Predictive models of benthic invertebrate methylmercury in Ontario and Quebec lakes

M.D. Rennie, N.C. Collins, C.F. Purchase, and A. Tremblay

Abstract: Multivariate analyses on benthic invertebrate methylmercury concentrations ([MeHg]) and water chemistry from 12 Quebec water bodies were used to guide the construction of simple, predictive models of benthic invertebrate [MeHg] in 23 lakes in Ontario and Quebec. Separate predictive models for collector–shredder and predatory benthic invertebrates were constructed using multiple regression and were assessed for their predictive utility by cross-validation. Predatory benthic invertebrate [MeHg] was negatively related to pH and positively related to dissolved organic carbon (DOC) concentration (cross-validation $r^2 = 0.31$). Collector–shredder [MeHg] was positively related to [DOC] only (cross-validation $r^2 = 0.13$). Predictive utility of our models is similar to or surpasses that observed in previously published zooplankton MeHg models tested against independently collected data. Significant environmental variables and their contribution to the overall explanatory power of benthic invertebrate MeHg models are similar to those found in zooplankton models, suggesting that in both pelagic and benthic food webs, pH and DOC are important indicators of MeHg bioavailability. Although seasonal patterns in invertebrate [MeHg] were examined, none was detected. These models represent an effective means of identifying water bodies of interest for researchers and for reconstructing past benthic invertebrate [MeHg] patterns using archived water chemistry data.

Résumé : Des analyses multidimensionnelles des concentrations de méthylmercure ([MeHg]) chez les invertébrés benthiques et de la chimie de l'eau de 12 milieux aquatiques du Québec nous ont guidés dans l'élaboration de modèles simples et prédictifs des concentrations de [MeHg] chez les invertébrés benthiques de 23 lacs de l'Ontario et du Québec. Nous avons construit des modèles distincts pour les invertébrés benthiques collecteurs–déchiqueteurs et les prédateurs à l'aide de régressions multiples et nous avons évalué par validation croisée leur utilité pour la prédiction. Il y a une relation négative entre [MeHg] chez les invertébrés benthiques prédateurs et le pH et une relation positive avec le carbone organique dissous (DOC; r^2 de validation croisée = 0,31). [MeHg] des collecteurs–déchiqueteurs est seulement en corrélation positive avec [DOC] (r^2 de validation croisée = 0,13). Les potentiels de prédiction de nos modèles sont semblables ou supérieurs à ceux des modèles de MeHg du zooplancton tirés de la littérature et évalués avec des données récoltées de façon indépendante. Les variables significatives du milieu et leur contribution au pouvoir explicatif global des modèles de MeHg des invertébrés benthiques sont semblables à ceux trouvés dans les modèles de zooplancton, ce qui laisse croire que le pH et DOC sont d'importants indicateurs de la biodisponibilité de MeHg, tant dans les réseaux alimentaires pélagiques que benthiques. Bien que nous les ayons recherchées, nous n'avons pas trouvé de tendances saisonnières du [MeHg] chez les invertébrés. Ces modèles sont des moyens efficaces pour identifier les milieux aquatiques intéressants pour les chercheurs et pour reconstituer les patterns de [MeHg] du passé chez les invertébrés benthiques à partir de données de qualité de l'eau accumulées en archives.

[Traduit par la Rédaction]

Introduction

Significant mercury (Hg) contamination of lakes that do not receive obvious point-source Hg inputs has been reported in North America and Europe (Cope et al. 1990; Bodaly et al. 1993; Parkman and Meili 1993). Concern over high contaminant levels (including Hg) detected in waterfowl and fish in the latter part of the 20th century initiated numerous government-issued advisories on safe levels of fish and wildlife consumption for humans (e.g., Ontario Ministry of the Environment 2003). The primary source of Hg in these otherwise "pristine" lakes is direct through atmospheric deposition (Rudd 1995) or indirect via terrestrial runoff (Mierle and Ingram 1991; Hintelmann et al. 2002).

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Yet, organisms in lakes that likely receive similar rates of atmospheric inputs (i.e., are geographically close together) may vary remarkably in Hg concentrations across the same or functionally similar species (Wren and Stephenson 1991; Tremblay et al. 1995; Sonesten 2003). Therefore, factors other than atmospheric deposition must influence bioavailability and accumulation of Hg in aquatic organisms.

The primary means of Hg accumulation for organisms occupying higher trophic positions (such as fish, avian, and mammalian predators) is dietary through the assimilation of organic forms of Hg (principally methylmercury (MeHg)) (Lock 1975; Hall et al. 1997; Lawson and Mason 1998). As well, concentrations of contaminants in aquatic organisms typically increase with trophic position (Jackson 1986; Rowan and Rasmussen 1994; McNicol et al. 1997). There is evidence that Hg concentrations ([Hg]) in fish often correlate strongly with environmental correlates such as pH (Qian et al. 2001; Ikingura and Akagi 2003), dissolved organic carbon concentration ([DOC]) (Cope et al. 1990; Suns and Hitchin 1990; Driscoll et al. 1995), and influx from wetlands (Greenfield et al. 2001). However, because fish and waterfowl assimilate most of their Hg burden from their diet, the correlations detected between environmental factors and their Hg loads cannot be direct. Rather, it is more likely that these correlations reflect causal connections between environmental factors and MeHg assimilation in the prey of fish and waterfowl (Westcott and Kalff 1996; Lawson and Mason 1998).

Despite this observation, comparatively little is known about MeHg accumulation in organisms occupying lower trophic positions, particularly benthic invertebrates, which are common prey for a number of waterfowl and fish species for which consumption advisories have been issued. There is evidence that organic [Hg] in organisms occupying lower trophic levels may be correlated with variation in lake water chemistry, as in fish (Westcott and Kalff 1996). MeHg concentrations ([MeHg]) in primary producers and lake seston also seem to depend on ambient environmental conditions (Lawson and Mason 1998; Morrison and Watras 1999). Freshwater invertebrates occupying lower trophic levels are short-lived relative to fish; therefore, [MeHg] in invertebrates may respond more rapidly to variations in water chemistry compared with that in longer-lived organisms.

Benthic invertebrates play an important role in the transport of Hg in lake food webs, as burrowing species may help clear Hg from sediments (Boddington et al. 1979). Benthic invertebrates are a key food source for a variety of fish and can make up a significant energetic subsidy for piscivorous fish when prey fish are unavailable (Vander Zanden and Vadeboncoeur 2002). Thus, the ability to predict [MeHg] in benthic organisms from easily obtained environmental data could be useful in studying ecosystem Hg loads, rates of Hg transport, and food web dynamics. This is particularly true for examinations of historical data, since measures of benthic invertebrate [MeHg] are scarce prior to 1980.

Environmental factors are likely to explain some degree of variation in benthic invertebrate [MeHg] within or across water bodies. Using data from 12 Quebec water bodies, Tremblay and Lucotte (1997) found that benthic invertebrates in reservoirs had higher [MeHg] than those in natural lakes, and [MeHg] was typically higher in predatory versus nonpredatory invertebrates. Hall et al. (1998) have corroborated these findings. However, reservoir age correlates not just with fish and benthic invertebrate MeHg, but also with lake chemistry parameters such as pH (Ikingura and Akagi 2003). Thus, nonindependence between the status of a water body (lakes versus reservoirs) and its associated water chemistry may have confounded past investigations of relationships between lake water chemistry and invertebrate [MeHg] where data sets included both natural lakes and impoundments.

Although they exist for fish, zooplankton, water, and seston, simple models that describe the relationship between [MeHg] in benthic macroinvertebrates and lake water chemistry are lacking. Past attempts to find such relationships have been limited by a lack of relevant data (Wren and Stephenson 1991) and the wide variation in [MeHg] among benthic organisms observed even within a lake (Back and Watras 1995). The aim of our study was to generate models describing invertebrate [MeHg] based on environmental data typically collected by water-monitoring agencies. Our specific goals were to (*i*) determine the appropriate level of taxonomic or functional resolution for generating benthic invertebrate MeHg models, (*ii*) identify environmental variables most strongly correlated with benthic invertebrate [MeHg], (*iii*) construct empirical models using these correlates, and (*iv*) test the predictive efficacy of these models using cross-validation and by comparing the ability of our models to predict independent data in benthic invertebrate [MeHg] against the predictive efficacy of existing zooplankton [MeHg] models to explain independent zooplankton [MeHg] data. In addition, to determine the effect of exposure to fish digestive enzymes on invertebrate [MeHg], we compared predicted invertebrate [MeHg] estimates with [MeHg] of organisms collected from yellow perch (*Perca flacvescens*) stomachs.

Methods

Data sources

The data used in this study include (*i*) published invertebrate [MeHg] and previously unpublished environmental data from 12 water bodies collected by Tremblay and Lucotte (1997) in northwestern Quebec (53–54°N, 70–75°W), (*ii*) published invertebrate [MeHg] data from three lakes at the Experimental Lakes Area in northwestern Ontario (49°35′–49°37′N, 93°36′–93°52′W) collected by Hall et al. (1998), with unpublished environmental data obtained through the Government of Canada Department of Fisheries and Oceans, and (*iii*) invertebrate [MeHg] data collected and analyzed from seven lakes in central and northern Ontario (45–48°N, 78–81°W) in combination with surface water pH and [DOC] collected by either the Ontario Ministry of the Environment or the Ontario Ministry of Natural Resources.

Together, these data represent a wide geographical range of typical lakes and reservoirs found on the Canadian Shield (Appendix A). Variables of interest included organism [MeHg], organism [Hg], sediment [MeHg] and [Hg] (all expressed as nanograms of Hg per gram dry weight), year and month of collection, lake temperature (degrees Celsius), pH, Eh (redox potential reported in millivolts), [DOC] (milligrams per

litre), ammonium (micromoles per litre), and phosphate (micromoles per litre). [DOC] has been shown to approximate 90%–100% of total organic C concentration in boreal lakes (Kolka et al. 1999; Brunberg et al. 2002); therefore, when only total organic C concentration was reported, we estimated [DOC] as equivalent to total organic C (Rennie 2003). [DOC] was also estimated using a relationship between lake colour (measured at 425 nm, converted to platinum colour units (PCU) and reported as such) and [DOC] ($F_{[1,6]} = 18.5$, $p = 0.008$, $r^2 = 0.78$) (Rennie 2003). The relationship can be expressed as

$$(1) \qquad [\text{DOC}] = 0.0985 \times \text{colour} + 3.1715$$

and is similar to those published for other lakes from within our study region (Molot and Dillon 1997).

Invertebrate collections

Benthic invertebrate sampling is described in Tremblay and Lucotte (1997) for Quebec lakes and in Hall et al. (1998) for northwestern Ontario Experimental Lakes Area lakes. Clean techniques were used in both studies to avoid Hg contamination. Invertebrates from the central and northern Ontario lakes were obtained during daylight hours in July 2001 from all seven lakes and additionally in May, July, and August in 2002 from Plastic and Shoe lakes (Rennie 2003). When possible, lakes were sampled on alternate days during each collection period, and equipment was cleaned between visits to avoid cross-contamination. Benthic invertebrates were collected using clean kick-and-sweep nets from four to eight sites around each lake to obtain sufficient mass for analyses. Invertebrates and associated debris were stored in acid-washed 591-mL glass jars sealed with acid-washed polyethylene lids and kept on ice until they were returned to the laboratory for sorting and identification.

Benthic invertebrates were separated into coarse taxonomic groups using clean acid-washed Teflon utensils within 2–4 h of collection. Anisopterans, corixids, gerrids, and gyrinids were classified functionally as predators and amphipods, chironomids, ephemeropterans, and trichopterans as collectors–shredders. Composite samples were produced for each sampling date by grouping organisms from common taxonomic groups across all sites of a lake on a given day to achieve sufficient mass for [MeHg] determination. Samples were rinsed with deionized water, blotted with ultralow Hg Kimwipes™, placed in acid-washed 20-mL glass scintillation vials, and frozen at –20 °C for later analysis. Because no effort was made to allow benthic invertebrates to clear their guts before analysis, organism [MeHg] may include [MeHg] from gut contents.

Stomach contents were also collected from yellow perch in 2001 and 2002 from Shoe and Plastic lakes (Rennie 2003). Fish were euthanized, stomachs were removed, and contents were carefully placed into clean acid-washed 1.5-mL scintillation vials. Vials were sealed and stored at –20 °C until analysis. Samples were examined to determine the primary constituents of gut contents before analysis.

Organic Hg analyses

Only organic forms of Hg (principally monomethylmercury) are biomagnified in aquatic food chains (Mason et al. 1995; Lawson and Mason 1998; Lawrence et al. 1999). Although the fraction of MeHg in the total Hg pool of aquatic ecosystems is relatively small (Watras et al. 1995*b*), the MeHg fraction of total Hg in fish occupying higher trophic positions often approaches 90%–100% MeHg (Monteiro et al. 1991; Bloom 1992; Kannan et al. 1998). Thus, studies of Hg transfer focusing on upper trophic organisms can use comparatively simple total Hg measurements as a reliable index of MeHg. However, the organic Hg fraction in both fish and invertebrates occupying lower trophic positions is much more variable and rarely equivalent to total [Hg] values (Lasorsa and Allengil 1995; Tremblay and Lucotte 1997; Mason et al. 2000). Therefore, organic forms of Hg must be measured directly in food web biomagnification studies involving zooplankton and benthos.

[MeHg] in invertebrates has been shown to be equivalent to measures of organic [Hg] (Paterson et al. 1998). Therefore, we chose to analyze samples for organic [Hg] as an estimate of [MeHg] in invertebrates, consistent with the methodology reported elsewhere (Hall et al. 1998). Benthic invertebrate MeHg analyses are as described in Tremblay and Lucotte (1997) for Quebec lakes and in Hall et al. (1998) for Ontario Experimental Lakes Area lakes.

Organic [Hg] for invertebrates collected from our seven Ontario lakes and for those collected from fish stomach contents was determined using methods similar to those reported by Paterson et al. (1998). Briefly, invertebrates were placed in 20-mL preweighed, acid-washed glass scintillation vials and weighed. Wet tissues were dried at 50 °C to constant weight. To homogenize the samples, dried tissues were ground with a glass rod. To avoid contamination between samples, glass rods were rinsed with Hg-free deionized water, soaked in concentrated H_2SO_4 for approximately 30 s, rinsed again in deionized water, and then wiped dry with a Kimwipe™. Homogenized invertebrate tissues were suspended in 5 mL of deionized water to which 2 mL of 3 mol $KBr \cdot L^{-1}$ and 3 mL of 0.65 mol $CuSO_4 \cdot L^{-1}$ were added. The mixture was then shaken for 30 min to release bound organic Hg from invertebrate tissues. A 5-mL aliquot of 3:2 dichloromethane–hexane was added. Samples were shaken for 24 h to extract organically bound Hg from the samples and left overnight to settle. Four millilitres of the organic layer (containing the organic Hg from the sample) was removed and placed in 20-mL acid-washed glass digestion tubes. A 2-mL aliquot of 4:1 HNO_3–H_2SO_4 was then added to the extract, which was then digested at 250 °C for 4–6 h to convert organic Hg to inorganic Hg. Samples were then diluted with 10 mL of Hg-free deionized water and preserved with 200 μL of concentrated BrCl (Bloom and Crecelius 1983) until analysis. Samples were treated with 40 μL of 30% hydroxylamine hydrochloride immediately before analysis. Total [Hg] values were determined using an automated Tekran model 2600 mercury analyzer compliant with US Environmental Protection Agency (2000) method 1631. Reagent and analytical blanks were determined and subtracted from all samples and biological reference standards.

To assess the quality of MeHg data, we also ran a certified biological reference standard from the National Research Council of Canada, DORM-2. The average value (±1 SE) for DORM-2 over all organic Hg analyses, based on 27 runs, each consisting of two to five replicate digests, was 4.06 ± 0.14 μg $Hg \cdot g$ dry $weight^{-1}$, which is within 10% of the nomi-

Table 1. Distances from discriminant analysis between taxa.

	Collectors–shredders				Predators			
Taxa	Amp	Chi	Eph	Tri	Ani	Cor	Ger	Gyr
Amp	—	0.0170	0.0366	0.0014	*0.0001*	*0.0001*	*0.0001*	*0.0001*
Chi	0.9601	—	0.5022	0.2956	*0.0001*	*0.0001*	*0.0001*	*0.0001*
Eph	0.7285	0.1362	—	0.6507	*0.0001*	*0.0001*	*0.0001*	*0.0001*
Tri	1.2123	0.1945	0.0621	—	*0.0001*	*0.0001*	*0.0001*	*0.0001*
Ani	*5.6216*	*2.776*	*2.4095*	*1.7172*	—	0.0028	0.0497	0.0754
Cor	*7.2801*	*3.2034*	*3.4143*	*2.5812*	0.6838	—	0.6426	0.0001
Ger	*7.3745*	*3.4318*	*3.4681*	*2.6072*	0.4053	0.0597	—	0.0001
Gyr	*4.3121*	*2.5261*	*1.8472*	*1.3474*	0.3214	1.8684	1.4355	—

Note: The bottom triangular matrix consists of distance measures between taxa, and the top triangular matrix contains probabilities of similarities between taxa. Taxa are represented by the first three letters in the group name: Amp, amphipods; Chi, chironomids; Eph, ephemeropterans; Tri, trichopterans; Ani, anisopterans; Cor, corixids; Ger, gerrids; Gyr, gyrinids. Comparisons between functional groups (predators versus collectors–shredders) are italicized.

nal value reported by the National Research Council of Canada (4.47, SD = 0.32).

Statistical analyses

Data for the 12 Quebec water bodies were used to determine the appropriate taxonomic resolution of models and to identify which environmental variables were most important in explaining variation in organism [MeHg]. Pearson and Spearman correlation coefficients for all pairwise comparisons of variables were compared to help identify nonlinear relationships and variables in need of transformation. Organism [Hg] and [MeHg], sediment [Hg] and [MeHg], ammonium, and phosphate were all log-transformed to establish normality in each of the individual variables of interest. Linearity between variable pairs was assessed both visually and through agreement of Spearman with Pearson correlation coefficients.

Objective 1: appropriate resolution for benthic invertebrate [MeHg] models

To achieve this objective, we applied a discriminant analysis to benthic invertebrate [Hg] and [MeHg] (independent variables) to determine the degree to which we could classify organism types (dependent variables) from our observations in multivariate space. We examined the efficacy of this discrimination on two taxonomic levels: first, on the eight taxa of organisms encountered in this data set and, second, based on organism trophic status. Discriminant analyses were carried out using SAS (SAS Institute Inc. 1989). Collectors–shredders made up 42.6% of the total 225 observations, and predators made up 57.3% of all observations.

Objective 2: environmental variables associated with benthic invertebrate [MeHg]

To identify environmental variables most strongly correlated with benthic invertebrate [MeHg], the data were examined in a canonical redundancy analysis (CRA) to examine relationships between our multiple levels of invertebrate [MeHg] (separated by taxonomy based on results from discriminant analysis in objective 1) and the environmental variables that they experienced across invertebrate trophic levels (see Methods, Data sources for a list of environmental variables examined in the analysis). A binary variable was also included to describe water bodies based on their flooding history. Values for organism [MeHg] were sorted by site, and site-specific averages were obtained for groups of benthic invertebrates under study (both by taxa and by functional group). This reduced our total number of observations from 225 to 42. Of the 42 sites, 20 were from lakes and 22 were from reservoirs. Thus, the CRA included as many dependent variables as organisms under study and 11 environmental variables. Observations with missing values for organism [MeHg] were removed from the analysis. CRA were performed using SAS (SAS Institute Inc. 1989); Monte Carlo simulation probabilities and plots from CRA were obtained using redundancy analysis in CANOCO version 4.02 (Centre for Biometry, Wageningen, Netherlands), which is an equivalent procedure to CRA in SAS. Lastly, models were simplified to include only those environmental variables showing a large degree of explanatory power on invertebrate [MeHg] based on the magnitude of correlations with the first axis describing organism [MeHg]. Because our objective was to generate predictive models based on readily available data, we limited the selection of environmental variables to those that are commonly measured and available to researchers.

Objective 3: constructing empirical models

We added data from 11 water bodies in Ontario to our 12 Quebec water bodies and compiled a database consisting of organism [MeHg], lake pH, and lake DOC. As in the previous analysis, organism [MeHg] was log-transformed. Multiple observations within lakes were initially averaged such that each lake would have a single value for pH, DOC, and functional group MeHg (Appendix A). Each observation of either predator or collector–shredder [MeHg] represents the average over one to four taxonomic groups, each of which is the average of one to nine measurements of organism [MeHg] from a given sampling event (Appendix A). Taxonomic groups were weighted equally within a given functional group. Multiple regressions (linear, ordinary least squares) were applied to examine empirical relationships in which log-transformed organism [MeHg] was considered a function of pH, [DOC], and the interaction between the two. Nonsignificant interaction terms and then independent variables were removed in a stepwise fashion until all remaining terms in the model were significant (hereafter referred to as

independent models). Multiple regressions were carried out using SYSTAT (Systat Software Inc. 1998).

Objective 4: testing predictive efficacy

For cross-validation, we expanded the data set to include multiple samples taken from lakes, where multiple samples differed in the month and year in which they were obtained (Appendix A). We repeated the procedure outlined above to find the models of benthic invertebrate [MeHg] in these expanded data sets that included only significant independent variables. This procedure increased the sample size from 23 to 70 and facilitated the cross-validation of our models (hereafter referred to as expanded models). For approximately 30% of the observations in the expanded data sets, measurements of either pH or [DOC] were not made on the date that organisms were sampled. In these cases, we replaced missing values with monthly averages of pH and [DOC] calculated from within the lake in question. pH and [DOC] were not significantly correlated with one another (independent data set, $r = -0.12$, $p = 0.62$; expanded data set, $r = -0.07$, $p = 0.57$).

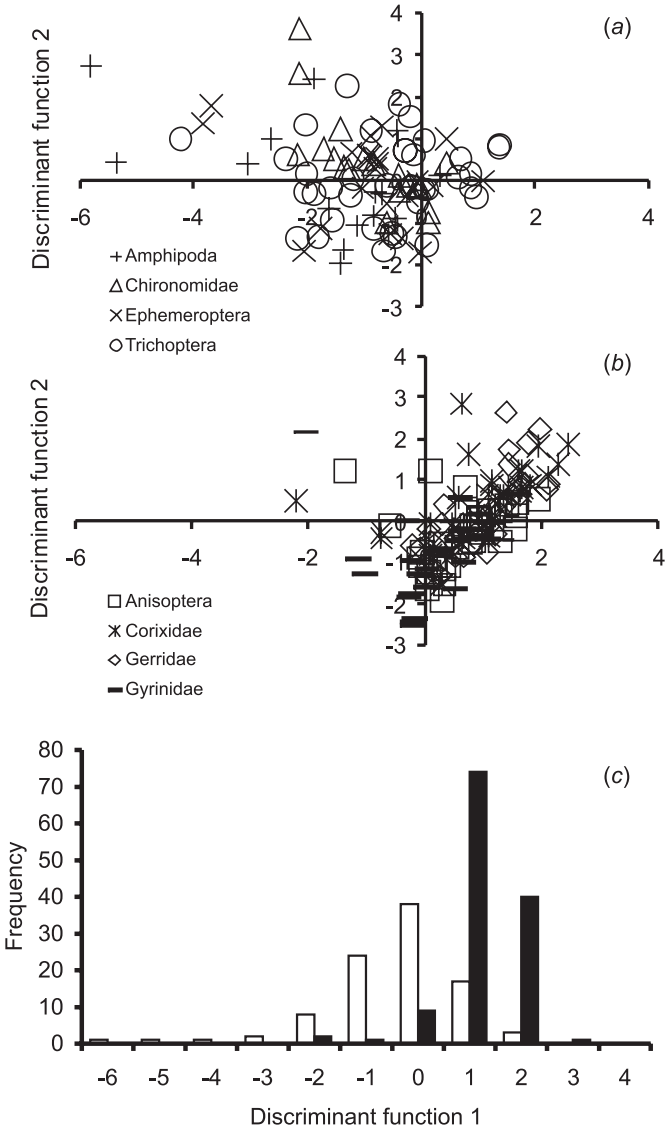
To cross-validate our models, all observations from one selected lake were removed from the data set and data from the remaining lakes were used to generate a statistical model. This model was then tested for its ability to accurately predict the data from the lake that was excluded from the model generation. This procedure was repeated sequentially so that data from each lake were in turn excluded from model generation and predicted independently. After independent predicted values were accumulated for every data point, we estimated the r^2 for cross-validation, Xr^2, as

$$(2) \qquad Xr^2 = 1 - (\Sigma[y_{obs} - y_{predicted}]^2 / SS_T)$$

where y_{obs} is the observed value of organism [MeHg] excluded from model generation, $y_{predicted}$ is the value of organism [MeHg] predicted from the subset of data from which the observed values were excluded, and SS_T is the total sum of squares estimated as the variance of organism [MeHg] times $N - 1$, where N is the number of observations used to generate the model.

Lastly, we used 22 independently collected zooplankton [MeHg] and water chemistry data to test the predictive efficacy of two previously published zooplankton models (Westcott and Kalff 1996; Garcia and Carignan 1999) so as to compare the relative performance of our models against others that also relate invertebrate [MeHg] to pH and [DOC]. Previously published zooplankton and (or) environmental data were obtained from Tremblay et al. (1998), Paterson et al. (1998), Back et al. (2003), Gorski et al. (2003), and Watras and Bloom (1992). Daytime samples of zooplankton were taken from four central/northern Ontario lakes using a 0.5-m-diameter plankton net with a 150-μm mesh. Nets were cleaned with Citranox between lakes to avoid cross-contamination. Predatory organisms were removed from samples of two lakes (Plastic and Shoe lakes) but not removed from the other two. Values of Xr^2 were calculated using eq. 2 using the difference between the predicted values of zooplankton [MeHg] calculated from published models compared with actual values observed in independent zooplankton [MeHg] data. Prediction limits (95%) were calculated around predicted organism [MeHg] for all multiple regression models (Sokal and Rohlf 1995).

Fig. 1. Comparisons between invertebrate taxa along the first two discriminant functions: (*a*) collectors–shredders and (*b*) predators separated for clarity. (*c*) Frequency of scores along discriminant function 1 showing separation between invertebrate trophic levels. Open bars, collectors–shredders; solid bars, predators. Invertebrates collected from Quebec reservoirs and lakes, 1992–1995.



Results

Objective 1: appropriate level of resolution for MeHg models

Using our eight taxa as our classification variable, the discriminant function demonstrated a poor but significant separation between taxa (Wilks' $\lambda = 0.4929$, $F_{[14,426]} = 12.91$, $p < 0.0001$). Statistical similarities always occurred among organisms belonging to the same functional groups (Table 1; Figs. 1*a* and 1*b*). Although some differences were observed between organisms in the same functional group, differences between functional groups were always significant (Table 1). SAS cross-validates each observation in the data set by clas-

Fig. 2. Ordination plot from CRA of invertebrate trophic level on environmental factors form Quebec lakes and reservoirs, 1992–1995. Points are individual site scores. Thick lines are invertebrate MeHg concentration; thin lines are environmental variables. Sites in the upper left diagonal of the plot are from reservoirs, and sites in the lower right diagonal are from natural lakes.

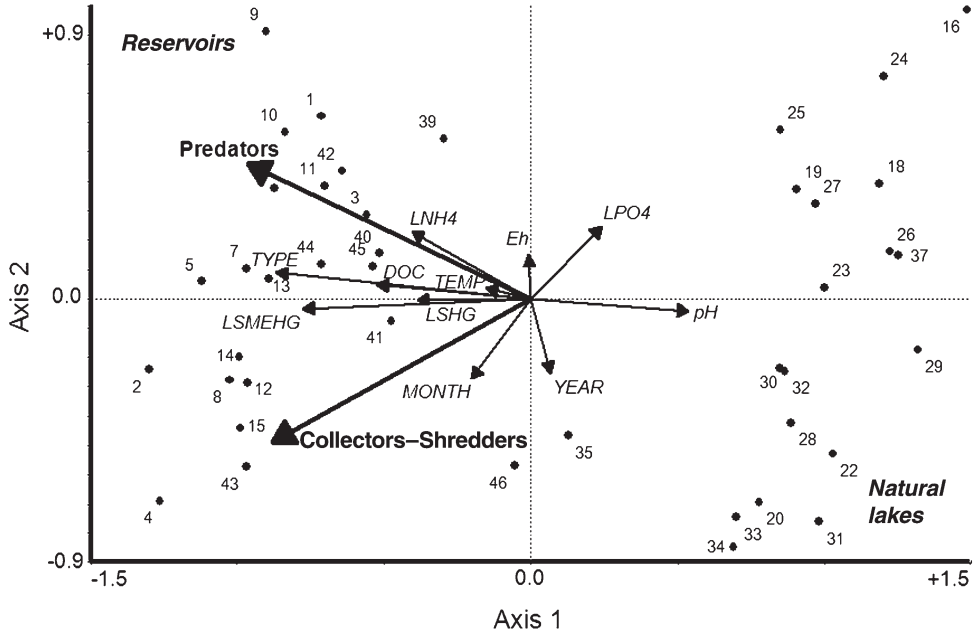


Table 2. Correlations between environmental variables and first two axes describing organism [MeHg] in CRA, with lake type removed.

	Organism [MeHg]	
Environmental variable	Axis 1	Axis 2
Year	–0.0832	0.2863
Month	0.1945	0.2942
Temperature	0.1586	–0.0475
pH	**–0.5526**	**0.0693**
Eh	0.0158	–0.1685
DOC	**0.5429**	**–0.0746**
$\log_{10}$(sediment [Hg])	0.3977	–0.0124
$\log_{10}$(sediment [MeHg])	***0.7939***	***0.01***
$\log_{10}(NH_4)$	0.4272	–0.2703
$\log_{10}(PO_4)$	–0.2366	–0.2626

Note: Bold/italicized values are significant regression coefficients in univariate multiple regression models. Bold variables appear to be most important in all factors examined based on magnitude of correlations with the first axis describing organism [MeHg]. Eh, redox potential.

sifying it using a discriminant function calculated from all observations in the data set but excluding the observation in question. No group of organisms fared well under this cross-validation model; the lowest classification error rate observed was for corixids, which were misclassified only 46% of the time, and all other groups of organisms had classification error rates greater than 56%.

The discriminant analysis was rerun after classifying organisms into only two groups: predators and collectors–shredders. Again, the overall discriminant model was significant (Wilks' $\lambda = 0.6201$, $F_{[2,219]} = 67.09$, $p < 0.0001$) (Fig. 1*c*). The standardized distance between the two groups was highly significant ($p < 0.0001$). The cross-validation model fared much better under this broader classification, indicating large differences between these groups. Collectors–shredders had approximately three times the error rate in classification than did predators, but at 24% and 8%, respectively, the amount of overlap between functional groups was dramatically less compared with differences among taxa (Fig. 1). Organism [MeHg] explained almost three times more variation in the discriminant function than did organism [Hg] (organism [MeHg], $r^2 = 0.26$; organism [Hg], $r^2 = 0.08$). Mean organism [MeHg] and [Hg] were both significantly different between functional groups ($p < 0.0001$). Based on the clear distinction between benthic invertebrate functional group [MeHg], we chose to develop separate predictive models for predators and collectors–shredders.

Objective 2: selection of environmental variables

The CRA of organism [MeHg] on environmental variables was significant (Wilks' $\lambda = 0.073$, $F = 7.08$, df = 22, $p < 0.0001$). A Monte-Carlo simulation testing the significance of the first canonical axis yielded a p value of 0.005 (based on 199 iterations). Univariate multiple regressions were also significant for both groups of organisms based on environmental variables (collectors–shredders, $r^2 = 0.72$, $p < 0.0001$; predators, $r^2 = 0.84$, $p < 0.0001$). Environmental variables that were important in correlations with [MeHg] of both collectors–shredders and predators were water-body type, pH, [DOC], and sediment [MeHg] (Fig. 2). These variables represent the longest vectors of all environmental variables in the triplot, and all occur along the same plane (Fig. 2). These variables were also strongly correlated with their own principal canonical axis, as well as with the principal canonical axis describing [MeHg] of benthic invertebrates (Table 2). The first environmental canonical variable explained 72.2% of the variation in organism [MeHg], the second added only 5.5% to the explained variation.

Initially, lake type appeared to be the most important variable considered in explaining benthic invertebrate [MeHg], being the only one to yield statistically significant regression coefficients in univariate multiple regressions of environmental variables on organism [MeHg] ($p < 0.0001$). As well, sites were well separated based on lake type alone; organisms from reservoirs occurred on the left-hand side of the triplot, whereas organisms from natural lakes occurred on the right (Fig. 2). We attempted to examine these models after adjusting for the effect of lake type using partial CRA. However, doing so made the statistical model insignificant (Wilks' $\lambda = 0.4122$, $F = 1.162$, df = 20, $p = 0.07$). Additionally, correlations between all environmental variables and organism [MeHg] proved weak. This was due to the fact that lake type is strongly correlated with lake pH, [DOC], and sediment [MeHg] (Fig. 3) (t tests between lakes and reservoirs: pH, $p << 0.0001$; [DOC], $p = 0.0007$; $\log_{10}$ sediment [MeHg], $p << 0.0001$; all tests, df = 44, $p_{crit} = (0.025/3) = 0.008$).

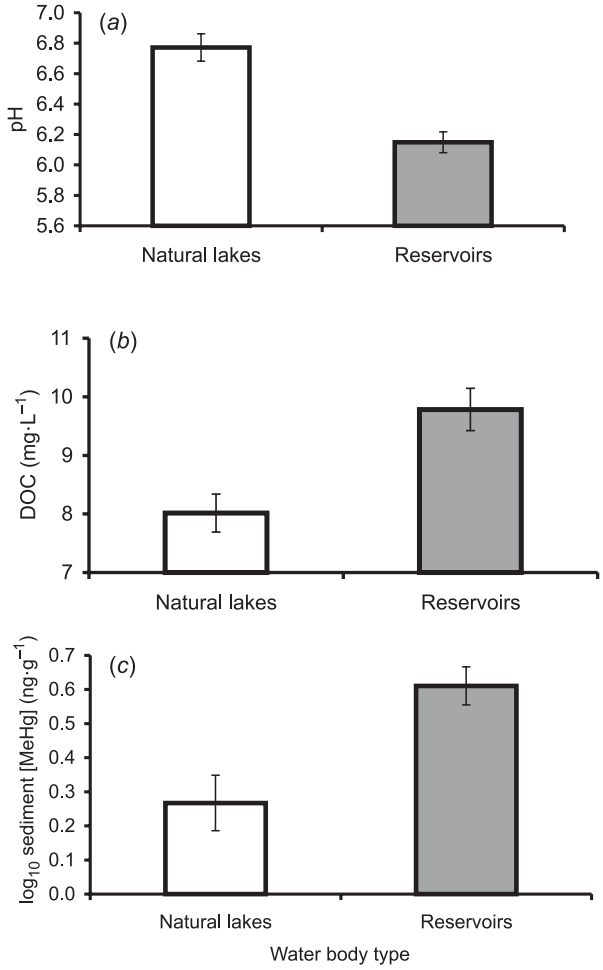
Repeating the CRA with the exclusion of the variable describing lake type yielded a significant model (Wilks' $\lambda = 0.200$, $F = 3.7088$, df = 20, $p < 0.0001$), although reduction in the estimated F statistic reflected a loss of explanatory power due to the loss of the lake type variable. Values of r^2 in the univariate models when lake type was removed fell to 62% and 66%, respectively, for collectors–shredders and predators. From this analysis, we concluded that the inclusion of the lake type variable was masking the effects of other important environmental variables (as per Graham 2003), as the majority of the explanatory power of the lake type variable appeared to be explained by correlation with the other environmental variables listed above. Without lake type, sediment [MeHg], pH, and [DOC] once again appeared as the strongest variables on the first environmental axis (Table 2), each explaining about 20% more variation than in the model including lake type, although sediment [MeHg] was the only significant variable in univariate multiple regressions ($p = 0.0016$) (Fig. 2) (Appendix B).

Sediment [MeHg] occurs infrequently at best in published data or government databases relative to other strongly correlated variables in this analysis. Therefore, to generate models that would be practical given existing available data, we chose to limit our CRA to variables that were strong correlates in the initial analyses and common in lake databases. Models that included only month of collection, pH, and [DOC] were significant (Wilks' $\lambda = 0.467$, $F = 5.67$, df = 6, $p < 0.0001$), and pH and [DOC] were significant terms in models with sediment [MeHg] removed ($p < 0.1$) (as per Graham 2003). r^2 values of univariate models describing invertebrate [MeHg] for collectors–shredders and predators with only these variables were 0.34 and 0.46, respectively. Because these variables alone explained more than half the variation of models with all environmental variables included, and because they are among the most commonly collected of the environmental variables considered here, we chose them to build our predictive models.

Objective 3: empirical models

Both independent models of organism [MeHg] were statistically significant based on only one or two water chemistry variables (Table 3). Coefficients for models based on independent and expanded data sets were similar, and standard errors of coefficient estimates for both procedures overlapped considerably within functional groups. The predator model explained 43% of the overall variation in predatory benthic invertebrate [MeHg] from pH and [DOC] in expanded data sets, representing our most conservative estimate of explained variation (Table 3; Fig. 4*a*). Partial r^2 values were 0.27 for [DOC] and 0.15 for pH, indicating that [DOC] played the major role in predicting predator [MeHg]. The collector–shredder model explained 26% of the overall variation in collector–shredder [MeHg] from [DOC] in expanded data sets (Table 3; Fig. 4*b*). The month of collection did not affect [MeHg] of invertebrate functional groups in expanded data sets (Appendix B); therefore, the month of collection was not considered further.

Fig. 3. Patterns of (*a*) pH, (*b*) [DOC], and (*c*) sediment [MeHg] between natural lakes and reservoirs in Quebec. Error bars are ±1 SE.



Objective 4: model cross-validation and comparison

Our models describe independent data as well as or better than other existing models describing invertebrate [MeHg] based on pH and [DOC]. Cross-validation demonstrated that our models explained 31% of the variation in independent predator data and 13% of the variation in independent

Table 3. Predictive equations of organism [MeHg] from lake chemistry parameters and associated statistical parameters.

Model No.	Model	df	F	p	r^2	r^2_{adj}	Xr^2	SE_{est}
1	$\log(MeHg_p) = 3.461(\pm0.546) - 0.261(\pm0.079)pH + 0.043(\pm0.014)DOC$	2, 19	10.78	<0.001	0.531	0.482	—	0.180
2	$\log(MeHg_p) = 3.113(\pm0.364) - 0.214(\pm0.054)pH + 0.053(\pm0.010)DOC$	2, 65	24.11	<<0.0001	0.426	0.408	0.312	0.240
3	$\log(MeHg_{cs}) = 1.184(\pm0.192) + 0.055(\pm0.022)DOC$	1, 19	6.59	0.019	0.257	0.218	—	0.180
4	$\log(MeHg_{cs}) = 1.209(\pm0.116) + 0.062(\pm0.013)DOC$	1, 62	22.22	<<0.0001	0.264	0.252	0.126	0.305
5	$MeHg_z = 449.64 - 47.41pH + 146.35\log(\text{water colour})$	2, 22	39.29	0.0001	0.73	nr	np	65.61
6	$\log(MeHg_z) = 3.52(\pm0.25) + 0.62(\pm0.15)\log(DOC)$	1, 23	nr	0.0005	0.43	nr	0.244	0.23

Note: r^2_{adj}, adjusted r^2 values according to statistical software; Xr^2, cross-validated r^2 values. Models 1 and 3 are based on lake-wide averages, and models 2 and 4 are based on repeated measures within lakes. Cross-validation was carried out on expanded data sets only for benthic invertebrate models (models 2 and 4). Model 5 is from Westcott and Kalff (1996); model 6 is from Garcia and Carignan (1999). Partial r^2 values for the cross-validation predator model (model 2) are 0.27 and 0.15 for DOC and pH, respectively. Subscripts: p, predator; cs, collector–shredder; z, zooplankton; nr, not reported by the authors of the model; np, not possible to calculate given independent data (value less than zero).

collector–shredder data (Table 3). These values are comparable with those obtained for existing zooplankton models predicting independent zooplankton data ($N = 22$) (Table 3; Fig. 5). Xr^2 could not be calculated for the Westcott and Kalff (1996) model owing to large differences between observed and predicted values in independent zooplankton data (Fig. 5*d*). The Garcia and Carignan (1999) model had an $Xr^2 = 0.24$, which falls between the values calculated for our predator and collector–shredder models. However, a large proportion (at least 50% of observations) of zooplankton [MeHg] data for both previously published models fall outside 95% prediction limits (Figs. 5*c* and 5*d*). In contrast, two independent data points of benthic invertebrate [MeHg], pH, and [DOC] obtained from Gorski et al. (2003) fell within the 95% prediction limits of our relationships (Fig. 5).

Although our models appear to underestimate slightly collector–shredder benthic invertebrate [MeHg] from reservoirs, no such bias is evident for predators, and both models appear to accurately describe benthic invertebrates from natural lakes (Figs. 5*a* and 5*b*). Data from reservoirs were consistently underestimated by the Garcia and Carignan (1999) model, with all observations from reservoirs falling above 95% confidence intervals (Fig. 5*c*). In contrast, the Westcott and Kalff (1996) model described reservoirs well, with all observations from reservoirs falling within 95% confidence intervals (Fig. 5*d*).

Observed values of [MeHg] for benthic invertebrates collected from perch stomach contents fall within the general distribution around predictive models as the invertebrate MeHg data (Figs. 5*a* and 5*b*). Some variation was observed between years in collectors–shredders collected from fish stomachs, but considered together, invertebrates collected from stomachs generally fall within the range of predicted values for a given DOC concentration in those lakes. Neither zooplankton model accurately predicts zooplankton [MeHg] collected from fish stomachs (Fig. 5), as at least half of these observations fell outside the 95% confidence intervals of these models.

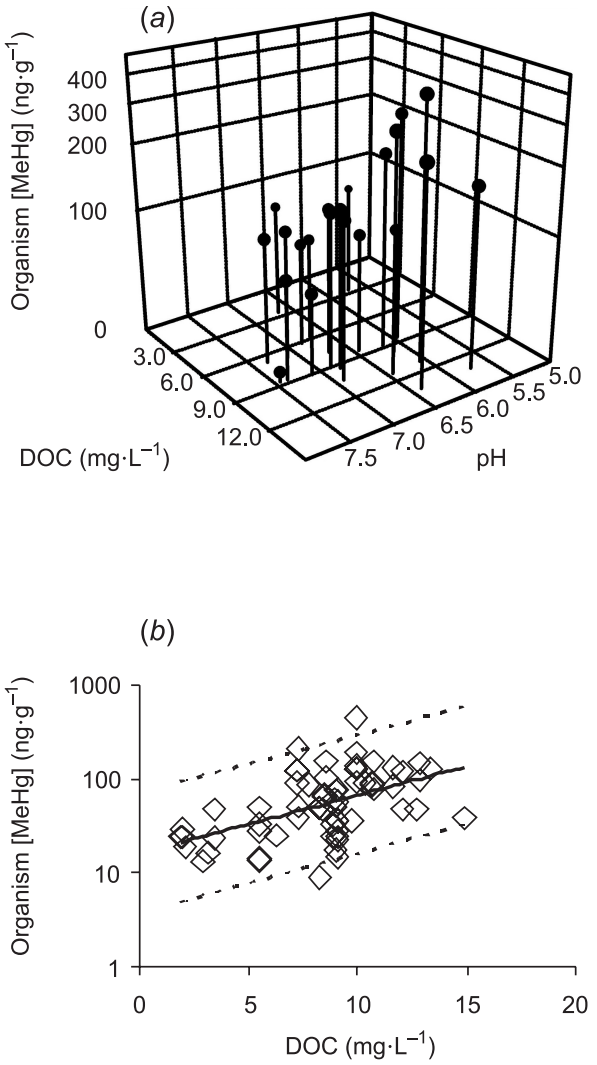
Discussion

Selection of appropriate model resolution

Benthic invertebrate [MeHg] values were most distinct among functional groups, whereas differentiation based on taxa proved to be generally unreliable. This supports previous approaches that make comparisons between benthic invertebrate [MeHg] at the functional group level (Tremblay and Lucotte 1997; Hall et al. 1998). Previous studies have also shown a significant effect of reservoir age on organism [MeHg] (Tremblay and Lucotte 1997; Hall et al. 1998; Ikingura and Akagi 2003). Our data indicate that lake chemistry alone may explain much of the variation in benthic invertebrate [MeHg] previously attributed to water-body status (lakes versus reservoirs), as excluding lake type caused the explanatory power in our CRA models to fall by only 18% and 10% for the predator and collector–shredder models, respectively. This was likely a result of lake type being significantly correlated with the three environmental variables that also explained the largest amount of variation in benthic invertebrate [MeHg] (Graham 2003). Unfortunately, because of the observed colinearity between impoundment and other environmental variables observed in this and other studies (Ikingura and Akagi 2003), as well as a current lack of alternative data sets with which to evaluate these models, we are unable to distinctly rule out the possibility that some other factor besides pH or [DOC] also associated with lake type may be a contributing factor to our results. We hope that this research stimulates further investigation among systems with wider ranges of pH, [DOC], and benthic invertebrate [MeHg] within each lake type (reservoirs only or natural lakes only) to better evaluate the generality of these models to many aquatic systems.

As expected, [DOC] and pH were important explanatory variables in relationships with benthic invertebrate [MeHg]. Besides previously cited observations in zooplankton (Westcott and Kalff 1996; Garcia and Carignan 1999), [DOC] and pH have also been found to explain a significant

Fig. 4. Relationships describing benthic invertebrate [MeHg] with environmental correlates in (*a*) predators and (*b*) collectors–shredders. The broken lines in Fig. 4*b* represent 95% prediction limits for collector–shredder [MeHg] from a given DOC value.



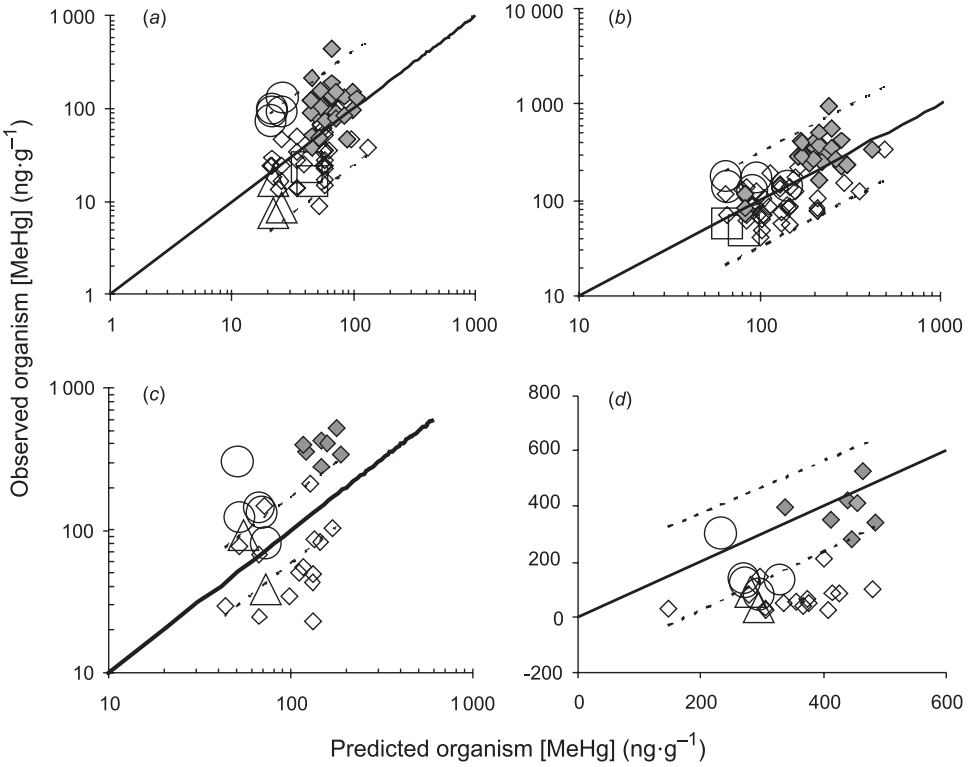
proportion of the variation in fish [Hg] standardized for size (Cope et al. 1990; Suns and Hitchin 1990; Qian et al. 2001). There is, however, disagreement as to whether water chemistry or lake productivity ultimately plays a more significant role in dictating [Hg] in fish (Kidd et al. 1999; Sonesten 2001; Essington and Houser 2003). [DOC] may also be important in binding free MeHg, which can in turn be taken up by filter-feeding organisms (Watras and Bloom 1992; Hintelmann et al. 1995; Morrison and Watras 1999). Other studies have identified pH and [DOC] as important correlates of [MeHg] values in lake water, where [DOC] and pH together explained 80%–90% of the variation in waterborne [Hg] and [MeHg] (Watras et al. 1995*b*). Concentrations of organic matter correlate positively with sediment [Hg] and methylation rates of sulfate-reducing bacteria (Jackson 1988*b*), and organic matter transported from catchments to lakes is a significant source of Hg in lakes (Mierle and Ingram 1991). Thus, greater inputs of humic matter from watersheds can increase the available pool of inorganic Hg for methylation by bacteria (Jackson 1988*a*). Lower pH also increases the binding capacity of MeHg to lake seston (Hintelmann et al. 1995), and pH is generally negatively correlated with waterborne [MeHg] (Hintelmann et al. 1995; Watras et al. 1995*a*; Morrison and Watras 1999). Similarly, low pH may stimulate methylation rates of bacteria (Xun et al. 1987). [DOC] and pH also appear to be important in the transport of waterborne MeHg into phytoplankton; uptake rates of MeHg by algae are positively affected by the presence of organic ligands possessing sulfhydryl groups, such as thiourea and cysteine (Lawson and Mason 1998), and the binding capacity of sulfhydryl groups for MeHg increases at low pH (Rabenstein and Fairhurst 1975).

The transfer of MeHg from primary consumers up aquatic food chains is largely dietary (Lawson and Mason 1998) because of the strong affinity of MeHg to sulfhydryl-containing amino acids in the bodies of organisms (Harris et al. 2003) and the slow rate of MeHg elimination from organisms (Trudel and Rasmussen 1997). Organisms exposed to unusually high levels of aqueous MeHg (i.e., Visman et al. 1995) may absorb MeHg directly from waterborne exposure, although concentrations required for this to occur are much greater than those observed in either natural lakes or reservoirs.

This evidence together suggests that water pH and DOC can directly affect both MeHg availability and rates of transfer at the base of aquatic food webs. Furthermore, if assimilation rates from one trophic level to the next are relatively constant across ecosystems, then pH and DOC may directly affect the [MeHg] of aquatic organisms at multiple trophic levels, including benthic invertebrates, by controlling MeHg uptake and availability at the base of aquatic food webs. Collector and shredder organisms typically feed on epiphyton and aquatic macrophtyes. Detritivores such as amphipods (also represented in the collector–shredder model) feed on dead organic matter, which is also capable of taking up waterborne MeHg (Lock 1975). Uptake of MeHg by dead organic matter is passive, as it is likely to be in both algae and seston (Lawson and Mason 1998). Therefore, dead organic matter, algae, and seston are likely to have similar rates of MeHg assimilation and may be affected similarly by water chemistry. If so, models of [MeHg] for zoobenthos based on water chemistry should be similar to those for zooplankton, as both benthic invertebrates and zooplankton assimilate MeHg at similar rates when feeding on diets to which they are adapted (Lawson and Mason 1998). A comparison of partial r^2 values between benthic invertebrate models and those reported by Westcott and Kalff (1996) support this hypothesis. Partial r^2 values for the zooplankton model indicated that water colour explained the majority of [MeHg] variation in zooplankton (Westcott and Kalff 1996). Similarly, DOC (a variable strongly correlated with water colour in these lakes) was three times more important than pH in explaining benthic predator [MeHg] and was the only significant variable in the benthic collector–shredder MeHg model.

The month of collection did not explain a significant proportion of the variation in benthic invertebrate [MeHg], indicating no seasonal trend (Appendix B). This contrasts with other studies that have found significant seasonal effects in zooplankton (Monson and Brezonik 1998; Garcia and Carignan 1999). Studies examining seasonal patterns of sta-

Fig. 5. Observed versus predicted values of organism [MeHg] (diamonds) from cross-validated models of invertebrate (*a*) collectors–shredders and (*b*) predators, as well as for independent zooplankton data predicted from published empirical relationships reported by (*c*) Garcia and Carignan (1999) and (*d*) Westcott and Kalff (1996). Note the differences in scale among axes. Triangles (2001 data) and circles (2002 data) are [MeHg] of organisms collected from fish stomachs. Squares are values from Gorski et al. (2003). The broken lines represent 95% prediction limits of organism [MeHg] for a given *X* or set of *X*. Shaded symbols, reservoirs; open symbols, lakes.



ble isotopes have also observed large variation in zooplankton isotopic ratios over seasonal time scales that were not observed in benthic organisms (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1996). This may be due in part to short generation times (i.e., weeks to months) and fast turnover rates in pelagic zooplankton compared with benthic invertebrates (i.e., months to years). Thus, compared with zooplankton, benthic invertebrate [MeHg] may be less sensitive to variations in water chemistry over seasonal time scales.

Predictive efficacy of benthic invertebrate MeHg models

Our empirical models of benthic invertebrate [MeHg] performed well in predicting independent data compared with existing models of zooplankton [MeHg]. Benthic invertebrate Xr^2 values were similar to those for zooplankton using the Garcia and Carignan (1999) model. Although our collector–shredder model appears to slightly underestimate benthic invertebrate [MeHg] in reservoirs, the model still accurately describes invertebrates from natural lakes, and the predator model appears to accurately describe organisms from both water-body types. Additionally, this bias is nowhere near as large as was observed in the two zooplankton models, where a large proportion of our independent data fell outside the 95% prediction limits. Data from two lakes in the immediate geographic region of lakes reported by Westcott and Kalff (1996) were overestimated (observed versus predicted: Plastic Lake, 77.8 versus 277.5 ng·g^{-1}; Shoe lake, 146.8 versus 297.2 ng·g^{-1}), with Plastic Lake falling outside the 95% prediction limits of their model. Although their model describes MeHg in cladocerans, the discrepancies that we observed could not be caused by the accidental inclusion of predator organisms in our samples, as inclusion of these organisms should produce a deviation in the direction opposite to that observed. Watras and Bloom (1992) reported individual MeHg concentrations for filter-feeding cladocerans, but predicted values for this water body are also overestimated by the Westcott and Kalff (1996) model by an order of magnitude (observed versus predicted: Little Rock Lake, acidified basin, 67.5 versus 373.7 ng·g^{-1}; reference basin, 25.0 versus 307.3 ng·g^{-1}) and also fall outside 95% prediction limits of the model. In comparison, two data points collected from benthic invertebrates in Michigan lakes (Gorski et al. 2003) fell within the 95% prediction limits of both of our benthic invertebrate models.

The poor fit of our independent zooplankton data to the two zooplankton models likely results from the fact that our independent observations span a much wider range both geographically and taxonomically than the data used to generate either of the zooplankton models and that our application of these models arguably goes beyond the scope of their initial purpose. For instance, the Garcia and Carignan (1999) model was originally designed to describe zooplankton from lakes that had sustained recent burns in their catchments. As

well, our data varied slightly in the taxonomic resolution of zooplankton analyzed compared with the models being tested. Westcott and Kalff (1996) analyzed MeHg in cladoceran filter-feeding zooplankton only. With the exception of the two lakes from the region sampled by Westcott and Kalff (1996) and data reported by Watras and Bloom (1992), all of our independent zooplankton data come from bulked zooplankton samples, as reported by Garcia and Carignan (1999). Regardless, no systematic variation was observed due to potential differences in the level of classification of zooplankton with either model.

Although a large proportion of the effect of impoundment on benthic invertebrate [MeHg] appears to be explained by water chemistry, the same cannot be said for zooplankton. In our independent data set, zooplankton [MeHg] from reservoirs was typically higher than predicted by the Garcia and Carignan (1999) model but was described well by the Westcott and Kalff (1996) model. However, zooplankton [MeHg] from natural lakes in our data were typically underestimated by both models (although less so for the Garcia and Carignan (1999) model). Given the large separation of zooplankton [MeHg] that we observed between lakes and reservoirs, impoundment effects on zooplankton (and thus perhaps on other organisms reliant on the pelagic food web) may go beyond simple changes in water chemistry due to upland flooding. An increase in the concentration of suspended particulate matter is often observed in reservoirs and may account for elevated levels of zooplankton [MeHg] that occupy them (Tremblay et al. 1998).

Of the two models that we report in this study, the overall better fit of the predator model may be due to the fact that the collector–shredder functional group designation assumes a much narrower range of feeding habits than actually occurs in nature. Predatory forms of both trichopterans and chironomids are known to inhabit lakes within the range of this study, but our coarse taxonomic identification assumed that all trichopterans and chironomids encountered were collectors–shredders. Despite these optimistically simple assumptions regarding the feeding habits of organisms in these groups, our models provide high predictive performance relative to the reported average ability of ecologists to explain variation in biological variables of interest (Moller and Jennions 2002).

These models, although statistically significant, offer at best ballpark estimates of benthic invertebrate [MeHg], as evidenced by reported Xr^2 and predictive 95% confidence limits around models. Although these models cannot substitute for actual measures of invertebrate [MeHg], they may allow researchers to identify potential benthic invertebrate MeHg "hotspots" (in lakes that do not receive obvious point-source contamination) based on very basic environmental data and may also allow estimation of benthic invertebrate [MeHg] from historic data sets where these data are absent.

Because [MeHg] in fish tissues is generally higher by an order of magnitude than that of their food, one might expect that Hg contamination of invertebrates would occur in the stomachs of fish. However, for the two lakes for which we have data, [MeHg] measured from stomach contents of fish were similar to estimates for invertebrates collected directly from the lake. This was not the case for zooplankton collected from fish stomachs when predicted with existing zooplankton models, where only half of these observations fell within the 95% confidence intervals of the models. However, this rate is similar to that observed for independent zooplankton data collected directly from the lake. Therefore, we can only say that these existing zooplankton models predict independent zooplankton [MeHg] data collected from fish stomachs, as well as independent zooplankton [MeHg] data collected from lakes.

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Appendix A

Table A1. Averages of environmental characteristics for 23 water bodies under study and number of observations from each lake included in cross-validated data sets.

Water body	Water-body code[a]	Location[b]	pH	[DOC] ($mg \cdot L^{-1}$)	No. of observations in expanded data set
D24N (north basin)	R	1	6.37	10.68	8
D24S (south basin)	R	1	6.21	10.74	7
Detcheverry	L	1	6.97	8.88	3
Des Voeux	L	1	6.90	5.52	5
Duncan	L	1	6.55	9.05	8
km 17	R	1	7.36	7.28	3
Laporte	L	1	6.30	8.49	1
Evans	L	1	6.85	10.46	1
Jobert	L	1	6.80	6.30	1
Koury	L	1	6.40	8.27	1
LA40	R	1	5.84	7.80	7
Opinaca	L	1	6.24	10.36	1
Plastic	L	2	5.57	2.05	4
Shoe	L	2	6.62	3.24	4
Ashley	L	2	6.7	8.2	1
Lady Ruth	L	2	6.5	8.4	1
Margueratt	L	2	6.8	8.5	1

Table A1 (*concluded*).

Water body	Water-body code[a]	Location[b]	pH	[DOC] (mg·L^{-1})	No. of observations in expanded data set
Melick	L	2	7.3	9.0	1
Trailer	L	2	7.4	9.0	1
L240	L	3	7.17	6.69	2
L632	L	3	5.79	13.20	3
L979	L	3	6.66	9.34	2
L979R (postflooding)	R	3	6.29	13.94	4

[a]R, reservoir; L, lake.
[b]1, northwestern Quebec; 2, north-central Ontario; 3, northwestern Ontario.

Appendix B

We evaluated relationships between organism [MeHg] with sediment [MeHg] and the season in which organisms were collected. Correlations between collector–shredder [MeHg] and sediment [MeHg] were strong and significant (Pearson $r = 0.72$, $p = 0.012$, $p_{crit} = 0.025$). Correlatons between predator [MeHg] and sediment [MeHg] were similar but insignificant after Bonferroni correction (Pearson $r = 0.63$, $p = 0.038$, $p_{crit} = 0.025$). However, patterns of sediment [MeHg] with [MeHg] for both functional groups were largely similar over the range of variation observed (Fig. B1*a*). We also performed a three-factor ANOVA on organism [MeHg] treating functional group and month as fixed factors and lake as a randomized block factor. Removing the effect of lake, the month of collection did not explain a significant component of variation in organism [MeHg] ($F_{[3,98]} = 0.49$, $p = 0.69$), and there was no significant interaction between functional group and month ($F_{[3,98]} = 0.27$, $p = 0.27$) (Fig. B1*b*). This supports insignificant findings of single-factor ANOVAs performed on the month of collection for each functional group [MeHg], disregarding potential lake effects (ANOVA: predators, $F_{[3,62]} = 1.05$, $p = 0.38$; collectors–shredders, $F_{[3,58]} = 0.92$, $p = 0.43$).

Fig. B1. Relationships of organism [MeHg] with (*a*) sediment [MeHg] and (*b*) month of invertebrate collection, variables not considered in empirical models. Circles, predators; diamonds, collectors–shredders. In *b*, error bars are ±1 SE, and the numbers beside each data point represent the number of individuals used to generate means and standard errors.