Adjusting Mercury Concentration for Fish-Size Covariation: A Multivariate Alternative to Bivariate Regression

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Regression-based methods like analysis of covariance (ANCOVA) are frequently used to adjust one variable for the correlated influence of a second less interesting variable (e.g., mercury concentration and fish size). However, the influence of the covariate (i.e., fish size) is not removed unequivocally when regression slopes are not parallel. Using data on tissue-mercury concentration and fish size from 30 populations of lake trout (*Salvelinus namaycush*), we show that data adjusted to a common size with bivariate regression can retain information associated with the original size differences. As an alternative, we use univariate and bivariate summary statistics from each population as raw data in a multivariate analysis to search for differences among populations. Ordination axes resulting from this analysis exhibited both small- and large-scale spatial autocorrelation. Localized spatial patterns probably reflect similar geochemical features of the watersheds of neighbouring lakes in small geographic areas. In contrast, regional spatial autocorrelation suggested broad-scale patterns that may implicate atmospheric inputs of mercury. As an extension of this multivariate approach, both regional and local patterns could be compared with environmental variables to reveal correlations that may suggest new cause-and-effect hypotheses.

Des méthodes fondées sur la régression comme l'analyse de la covariance sont souvent utilisées pour ajuster une variable pour tenir compte de l'influence corrélée d'une deuxième variable moins intéressante (p.ex., la concentration de mercure et la taille du poisson). Toutefois, l'influence de la covariable (c.-à.-d la taille du poisson) n'est pas supprimée de façon univoque lorsque les pentes de régression ne sont pas parallèles. À l'aide de données sur la concentration de mercure dans les tissus et la taille des poissons de 30 populations de touladi (*Salvelinus namaycush*), nous avons montré que les données ajustées à une taille courante au moyen de la régression à deux variables peuvent garder des informations associées aux différences de taille originales. Comme autre possibilité, nous utilisons les statistiques sommaires à une variable et à deux variables de chaque population comme données brutes dans le cadre d'une analyse à plusieurs variables afin de déceler des différences entre les populations. Les axes d'ordination obtenues à partir de cette analyse démontraient une autocorrélation spatiale sur une petite échelle et sur une grande échelle. Des configurations spatiales localisées reflètent probablement des caractères géochimiques similaires de bassins hydrographiques des lacs voisins dans de petites zones géographiques. Par contraste, une autocorrélation spatiale régionale semblait indiquer des configurations sur une vaste échelle susceptibles de comporter des apports atmosphériques de mercure. Pour élargir cette approche à plusieurs variables, des configurations régionales et locales pourraient être comparées à des variables environnementales afin de mettre à jour des corrélations qui peuvent indiquer de nouvelles hypothèses cause–effet.

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The proper method to correct or adjust one variable for relatively uninteresting variation in a second variable is a long-standing issue in biological studies (e.g., Atchley 1978; Smith 1984; Reist 1985, 1986; Packard and Boardman 1988). The use of indices or ratios was once widely accepted as one such adjustment (e.g., condition factor, gonadosomatic index (GSI); liver somatic index (LSI), and so on; see Leatherland and Sonstegard 1987; Munkittrick and Dixon 1988; Willis 1989). But the recognition of statistical problems with ratios (e.g., Pearson 1897; Chayes 1971; Atchley et al. 1976; Atchley and Anderson 1978; Kenney 1982; Phillips 1983; Gibson 1984; Packard and Boardman 1987, 1988) has prompted the evaluation and use of numerous alternatives (e.g., Corruccini 1977; Strauss 1985; Craig and Babaluk 1989; Cone 1989; dos Reis et al. 1990; Jackson et al. 1990; Murphy et al. 1990).

Many of these alternatives incorporate regression-based procedures (e.g., Atchley 1978; Smith 1984; Green 1986); however, interpretive problems arise when comparing populations if regression slopes differ (Thorpe 1976; Reist 1985). Frequently, such problems emerge in studies of contaminants in animal tissues where contaminant concentration increases with animal size (e.g., mercury in fish; Wren and MacCrimmon 1983; Rada et al. 1986; McMurtry et al. 1989; Wiener et al. 1990; Wren et al. 1991) or with lipid concentration (e.g., Roberts et al. 1977; Borgmann and Whittle 1983; Möller et al. 1983). At issue is how to remove the correlated influence of animal size or lipid concentration so differences in contaminant concentrations can be examined and better understood.

One common approach employs analysis of covariance (ANCOVA) to compare slopes and intercepts of several regres-

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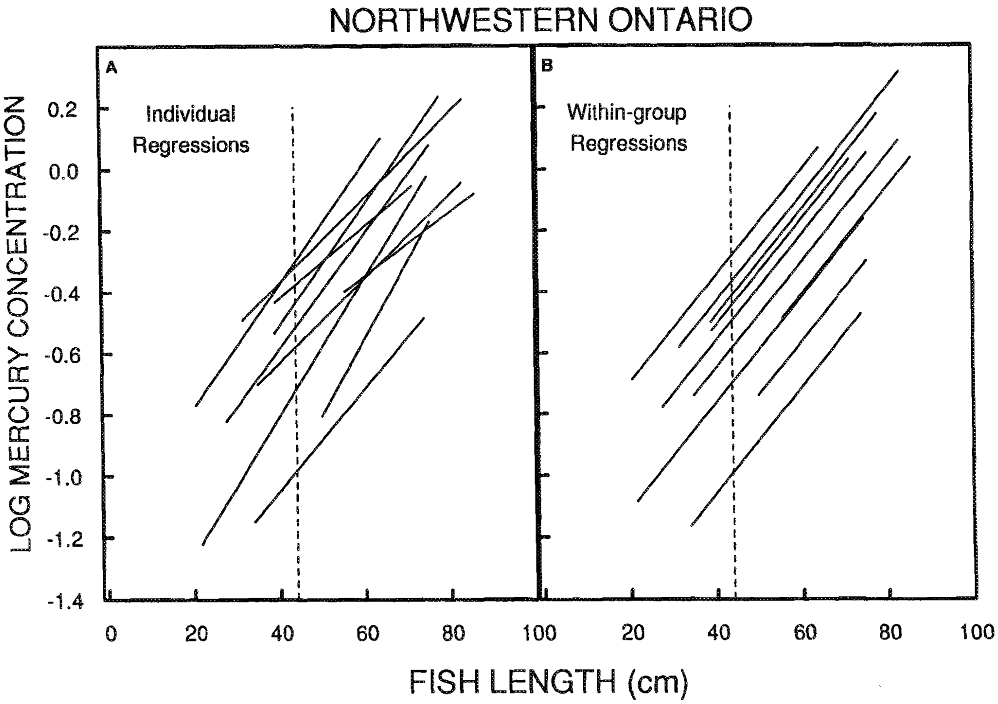

FIG. 1. Regressions of mercury concentration on fish length for 10 populations of lake trout from northwestern Ontario lakes. The adjusted mercury concentration for a standardized length of 44 cm is indicated (by the broken vertical line) for each population based on (A) individual regressions and (B) common within-groups regressions. Line lengths span the range of lengths in the collection for each population.

sions, thereby using bivariate regression to adjust for uncontrolled variation in the independent variable (i.e., X) (e.g., Green 1986; Packard and Boardman 1987, 1988; Tracy and Sugar 1989). An ANCOVA is relatively simple with a small number of regressions, but with large data sets and many regressions, the revelation that slopes differ leaves much to be desired. Differing slopes confound further analysis, much like a significant interaction between main effects in an analysis of variance (ANOVA) (see Sokal and Rohlf 1981, p. 414–417). As a result, the ANCOVA will not reliably evaluate whether intercepts differ (i.e., the usual test of interest) when regressions are not parallel. This limitation is particularly frustrating when the covarying X variable could not be "controlled" at the time of data collection (e.g., Reist 1985; Packard and Boardman 1987; Meffe et al. 1988).

The problem of size adjustment only materializes when slopes are not parallel because ANCOVA automatically adjusts for the covariate and compares intercepts (or adjusted means) when the slopes are not different. However, when slopes differ, the common within-groups slope is recommended as the best estimate of the common slope (Thorpe 1976; Thorpe and Leamy 1983; Reist 1985) (e.g., see Baltz and Moyle 1981; Ihssen et al. 1981; Reist and Crossman 1987). This estimated slope is then used to calculate the intercept and adjusted mean concentation for each population, or the predicted concentration at some arbitrarily selected standard size. However, Reist (1985) cautioned that results from this type of adjustment may vary depending on the particular data set being analysed (e.g., see Thorpe 1976; Rayner 1985; Shea 1985).

In essence, the within-groups approach ignores the fact that slopes differ significantly among populations and simply estimates a common slope across all populations (see Fig. 1B). The suitability of this estimate depends on the relative variation of each population, since varying populations can have undue influence on the estimate of the common slope (e.g., see Thorpe 1976; Pimentel 1979, p. 81–91; Thorpe and Leamy 1983).

An alternative approach uses the individual regression equations to estimate mercury concentration at a common size for each population (see Fig. 1A) (e.g., Taylor and McPhail 1985; Johnson 1987; McMurtry et al. 1989). By using individual equations, significant differences in slopes are ignored although these differences are incorporated into the standardization. As a result, this method may combine differences in slopes with differences in intercepts (see below). The relative contributions of the slope and intercept depend on the scale of the original axes (e.g., the size of the slope relative to the intercept) and the common size selected. Because the choice to use the common, within-groups approach may also affect the results, we must choose between two potential distortions in order to examine other factors that correlate with observed differences among populations.

McMurtry et al. (1989) provided a recent example in an examination of the concentrations of mercury in dorsal muscle of populations of lake trout (*Salvelinus namaycush*) and smallmouth bass (*Micropterus dolomieu*) from approximately 90 lakes in Ontario. M.J. McMurtry (personal communication) first used ANCOVA to compare regressions of logarithmically transformed mercury concentration on fish length for each population because mercury concentration in fish muscle tends to covary with fish size (e.g., Scott and Armstrong 1972; Scott 1974; MacCrimmon et al. 1983; Sprenger et al. 1988). Unfortunately, the regression slopes differed among populations (e.g., see Fig. 1A). To explore environmental factors that correlated with these differences, McMurtry et al. (1989) subsequently chose to adjust observed mercury concentrations to a standard lake trout size of 44 cm using individual regression equations for each population (e.g., Fig. 1A). These adjusted values were then correlated with physical and chemical variables from each lake to determine which environmental factors best predicted size-adjusted tissue-mercury concentrations.

The estimated mercury concentration used by McMurtry et al. (1989) is a composite index based on the observed regression intercept plus the slope multiplied by the adjusted size (compare Fig. 1A and 1B). Although means and standard deviations of the original variables (i.e., fish length and mercury concentration) differed among populations, these differences were ignored because differences in the size distributions of the samples likely reflected collecting biases (M.J. McMurtry, personal communication).

By using either the within-groups or the individual regression adjustment, the bivariate relationships are reduced to a single index for each population (see Fig. 1). As a result, the means and standard deviations for each variable are combined with the regression slopes, intercepts, and correlations (i.e., weighted by the correlation; see Jensen 1986) to estimate a mercury concentration that should be independent of fish length. Unfortunately, this data-reduction approach pools information inconsistently because different weights are used for each population. As a result, similar size-adjusted values may arise even though the original values differed considerably. Henceforth, this difference is lost from subsequent analyses.

Alternatively, an analysis using univariate and bivariate summary statistics as new variables for each population may provide insight with less information loss (e.g., Atchley and Rutledge 1980; Rohlf and Archie 1984; Ferson et al. 1985). Because the summary statistics are estimated from the same variables, these statistics are not independent and hence, a multivariate analysis is warranted. If a priori groups or classes of populations exist (e.g., geographic regions), a multivariate analysis of variance (i.e., MANOVA) might be employed. Otherwise data exploration techniques such as principal components analysis would summarize covarying patterns among variables.

This paper focuses on the recurring problem of adjusting for a covariate when regression slopes differ (e.g., Whittle and Fitzsimons 1983; Johnson et al. 1987; Allard and Stokes 1989) by (1) illustrating the implications of such adjustments on analytical results and subsequent data interpretation, and (2) demonstrating an alternative multivariate approach to this problem. We use a fish-and-mercury example although this is a common problem relevant to physiology, morphometrics, and many other subdisciplines (e.g., see Reist 1985; Packard and Boardman 1987, 1988). To demonstrate the utility of our multivariate approach, we analyse data from 10 lake trout populations from each of three regions in Ontario to search for large-scale regional patterns as possible evidence of atmospheric deposition (i.e., spatial autocorrelation) (e.g., Jumars et al. 1977; Sokal and Oden 1978; Mackas 1984; Fortin et al. 1989).

Future studies should contrast these multivariate results with lake physical and chemical features to search for potential cause-and-effect relationships (as in McMurtry et al. 1989; Cope et al. 1990; Wiener et al. 1990). For example, spatial patterns in the multivariate results could be compared with patterns in the physical and chemical variables using Mantel tests (e.g., Jackson and Harvey 1989; Legendre and Fortin 1989) and Procrustes analysis (e.g., Digby and Kempton 1987, p. 112–123; Rising and Somers 1989; Rohlf and Slice 1990). Similarly, localized patterns could be explored with partial Mantel tests that first remove large-scale regional patterns (e.g., Hubert 1985; Manly 1986; Smouse et al. 1986; Bocquet-Appel and Sokal 1989).

Methods

To explore this problem with a balanced design focusing on three geographic regions in Ontario, we selected 30 of the 91 regressions for lake trout populations that were described by McMurtry et al. (1989). Ten lakes were chosen from northwestern Ontario (i.e., with latitude >48° and longitude >89°), 10 from the Algoma area (i.e., 46–48° latitude and 82–85° longitude), and 10 from the Algonquin area (i.e., 44–46° latitude and 77–81° longitude) (see Table 1). Individual lakes were selected if at least 20 fish were sampled. Lakes with samples of at least 15 and 13 fish, respectively, were included to obtain 10 lakes from northwestern Ontario and the Algoma region.

Raw data for fish size and mercury concentration were summarized with the mean, standard deviation, minimum, and maximum to provide eight univariate summary variables (see Table 1). Regressions of the logarithm of mercury concentration in dorsal muscle and fish length (as in McMurtry et al. 1989) generated three bivariate statistics for each population (i.e., slope, intercept, and correlation). One-way ANOVAs were used to contrast these 11 variables from the three regions to assess geographic differences (SAS Institute Inc. 1988). A posteriori differences were identified with the least-significant-difference test.

The impact of subjectively choosing an adjusted size was explored by calculating predicted mercury concentration at fish lengths of 25, 44, 100, and 200 cm using individual population regressions, within-group regressions, and the common regression across all populations (i.e., approximately 0.5, 1, 2, and 4 times the value used by McMurtry et al. 1989). The resultant predicted mercury concentrations were correlated with the original population parameters using Spearman rank correlations.

Multivariate differences were assessed with a MANOVA and canonical variates analysis (CVA) (SAS Institute Inc. 1988). We then summarized patterns among the lakes with an ordination. The eight univariate and three bivariate summary statistics were each standardized to a 0–1 range to weight each variable equally without changing the distribution of the values. A Euclidean distance matrix was then calculated between the 30 lakes using these 11 standardized variables. This distance matrix was rotated to the first five principal coordinate axes with principal coordinates analysis (PCoA) (Gower 1966). Loadings of the 11 variables on these new axes were assessed with Spearman rank correlations because of nonnormality in the data. Spearman rank correlations were also calculated between the predicted-mercury variables and the PCoA axes.

To test for spatial autocorrelation, the Euclidean distance matrix based on the first five principal coordinates was compared with an interlake geographic distance matrix using a Mantel test and associated correlogram (e.g., see Sokal 1979; Legendre and Troussellier 1988; Bocquet-Appel and Sokal 1989; Legendre and Fortin 1989). We only used the first five PCoA axes to omit uncorrelated variation embedded in the entire data set (i.e., random noise) (see Gauch 1982). Both Pearson and Spearman correlations were evaluated in the Mantel tests (and associated

TABLE 1. Location, sample sizes, and univariate and bivariate summary statistics for 30 lake trout populations from three regions in Ontario.

Region			Regression			Fish length (cm)				Mercury concn. ($\mu g \cdot g^{-1}$)			
Lake name	Lat., long.	N	r^2	Slope	Intercept	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
Northwestern Ontario													
Bending	49°19′, 92°08′	20	0.447	0.012	−0.89	56.5	11.3	39.2	71.5	0.67	0.420	0.30	2.20
Greenwater	48°34′, 90°26′	24	0.759	0.023	−1.71	48.2	13.6	21.6	74.9	0.34	0.388	0.05	2.00
Pickerel	48°37′, 91°19′	23	0.618	0.014	−0.93	57.4	14.7	31.6	83.3	0.89	0.667	0.31	3.20
Snook	50°12′, 94°41′	23	0.721	0.020	−1.18	51.0	13.4	20.5	64.2	0.86	0.581	0.12	2.70
Basswood	48°05′, 91°35′	18	0.772	0.014	−1.18	45.4	11.2	35.0	83.0	0.31	0.202	0.20	1.05
Clearwater	49°00′, 91°57′	17	0.728	0.025	−2.06	59.0	7.4	50.0	75.4	0.29	0.134	0.10	0.60
Paguchi	49°34′, 91°32′	16	0.752	0.017	−1.71	58.5	11.4	34.0	74.0	0.21	0.108	0.08	0.43
Quetico	48°34′, 91°55′	17	0.820	0.020	−1.30	54.3	9.7	39.0	78.0	0.67	0.430	0.30	1.91
Sandy Beach	49°49′, 92°21′	16	0.276	0.011	−0.98	68.6	8.2	55.6	85.8	0.58	0.214	0.27	0.98
Trout	50°14′, 94°55′	16	0.844	0.019	−1.34	57.3	12.6	27.6	75.5	0.65	0.414	0.18	1.60
Algoma													
Burns	46°36′, 83°07′	59	0.838	0.031	−2.37	34.1	11.6	20.2	75.0	0.08	0.090	0.01	0.50
Esten	46°22′, 82°40′	20	0.939	0.024	−1.75	46.0	19.4	20.6	87.0	0.38	0.440	0.04	1.40
Wawa	48°01′, 84°43′	37	0.765	0.014	−1.34	34.0	16.3	13.6	67.9	0.16	0.087	0.03	0.39
Cobre	46°38′, 82°48′	15	0.411	0.013	−1.53	43.3	8.1	28.5	52.9	0.12	0.054	0.07	0.25
Como	47°55′, 83°30′	15	0.567	0.021	−1.39	68.2	9.6	53.0	89.5	1.29	0.615	0.32	2.21
Flack	46°35′, 82°47′	17	0.257	0.013	−1.31	40.1	5.0	29.0	48.0	0.17	0.046	0.10	0.25
Lac Aux Sables	46°47′, 82°20′	17	0.801	0.019	−1.42	51.2	10.3	39.6	74.5	0.41	0.264	0.20	1.19
Matinenda	46°22′, 82°57′	15	0.880	0.020	−1.52	48.7	13.7	19.5	68.4	0.34	0.231	0.11	0.81
North Hubert	47°20′, 84°27′	17	0.443	0.018	−1.54	46.8	10.9	31.0	64.5	0.24	0.168	0.08	0.60
Saymo	46°59′, 83°31′	13	0.856	0.018	−1.19	44.0	11.6	30.6	69.4	0.45	0.299	0.20	1.14
Algonquin													
Bark	45°27′, 77°51′	20	0.464	0.015	−0.81	66.2	6.2	52.3	79.1	1.61	0.658	1.10	3.50
Big Porcupine	45°27′, 78°37′	24	0.751	0.037	−2.18	36.2	6.5	25.5	45.3	0.18	0.102	0.05	0.39
Bonnechere	45°28′, 78°35′	26	0.787	0.026	−1.61	40.7	8.2	21.2	54.1	0.33	0.177	0.12	0.69
Burn's	45°19′, 77°05′	29	0.254	0.019	−1.81	30.5	5.4	23.0	43.0	0.06	0.024	0.01	0.13
Camp	45°26′, 78°55′	21	0.672	0.018	−1.49	34.4	10.1	26.0	59.0	0.15	0.084	0.04	0.40
Drag	45°05′, 78°24′	35	0.663	0.025	−1.69	38.1	11.0	24.0	74.0	0.27	0.330	0.04	1.50
Fletcher	45°21′, 78°47′	21	0.851	0.021	−1.64	41.1	16.1	19.5	69.0	0.25	0.220	0.05	0.71
Gull	44°51′, 78°47′	20	0.366	0.010	−0.98	31.7	5.4	21.0	40.2	0.23	0.050	0.15	0.36
Haliburton	45°12′, 78°24′	20	0.524	0.017	−1.00	60.5	8.1	39.5	73.8	1.07	0.426	0.43	2.01
Kennisis	45°13′, 78°38′	20	0.850	0.024	−1.82	41.7	10.2	21.4	66.9	0.19	0.198	0.07	0.99

correlograms) using two-tailed probabilities and 10 000 randomizations (as recommended by Jackson and Somers 1989; also see Legendre and Fortin 1989).

Results

One-way ANOVAs of the raw data confirmed concerns expressed by McMurtry et al. (1989). The mean length of lake trout collected from northwestern Ontario lakes was significantly greater than the mean for lake trout collected from Algoma and Algonquin lakes ($F = 5.29$ with 2, 27 df, $P < 0.012$, $r^2 = 0.282$). In addition, the maximum length of lake trout differed among the three areas ($F = 4.77$ with 2, 27 df, $P < 0.017$, $r^2 = 0.261$). Maximum fish sizes from northwestern Ontario lakes were significantly larger than the Algonquin samples, and the Algoma fish were intermediate. None of the other summary statistics differed significantly among the regions ($P > 0.1$).

Regressions of the logarithm of mercury concentration and fish length displayed significantly different slopes across the 30 populations (ANCOVA, $F = 4.55$ with 29, 620 df, $P < 0.001$; with a common within-group regression slope of 0.0201). Significant heterogeneity of variance was also evident ($F_{max} = 8.93$ with 15, 18 df, $P < 0.001$). In addition, regression slopes were significantly different within regions (i.e., northwestern Ontario: $F = 2.33$ with 9, 179 df, $P < 0.025$, see Fig. 1A; $F_{max} = 4.15$ with 22, 16 df, $P < 0.005$; Algoma: $F = 7.93$ with 9, 214 df, $P < 0.001$; $F_{max} = 6.41$ with 15, 11 df, $P < 0.005$; Algonquin: $F = 2.69$ with 9, 225 df, $P < 0.01$; $F_{max} = 7.18$ with 33, 18 df, $P < 0.005$). The common, within-group regression slope was largest for the set of Algonquin lakes (0.0222) and decreased for the Algoma (0.0209) and northwestern Ontario lakes (0.0173).

Predicted mercury concentrations at lengths of 25, 44, 100, and 200 cm showed no significant differences among the regions (ANOVA, $P > 0.2$). Mercury values at 25 and 44 cm that were estimated with the individual regressions were significantly correlated with mean fish length, mean mercury concentration, and the regression intercept ($P < 0.05$) (Table 2). Mercury concentrations at a 25-cm length were also correlated with the regression slope. Correlations with mean fish length suggest that the adjusted values for fish 25 or 44 cm in length based on the individual regressions failed to remove the influence of fish size. In contrast, predicted mercury concentrations at 100 and 200 cm were correlated with the regression slope and correlation, but not mean size. Adjusted mercury at 100 cm was also correlated with mean mercury concentration whereas the 200-cm mercury concentration was correlated with the regression intercept.

These findings changed when using the common-slope adjustment or the comon, within-groups regression adjustment

TABLE 2. Spearman rank correlations (×1000) between common within-group-adjusted and individual-regression-adjusted mercury concentrations and univariate, bivariate, and multivariate summaries for lake trout populations from 30 Ontario lakes (r_s = 0.375 at $P < 0.05$, 0.483 at $P < 0.01$, 0.598 at $P < 0.001$).

Regression adjustment and associated size for [Hg] prediction	Spearman rank correlation (×1000)									
	Mean fish length	Mean Hg concn.	Regression			Principal coordinate axis				
			Slope	Intercept	Correlation	I	II	III	IV	V
Common slope across groups										
[Hg] at 44 cm	305	758	−178	742	002	576	013	325	380	395
Common within-group slope										
[Hg] at 25 cm	439	832	−256	810	−051	694	029	291	285	315
[Hg] at 44 cm	325	766	−213	776	−024	591	031	318	360	388
[Hg] at 100 cm	053	524	000	508	−018	289	056	231	540	377
[Hg] at 200 cm	−272	104	101	156	−013	−113	110	135	509	325
Individual regression slope and intercept										
[Hg] at 25 cm	453	767	−538	956	−224	687	242	347	114	315
[Hg] at 44 cm	451	840	−173	747	−040	683	042	202	345	301
[Hg] at 100 cm	096	425	730	−147	394	246	−497	−278	654	046
[Hg] at 200 cm	−047	089	974	−606	567	−039	−650	−407	449	−083

TABLE 3. Spearman rank correlations (×1000) between univariate and bivariate summary statistics and the first five principal coordinate axes for lake trout populations from 30 Ontario lakes (r_s = 0.375 at $P < 0.05$, 0.483 at $P < 0.01$, 0.598 at $P < 0.001$). Dashes within the body of the table represent correlations between a variable and itself.

Univariate and bivariate summary statistics	Spearman rank correlation (×1000)									
	Mean fish length	Mean Hg concn.	Regression			Principal coordinate axis				
			Slope	Intercept	Correlation	I	II	III	IV	V
Mean length	—	798	−151	392	−052	893	075	−313	−218	021
Length SD	053	159	185	−063	645	182	−814	429	−366	−150
Min. length	722	494	−339	397	−410	639	557	−494	−172	094
Max. length	690	619	069	181	262	757	−305	−285	−301	−073
Mean [Hg]	798	—	−078	620	074	937	−093	021	101	139
[Hg] SD	686	917	136	415	250	883	−379	061	146	−026
Min [Hg]	742	834	−396	769	−171	802	318	−081	036	380
Max [Hg]	678	906	095	461	181	891	−322	046	200	−007
Slope	−151	−078	—	−744	565	−186	−647	−422	384	−141
Intercept	392	620	−744	—	−346	593	378	434	−036	305
Correlation	−052	074	565	−346	—	−002	−846	022	−150	382

(Table 2). Correlations between predicted mercury concentration using the within-groups adjustment and mean fish length or mean mercury concentration resembled correlations based on the individual-regression adjustment. However, correlations with the regression statistics differed (i.e., depending on the adjusted size), with no significant correlations with the regression slope or associated correlation coefficient. Clearly, adjusting data to a common size affects subsequent results and interpretations when regression slopes differ.

A PCoA of the univariate and bivariate statistics summarized 95% of the standardized variation in the first five axes (Table 3). The first axis accounted for 50% of the variation and included seven of the univariate variables (excluding the standard deviation of fish length) and the regression intercept. The second PCoA axis contrasted the standard deviation of length, plus the regression slope and correlation with minimum fish length and intercept (i.e., 24.6% of the variation).

One-way ANOVAs of the five sets of PCoA scores indicated that the three regions differed on the first axis (F = 2.64 with 2, 27 df, $P < 0.09$, r^2 = 0.164) and differed significantly on the fourth axis (F = 5.98 with 2, 27 df, $P < 0.008$, r^2 = 0.307). This fourth axis contrasted the maximum and standard deviation of fish length with the regression slope (Table 3; i.e., 7.9% of the variation). The Algonquin lakes had greater mean scores compared with the Algoma and northwestern Ontario lakes.

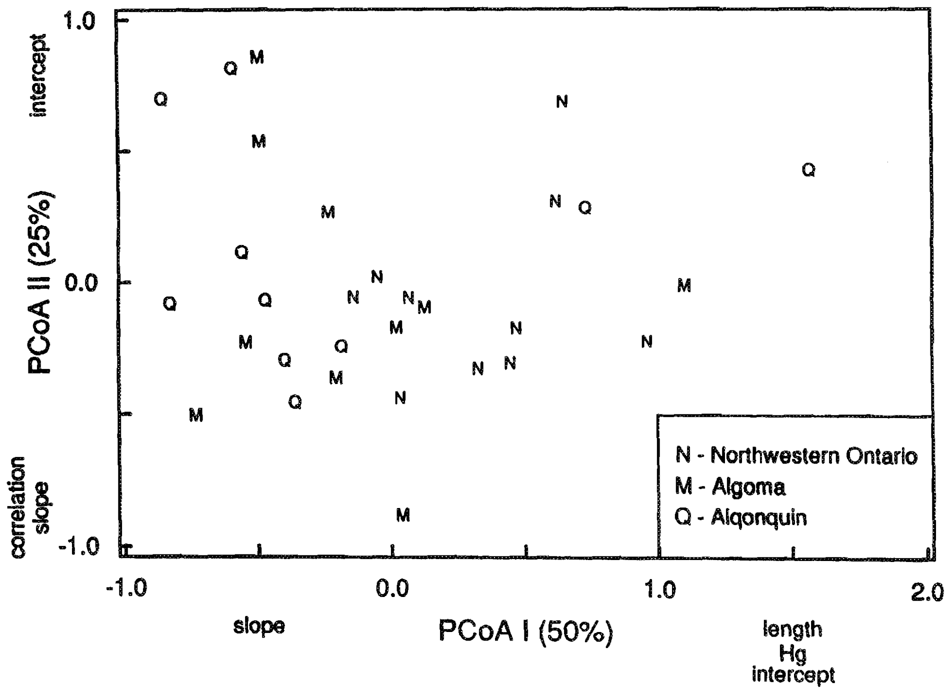

FIG. 2. Scatterplot of the first and second PCoA axes derived from the analysis of Euclidean distances among 30 lakes based on eight univariate and three bivariate summary statistics from data on lake trout length and tissue-mercury concentrations.

A MANOVA confirmed the univariate findings by revealing significant differences among the regions using the 11 variables (Wilk's lambda = 0.165, F = 2.25 with 22, 34 df, $P < 0.017$; Roy's Greatest Root = 2.98, F = 4.87 with 11, 18 df, $P < 0.002$). The CVA (not shown) emphasized that the multivariate differences arose because fish from northwestern Ontario were larger than the Algoma or Algonquin samples. A MANOVA using the five PCoA axes was also significant (Wilk's lambda = 0.490, F = 1.97 with 10, 46 df, $P < 0.059$; Roy's Greatest Root = 0.868, F = 4.16 with 5, 24 df, $P < 0.008$), emphasizing that the standardization and the choice to use only the information in the first five axes reduced the multivariate separation among the three regions.

The three sets of lakes overlap in the space defined by the first two PCoA axes (Fig. 2). Most of the northwestern Ontario lakes lie on the right side of the ordination, although this space is shared with four Algoma and two Algonquin lakes. These populations are characterized by larger fish with higher mercury concentrations and larger regression intercepts (Table 3). The remaining Algoma and Algonquin populations are intermixed, suggesting similarities among these populations with respect to the mercury and fish-length data.

The MANOVA based on the ordination revealed that populations of lake trout from the northwestern Ontario lakes show modest separation from the other populations (see Fig. 2). The overlap among lakes from the various regions suggests that factors contributing to differences in tissue mercury vary within a region (e.g., mean and maximum fish length). However, this observation does not discount a local-effects hypothesis that populations from nearby lakes (e.g., within the same watershed) exhibit similar mercury fish-size relationships.

The Mantel test indicated a significant correlation between distances among lakes on the first five PCoA axes and the geographic distances separating those same lakes (r = 0.116,

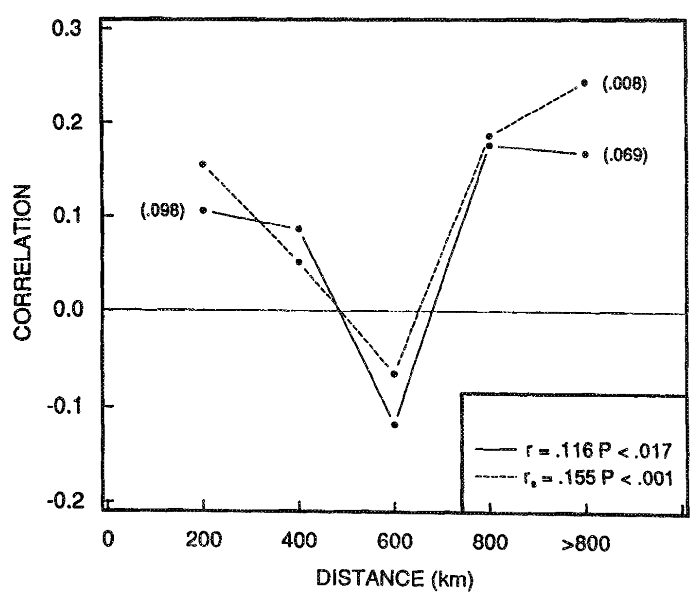

FIG. 3. Mantel test correlogram contrasting interlake distances from the first five PCoA axes and geographical distances. Overall Pearson and Spearman correlations are presented in the legend whereas class values are plotted (along with probabilities when less than 0.1). Class sample sizes are 115, 70, 66, 64 and 120, respectively. Distances represent class marks, not midpoints.

$P < 0.017$; r_s = 0.155, $P < 0.001$). The larger rank correlation suggests that the relationship between the two distance matrices is curvilinear, with differing local and regional effects.

A correlogram showed that positive spatial autocorrelation exists among the nearest lakes, as well as the most distant lakes (Fig. 3). This observation suggests that mercury fish-size relationships in lake trout populations in neighbouring lakes are similar ($r_s = 0.155$, $P < 0.098$; i.e., supporting the local-effects hypothesis). However, the strongest association existed between the most distant lakes (i.e., between regions; $r_s = 0.243$, $P < 0.008$), indicating significant large-scale regional patterns (i.e., the weak geographic clusters observed in Fig. 2). The shape of this correlogram is characteristic of a surface with a central peak or depression (e.g., see Legendre and Fortin 1989), although this pattern incorporates covariation associated with differences in fish length (i.e., length-related information in PCoA axis I that varies across regions; see Table 3).

A Mantel test and associated correlogram based on a distance matrix using the second through fifth PCoA axes omits a multivariate analogue of these length-correlated effects (e.g., see Somers 1989) and examines the spatial pattern of this four-dimensional configuration (i.e., 46.1% of the standardized variation in the original 11 variables). The overall Mantel test was not significant ($r = 0.023$, $P < 0.637$; $r_s = 0.051$, $P < 0.299$), emphasizing that the large-scale regional pattern was mainly based on the fish-size information in the first PCoA axis (see Table 3). The correlogram (not shown) was similar to the one based on all five axes (Fig. 3), with both small-scale (i.e., <200 km: $r_s = 0.164$, $P < 0.085$) and large-scale spatial autocorrelation approaching significance (600 km: $r_s = 0.213$, $P < 0.086$; 800 km: $r_s = 0.232$, $P < 0.068$; >800 km: $r_s = 0.151$, $P < 0.098$). The MANOVA using these four ordination axes provided similar results (Wilk's lambda = 0.614, $F = 1.65$ with 8, 48 df, $P < 0.135$; Roy's Greatest Root = 0.625, $F = 3.90$ with 4, 25 df, $P < 0.014$), given that Roy's Greatest Root has greater power to detect a gradient than Wilk's lambda (Clarke and Green 1988). These findings suggest that both local and regional patterns remained once the dominant fish-size effects were removed (i.e., patterns within areas, as well as among areas).

Discussion

McMurtry et al. (1989) assumed that their standardized mercury concentration represented a size-adjusted concentration for each lake trout population. Our analysis of a subset of their data substantiates this assumption (see Table 2). However, our size-adjusted data were also correlated with mean fish length and mean mercury concentration, suggesting that the standardization failed to remove the confounding influence of fish size. Had McMurtry et al. (1989) chosen an adjusted length of 25, 100, or 200 cm (recognizing that these lengths would be extrapolations beyond the data), their results would have probably differed. The choice of an alternative regression adjustment (e.g., the within-groups regression) would also affect their results.

Given these inconsistencies with size adjustments when slopes differ, we propose that univariate and bivariate summary statistics should be analysed with multivariate methods as an alternative to the use of a single adjusted value. Similar approaches are common in morphometrics (e.g., Atchley and Rutledge 1980), especially when using Fourier analysis methods (e.g., Rohlf and Archie 1984; Ferson et al. 1985). An ordination of univariate and regression statistics provides less ambiguous results and summarizes covarying patterns into uncorrelated axes for comparison with other suites of variables.

Since regressions are influenced by sample sizes, plus the standard deviations and ranges of the variables, the discovery of clusters of populations or outliers in the resultant ordination will assist in resolving differences among populations. The ordination in this example revealed patterns reflecting geographical proximity (i.e., spatial autocorrelation), perhaps due to both local watershed or lake basin geology, and large-scale patterns that might be attributable to atmospheric inputs (e.g., Rada et al. 1989; Mierle 1990). In addition, prey type can influence contaminant accumulation (e.g., MacCrimmon et al. 1983; Mathers and Johansen 1985; Cope et al. 1990), so biogeographical patterns associated with the presence or absence of particular prey species could also contribute to observed groupings of lakes (Rasmussen et al. 1990).

To extend the original study of McMurtry et al. (1989), the resultant ordination axes could be compared with patterns from ordinations of physical or chemical variables using a Mantel test or Procrustes analysis (e.g., Digby and Kempton 1987, p. 112–123; Jackson and Harvey 1989). Such methods have fewer restrictive assumptions than multiple regression, especially stepwise procedures (Reckhow et al. 1987; James and McCulloch 1990).

Perhaps one drawback to this approach is the difficulty in resolving or interpreting multivariate axes (James and McCulloch 1990), although the reification of such axes is now widely accepted (Pimentel 1979, p. 7; Reyment 1990). Given that these types of studies are inherently multivariate (i.e., based on several response variables), single summary indices (such as the adjusted mercury concentration described herein) are inappropriate and can contribute to misleading results and interpretations (e.g., see Jackson et al. 1990).

To summarize, we caution against the routine use of individual or common within-group regressions to adjust data to remove the influence of a predictor variable when regression slopes differ. These types of adjustments can produce misleading results reflecting differences in regression slopes rather than intercepts, or retaining information correlated with the original size variable. As an alternative, we illustrate that multivariate procedures can be used to examine univariate and bivariate summary statistics to resolve and interpret patterns with less information loss. In addition, the resultant ordination axes can be used to evaluate correlations with various environmental factors, including geographic location (i.e., spatial autocorrelation).

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