Mercury in the Northern Crayfish, *Orconectes virilis* (Hagen), in New England, USA

CHRIS M. PENNUTO,[1,]* OKSANA P. LANE,[2] DAVID C. EVERS,[2] ROBERT J. TAYLOR[3] AND JEFF LOUKMAS[4]

[1]*Department of Biology & Center for Great Lakes Research, Buffalo State College, Buffalo, NY, USA, 14222*
[2]*BioDiversity Research Institute, Gorham, ME, USA04038*
[3]*Trace Element Research Lab, Texas A & M University, College Station, TX, USA77843*
[4]*New York State Department of Environmental Conservation, 625 Broadway, Albany, NY12233-4756*

Abstract. Biologists and policy makers continue to seek environmental correlates of mercury bioavailability in aquatic ecosystems. In this study, we assessed the effects of drainage basin, habitat type, size class, and sex on mercury concentrations in the northern crayfish, *Orconectes virilis* (Hagen). Drainage basin, habitat type, and size class had significant effects on mercury concentration in crayfish tail muscle even though animals from roughly half the sites examined had mean mercury values at or below expected background levels. The low observed mercury values in crayfish tail muscle indicate a low consumptive risk. Contrary to expectations, crayfish from brooks had higher mercury concentrations than animals from other habitat types, possibly as a result of point source contamination or varying diet compositions among habitats. We suggest that crayfish represent a good indicator of mercury bioavailability in aquatic ecosystems and provide a synthesis for lower food webs. Our understanding of mercury dynamics in lower food webs has been hindered by an under appreciation of the complexity in foraging habits of macroinvertebrates. Further studies focusing on benthos with well-understood life histories and foraging behavior are essential to improve our understanding of mercury transfer and bioavailability through aquatic systems.

Keywords: trophic level; lower food webs; benthic macroinvertebrates; mercury

Introduction

Mercury pollution remains a priority contaminant concern for resource managers and policy makers. There have been numerous studies over the last 30 years investigating the health and ecological risks associated with mercury exposure (EPA, 1998). In 2002, every state except Alaska and Wyoming issued a fish consumption advisory for at least some waters within the state and 28 states had general statewide advisories related to mercury. The total lake area under advisory restrictions continues to increase (EPA, 2002). More recently, the environmental and wildlife risks associated with mercury and methylmercury contamination have also been documented (Wren et al., 1983; Lucotte et al., 1999; Evers et al., 2003). Clearly, mercury contamination in aquatic ecosystems is a significant human and environmental

*To whom correspondence should be addressed:
E-mail: pennutcm@buffalostate.edu.

health issue. Determining the environmental factors responsible for mercury levels observed within organisms remains elusive although several key parameters have been correlated with high mercury or methylmercury levels in fish. For example, increasing trophic position (Spry and Weiner, 1991; Jackson, 1998), body size (Scott and Armstrong, 1972; Scott, 1974; Wren et al., 1983), age (Simonin et al., 1994), dissolved organic carbon (DOC) concentration (Driscoll et al., 1995; Wiener and Spry, 1996), pH (Cope et al., 1990; Grieb et al., 1990; Haines et al., 1992), food web complexity (Futter, 1994; Wong et al., 1997; Chen et al., 2000), degree of flooding (Jackson, 1991; Johnston et al., 1991; McNicol et al., 1997), and percent of watershed with wetland habitats (Jackson, 1988; McMurty et al., 1989), have all been variously associated with mercury levels in fish or fish-eating birds and mammals.

Whereas there has been much research on mercury levels in fish, comparatively little is known about the patterns of mercury contamination in benthic macroinvertebrates. Yet, most research suggests that mercury uptake occurs primarily through consumption (Rogers, 1994), and benthic organisms comprise an important energy link in the diet of many fish species for which advisories are posted. For example, yellow perch (*Perca flavescens* Mitchill) remain primarily insectivorous until roughly 4 year, and the stream-dwelling salmonids maintain macroinvertebrate-biased diets throughout their lives. Even the large piscivores (*e.g.*, northern pike (*Esox lucius* L.), walleye (*Scizostedion vitreum* Mitchill), smallmouth (*Micropterus dolomieui*), and largemouth bass (*Micropterus salmoides*), are opportunistic predators of macroinvertebrates, consuming crayfish, large insects, or annelids. Further, macroinvertebrates, especially aquatic insects, represent a high proportion of the diets of many of the forage fish species that are eaten by piscivores, and they are also important food items of fry and juvenile piscivores (Cooper, 1983). In addition to their importance in fish diets, benthic macroinvertebrates are critical components in ecosystem energy flow and matter cycling (*e.g.*, Resh and Rosenberg, 1984), and contaminant release from sediments via their bioturbation activities can be significant (Matisoff and Wang, 1998). Thus, it is essential that we understand the movement of mercury through lower food webs to provide support for patterns in higher food webs.

Benthic invertebrates maintain an intimate association with lake and river sediments and sediments often harbor the highest mercury concentrations in aquatic systems (Meili, 1991; Ullrich et al., 2001). Indeed, invertebrates from mercury-contaminated lakes sometimes have very high Hg concentrations and concentrations that may exceed those of the top predators within the system (*e.g.*, Parkman and Meli, 1993; Tremblay et al., 1995). Suchanek et al. (1995) reported total Hg concentrations in benthic oligochaetes as high as 41.7 μg/g and chironomid midges as high as 27.7 μg/g in Clear Lake, California, a site acutely contaminated by mine-effluents. However, there is not a strong relationship between sediment Hg concentration and invertebrate Hg concentration (Verta et al., 1986; Parkman and Meili, 1993; Tremblay et al., 1995). Substrate geochemistry and particle size distribution, not sediment Hg concentration *per se*, seem to correlate with Hg content in the benthos (Parkman and Meili, 1993; Tremblay et al., 1995). Mercury content in macroinvertebrates does not always increase with increasing body size, unlike the relationship in fish.

The relationship between invertebrate body size and mercury concentration appears to be governed by food web dynamics and the type of mercury measured. Parkman and Meili (1993) and Wong et al. (1997) found a correlation between total Hg concentration and predatory insect weight when compared across all taxonomic groups; however, they did not find a relationship within taxa. They suggested that life cycle duration had little to do with mercury accumulation; rather, trophic status of the invertebrate was the key factor in determining mercury levels. For example, large predatory taxa were more likely to accumulate Hg than small predators of a different taxon, because they were more likely to feed higher on the food chain. However, they also found lower trophic level invertebrates like detritivores and grazers with higher mercury levels than predators (Table 1), indicating that the relationship between total mercury concentration and food chain status is not always valid. Tremblay (1999) found no relationship between size and methylmercury concentrations (which averaged about 80% of the total mercury concentrations) of odonates from boreal

Table 1. Patterns in total mercury concentration of benthic macroinvertebrates in lake and river habitats

	Habitat	Source
Phylogenetic effects on total mercury		
Oligochaeta > Nematoda > Pelecypoda > Chironomidae	Lentic	Jackson (1988)
Heteroptera > Odonata > Coleoptera > Trichoptera > Ephemeroptera > Diptera	Lentic	Tremblay and Lucotte (1997)
Gastropoda > Coleoptera > Oligochaeta > Chironomidae > Amphipoda > Ephemeroptera > Odonata > Trichoptera > Hirudinea	Lentic	Wong et al. (1997)
Megaloptera > Diptera > Trichoptera > Decapoda	Lotic	Hildebrand et al. (1980)
Ephemeroptera = Odonata > Trichoptera	Lentic	Gorski et al. (2003)
Odonata > Ephemeroptera > Decapoda > Trichoptera > Isopoda	Lentic	Loukmas (unpubl. data)
Diptera > Megaloptera > Odonata > Decapoda > Trichoptera > Ephemeroptera > Isopoda > Plecoptera	Lotic	Loukmas (unpubl. data)
Feeding mode effects on total mercury		
Predators > Predator/grazer > Detritivore/grazer > Detritivore	Lentic	Tremblay et al. (1995)
Predators > Herbivores/Detritivores	Lotic	Mason et al. (2000)
Detritivores > Predators	Lentic	Parkman and Meili (1993)
Predators > Detritivores	Lentic	Loukmas (unpubl. data)
Predators > Detritivores	Lotic	Loukmas (unpubl. data)

lakes and reservoirs in Quebec. He also found an increase in MeHg levels with increasing food chain status among all invertebrates, suggesting that, for MeHg accumulation, trophic level is more important than body size, which is a concept supported by other studies (Table 2). He concluded that, because of the highly bioaccumulative nature of MeHg, its availability to benthic invertebrates could play an important role in determining MeHg concentrations throughout the entire aquatic community. An understanding of the uptake, assimilation and depuration rates and pathways within macroinvertebrates is essential to unraveling the bioavailability of mercury in lower food webs.

Inorganic mercury does not easily cross the gut lining of most insects (Boudou et al., 1991), whereas methylmercury crosses readily. Both forms can pass through the gill membranes of invertebrates (*e.g.*, Wright et al., 1991; Odin et al., 1995). This differential membrane discrimination and uptake of mercury species probably plays a

Table 2. Patterns in methylmercury content of benthic macroinvertebrates from lake and river habitats

	Habitat	Source
Phylogenetic effects on methylmercury		
Odonata > Ephemeroptera > Decapoda > Isopoda > Trichoptera	Lentic	Loukmas (unpubl. data)
Megaloptera > Odonata > Decapoda > Trichoptera > Diptera/Isopoda > Plecoptera > Ephemeroptera	Lotic	Loukmas (unpubl. data)
Heteroptera > Odonata > Coleoptera > Trichoptera > Ephemeroptera > Diptera	Lentic	Tremblay and Lucotte (1997), Tremblay (1999)
Diptera > Trichoptera	Lentic	Weins et al. (unpubl. data)
Odonata > Trichoptera = Ephemeroptera	Lentic	Gorski et al. (2003)
Feeding mode effects on methylmercury		
Predators > Detritivores	Lentic	Loukmas (unpubl. data)
Predators > Detritivores	Lotic	Loukmas (unpubl. data)
Predators > Detritivores	Lentic	Gorski et al. (2003)
Predators > Herbivores/Detritivores	Lotic	Mason et al. (2000)
Predators > Detritivores	Lentic	Tremblay (1999)
Predators > Collector/shredders	Lentic	Hall et al. (1998)

significant role in preventing a general sediment Hg/body concentration relationship and reduces the likelihood of a biomagnification pattern for total mercury within benthic food webs. Conversely, methylmercury exhibits biomagnification within benthic food webs, but with extreme variability in the contribution of methylmercury to whole body mercury burdens among different taxa. The percent MeHg of THg in invertebrates ranges from 0 to 100%, with an average across multiple orders and feeding modes of ~65% (e.g., ~40% in grazing mayflies and ~75% in predatory odonates; Huckabee et al., 1979; Hildebrand et al., 1980; Odin et al., 1995; Tremblay et al., 1995; Tremblay and Lucotte, 1997; Fischer and Gustin, 2002). Several studies suggest benthic insects compartmentalize mercury, with high concentrations found in the gut and adsorbed to the exoskeleton (Ellwood et al., 1976; Hildebrand et al., 1980). Because of this, allowing sample organisms to clear their guts and including clean water rinses to collection protocols is recommended for Hg investigations in macroinvertebrates.

Mercury and methylmercury dynamics are better understood in crayfish than any other common freshwater macroinvertebrate. Crayfish are among the largest, longest-lived benthic invertebrates [2–3 years in northern climates (Martin, 1997)], are intimately associated with the substrate, have a ubiquitous distribution, and are an important food item for many organisms. Large, predatory fish species (*e.g.*, smallmouth and largemouth bass and northern pike), multiple fish-eating bird species (*e.g.*, herons, bitterns, loons, or kingfishers), and carnivorous, semi-aquatic mammals (*e.g.*, raccoons, mink, and otters) consume them (Casson and Klimstra, 1983; Martin, 1997). Thus, they represent an important trophic link between benthic and water-column food webs in lakes and streams and have been suggested as excellent indicator species for mercury bioavailability studies (Verneer, 1972; Armstrong and Hamilton, 1973; Allard and Stokes, 1989; Parks, 1988; Parks et al., 1991). They exhibit high site fidelity with small home ranges (Hazlett et al., 1974), thus do not disperse as widely as fish. As omnivores, they function at multiple trophic levels simultaneously and are known to accumulate Hg (Armstrong and Hamilton, 1973; Parks, 1988; Allard and Stokes, 1989; Parks et al., 1991; Evers et al., 2003).

Several studies have indicated Hg concentrations are highest in crayfish abdominal muscle relative to other body compartments (Stinson and Eaton, 1983; Allard and Stokes, 1989; Headon et al., 1996), but the gills are also an important accumulation site (Wright et al., 1991; Simon and Boudou, 2001). Mercury concentrations increase with crayfish size (Armstrong and Hamilton, 1973; Ricon et al., 1987; Parks, 1988; Parks et al., 1991; Evers et al., 2003). Scheuhammer and Graham (1999), however, did not detect a significant body size:Hg concentration correlation when examining *Orconectes virilis* in two Ontario lakes and Lafrancois and Carlisle (2004) found no correlation between THg and body weight in *O. virilis* in lakes from Voyageurs National Park. Knowledge of Hg depuration rates and tissue specificity is essential for understanding accumulation rates and body size:concentration correlations. Headon et al. (1996) reported that crayfish assimilated ~ 96% of the Hg in their food, but the assimilation rate differed between size classes. They suggested that small crayfish assimilated Hg more rapidly than large crayfish and that very little Hg was lost after assimilation, estimating a half-life in abdominal muscle of 3–5 years. Wright et al. (1991), however, suggested a much shorter half-life of only about 2 days for laboratory crayfish fed $HgCl_2$ as part of their diet. Methylmercury half-lives within abdominal muscle are very long, leading to bioaccumulation within individuals, and MeHg may account for >75% of the total mercury found in crayfish (Headon et al., 1996). In northeastern North America, background mercury concentrations in crayfish tail muscle are expected to be ≤ 0.1 μg/g based on surveys of natural lakes in remote Canadian locations removed from any potential point-source contamination (Parks and Hamilton, 1987).

In this report, we summarize four years of data on the mercury content in the northern crayfish, *Orconectes virilis* (Hagen), from multiple habitat types and drainage basins in New England. We sought to determine if total mercury in crayfish tail muscle was predicted by drainage basin, habitat type, or sex. We also investigated size:Hg content relationships across a broad range of habitats to see if a general size class might represent maximum risk to Hg exposure. Further, we examined methyl and total mercury distribution patterns in crayfish

tail muscle and body tissues to determine if health risks from crayfish consumption might differ between humans and wildlife, since humans tend to eat only tail muscle whereas wildlife tend to consume the entire crayfish. Based on previous work, we anticipated a significant body size:Hg concentration correlation. We also anticipated the highest mercury concentrations would be found in crayfish from reservoirs, and the lowest concentrations in crayfish from natural ponds or small brooks due to effects of water level fluctuation and the small watershed drainage area associated with low order streams. Lastly, we expected methylmercury to represent >75% of the total mercury in crayfish and tail muscle tissue to contain greater Hg and MeHg than the remaining body parts.

Materials and methods

Crayfish collections

Northern or virile crayfish, *Orconectes virilis* (Hagen), were collected as part of various monitoring programs and studies conducted by Bio-Diversity Research Institute (BRI) in New Hampshire, Vermont, and Maine (Fig. 1). Animals were collected from brooks ($n = 12$), ponds ($n = 8$), rivers ($n = 5$), and reservoirs ($n = 3$) from four major drainage basins (Connecticut, Passumpsic, Dead, and Androscoggin Rivers) (Table 3). Brooks and rivers were sampled primarily with baited minnow traps, whereas crayfish from ponds and lake coves were obtained primarily by hand capture. All animals were collected between August and October 2000–2003 and upon capture, blotted wet weight, total length (cm), carapace width and length (cm), and sex were determined. We used three size classes to investigate body size:Hg concentration relationships: small (4–6 cm TL), medium (6–8 cm), and large (>8 cm). These lengths correspond roughly to 2, 3, and 3+ year old animals (Armstrong and Hamilton, 1973). Crayfish were double-encased in plastic wrap, placed in ziptop plastic bags, and frozen until analysis.

Most animals from the VT and NH sampling locations were analyzed as individuals, but ME crayfish were generally analyzed as composite samples of 2–9 animals. Composites only included same-sized animals, thus these data were used in the size class analyses. However, sex effects were investigated without the composite samples since composites often contained mixed collections.

Chemical analysis

Analyses of Hg in crayfish abdominal muscle were performed at Texas A & M University Trace Element Research Laboratory (TAMU), College Station, Texas. Tail muscle was removed from each crayfish, homogenized wet with an OMNI Mixer equipped with titanium blades, lyophilized in a Labconco Lyph Lock 12 freeze dryer, and powdered in a Spex Mixer Mill. Moisture was determined by weight loss upon freeze-drying. All VT and NH animals were analyzed individually whereas ME animals were composites of 2–9 animals of similar size. Total Hg (THg) was determined for all crayfish and methylmercury (MeHg) was determined for a subset of animals from the Connecticut River system (Moore Reservoir, $n = 13$). Methylmercury was determined in both tail muscle and remaining body parts, exclusive of the exoskeleton, for this subset.

Samples were analyzed for Hg by cold-vapor atomic absorption (CVAA) using the method of Hatch and Ott (1968). Digestion procedures incorporated concentrated sulfuric and nitric acids and heating at 95 °C and used both potassium permanganate and potassium persulfate. Following digestion, excess permanganate was reduced with hydroxylamine hydrochloride and samples were diluted to final volume (50 ml).

Mercury content of crayfish digests was measured using an LDC Hg Monitor equipped with a 30 cm cell. One ml aliquots of sample digests were added to a closed reaction cell where the Hg^{2+} was reduced to volatile Hg^0 with 1 ml of 10% w:v $SnCl_2$ in 10% HCl. Mixing and release of Hg^0 to the headspace were facilitated by vortexing the reaction cell on a Vortex Genie 2. The headspace was introduced to the CVAA absorption cell using a syringe pump set to deliver 20 ml of deionized water to the bottom of the reaction cell. This injection approach avoided dilution of the Hg^0 in the headspace that would have resulted from the use of a sweep gas. Calibration was accomplished using commercial standards (CPI) diluted as necessary with 5% HNO_3/1% HCl, and included a
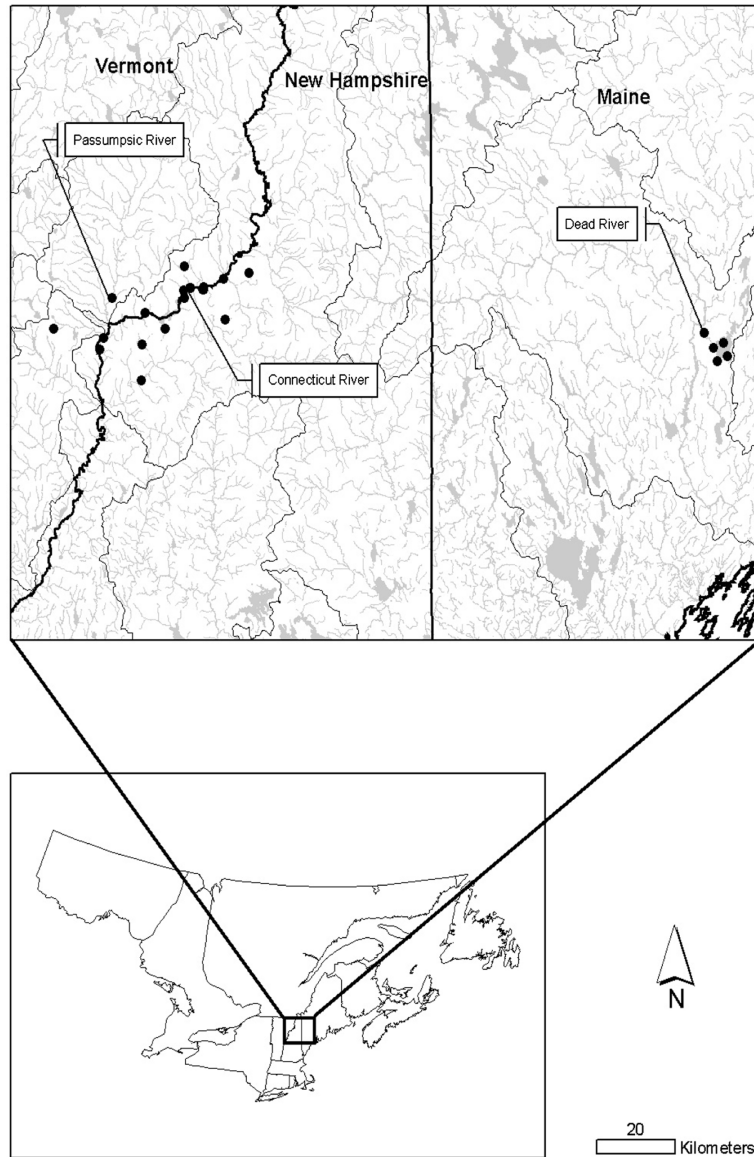
Figure 1. *Orconectes virilis* collection sites. Watershed boundaries are indicated.

blank and five standards that ranged from 1 to 10 ppb. The calibration line was calculated using unweighted linear regression and verified using an independent standard and a blank that were analyzed following calibration, after every 10 samples, and at the end of the analysis. Hg absorbance was measured using a Shimadzu CR601 integrator in peak height mode. Each batch of Hg samples included a method blank, spiked blank, reference material (NRCC DORM-2), duplicate sample,

and spiked sample. The detection limit for total Hg was 0.002 μg/g on a wet weight basis.

A subset of 13 crayfish was analyzed for MeHg using the method of Wagemann et al. (1997). MeHg was extracted from freeze dried samples with acidic KBr and CuSO4 solutions into a 2:3 mixture of hexane:methylene chloride. Extracted MeHg was digested and measured by element-specific detection of Hg via CVAAS, using methods similar to those described above but modified

Table 3. Orconectes virilis collection sites

Drainage basin	Sampling site	State	Habitat type	Number of *O. virilis* analyzed[a]	Mean THg concentration (µg/g, ww)
Amonoosuk	Amonoosuk	NH	1	2	0.044
Androscoggin	Androscoggin River	ME	1	5[a]	0.120
Connecticut	Carpenter Brook	VT	4	1	0.216
	Comerford Reservoir	NH	3	12	0.101
	Connecticut River	NH	1	14	0.163
	Cow Brook	VT	4	2	0.094
	Cutting Brook	VT	4	10	0.099
	Dodge Pond	NH	2	2	0.038
	Forest Lake	NH	2	2	0.044
	Martin Meadow Pond	NH	2	7	0.034
	Martin's Pond	VT	2	10	0.023
	McIndoe Reservoir	NH	3	11	0.089
	Miles Brook	VT	4	10	0.155
	Miles Pond	NH	2	1	0.122
	Moore Reservoir	NH	3	15	0.186
	Mulliken Brook	NH	4	11	0.070
	Roaring Brook	VT	4	11	0.188
	Un-named Brook	NH	4	4	0.091
Dead	Androscoggin Lake	ME	2	9[a]	0.119
	Bear brook	ME	4	2[a]	0.164
	Bog Brook	ME	4	1[a]	0.045
	Cove Road Brook	ME	4	3[a]	0.550
	Dead River	ME	1	11[a]	0.218
	Decoster's Stream	ME	4	2[a]	0.186
	Gould Brook	ME	4	8[a]	0.161
	Mill Pond	ME	2	4[a]	0.063
	Pocasset Lake	ME	2	6[a]	0.091
Passumpsic	Passumpsic River	VT	1	6	0.044

[a] Indicates composite sample of 2–9 individuals.

as necessary for reduced sample sizes. MeHg QC samples were similar to those included for total Hg analysis, with the difference that standards and spikes were prepared from CH_3HgCl (Johnson Matthey). MeHg is certified in DORM-2 at 4.47 µg/g. The detection limit for MeHg was 0.0008 µg/g on a wet weight basis.

Statistical procedures

All statistical analyses were carried out on log-transformed THg data to meet variance assumptions. We used a MANOVA to determine whether drainage basin (n = 4), habitat type (n = 4), or size class (n = 3) provided significant information to explain the mercury concentration in crayfish tail muscle. A one-way ANOVA followed by Tukey's HSD *post-hoc* multiple comparison test was applied to all variables with significant effects

on mercury concentration. Compartmentalization of mercury (tail muscle versus body) and percent methylmercury in the two body regions was assessed with separate *t*-tests and the crayfish size:Hg concentration relationship was examined using linear regression. All analyses were performed using Statistix$^{©}$ for Windows (Analytic Software, Tallahassee, FL) with $\alpha = 0.05$.

Results

Crayfish were collected from 28 sites and total mercury in tail muscle was determined for 182 samples among all sites, 41 of which were composite samples (Table 3). There was no significant difference in mean weight of animals among the four drainage basins ($F_{3,178} = 0.44$; $p = 0.722$) and length explained over 91% of the variance in body

mass (Fig. 2). Crayfish from the Passumpsic River basin grew more rapidly than crayfish from the remaining basins as indicated by the much steeper regression slope (*b*) in the weight/length plots (*b* = 0.608, 0.199, 0.216, 0.221 for Passumpsic, Androscoggin, Connecticut, and Dead River basins, respectively). Quality assurance and quality control results for both total and methylmercury were well within accepted levels, generally resulting in % recoveries in spiked samples between 93 and 103% and RPD under 10% (Table 4).

There was a significant relationship between *O. virilis* length (cm) and THg concentration in tail muscle ($F_{1,180} = 18.93$; $p \ll 0.001$), but length only explained a small proportion of the observed variance in mercury ($R^2 = 0.095$; Fig. 3). Even though mercury concentration in tail muscle was variable across sites, there were significant drainage basin, habitat type, and size class effects on mercury concentration in crayfish (all $p < 0.05$). There was not a significant sex effect on mercury concentration ($p > 0.05$). One-way ANOVAs and *post-hoc* Tukey tests confirmed the lack of a sex effect on mercury content in crayfish and significance for remaining effects (Table 5, Fig. 4). Crayfish from the Dead River drainage basin in Maine contained significantly higher mercury than crayfish from the Connecticut and Passumpsic drainages, but were not significantly different from crayfish in the Androscoggin (Fig. 4). Thus, the eastern most sites had mercury levels higher than the western sites. Among habitat types, crayfish from natural ponds had significantly less mercury than crayfish from streams, rivers, or reservoirs (Fig. 4), regardless of drainage basin. The largest crayfish size class had significantly higher total mercury content than the medium and the small size classes (Fig. 4).

Table 4. Mean percent recovery (spiked blanks, spiked samples, and SRM's) and mean relative percent difference (RPD; duplicate samples) results for total and methylmercury QA/QC testing

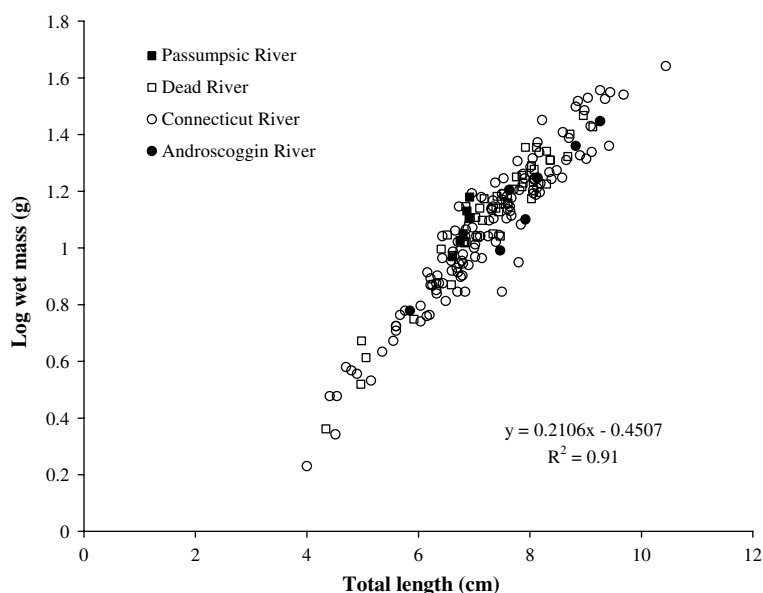|	Spiked blanks	SRM	Duplicate samples	Spiked samples
Total mercury				
Mean	102.5	95.5	8.1	101
Standard error	0.96	1.04	1.86	2.44
Sample size	13	14	14	13
Maximum	107	100	18	108
Minimum	95	88	0	76
Methylmercury				
Mean	99	96.5	3	93
Standard error		0.50		2.00
Sample size	1	2	1	2
Maximum		97		95
Minimum		96		91

$$y = 0.2106x - 0.4507$$
$$R^2 = 0.91$$

Figure 2. Length/mass relationship for crayfish collected in four drainage basins in New England.

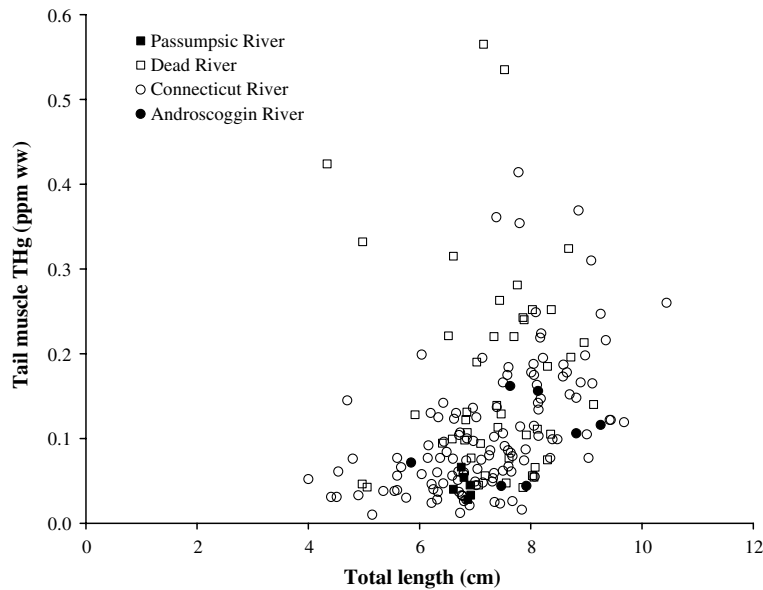*Figure 3*. Scatterplot of tail muscle mercury content (µg/g ww) as a function of crayfish length from four New England drainage basins.

Table 5. Results of one-way ANOVAs determining effects on tail Hg concentration (µg/g, ww) in crayfish (*Orconectes virilis*) across New England

	df	F	P
Drainage basin	3.178	7.34	< 0.001
Habitat type	3.178	11.76	≪ 0.001
Size category	2.179	9.19	< 0.001
Sex	1.143	0.01	n.s.

Drainage basin, habitat type, and size categories identified in Methods. Data include site composite samples for drainage basin, habitat type, and size categories, but not sex.

The % methylmercury in tail muscle tissue was not significantly different from the % methylmercury in the remaining body parts ($t = 0.64$, df = 24, $p = 0.528$), nor was there any difference in the concentration of MeHg in the two body regions ($t = 1.39$, df = 24, $p = 0.176$; Fig. 5). MeHg averaged 88% of the total mercury in crayfish and did not show any correlation with crayfish size ($r = 0.455$, df = 11, $p = 0.159$). Tail muscle THg (µg/ g ww) was not significantly different from the remaining body THg concentration ($t = 1.31$, df = 24, $p = 0.202$; Fig. 5).

Discussion

Mercury concentrations exhibited high variability in the crayfish *Orconectes virilis* across three New England states, and half of the sites (14 of 28 or 50%) had representative animals with mercury levels at or above expected background concentrations (≤ 0.1 µg/g; Parks and Hamilton, 1987). This background concentration represents mercury levels detected in crayfish from isolated water bodies located in pristine habitats of Canada with geology and vegetation similar to our study areas. Three of the four variables examined had significant effects on mercury concentration in crayfish tail muscle: drainage basin, habitat type, and crayfish size class. The remaining variable, sex, had no significant effect on mercury concentration in crayfish. Whereas other studies have found strong correlations between water chemistry variables and mercury content in organisms (e.g., pH and DOC; Allard and Stokes, 1989; Cope et al., 1990; Gorski et al.,
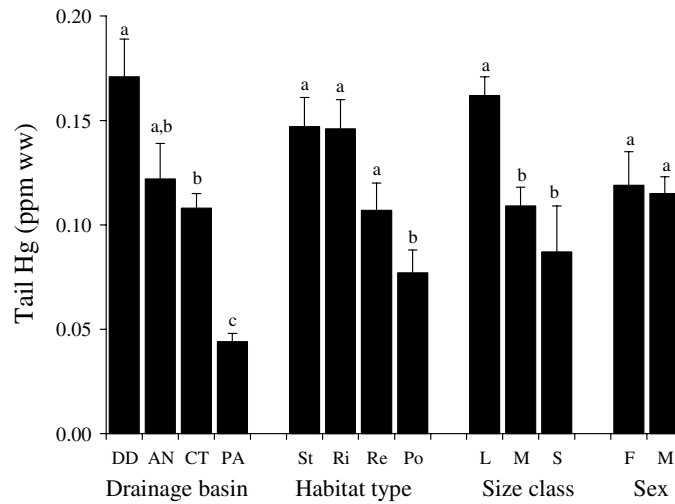
Figure 4. Tukey LSD *post-hoc* results for drainage basin, habitat type, and size class effects on mercury concentration in crayfish. Same letters within each main effect are not significantly different from each other at $p < 0.05$. For drainage basins, DD = Dead River, Maine; AN = Androscoggin River, Maine; CT = Connecticut River, Vermont/New Hampshire; PA = Passumpsic River, Vermont. For habitat type, St = stream or brook; Ri = river; Re = reservoir; Po = pond/natural lake. For size class, L = large (≥8 cm); M = medium (6 8 cm); S = small (4–6 cm). Bars represent 1 SE.
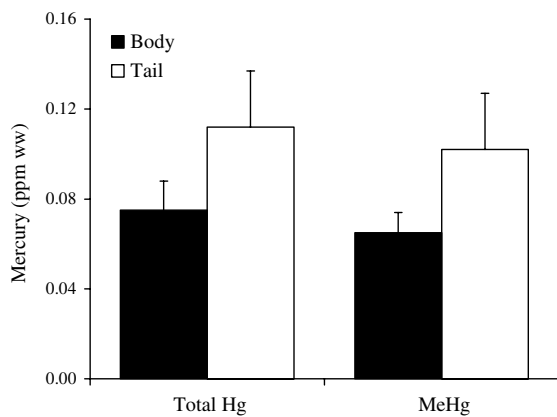

Figure 5. Total and methyl mercury concentration in tail muscle and the remaining body for the crayfish *Orconectes virilis* from Moore Reservoir, VT/NH.

2003), we focused on larger-scale variables across a large geographic area.

In Maine, 70% (7 of 10) of the sample sites exceeded the expected background level, but only a single site had mercury levels exceeding 0.3 µg/g, the lowest observed effects level (LOEL) concentration for reproductive impairment in Common Loons (*Gavia immer*) (Barr, 1986). The mercury concentration in crayfish from this site (Cove Road Brook) averaged 0.55 µg/g ($n = 3$ composite

analyses), a value well above the 0.1 µg/g expected background level. In NH and VT, 50% of the sites exceeded background expectations, but no sites exceeded the 0.3 µg/g LOEL concentration for Common Loons. These data indicate that crayfish in three of four drainage systems would not pose significant risks to foraging wildlife and that *O. virilis* serves as a good site indicator for Hg, identifying a potential point-source contamination site at Cove Road Brook. Since drainage basins, by definition, have hydrologic connectivity, it is not surprising to observe a significant drainage effect on mercury content as within drainage concentrations should be more similar than between drainage concentrations, especially over the rather short geographic distance sampled during this study. Even so, there was a suggestion of a west-to-east increase in Hg concentration similar to that detected by Evers et al. (1998) for mercury concentrations in loons across a much larger geographic area of northern North America. Lafrancois and Carlisle's (2004) recent finding of a low average mercury level in *O. virilis* of Voyageur National Park (0.06 µg/g) also supports an easterly increase in crayfish mercury content. Our sites did not differ appreciably in elevation or geology (based on surficial geology maps), thus landscape position and watershed geochemistry

likely do not account for the spatial pattern of increased mercury.

The pattern of mercury concentration among habitat types differed from our expectation that reservoir crayfish would contain the highest mercury and stream-dwelling or pond crayfish would contain the lowest. Although crayfish from ponds and natural lakes contained the lowest mercury, stream and river animals exhibited higher levels than we expected. We anticipated that the low watershed area associated with small order streams would reduce the residence time of water within a basin, thus reducing time available for mercury accumulation or methylation. Perhaps the intimate association between small brooks and their surrounding forest soils, prior to significant dilution from larger water bodies, affects mercury retention in these systems (Allan, 1995). Alternatively, Hogger (1988) suggests pond-dwelling crayfish may be more herbivorous than lotic crayfish. If pond-dwelling *O. virilis* are more herbivorous than stream-dwellers, they might be expected to have lower Hg levels since plant tissue is low in Hg relative to animal tissue. Mercury isotopic analyses have been used to detect the sources of mercury for sediments and food webs in Lake Ontario (Jackson, 2001), and would prove valuable in determining the dietary Hg source (animal or vegetable matter) for animals from different habitat types.

Large crayfish (≥8 cm TL), regardless of habitat type or drainage, had higher total mercury concentrations than medium or small crayfish, indicating Hg accumulation occurs and that depuration rates are very low. This observation supports the conclusion of Headon et al. (1996) that Hg half-life in crayfish muscle is very long (3–5 years). Methylmercury represented nearly 90% of total mercury in all the crayfish examined, in agreement with our expectation and work of Lafrancois and Carlisle (2004) (88% MeHg in *O. virilis* from VNP). However, we did not find a significant correlation between % MeHg and crayfish size for animals from Moore Reservoir (the only site where methylmercury was measured), even though this subset of animals spanned nearly the entire size range across the whole study area. Additionally, the mercury concentration (both MeHg and THg) in tail muscle did not differ from the remaining body parts. This observation is contrary to the findings of several others (Stinson and Eaton, 1983; Allard and Stokes, 1989; Headon et al., 1996). Greater understanding of the foraging behavior of these animals may help explain the observed patterns. Crayfish diet composition often shifts from a dominance of animal matter in juveniles to plant matter in adults ≥2.5 year old (Goddard, 1988). Given the apparent long half-life of MeHg in these animals, if juveniles consume a larger fraction of animal matter than adults and if animal matter contains higher methylmercury content than plant material (Watras et al., 1998), then MeHg concentrations should reach high values early and remain stable with age or show only a slight increase since animal matter is not completely eliminated from the diet of mature crayfish. We recommend that further studies examine methylmercury and diet composition across a range of crayfish sizes within a system to evaluate this expectation.

The lack of a significant compartmental difference in both MeHg and THg was surprising since other studies have shown large differences in mercury concentrations in multiple body parts (Stinson and Eaton, 1983; Allard and Stokes, 1989; Wright et al., 1991; Headon et al., 1996; Simon and Boudou, 2001). Potentially, our decision to quantify Hg in just two body compartments (i.e., tail muscle vs rest of the body, exclusive of the exoskeleton) masked some of the variability. For example, gill tissue is a significant Hg accumulation site (Simon and Boudou, 2001) that was analyzed with the 'body' fraction in our study, potentially elevating observed body burdens. The similarity in body and tail muscle mercury concentrations suggests wildlife dietary exposure per unit tissue mass consumed is roughly the same as the dietary exposure experienced by humans consuming *O. virilis* since there was no apparent compartmentalization of mercury. Our findings also indicate that tail muscle concentrations are a good index of whole animal mercury and methylmercury, providing a good parameter for mass balance investigations within lower food webs.

Conclusions

As a polytrophic consumer, crayfish are in a unique position in benthic aquatic food webs,

occupying a large range of feeding levels at the same time, from detritivore to carnivore (Hogger, 1988; Creed, 1994). By consuming detritus, macrophytes, and animal tissue, Hg concentrations in crayfish are likely directly dependent on abiotic and microbial processes, as well as aquatic food web dynamics. Their generic feeding habits and desirability as a food item make crayfish an important link in the transfer of mercury in aquatic systems. Their high site fidelity, relatively long life, ubiquitous distribution throughout the northeastern United States and southeastern Canada, and consistently high percent MeHg enhance the value of *O. virilis* as an indicator of local mercury bioavailability. However, our unexpected results regarding habitat-, body size-, and compartmentalization-Hg relationships illustrate the need to further our understanding of mercury movement in aquatic food webs.

The details of mercury movement through lower trophic webs have been underappreciated until recently. The large and often dominant biomass that benthic macroinvertebrates represent in aquatic systems, plus their essential role as prey for higher consumers, suggests they will be critical to a complete understanding of mercury transfer in freshwaters. The large variability in functional feeding modes with major invertebrate groups precludes finding strong phylogenetic relationships between mercury content and benthic macroinvertebrates. However, a focus on individual taxa with well-documented life history and feeding behavior may prove valuable in generating patterns of mercury contamination within the benthos. For example, the Odonata and Belostomatidae represent some of the largest insect predators in freshwater lakes and ponds. The Odonata (all Families) are obligatory macrophagous predators consuming their prey whole, whereas the Belostomatidae are obligatory, fluid sucking predators within the Hemiptera. The odonates should receive a larger mass of mercury per prey consumed since they eat the entire prey item. The belostomatids, by consuming the liquefied internal parts of their prey, should not receive any mercury adsorbed to the exoskeleton nor any mercury adsorbed to detritus within the prey's gut, a significant mercury compartment in benthic organisms (Ellwood et al., 1976; Hildebrand et al., 1980). However, belostomatid diets should be

higher quality diets with lower C:N:P ratios since chitin is a nitrogen and phosphorous-deficient biomolecule (Sterner and Elser, 2002). The diet mass also is not diluted by the disproportionately large mass of the exoskeleton of insect prey. Together, these diet qualities should make their ingested mercury concentration higher per prey mass consumed. Future research focusing on mercury concentrations and bioaccumulation in benthic consumers with clearly detailed dietary habits, like odonates and belostomatids, will shed light on mercury transfer pathways and accumulation in lower food webs of aquatic ecosystems.

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