species and supports use of toe/tail clip MeHg as the preferred measure to estimate whole body burdens of the more toxic form of Hg. Previous studies demonstrating the utility of non-lethal techniques (Bergeron et al. 2010; Todd et al. 2012; Haskins et al. 2019; Pfleeger et al. 2016) only measured THg, but we improved on the accuracy of all-species anuran and salamander toe/tail clip to whole body estimates and were able to compare the efficacy of THg versus MeHg measurements. While our study indicates that measuring MeHg in toe or tail clip tissue is the best indicator for whole-body MeHg estimates, this might not always be as accessible to researchers or natural resource managers due to analysis cost. However, while it is more expensive to analyze MeHg versus THg, there is the tradeoff risk of decreased accuracy and precision due to limited tissue size and/or low tissue Hg content for THg analysis. For our THg analyses, many of our samples (i.e., 44% and 22% of toe and tail clips, respectively) were considered either low mass (<5 mg dw) or low Hg (<1 ng). Methylmercury analysis allows for a greater range of tissue sizes and Hg content and, as our results indicate, greater accuracy and precision with low-Hg samples. This information can be used to plan monitoring efforts that optimize resources as well as potential data output.

These non-lethal sampling techniques not only allow for monitoring and research of sensitive populations but also influence the scale of investigations. For instance, use of this methodology has led to a greater understanding of MeHg bioaccumulation across multiple species at the landscape level (Tornabene et al. 2023). These techniques also allow for repeated sampling of individuals, expanding avenues to test exposure and body burden while also monitoring sublethal effects. It follows that extending and validating these non-lethal methods to amphibian larvae is an important next step that would further broaden our ability to assess MeHg contamination and investigate the effects of MeHg on populations. Through small and large-scale monitoring efforts, our understanding of individual and population health risks related to Hg exposure improve our ability to respond to threats leading to global amphibian declines.

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Data availability Data are available via ScienceBase https://doi.org/10.5066/P9LSR4HY.

Declarations

Conflict of interest The authors declare no competing interests.

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