Bulletin of Environmental Contamination and Toxicology (2023) 111:21
https://doi.org/10.1007/s00128-023-03780-y

Investigating the Allometric Relationship for Total Mercury in Male and Female Signal Crayfish (*Pacifastacus leniusculus*) from Three Regions in the Columbia River Basin, USA

Tate E. Libunao[1] · Alan S. Kolok[2]

Received: 2 May 2023 / Accepted: 28 July 2023

Abstract
The objective of this study was to evaluate the relationship between clawless body mass (CBM) and total mercury concentration (THg) in male and female crayfish captured from three different sites in the Columbia River Basin: the Spokane River, Boise River and Hangman Creek. It was found that the allometric relationships for THg in male and female crayfish did not differ from one another at any of the three locations (Spokane River: ANCOVA, $F_{1,49} = 0.001$, $p = 0.63$; Hangman Creek: ANCOVA, $F_{1,73} = 0.007$, $p = 0.93$; Boise River: ANCOVA, $F_{1,38} = 0.02$, $p = 0.88$. Furthermore, the slopes of the CBM and THg regression lines were not significantly different from one site to the next (ANCOVA, $F_{2,166} = 1.5$ $p = 0.24$), despite considerable differences in mean mass-adjusted THg across locations (Spokane River: 26.8 ± 2.6; Hangman Creek: 75.2 ± 2.1; Boise River: 99.0 ± 2.9). For signal crayfish (*Pacifastacus leniusculus*) from the Columbia River Basin, size and sex standardization can be accomplished with a single linear relationship.

Keywords Sentinel organism · Mercury · Standardization · Crayfish

Introduction

The use of crayfish in environmental monitoring for metals has grown substantially over the past few decades (Aluma et al. 2017; Anderson et al. 1997; Goretti et al. 2016; Hothem et al. 2007; Johnson et al. 2014; Kouba et al. 2010; Kuklina et al. 2014; Mueller et al. 2002; Pennuto et al. 2005). Such use, however, can be problematic, as there are biotic factors such as, sex (Hothem et al. 2007), species (Johnson et al. 2014), and size (Pennuto et al. 2005), as well as abiotic factors such as water quality (Millard et al. 2020) and geographic variation (Eagles-Smith et al. 2020) that influence metal body burden in sentinel organisms.

Currently, the literature is not consistent with respect to whether size or sex influences mercury concentration in crayfish. Pennuto et al. (2005) found a significant, positive relationship between size class and total mercury concentration in the adductor muscle (THg) of northern virile crayfish (*Faxonius virilis*), however, Hothem et al. (2007) did not find that relationship in signal crayfish (*Pacifastacus leniusculus*). The same is true for sex. While Hothem et al. (2007) reported significantly higher methylmercury concentrations in the adductor muscle of female red swamp crayfish (*Procambarus clarkii*) relative to males, neither Pennuto et al. (2005) nor Johnson et al. (2014) found such differences in four different species of crayfish, including *P. clarkii*.

The objective of this study was to determine the relationship between clawless body mass (CBM) and THg for signal crayfish collected at three different sites within the Columbia River Basin. A second goal was to determine the degree to which these relationships were influenced by site location and sex.

✉ Alan S. Kolok
akolok@uidaho.edu

[1] Department of Environmental Sciences, University of Idaho, Moscow, ID, USA

[2] Department of Fish and Wildlife Sciences, University of Idaho, Moscow, ID, USA

Published online: 10 August 2023

Table 1 Summary table of signal crayfish captured from June-September in 2021–2022 from the Spokane River and Hangman Creek, Washington, and the Boise River, Idaho. Differences in subscript letters indicate a statistically significant difference among the three site locations. Associated r^2 and significance values are derived from the linear regressions between mass-adjusted THg concentrations

Site Name	Unadjusted THg (µg/kg) w.w.± SE	Mass-adjusted THg (µg/kg) w.w.± SE	Mean Mass (g) ± SE	(n)	r^2	p value	Male (n)	Female (n)
Spokane River	$28.9_A \pm 1.1$	$26.8_A \pm 2.6$	$27_A \pm 1.1$	53	0.16	0.0003	14	39
Hangman Creek	$76.9_B \pm 2.2$	$75.2_B \pm 2.1$	$23.4_B \pm 0.9$	77	0.16	0.0082	49	28
Boise River	$88.3_C \pm 4.3$	$99.0_C \pm 2.9$	$13.2_C \pm 0.8$	42	0.12	0.0097	20	22

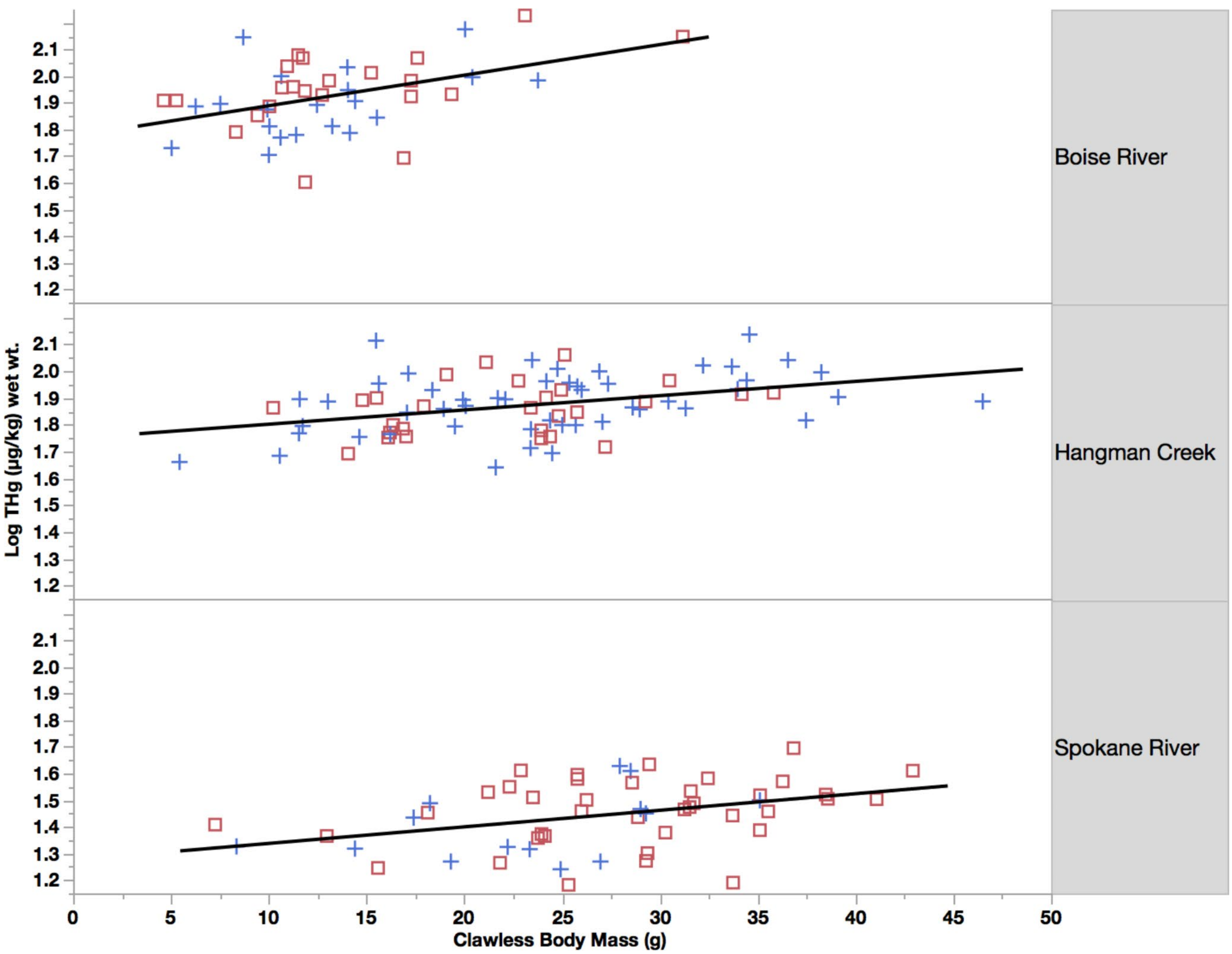

Fig. 1 Relationship between log THg (µg/kg) and CBM (g) in signal crayfish captured from the Spokane River, Hangman Creek, and Boise River. Female crayfish are displayed as red squares while male crayfish are displayed as blue crosses

Methods

During 2021 and 2022, a total of 172 signal crayfish were collected from three different locations in the Columbia River Basin. Animals were collected in Washington from the Spokane River at Plese Flats (47°43'01" N, 117°40'51" S), and from Hangman Creek (50°15'01" N, 117°40'51" S), a tributary of the Spokane River. Animals were also collected from the Boise River, Idaho at West Morland Park (43°39'36" N, 116°16'39 S) (Table 1). All animals were captured using a mix of hand collection and baited wire traps. Once captured, crayfish were placed into individual Zip-Loc bags, labeled with unique numerical identifiers and geospatial coordinates, then placed on ice and transported back to the University of Idaho. Upon arrival at the laboratory the crayfish were frozen whole at -80°C for a minimum of 24 h prior to dissection.

Animals were partially thawed and weighed (±0.01 g), the claws were removed, then weighed again to determine CBM. CBM was then used in all subsequent analyses, as the presence and mass of the claws varied considerably from one animal to another. Male crayfish were distinguished from females by the presence of developed gonopods. For each crayfish, the entire adductor muscle was removed with acid-washed instruments, placed into a 5ml cryovial, and stored within a -80 °C freezer prior to total mercury analysis.

Crayfish adductor muscle (0.5 g±0.01) was submitted to Anatek Laboratories in Moscow, Idaho for THg analysis, which was determined using an Agilent 7850 Series Mass-Hunter ICP-MS (Agilent Technologies, Santa Clara, CA, USA) following EPA Method 6020B. The quantitation limit on the ICP-MS was 0.5 µg/L, while the detection limit was 0.1 µg/L. A matrix spike (in duplicate) was analyzed as a measure of replicate precision. The replicate pair showed results of 0.6% relative percent difference (RPD) between the duplicates. A laboratory fortified sample with a known concentration of inorganic mercury was used as a reference material and ran once every 20 samples per batch. A continuing calibration blank of 18 MΩ DI water were run in between every 10 samples to verify the continued absence of instrumental interferences. Matrix spikes and spike-duplicates were spiked with inorganic mercury at known concentrations of 25 µg/kg and were ran every 20 samples. Recoveries from spikes and duplicates were well within the acceptable recovery range, ranging from 95.1 to 101% and 90.7 – 102%, respectively. The average RPD among the duplicates was 12.5%.

All results were reported in µg/kg wet weight. Briefly, a mass of adductor muscle (approximately 0.5 g) was weighed and placed into a digestion vessel along with 10 mL of 18 MΩ DI water, 10 mL of concentrated trace metal grade nitric acid, and 5 mL of concentrated trace metal grade hydrochloric acid. The solution was then heated for 2 h at 80–85 °C. Two mL of 30% hydrogen peroxide was then added to solution and heated for an additional 1 h. After tissue digestion, the solution was diluted to a final volume of 50 mL using 18 MΩ DI water. A 2 mL aliquot of the solution was then brought to 10 mL and injected onto the ICP-MS instrument.

Statistical analysis was performed using JMP statistical software (Ver 14.0, SAS Institute Inc., Cary, North Carolina, USA). THg data were not normally distributed and therefore were log transformed prior to analysis to meet the assumptions of parametric statistics as per Eagles-Smith et al. (2020). THg values in Table 1 are reported as back-transformed least-squares means.

For all statistical analyses, statistically significant differences were inferred when $p < 0.05$. Given that there was a significant relationship between CBM and THg, statistical comparisons among the locations were determined using ANCOVA to produce mass adjusted concentrations. To determine differences in mass-adjusted THg and CBM among the three locations, a series of one-way ANOVAs and post-hoc Tukey-HSD pairwise multiple comparison analyses were carried out. Differences in the regression slopes between the sexes at each site and among the three site locations were evaluated using ANCOVA. Significant interaction effects were evaluated as per Başusta and Khan (2021).

Results and Discussion

Statistically significant differences in mass adjusted THg (ANOVA, $F_{2,169} = 192.7$, $p < 0.0001$) and CBM (ANOVA, $F_{2,169} = 50.8$, $p < 0.0001$) were found at each of the three field sites (Table 1). Pairwise post hoc comparisons found that THg was lowest in animals from the Spokane River, followed by animals from Hangman Creek, and then from the Boise River, whereas the CBM was lowest in crayfish from the Boise River, followed by animals from Hangman Creek, and then from the Spokane River (Table 1). It was assumed that larger crayfish would have an elevated THg relative to smaller individuals (Pennuto et al. 2005), as larger crayfish may be older (France et al. 1991) and consequently have more time to accumulate mercury. While the differences in mean CBM were not positively associated with the differences in mean THg (Table 1), it appears that that there is considerable geographic variation in THg. Site-specific factors such as dissolved organic carbon (Thomas et al. 2020) and nutrient input (Wright and Hamilton 1982) may contribute to the observed differences among sites.

A second objective of this study was to determine if the slope of the relationship between CBM and THg differed between males and females at any of the three sampling locations. When males and females were analyzed separately, the interaction term between CBM and sex was not significant, nor were there any statistical differences among the slopes of the three regression lines (Spokane River: ANCOVA, $F_{1,49} = 0.001$, $p = 0.63$; Hangman Creek: ANCOVA, $F_{1,73} = 0.007$, $p = 0.93$; Boise River: ANCOVA, $F_{1,38} = 0.02$, $p = 0.88$).

The relationship between sex and mercury concentration in crayfish adductor muscle is not consistent across different studies, as some have found a significant impact of sex (Heit and Fingermann 1977; Hothem et al. 2007), while others have not (this study, Pennuto et al. 2005, Johnson et al. 2014). The lack of a difference in THg between males and female crayfish is curious (Fig. 2) in that it was assumed that mercury would be present in higher concentrations in animals with greater lipid content (Ghaeni et al. 2015). While

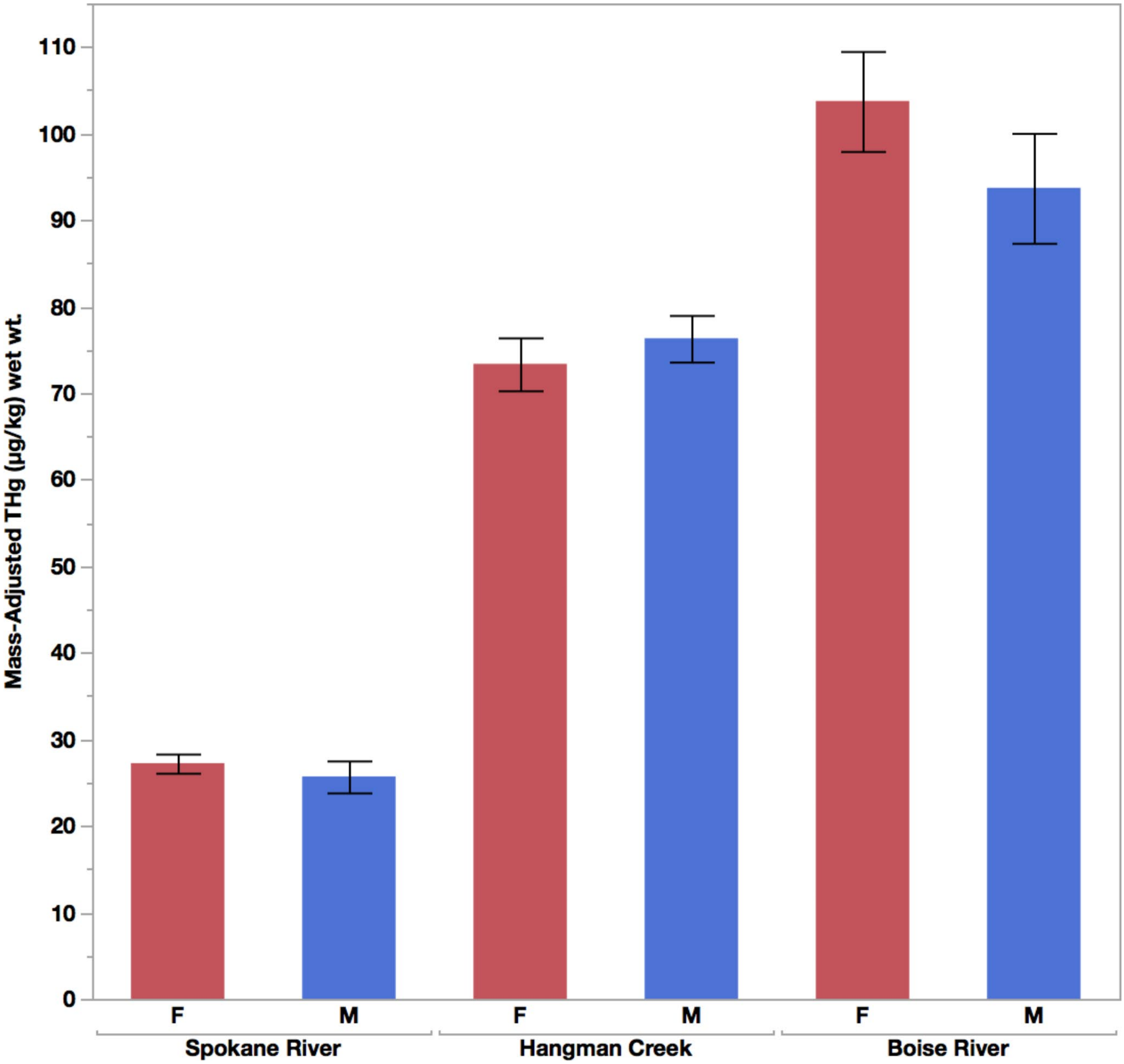

Fig. 2 Mass-adjusted THg (µg/kg) wet wt. ± SE for male (blue) and female (red) signal crayfish captured from the Spokane River, Boise River, and Hangman Creek

the production of eggs necessitates that females will have higher lipid content than males, at least for a portion of the year, our results do not suggest that there is an appreciable difference in the kinetics of THg within male and female signal crayfish.

When male and female signal crayfish were analyzed together, the relationships between THg and CBM were statistically significant for animals collected from the three rivers (Fig. 1; Table 1). When the three relationships were analyzed together, the interaction term between mass and site location was not significant, nor were there any statistical differences in the slopes of the three regression lines (ANCOVA, $F_{2,166} = 1.5$ $p = 0.24$).

While the current study found a significant relationship between THg and body size, Hothem et al. (2007) did not. One difference between the two studies was sample size, as the maximum number of animals collected by Hothem et al. (2007) was 10, whereas the minimum sample size in the current study was 42. While it is common practice to size adjust the animal in studies comparing mercury concentrations among sites and taxa (Sonesten 2003; Selch et al. 2019; Katner et al. 2010), the discrepancy between our results and Hothem et al. (2007) may be due to differences in sample size. Given that the explanatory power (r^2, Table 1) of these linear relationships is quite low, it may be necessary to acquire sample sizes considerably larger than 10 to demonstrate the significant relationship between size and THg in signal crayfish.

In this study, we found that the relationships between CBM and THg were consistent from one site to the next, even though the mean THg varied dramatically across site locations. We are unaware of any other study that compared the allometric relationship in THg across multiple sites for signal crayfish. One study (Fowlie et al. 2008) featuring four teleost fish species, found that allometric relationships in THg were not consistent across contaminated and uncontaminated sites. They speculated that the observed variation was due to differential feeding habits between fish from the two locations. While evaluating the feeding habits of crayfish was outside the scope of this paper, the fact that crayfish are opportunistic scavengers suggests that they consume dead animal protein from across many different trophic levels. Consequently, the omnivorous nature of crayfish feeding (Parkyn et al. 2001) reduces the likelihood that trophic complexity influences mercury bioaccumulation. This may be a fundamental difference between crayfish and teleost fishes.

The use of invertebrates to monitor the environment for mercury is becoming common place (Eagles-Smith et al. 2020; Hannappel et al. 2021). However, such use necessitates standardization for factors such as sex and mass. For signal crayfish from the Columbia River Basin, such standardization can be accomplished with a single linear relationship.

Acknowledgements This project was funded in part by the US Environmental Protection Agency assistance agreements (RB 01J73101-0 and 44-02J15801-0). Volunteers from the Boise River Enhancement Network, Spokane Riverkeepers and the Salish School of Spokane assisted in the collection of these crayfish.

Authors' contributions Conceptualization, Methodology, Analysis and Investigation, Writing-Review-Editing, TEL, ASK; Supervision: ASK.

References

Aluma E, Johnson KS, Hassett P (2017) Mercury bioaccumulation in crayfish in acid mine-impaired Appalachian streams. Water Air Soil Pollut 228:1–13

Anderson MB, Preslan JE, Jolibois L, Bollinger JE, George WJ (1997) Bioaccumulation of lead nitrate in red swamp crayfish (*Procambarus clarkii*). J Hazard Mater 54(1–2):15–29

Başusta N, Khan U (2021) Sexual dimorphism in the otolith shape of shi drum, *Umbrina cirrosa* (L.), in the eastern Mediterranean Sea: Fish size–otolith size relationships. J Fish Biol 99(1):164–174

Eagles-Smith CA, Willacker JJ, Nelson SJ, Flanagan Pritz CM, Krabbenhoft DP, Chen CY, Ackerman JT, Grant EHC, Pilliod DS (2020) A national-scale assessment of mercury bioaccumulation in United States National Parks using dragonfly larvae as biosentinels through a citizen-science framework. Environ Sci Technol 54:8779–8790. https://doi.org/10.1021/acs.est.0c01255

Fowlie AR, Hodson PV, Hickey MBC (2008) Spatial and seasonal patterns of mercury concentrations in fish from the St. Lawrence River at Cornwall, Ontario: implications for monitoring. J Great Lakes Res 34(1):72–85

France R, Holmes J, Lynch A (1991) Use of size–frequency data to estimate the age composition of crayfish populations. Can J Fish Aquat Sci 48(12):2324–2332

Ghaeni M, Pour NA, Hosseini M (2015) Bioaccumulation of polychlorinated biphenyl (PCB), polycyclic aromatic hydrocarbon (PAH), mercury, methyl mercury, and arsenic in blue crab Portunus segnis from Persian Gulf. Environ Monit Assess 187:1–9

Goretti E, Pallottini M, Ricciarini MI, Selvaggi R, Cappelletti D (2016) Heavy metals bioaccumulation in selected tissues of red swamp crayfish: an easy tool for monitoring environmental contamination levels. Sci Total Environ 559:339–346

Hannappel MP, Chumchal MM, Drenner RW, Kennedy JH, Barst BD, Castellini JM, Nolan AR, Willoughby FM, Trauffler LP (2021) Effect of body size on methyl mercury concentrations in shoreline spiders: implications for their use as sentinels. Environ Toxicol Chem 40:1149–1154. https://doi.org/10.1002/etc.4964

Heit M, Fingerman M (1977) The influence of size, sex and temperature on the toxicity of mercury to two species of crayfishes. Bull Environ Contam Toxicol 18:572–580

Hothem RL, Bergen DR, Bauer ML, Crayon JJ, Meckstroth AM (2007) Mercury and trace elements in crayfish from Northern California. Bull Environ Contam Toxicol 79:628–632

Johnson BL, Willacker JJ, Eagles-Smith CA, Pearl CA, Adams MJ (2014) Invasive crayfish as vectors of mercury in freshwater food webs of the Pacific Northwest. Environ Toxicol Chem 33(11):2639–2645

Katner A, Sun MH, Suffet M (2010) An evaluation of mercury levels in Louisiana fish: trends and public health issues. Sci Total Environ 408(23):5707–5714

Kouba A, Buřič M, Kozák P (2010) Bioaccumulation and effects of heavy metals in crayfish: a review. Water Air Soil Pollut 211:5–16

Kuklina I, Kouba A, Buřič M, Horká I, Ďuriš Z, Kozák P (2014) Accumulation of heavy metals in crayfish and fish from selected Czech reservoirs. *BioMed research international*, *2014*

Millard GD, Driscoll CT, Montesdeoca M, Yang Y, Taylor M, Boucher S, Shaw A, Richter W, Paul E, Parker C, Yokota K (2020) Patterns and trends of fish mercury in New York State. Ecotoxicology. https://doi.org/10.1007/s10646-020-02163-x

Mueller KW, Serdar DM (2002) Total mercury concentrations among fish and crayfish inhabiting different trophic levels in Lake Whatcom, Washington. J Freshw Ecol 17(4):621–633

Parkyn SM, Collier KJ, Hicks BJ (2001) New Zealand stream crayfish: functional omnivores but trophic predators? Freshw Biol 46(5):641–652

Pennuto CM, Lane OP, Evers DC, Taylor RJ, Loukmas J (2005) Mercury in the northern crayfish, *Orconectes virilis* (Hagen), in New England. USA Ecotoxicol 14:149–162

Selch TM, Chipps SR, Blackwell BG, Hanten RP (2019) Influence of season, sex, age and diet composition on mercury concentration in walleye *Sander vitreus*. Arch Environ Contam Toxicol 77(3):336–343

Sonesten L (2003) Fish mercury levels in lakes—adjusting for hg and fish-size covariation. Environ Pollut 125(2):255–265

Thomas SM, Melles SJ, Mackereth RW, Tunney TD, Chu C, Oswald CJ, Bhavsar SP, Johnston TA (2020) Climate and landscape conditions indirectly affect fish mercury levels by altering lake water chemistry and fish size. Environ Res 188:109750

Wright DR, Hamilton RD (1982) Release of methyl mercury from sediments: effects of mercury concentration, low temperature, and nutrient addition. Can J Fish Aquat Sci 39(11):1459–1466