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Fish mercury levels in lakes—adjusting for Hg and fish-size covariation

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"Capsule": *Fish-size covariation can be circumvented by regression intercepts of Hg vs. fish length as lake-specific Hg levels.*

Abstract

Accurate estimates of lake-specific mercury levels are vital in assessing the environmental impact on the mercury content in fish. The intercepts of lake-specific regressions of Hg concentration in fish vs. fish length provide accurate estimates when there is a prominent Hg and fish-size covariation. Commonly used regression methods, such as analysis of covariance (ANCOVA) and various standardization techniques are less suitable, since they do not completely remove the fish-size covariation when regression slopes are not parallel. Partial least squares (PLS) regression analysis reveals that catchment area and water chemistry have the strongest influence on the Hg level in fish in circumneutral lakes. PLS is a multivariate projection method that allows biased linear regression analysis of multicollinear data. The method is applicable to statistical and visual exploration of large data sets, even if there are more variables than observations. Environmental descriptors have no significant impact on the slopes of linear regressions of the Hg concentration in perch (*Perca fluviatilis* L.) vs. fish length, suggesting that the slopes mainly reflect ontogenetic dietary shifts during the perch life span.

Keywords: Environmental pollutants; Mercury (Hg); Bioaccumulation; Fish-size covariation; Standardization (nomalization) of fishsize; Partial Least Squares regression (PLS)

1. Introduction

Covariation between noxious substances and fish body size is common in studies of environmental pollutants (Moriarty, 1983). The covariation makes it difficult to obtain accurate lake-specific estimates of bioaccumulating substances, such as mercury (Hg), in fish (e.g. Johnels et al., 1967; Verta et al., 1986; McMurtry et al., 1989; Somers and Jackson, 1993; Tremblay et al., 1998). Regression procedures are regularly used to circumvent the Hg concentration vs. fish body-size problem. Standardization (normalization) to an arbitrary fish size, including regression with a common slope for all lakes or individual regressions for each lake, is frequently used (e.g. Johnels et al., 1967; Verta et al., 1986; Håkanson et al., 1988; Lathrop et al., 1989; McMurtry et al., 1989; Harris and Bodaly, 1998). Other procedures include analysis of covariance (ANCOVA), where the fish-size dependence is considered being removed by using it as a covariate (Watras et al., 1998), and lake average residuals from regression on pooled data employed with a common slope (Richardson and Currie, 1995).

In all these methods, fish-size covariation will not be unequivocally removed if the slopes for different lakes are not parallel. In assessments of the environmental impact on Hg levels in fish, or if time series are examined for trends, the residual size dependence may present a problem (cf. Somers and Jackson, 1993). One serious implication is that the conclusions may be erroneous due to the fish-size distribution in the catch and/or the normalized fish size (Somers and Jackson, 1993; Tremblay et al., 1998). This could perhaps provide an explanation for the very divergent conclusions found in different studies on the influence of lake pH on Hg in biota (cf. Richman et al., 1988; Winfrey and Rudd, 1990; Downs et al., 1998; Watras et al., 1998).

The size covariation problem may be circumvented either by using fish within a very narrow size range or

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by using only small specimens such as 0^+ or 1^+ individuals (e.g. Nilsson and Håkanson, 1992; Post et al., 1996; Harris and Bodaly, 1998). Promising methods to avoid the size dependence have recently been presented, including multivariate methods (Somers and Jackson, 1993) and polynomial regression with indicator variables (Tremblay et al., 1998).

In this paper I will demonstrate that, in assessments of the environmental impact on Hg levels in fish, with pronounced Hg vs. size dependence, the intercept of the linearly regressed Hg concentration in fish vs. fish length is a good estimate of the lake-specific Hg level. In addition I will show that the application of common-slope (ANCOVA) or standardization techniques is improper for exploration of environmental effects on Hg levels in fish, since they do not fully remove the fish-size dependence. I will also point out that partial least squares regression (PLS), also called projections on latent structures, is a suitable evaluation tool to reveal environmental impact on Hg levels in fish (cf. Sonesten, 2001). The PLS method will be compared with the commonly used stepwise multiple linear regression (SMLR). PLS is a multivariate method that can be considered a biased regression extension of principal component analysis (PCA). The PLS method is especially applicable to collinear data (e.g. Geladi, 1988; Höskuldsson, 1988; Garthwaite, 1994), thus almost always to the kind of data considered here.

2. Materials and methods

Data consist of fish and lake water sampled in a regional survey of 79 lakes in the County of Uppsala, Sweden (latitude 59°30′–60°40′ N, longitude 16°40′–18°30′ E) during the period 1991–1993. All fish were caught between mid July and mid September. In total, the Hg content in 571 perch and 427 roach samples were analyzed (Appendices A and B). Catchment area characteristics (size, land use, etc.) were obtained from Brunberg and Blomqvist (1998). An extensive account of sample handling and analysis, as well as an area description is given in Sonesten (2001).

Six different approaches were used to investigate the applicability of the regression intercept as an estimate on the lake specific Hg level, environmental influence on the regression slope, and comparisons to fish-size standardization and stepwise multiple regression methods.

(1) Three different PLS models were compared to examine if the regression intercept of the Hg content vs. fish length is an accurate estimate in assessing the environmental impact on Hg levels in fish with an obvious Hg and fish-size dependency. Two single response variable models assessed the environmental impact on the Hg levels of perch (*Perca fluviatilis* L.) and roach (*Rutilus rutilus* L.). Theses PLS regression models were made on 48 and 46 environmental descriptors, respectively (cf. Sonesten, 2001, 2003). Theses environmental variables describe land use in the catchment area, various catchment area and lake characteristics, lake water chemistry and the fish stock. The respons parameters used to characterize the lake-specific Hg levels (response variables) were, for perch, the intercept of the linear regression of the log-transformed Hg concentration vs. fish length, and for roach, the geometric mean Hg concentration (Appendices A and B, respectively). These two single resonse PLS models were compared with a composite model. In this model, the intercept of Hg concentration in perch and the geometrical mean Hg concentration in roach were used as the two response variables, and the 46 environmental predictors employed in the roach model were explanatory variables (i.e. the perch growth rate and the lake specific Hg level in roach which were used in the single response perch model were excluded. All other descriptors were the same in the two single response models). The outcome of the composite model was graphically compared with the two individual models where the response variables were treated separately.

(2) The influence of environmental variables on the regression slope of the Hg concentration in perch vs. fish length (Appendix A) was also assessed by applying a PLS regression model. The 48 environmental predictors from the survey were used as explanatory variables (cf. Sonesten, 2003). In addition to the 46 environmental variables used in the combined PLS model, perch growth rate and Hg concentration in roach were also included.

Two tests were applied to examine the efficiency of commonly used methods in reducing the Hg and fish-size covariance.

(3) The common regression slope technique, as in ANCOVA, was exemplified by comparing the observed Hg concentrations in perch from Lake Vikasjön with predicted Hg concentrations achieved from a linear regression model on the Hg concentration in perch vs. fish length in all 78 lakes with perch in the survey ($\text{Ln[Hg]} = 0.0866 \times \text{perch length} \times 3.24$; $n = 571$, $R^2 = 0{,}64$, $P < 0.0001$).

(4) The effect of standardization of fish size on model stability and interpretation was evaluated by using different standardized length classes (0–40 cm) as response variables in 9 consecutive PLS models with the 48 environmental predictor variables used previously. The lake-specific linear regression models (Appendix A) were used to calculate the standardized Hg concentrations. Calculations were made on 5-cm fish length intervals within 5–40 cm total lengths. All fish-length intervals were within the size range encountered in the survey (5.0–44 cm).

(5) The interdependence between the standardized Hg concentrations used above and the lake-specific fish-size distribution of the analyzed samples were examined in a composite PLS model. The standardized Hg concentrations, the intercept, and the slope of the lake-specific Hg vs. fish-length regressions, were used as response variables and lake-specific fish-catch characteristics (minimum, maximum, mean, and standard deviation of fish length and weight) as explanatory variables (Appendix A).

(6) The PLS model on the environmental impact on Hg level in perch was also compared to the results from a SMLR using the same data as in Sonesten, 2003.

In each PLS model, cross validation was used to extract significant PLS components and to explore the predictive ability of the model (Eriksson et al., 1995; Lindgren at al., 1996). To ascertain that there was no serious background correlation in the data set due to latent structures, permutation tests were performed (Lindgren et al., 1996). The background correlation is given by the intercepts of the R^2 and Q^2 (cross-validated R^2) regressed against the R^2 of the real observed y and the 25 times rearranged **y** vectors. The statistical analyses were performed with Simca-P® 7.0 (Umetrics Inc.) and SAS® (V612 for the Macintosh, SAS Institute Inc.).

3. Results and discussion

3.1. Comparing the intercept and geometric mean as lake-specific Hg level estimates

The two different methods to estimate the Hg levels in perch and roach (i.e. the intercept of the Hg concentration vs. fish-length regression and the geometric mean Hg concentration) gave similar results. The PLS weights plot of the combined model (Fig. 1), shows that the correlation structure between the environmental predictors and the Hg levels is similar to the weights plots of the separate PLS models (Sonesten, 2001, 2003). Furthermore, the weights (c) for the two Hg level estimates are very close to each other (Fig. 1), which indicates their similarity, even though a separate correlation analysis only reveals a modest relationship ($r^2 = 0.46$, $P < 0.0001$). The directions of the main environmental influence, as well as the positions of various predictors (arrows and points respectively, in Fig. 1), in the joint model are also very similar to the separate models (op. cit.). The analogy of the different PLS models indicate that they extract virtually the same information from the environmental matrix, thus describing the same thing, viz. the environmental influence on the lake-specific Hg levels of the two different species. The application of the "intercept method" requires fish species with size dependence of the Hg concentration and a sample with a wide fish-size range. Small specimens in particular are needed to obtain an accurate estimate of the intercept.

3.2. Between-lake variation of the Hg vs. fish-size regression slope

Partial least square regression analysis failed to find any significant influence of the environmental predictors on the regression slope of the Hg concentration vs. fish size. Actually, the explained variation ($R^2 = 0.14$; Table 1) is very close to the background correlation ($R^2 = 0.13$) in the data set as measured by a permutation test. The cross-validated R^2 (Q^2) is even less than 0 (Table 1), which supports the conclusion that this is a "nonsense model" without predictive power. Not even perch growth rate, which is indirectly affected by the environment, had any significant impact on the regression slopes. This suggests that the lake-specific bioaccumulation rate of Hg in perch, depending on the net effect of Hg content in food and various metabolic processes, merely reflect different Hg levels in the prevailing diet. The feeding behaviour of perch varies both spatially and temporally, generally including several dietary changes during its life span. As fry, the perch generally feed on zooplankton, turning successively to benthic and littoral invertebrates, and finally becoming piscivorous when sufficiently large (Collette et al., 1977; Persson et al., 2000). Hence, it follows that perch feed at different trophic levels during their development, generally feeding on prey with increasing Hg content as they grow.

3.3. Common slope as a means to remove fish-size dependence

The use of a common-slope approach to remove the fish-size dependence of the observed Hg concentration in perch was not successful. Considerable discrepancies between the observed and the predicted Hg concentrations were found (Fig. 2), which may be due to an incomplete removal of the size effect. Differences among lakes in the slopes of regression lines of Hg concentration vs. fish size presumably gave this residual variation. The common-slope method (ANCOVA) is frequently used to compare the intercepts, or adjusted means, between lakes, or in standardizing (normalizing) to an arbitrary fish size. This method has been commonly adopted in Swedish surveys, where the Hg concentration in northern pike (*Esox lucius* L.) is predominantly standardized to 1-kg specimens by dividing the Hg concentration by the fish weight (e.g. Johnels et al., 1967; Björklund et al., 1984; Håkanson et al., 1988). The underlying presumption is that the regression slopes are not significantly different, and fish within a narrow size range are often used to accomplish this. Another approach is to exploit the residuals between Hg concentrations predicted by a common

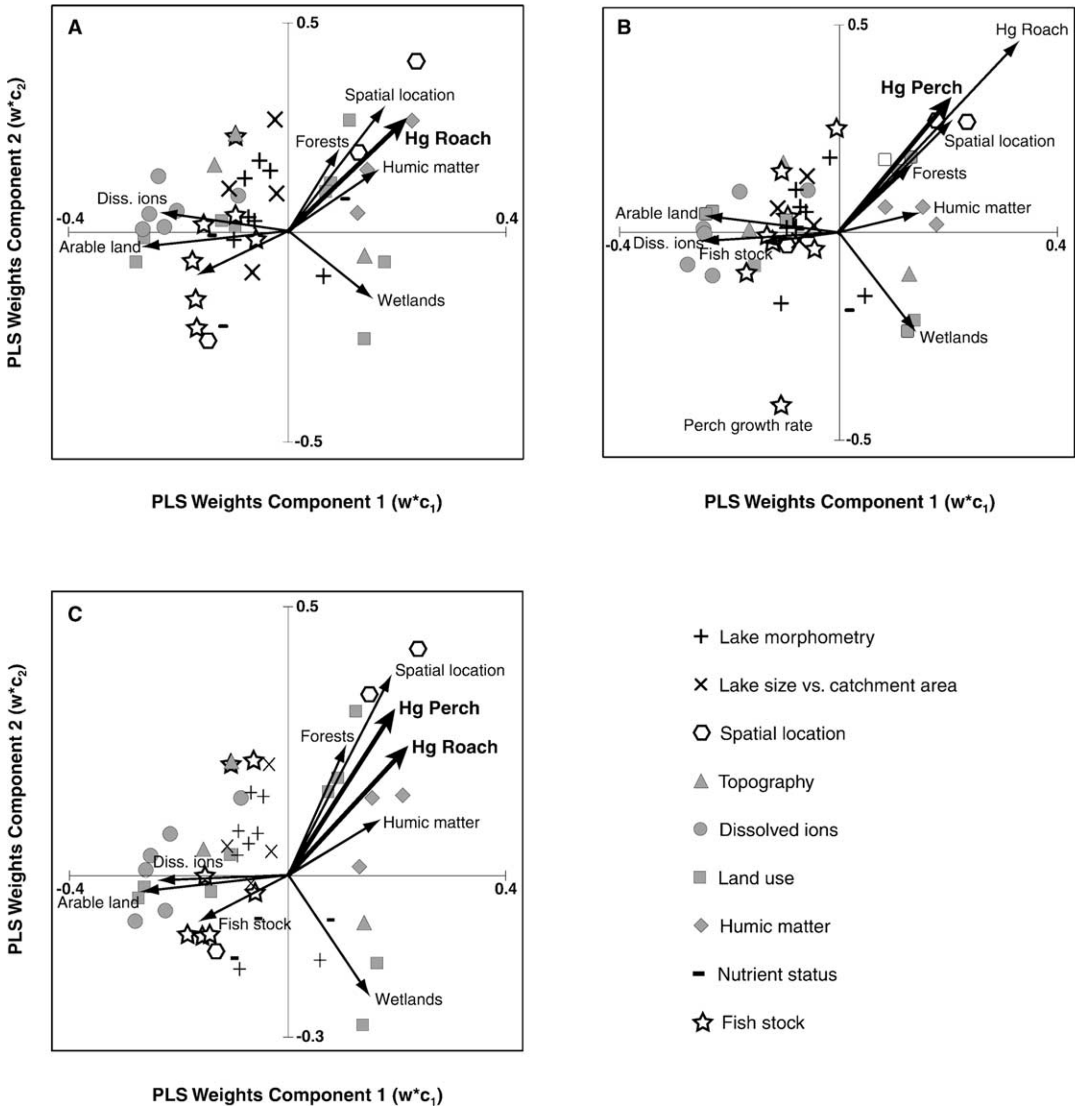

Fig. 1. The weights (w*c_1 vs. w*c_2) for the first and second latent components of three different PLS models on lake specific Hg levels in fish vs. various environmental descriptors. (A) Hg levels in roach (*Rutilus rutilus* L.) vs. 46 descriptors (after Sonesten, 2001), (B) Hg levels in perch (*Perca fluviatilis* L.) vs. 48 descriptors (after Sonesten, 2003), and (C) a combined model on the Hg levels in both perch and roach vs. 46 descriptors. The different models give the correlation structure between the environmental predictors and the observed Hg levels in the fish. To facilitate the interpretation the most important environmental variables have been subjectively divided into functional groups (arrows) giving the directions and strengths of their correlative relationships.

Table 1
Predictive ability, as measured by R^2 and Q^2 (cross-validated R^2), of the PLS model on the slope of Hg concentration in perch vs. fish length and 48 environmental predictors

PLS component	X	Percent variation		Q^2
		R^2	R^2adj.	
1	20	14	13	−9
2	31	29	27	−25

The amount of variation in the environmental matrix used by the model is also given (X).

slope and the observed concentrations. The lake average of the residuals is then considered to be the lake-specific deviation from the "average lake" in further statistical analysis (Richardson and Currie, 1995). However, if size dependence is not fully removed, the pronounced effect of the size distribution of the fish sample may be devastating (cf. Fig. 2). It is seldom easy to "control" the fish size during sampling, and with the strong size dependence still remaining after size adjustment (Fig. 2) the application of the common-slope approach is questionable.

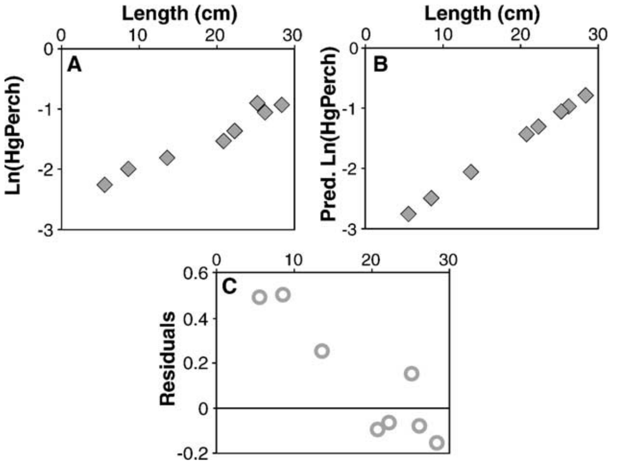

Fig. 2. The consequences of using a common-slope approach to adjust Hg concentration for fish-size covariation, when regression lines are not parallel. (A) Natural logarithms of Hg concentration vs. length of perch (*Perca fluviatilis* L.) from Lake Vikasjön, Sweden. (B) Predicted Hg concentration estimated by the common slope of Hg concentration vs. length of perch from 78 lakes in the County of Uppsala, Sweden, 1991–1993. (C) Residuals between the observed and the predicted Hg concentrations, illustrating the severe fish-size dependence remaining after the common-slope adjustment.

3.4. Fish-size effects on model stability of standardized Hg concentrations

Standardization of the Hg concentration to varying fish lengths showed that the fish-size covariation was unequivocally removed when there was a difference in regression slopes of the Hg concentration vs. fish size, in agreement with results of earlier studies (cf. Somers and Jackson, 1993; Tremblay et al., 1998). Accordingly, the model showed an increasing instability when several sequential perch sizes were analysed. The model degree of explanation (R^2) and predictive ability (Q^2) changes with standard fish size (Fig. 3). They are high for fish lengths up to approximately 25 cm, but are drastically reduced for larger standard sizes. As the standard fish size increases, there is a drop in predictability, indicative of ''nonsense'' models, probably caused by a lot of ''noise'' from the explanatory variables included. The interpretation of the PLS weights is strongly affected by standard fish size (Fig. 4). The correlative relationships between the environmental predictors and the Hg concentration in perch, as well as between the predictors themselves, varies greatly due to changes in standard size. The influence from the predictors varies notably in direction as well as in strength. Some variables (e.g. proportion of forested areas in the catchment area and lake-water Mg concentration) even shift signs when the fish size changes, i.e. the direction of their influence is altered (Fig. 4).

The interdependence between different standardized Hg concentrations and the fish-size distribution in the catch (or analyzed sub sample) was apparent (Fig. 5). In the model, where all the standardized Hg concentrations were used as explanatory variables and fish-size characteristics and the fish Hg concentration as response variables, there was a marked counterclockwise trend in the PLS weights as the standard fish size increased. The intercept (standard length = 0) was primarily affected by predictors that describe the perch Hg concentration in the first PLS component. When the standard fish size increased, the influence from the Hg concentration descriptors decreased and the Hg level was more correlated with the fish-size characteristics in the second PLS

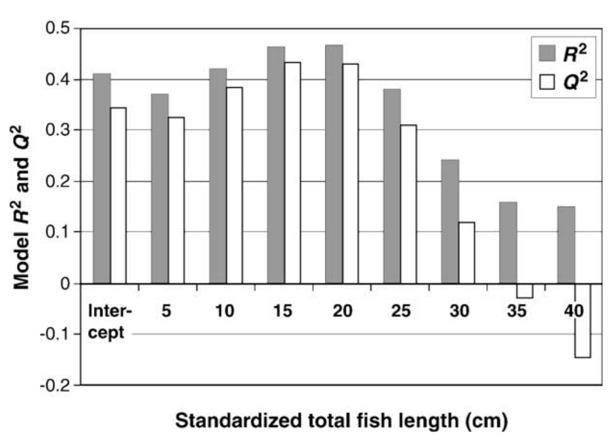

Fig. 3. The effect of standardized fish sizes on the PLS model stability. The degree of explanation (R^2) and predictive ability (Q^2) are illustrated for several separate PLS models with the Hg concentration standardized to various perch sizes (0–40 cm total length) vs. 46 environmental predictors.

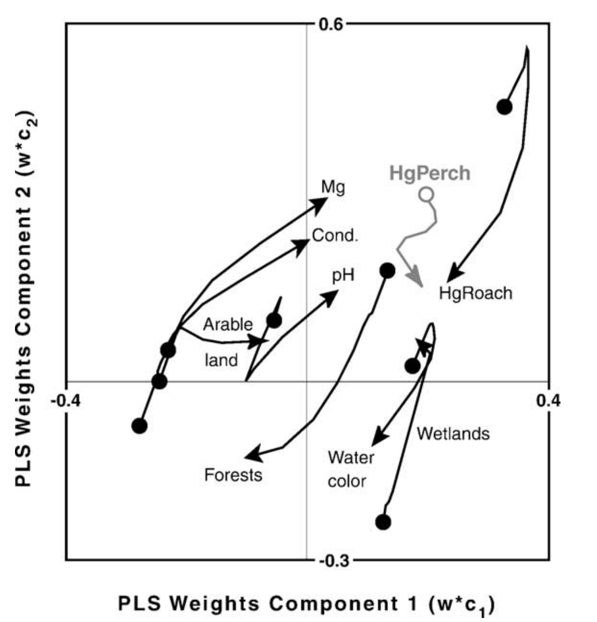

Fig. 4. Changes in PLS weight of Hg concentration in perch (*Perca fluviatilis* L.) vs. 48 environmental predictors, as an effect of changing standard fish size. The lines illustrate the changed influence, in strength as well as in direction, of selected predictors. Filled circles denote fish size 0-cm (intercepts) and arrowheads represent 40-cm standard fish length.

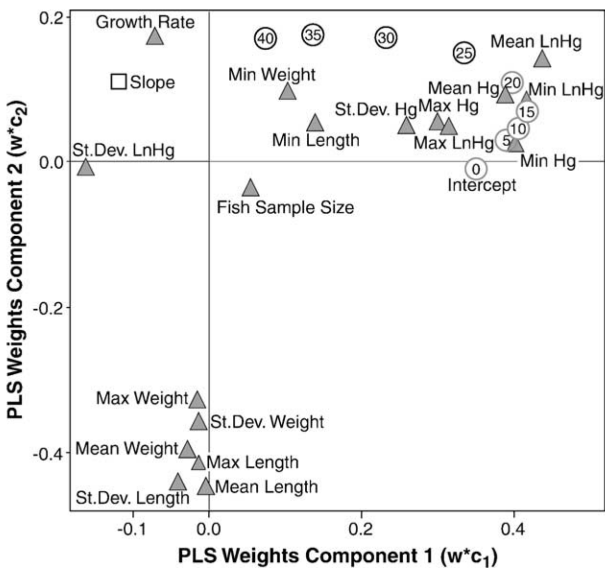

Fig. 5. The interdependence between Hg concentration in perch (*Perca fluviatilis* L.) standardized to various fish lengths, and fish-size distribution of sample. The PLS weights are from a combined PLS model on the standardized Hg concentrations vs. sample fish-size characteristics. Encircled numbers denote standardized Hg concentrations (number = total standard length in cm). Filled triangles give the positions of the fish-size and Hg concentration characteristics. The square marks the position of the regression slope of the Hg concentration vs. fish length.

component. For the largest standard fish sizes there were virtually no effects of the Hg concentrations. On the contrary, essentially all the variation in the standardized Hg level was explained by a negative relationship with samples containing large specimens, and consequently a positive association with samples with small specimens (Fig. 5). These correlations with various fish-size characteristics are illustrative of the incomplete removal of the Hg dependence on fish size.

The influence of pH on Hg bioaccumulation in aquatic food webs has been controversial during the past decade. Many investigations have shown a negative correlation between lake pH and Hg concentration in fish (Lathrop et al., 1989; Håkanson et al., 1990; Haines et al., 1995; Watras et al., 1998), whereas other studies have not been able to verify this relationship (Sonesten, 2001, 2003) or the results are inconsistent depending on species and/or time (Verta et al., 1986; Håkanson et al., 1988; McMurtry et al., 1989). It has also been proposed that the often-observed dependence may be explained by covariation in pH and the total lake biomass, since acidification results in a more oligotrophic ecosystem (Meili, 1994). I suggest that, apart from the problem of multicollinearity with other variables such as water color and the lake trophic status/biomass, some of these irregularities might stem from the instability of the models, caused by noise inclusion due to incomplete removal of the fish-size dependence.

3.5. Comparison of PLS and stepwise multiple regression

The SMLR of the Hg level in perch (the intercept) vs. the 48 environmental descriptors resulted in a model with nine explanatory variables (Table 2). The model, with $P<0.15$ as criteria for acceptance (SAS® default), is fairly unstable as indicated by Mallows C_p. With the most significant variable entered into the model, the C_p indicates that too few variables are included [C_p > number of included variables + 1 (cf. Freund and Littell, 1991)]. On the other hand, when two or more variables are included the low C_p values are indicative of an over-specified model with noise incorporated. The two most important predictors, according to the standardized regression coefficients, are the Hg levels in roach and the lake-water Mg concentration. This is in concordance with the PLS model, which identifies the same predictors as being the most important (Sonesten, 2003). Of the following seven variables included in the SMLR model, only two are found to offer significant contributions to the PLS model (geographical position in the northwest–southeast direction and lake-water Ca concentration). The lake-water Ca concentration illustrates one severe shortcoming in multiple linear regression (MLR) when applied to multicollinear data; the sign of the regression coefficient may shift depending on covariation with other explanatory variables (cf. Sokal and Rohlf, 1995). In the SMLR analysis, the regression coefficients of the Ca concentration and the closely related Mg concentration have opposite signs; whereas in the PLS analysis their influence was similar and with the same sign (Sonesten, 2003). Actually, out of the eight predictors with minor influence (partial $R^2<0.05$), four variables possess opposite signs compared to the PLS model (Table 2). This may lead to misleading conclusions about the influential direction of individual predictors in SMLR models, if underlying effects are not already known. Interestingly, the Mg concentration does not allow any inclusion of explanatory variables describing the influence of the catchment area or the amount of humic matter in the lakes. This gives the impression that the surrounding land and humic matter do not have major effects on the Hg level in perch (except via the Mg concentration), contradictory to conclusions earlier drawn (Sonesten, 2001, 2003). This discrepancy is explained by the severe multicollinearity between soil composition and land use, and e.g. the amounts of dissolved ions in lake water. It also displays the increased means of interpretation provided by multivariate projection methods like PLS that actually utilize the collinearity between predictors instead of being obstructed by it.

Table 2
Significant variables in stepwise multiple linear regression of the Hg level in perch vs. 48 environmental predictors (significance level: $P<0.15$). Regression coefficients in italic signify predictors possessing opposite direction of impact (different signs) on the Hg level compared with results from the PLS model (cf. Fig. 1). Environmental variables are explained inSonesten (2001, 2003)

Step	Variable entered into model	Regression coefficients		R^2		C_p	F	P
		Standardized	Ordinary	Partial	Model			
1	Hg level in roach	0.405	0.590	0.465	0.465	3.4	65.1	<0.001
2	Water Mg concentration	−0.567	−3.348	0.049	0.513	−1.5	7.4	0.008
3	O_2 depletion vulnerability[a]	*−0.213*	*−0.049*	0.035	0.548	−4.5	5.7	0.020
4	Perch growth rate	−0.181	−0.027	0.024	0.572	−5.9	4.0	0.050
5	CPUE Total weight of perch	*0.276*	*0.322*	0.023	0.595	−7.1	4.0	0.049
6	CPUE Total number of perch	−0.158	−0.003	0.016	0.611	−7.5	2.9	0.091
7	Z (geog. position in northwest–southeast)[b]	0.241	<0.000	0.017	0.628	−7.9	3.1	0.082
8	Maximum water depth	*0.205*	*0.055*	0.022	0.650	−9.1	4.4	0.040
9	Water Ca concentration	*0.197*	*0.227*	0.014	0.664	−9.0	2.7	0.106

[a] Water oxygen concentrations after 1 month of ice cover (data from Sonesten, 1989).
[b] cf. Sonesten (2001).

4. Conclusions

The proposed method to circumvent the Hg concentration dependence on fish size by using the intercept of lake-specific regressions of Hg concentration vs. fish size is shown to provide good estimates of Hg levels for evaluation of the environmental impact on Hg in lake ecosystems. The method is applicable to species like perch, showing evident Hg vs. fish-size dependence, and if a wide range of fish sizes has been collected. In the case of fish samples containing small specimens only, or with species like roach that lack a clear Hg vs. fish-size dependence, the geometric mean Hg concentration can be used instead as an accurate estimate of the lake Hg level.

This study also reveals that the Hg load dependence on fish size is still a prominent problem in environmental assessments, especially in carnivorous species that consume prey with high Hg contents. This size dependence is potentially a highly confounding factor, especially since the common measures for removing the fish-size effect are proven to be inefficient. The standardization of the Hg concentration to an arbitrary fish size is shown to still be dependent on the fish-size distribution of the sample, regardless of whether the standardization is accomplished by the common pooled-slope technique or by lake-specific regressions.

The study failed to identify any significant impact of the investigated environmental predictors on the regression slope of the Hg concentration vs. fish size. This lack of environmental impact on the accumulation of Hg with increasing size and/or age, suggests that the accumulation is mainly reflecting shifts in predominant prey during the perch life span. Thus, with time the perch will feed on prey from successive trophic levels, implying successively higher Hg contents in food sources due to biomagnification.

Stepwise multiple regression does not display the total complexity of the environmental impact upon the Hg levels in fish, as the method suffers severely from the inherent multicollinearity of the data. Instead, multivariate projection methods like PLS are recommended to reach a better understanding of the intricacy of environmental influence and processes.

Appendix A

Perch (*Perca fluviatilis* L.) size and Hg content range, and number of fish per lake used in lake-specific linear regressions of Ln(Hg content) vs. fish length. *Note! Regression coefficients are given for the log–linear relationship

Lake	N	Length (mm)		Weight (g)		Hg (mg/kg ww)		Intercept		Slope		R^2
		Min.	Max.	Min.	Max.	Min.	Max.	Coeff.*	P	Coeff.*	P	
Alsta sjö	7	53	265	1.3	231	0.045	0.594	−3.80	<0.0001	0.0130	0.0001	0.96
Assjösjön	7	80	298	6.3	346	0.035	0.253	−3.80	0.0011	0.0055	0.0984	0.45
Bredsjön	7	54	338	1.5	417	0.023	0.514	−3.27	0.0021	0.0093	0.0192	0.70
Bruksdammen	7	88	343	6.2	561	0.116	0.619	−2.68	<0.0001	0.0068	<0.0001	0.97
Dalarna	5	131	191	21.8	72	0.099	0.321	−4.66	0.0039	0.0186	0.0132	0.90

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Appendix A (*continued*)

Lake	N	Length (mm)		Weight (g)		Hg (mg/kg ww)		Intercept		Slope		R^2
		Min.	Max.	Min.	Max.	Min.	Max.	Coeff.*	P	Coeff.*	P	
Djupsjön, Alunda	6	51	275	1.2	224	0.046	0.357	−3.45	<0.0001	0.0087	0.0028	0.92
Djupsjön, Dalälven	6	117	314	15.7	326	0.213	1.197	−2.20	0.0085	0.0074	0.0250	0.75
Eckarfjärden	7	104	387	10.2	679	0.136	0.815	−2.39	0.0006	0.0054	0.0083	0.78
Edasjön	8	63	370	2.6	543	0.031	1.356	−3.41	0.0001	0.0093	0.0010	0.86
Ensjön	7	84	274	5.7	259	0.094	0.774	−3.22	0.0001	0.0100	0.0011	0.90
Fälaren	7	82	413	5.2	1003	0.099	1.338	−2.89	<0.0001	0.0083	0.0006	0.92
Fibysjön	7	56	376	1.5	717	0.046	1.359	−2.71	0.0020	0.0090	0.0064	0.80
Finnsjön	12	85	290	5.4	315	0.101	0.583	−2.68	<0.0001	0.0071	<0.0001	0.87
Fjärden	8	61	333	2.3	528	0.045	1.157	−3.41	0.0001	0.0096	0.0017	0.83
Funbosjön	7	52	289	1.5	300	0.029	0.480	−4.30	<0.0001	0.0137	0.0008	0.91
Gimo damm	7	84	307	5.0	365	0.101	0.846	−2.87	<0.0001	0.0079	0.0003	0.94
Gisslaren	7	77	376	4.5	764	0.035	0.503	−3.52	0.0007	0.0087	0.0084	0.78
Gårsjön	7	78	335	5.3	516	0.036	0.272	−3.28	0.0010	0.0062	0.0318	0.64
Hanelundsjön	7	63	363	2.4	632	0.076	0.495	−2.85	<0.0001	0.0064	0.0002	0.95
Huvsjön	6	62	247	2.2	157	0.054	0.368	−3.75	0.0004	0.0116	0.0044	0.89
Hålsjön	7	52	264	1.2	211	0.027	0.358	−3.56	0.0003	0.0090	0.0076	0.79
Kärven	7	50	368	1.0	712	0.075	0.362	−2.83	<0.0001	0.0045	0.0065	0.80
Lillsjön, Bålsta	7	65	338	2.5	519	0.024	0.455	−4.22	0.0003	0.0123	0.0034	0.84
Lillsjön, Österbybruk	7	65	278	2.5	248	0.035	0.537	−4.20	<0.0001	0.0128	0.0002	0.94
Lindsjön	6	72	165	3.3	41	0.082	0.300	−3.41	0.0005	0.0123	0.0091	0.85
Lissvass	7	89	320	7.1	373	0.086	1.211	−3.55	<0.0001	0.0121	0.0003	0.94
Lumpen	8	53	375	1.4	793	0.031	0.605	−2.74	0.0006	0.0066	0.0110	0.69
Långsjön, Almunge	8	59	279	2.0	247	0.057	0.699	−3.02	0.0004	0.0087	0.0058	0.74
Långsjön, Björklinge	7	85	298	5.3	295	0.018	0.271	−4.51	0.0001	0.0104	0.0038	0.84
Långsjön, Knutby	7	85	328	6.1	496	0.088	0.726	−3.10	0.0001	0.0077	0.0024	0.86
Långsjön, Länna	7	53	318	1.6	454	0.034	0.814	−3.2	0.0014	0.0092	0.0105	0.76
Löhammarsjön	7	56	276	1.3	263	0.047	0.363	−3.52	<0.0001	0.0090	0.0002	0.95
Mossaren	20	87	326	6.8	456	0.089	1.316	−2.55	<0.0001	0.0086	<0.0001	0.69
Mörtsjön, Fyrisån	7	56	437	1.4	1161	0.028	1.313	−3.95	0.0006	0.0114	0.0022	0.97
Mörtsjön, Järlåsa	7	67	397	2.6	678	0.039	1.580	−3.24	<0.0001	0.0086	<0.0001	0.87
N. Åsjön	7	95	338	8.0	511	0.204	1.256	−2.23	<0.0001	0.0066	0.0002	0.95
Norrsjön, Knutby	8	67	406	2.8	969	0.030	0.845	−3.07	0.0003	0.0075	0.0029	0.80
Norrsjön, Norreda	7	66	343	2.9	574	0.018	0.771	−4.22	0.0003	0.0125	0.0018	0.88
Norrsjön, Tysktorp	8	64	344	2.6	524	0.027	0.657	−3.84	<0.0001	0.0104	0.0003	0.91
Nävergården	4	156	345	37.7	580	0.331	1.051	−1.82	0.0327	0.0054	0.0572	0.89
Ramsen	8	62	378	2.5	761	0.023	0.748	−3.60	<0.0001	0.0099	0.0008	0.87
Ramsjön	7	51	399	1.0	972	0.071	1.766	−2.97	0.0003	0.0096	0.0008	0.91
Rastsjön	7	56	275	1.7	240	0.031	0.604	−3.57	0.0003	0.0117	0.0022	0.87
Ryssjön	7	55	372	1.5	785	0.019	0.293	−3.71	0.0005	0.0072	0.0185	0.70
S. Giningen	7	87	258	6.9	208	0.020	0.089	−4.66	<0.0001	0.0094	0.0037	0.84
S. Åsjön	8	84	329	5.7	450	0.200	1.363	−2.21	<0.0001	0.0075	<0.0001	0.97
Siggeforasjön	7	82	367	4.9	611	0.094	0.815	−2.59	0.0001	0.0071	0.0009	0.91
Skälsjön	7	95	290	8.5	322	0.173	0.889	−2.65	<0.0001	0.0077	0.0007	0.92
Skärsjön, Järlåsa	7	89	258	6.0	254	0.054	0.519	−2.84	0.0181	0.0077	0.1452	0.37
Skärsjön, Länna	7	90	430	7.0	1100	0.048	1.654	−3.6	<0.0001	0.0096	<0.0001	0.98
St. Agnsjön	7	90	340	7.2	531	0.113	1.406	−1.94	0.0017	0.0051	0.0041	0.86
St. Hallsjön	8	50	314	1.1	344	0.078	0.606	−2.84	<0.0001	0.0088	0.0003	0.92
St. Hållsjön	6	93	266	7.9	193	0.222	0.574	−2.72	0.0005	0.0067	0.0072	0.90
St. Hålsjön	10	56	438	1.6	1049	0.073	2.418	−2.51	<0.0001	0.0090	<0.0001	0.83
Stamsjön	6	154	279	41.3	289	0.037	0.331	−5.13	0.001	0.0145	0.0062	0.87
Stennässjön	7	51	350	1.2	588	0.028	0.493	−3.43	0.0005	0.0096	0.0048	0.82
Stordammen	7	55	291	1.6	330	0.035	0.260	−3.89	<0.0001	0.0095	0.0004	0.94
Storfjärden	6	90	200	7.7	98	0.121	0.271	−2.66	<0.0001	0.0064	0.0025	0.92
Storträsket	7	110	305	11.3	314	0.070	0.381	−3.43	<0.0001	0.0073	0.0003	0.94
Storvikarsjön	7	55	376	1.7	686	0.053	0.347	−3.28	<0.0001	0.0062	0.0001	0.96
Strandsjön	7	68	396	2.6	862	0.032	0.679	−3.66	0.0002	0.0093	0.0016	0.88
Strömmaren	11	96	337	8.3	483	0.044	0.386	−3.07	<0.0001	0.0060	0.0042	0.62
Strönningsvik	4	97	224	7.8	149	0.094	0.369	−3.20	0.0164	0.0088	0.0679	0.87
Säbysjön	7	53	331	1.3	444	0.020	0.451	−3.99	<0.0001	0.0098	0.0016	0.88
Sätersjön	7	86	313	5.5	385	0.204	1.200	−2.19	<0.0001	0.0079	0.0001	0.96

(*Continued on next page*)

Appendix A (*continued*)

Lake	*N*	Length (mm)		Weight (g)		Hg (mg/kg ww)		Intercept		Slope		R^2
		Min.	Max.	Min.	Max.	Min.	Max.	Coeff.*	*P*	Coeff.*	*P*	
Södersjön	8	64	288	2.6	339	0.027	0.556	−3.43	0.0004	0.0103	0.0048	0.76
Tarmlången	8	63	358	2.2	632	0.068	1.389	−3.02	<0.0001	0.0101	<0.0001	0.94
Testen	9	95	276	8.4	259	0.024	0.204	−4.32	<0.0001	0.0104	0.0018	0.77
Trehörningen	7	52	310	1.4	397	0.024	0.564	−4.12	<0.0001	0.0117	0.0002	0.95
Tvigölingen	8	60	294	2.0	308	0.059	1.071	−3.70	0.0011	0.0109	0.0243	0.60
Valloxen	7	67	353	2.9	589	0.015	0.177	−4.14	<0.0001	0.0077	0.0040	0.83
Velången	7	59	320	2.0	373	0.027	0.739	−3.71	<0.0001	0.0112	0.0007	0.92
Vikasjön	8	55	284	1.5	310	0.104	0.405	−2.58	<0.0001	0.0058	<0.0001	0.95
Vällen	7	68	381	2.9	796	0.018	0.595	−4.01	0.0002	0.0103	0.0024	0.87
Vällnoren	7	66	432	2.6	1043	0.032	0.728	−3.92	<0.0001	0.0091	0.0002	0.95
Åkerbysjön	7	88	325	6.5	407	0.100	0.904	−2.98	<0.0001	0.0089	<0.0001	0.98
Älgsjön	7	60	265	1.8	225	0.184	0.607	−2.15	<0.0001	0.0056	0.0012	0.90
Örsjön	7	65	340	2.8	437	0.057	0.963	−3.20	<0.0001	0.0096	0.0001	0.95

Appendix B

Roach (*Rutilus rutilus* L.) size range, number of fish, Hg content range, and lake-specific geometric mean Hg content (Ln[Hg]) in roach

Lake	*N*	Length (mm)		Weight (g)		Hg (mg/kg w.w.)		Ln[Hg] Mean
		Min.	Max.	Min.	Max.	Min.	Max.	
Alsta sjö	6	65	312	2.3	417	0.067	0.183	−2.26
Assjösjön	4	78	246	4.1	181	0.025	0.071	−3.20
Bredsjön	5	72	193	2.9	69	0.038	0.159	−2.58
Bruksdammen	5	94	220	5.3	119	0.111	0.249	−1.89
Bysjön	5	153	292	29.9	297	0.050	0.317	−2.03
Dalarna	5	88	239	5.5	153	0.067	0.285	−2.10
Djupsjön, Alunda	5	70	225	2.7	126	0.043	0.116	−2.65
Djupsjön, Dalälven	5	71	242	2.8	123	0.149	0.286	−1.58
Eckarfjärden	5	96	241	7.1	148	0.082	0.212	−1.95
Edasjön	6	76	269	3.9	275	0.067	0.200	−2.26
Ensjön	5	91	233	6.5	145	0.094	0.271	−1.93
Fibysjön	5	86	207	5.0	83	0.126	0.361	−1.42
Finnsjön	10	81	233	4.7	149	0.108	0.229	−1.85
Fjärden	5	86	254	5.9	257	0.047	0.214	−2.36
Funbosjön	6	85	307	5.7	359	0.030	0.263	−2.65
Fälaren	5	93	257	6.4	192	0.114	0.224	−1.77
Gimo damm	5	80	240	4.4	154	0.083	0.139	−2.29
Gisslaren	5	57	186	1.3	69	0.018	0.138	−2.90
Gårsjön	5	70	232	2.6	127	0.055	0.097	−2.64
Huvsjön	6	65	225	2.3	102	0.060	0.119	−2.60
Hålsjön	5	54	232	1.4	125	0.042	0.220	−2.27
Kärven	5	82	248	3.9	170	0.045	0.080	−2.82
Lillsjön, Bålsta	6	83	242	3.1	153	0.048	0.184	−2.56
Lillsjön, Österby	5	79	185	4.1	57	0.046	0.105	−2.49
Lindsjön	6	57	197	1.3	70	0.070	0.163	−2.25
Lissvass	5	92	233	6.6	156	0.106	0.392	−1.59
Lumpen	5	115	253	13.8	175	0.066	0.267	−1.91
Långsjön, Almunge	6	89	329	6.4	409	0.089	0.189	−1.95
Långsjön, Björklinge	6	68	338	2.6	463	0.026	0.121	−2.90
Långsjön, Knutby	5	89	247	5.8	191	0.098	0.139	−2.15
Långsjön, Länna	8	84	341	5.5	581	0.045	0.203	−2.62
Löhammarsjön	5	56	238	1.2	134	0.050	0.119	−2.53

(*Continued on next page*)

Appendix B (*continued*)

Lake	*N*	Length (mm)		Weight (g)		Hg (mg/kg w.w.)		Ln[Hg] Mean
		Min.	Max.	Min.	Max.	Min.	Max.	
Mossaren	12	80	246	4.6	184	0.063	0.481	−1.98
Mörtsjön, Fyris	6	123	198	17.3	78	0.046	0.181	−2.54
Mörtsjön, Järlåsa	5	68	245	2.3	138	0.061	0.260	−2.10
N. Åsjön	5	96	246	6.8	193	0.059	0.183	−2.15
Norrsjön, Knutby	7	71	290	2.8	279	0.047	0.193	−2.32
Norrsjön, Norreda	5	85	235	5.0	159	0.050	0.118	−2.68
Norrsjön, Tysktorp	5	74	248	3.3	180	0.052	0.329	−2.31
Nävergården	8	132	219	21.1	113	0.112	0.244	−1.66
Ramsen	5	88	231	6.2	139	0.061	0.207	−2.15
Ramsjön	5	57	249	1.2	167	0.114	0.310	−1.54
Rastsjön	5	67	275	2.6	242	0.062	0.191	−2.32
Ryssjön	5	74	258	3.4	182	0.033	0.050	−3.19
S. Giningen	5	78	226	4.1	131	0.040	0.107	−2.80
S. Åsjön	5	93	223	7.2	121	0.142	0.270	−1.52
Siggeforasjön	5	84	253	4.9	173	0.067	0.258	−2.23
Skälsjön	7	87	257	5.6	191	0.112	0.314	−1.65
Skärsjön, Fyris	5	88	261	7.0	195	0.036	0.153	−2.58
Skärsjön, Järlåsa	5	75	256	3.1	177	0.060	0.221	−2.21
St. Agnsjön	4	135	245	23.1	155	0.135	0.304	−1.54
St. Hallsjön	5	72	235	2.7	144	0.081	0.130	−2.22
St. Hållsjön	5	95	237	6.5	142	0.077	0.178	−2.10
St. Hålsjön	4	160	215	40.7	100	0.135	0.337	−1.45
Stamsjön	5	79	195	4.2	78	0.025	0.059	−3.12
Stennässjön	5	70	242	2.4	161	0.056	0.092	−2.61
Stordammen	5	68	250	2.4	159	0.059	0.087	−2.68
Storfjärden	5	70	249	2.7	143	0.074	0.326	−1.81
Storträsket	5	99	239	8.4	131	0.054	0.164	−2.49
Storvikarsjön	5	73	255	2.5	182	0.027	0.087	−2.86
Strandsjön	6	66	308	2.1	404	0.058	0.224	−2.08
Strömmaren	5	75	207	3.2	86	0.036	0.094	−2.77
Strönningsvik	3	169	175	50.2	62	0.091	0.163	−2.05
Säbysjön	6	62	292	1.6	351	0.025	0.148	−2.83
Sätersjön	6	77	251	3.5	166	0.136	0.466	−1.17
Södersjön	5	83	271	5.3	248	0.055	0.283	−2.09
Tarmlången	7	72	265	3.3	178	0.074	0.536	−1.80
Testen	7	69	243	2.6	158	0.025	0.052	−3.24
Trehörningen	5	93	254	7.2	170	0.046	0.120	−2.65
Tvigölingen	6	148	226	28.9	113	0.174	0.335	−1.41
Valloxen	5	69	223	2.9	120	0.019	0.265	−2.54
Velången	4	94	180	6.8	54	0.034	0.124	−2.73
Vikasjön	5	95	232	6.5	151	0.100	0.291	−1.95
Vällen	10	52	269	1.2	239	0.023	0.319	−2.45
Vällnoren	6	85	272	5.4	253	0.024	0.101	−2.96
Åkerbysjön	5	95	249	7.3	211	0.095	0.212	−1.95
Älgsjön	5	90	220	6.3	117	0.107	0.315	−1.99
Örsjön	4	113	220	11.8	113	0.089	0.204	−2.09

References

Björklund, I., Borg, H., Johansson, K., 1984. Mercury in Swedish lakes—its regional distribution and causes. Ambio 13, 118–121.

Brunberg, A.-K., Blomqvist, P., 1998. Vatten i Uppsala län. Upplandsstiftelsen Rapport 8. (Waters in the County of Uppsala, in Swedish). Available from: The Uppland Foundation, SE-751 48 Uppsala, Sweden.

Collette, B.B., Ali, M.A., Hokanson, K.E.F., Nagiec, M., Smirnov, S.A., Thorpe, J.E., Weatherley, A.H., Willemsen, J., 1977. Biology of percids. Journal of Fisheries Research Board of Canada 34, 1890–1899.

Downs, S.G., Macleod, C.L., Lester, J.N., 1998. Mercury in precipitation and its bioaccumulation in fish: a literature review. Water, Air and Soil Pollution 108, 149–187.

Eriksson, L., Hermens, J.L.M., Johansson, E., Verhaar, H.J.M., Wold, S., 1995. Multivariate analysis of aquatic toxicity data with PLS. Aquatic Sciences 57 (3), 217–241.

Freund, R.J., Littell, R.C., 1991. SAS® System for Regression, second ed.. SAS Institute Inc, Cary, North Carolina, USA.

Garthwaite, P.H., 1994. An interpretation of partial least squares. Journal of American Statistical Association 89 (425), 122–127.

Geladi, P., 1988. Notes on the history and nature of partial least squares (PLS) modelling. Journal of Chemometrics 2, 231–246.

Haines, T.A., Komov, V.T., Matey, V.E., Jagoe, C.H., 1995. Perch mercury content is related to acidity and color of 26 Russian lakes. Water, Air and Soil Pollution 85, 823–828.

Harris, R.C., Bodaly, R.A., 1998. Temperature, growth and dietary effects on fish mercury dynamics in two Ontario lakes. Biogeochemistry 40, 175–187.

Håkanson, L., Nilsson, Å, Andersson, T., 1988. Mercury in fish in Swedish lakes. Environmental Pollution 49, 145–162.

Håkanson, L., Nilsson, Å, Andersson, T., 1990. Mercury in fish in Swedish lakes—linkages to domestic and European sources of emission. Water, Air and Soil Pollution 50, 171–191.

Höskuldsson, A., 1988. PLS regression methods. Journal of Chemometrics 2, 211–228.

Johnels, A.G., Westermark, T., Berg, W., Persson, P.I., Sjöstrand, B., 1967. Pike (*Esox lucius* L.) and some other aquatic organisms in Sweden as indicators of mercury contamination in the environment. Oikos 18, 323–333.

Lathrop, R.C., Noonan, K.C., Guenther, P.M., Brasino, T.L., Rasmussen, P.W., 1989. Mercury levels in walleyes from Wisconsin lakes of different water and sediment chemistry characteristics. Wisconsin Department of Natural Resources, Madison, WI, Technical Bulletin 163.

Lindgren, F., Hansen, B., Karcher, W., Sjöström, M., Eriksson, L., 1996. Model validation by permutation tests: applications to variable selection. Journal of Chemometrics 10, 521–532.

McMurtry, M.J., Wales, D.L., Scheider, W.A., Beggs, G.L., Dimond, P.E., 1989. Relationship of mercury concentration in lake trout (*Salvelinus namaycush*) and smallmouth bass (*Micropterus dolomieui*) to the physical and chemical characteristics of Ontario lakes. Canadian Journal of Fisheries and Aquatic Sciences 46, 426–434.

Meili, M., 1994. Aqueous and biotic mercury concentrations in boreal lakes: Model predictions and observations. In: Watras, C.J., Huckabee, J.W. (Eds.), Mercury Pollution—Integration and Synthesis. Lewis Publishers, Boca Raton, FL, USA, pp. 99–106.

Moriarty, F., 1983. Ecotoxicology—The Study of Pollutants in Ecosystems, second ed.. Academic Press, London.

Nilsson, Å, Håkanson, L., 1992. Relationships between mercury in lake water, water colour and mercury in fish. Hydrobiologia 235, 675–683.

Persson, L., Byström, P., Wahlström, E., 2000. Cannibalism and competition in eurasian perch: population dynamics of an ontogenetic omnivore. Ecology 81 (4), 1058–1071.

Post, J.R., Vandenbos, R., McQueen, D.J., 1996. Uptake rate of food-chain and waterborne mercury by fish: field measurements, a mechanistic model, and an assessment of uncertainties. Canadian Journal of Fisheries and Aquatic Sciences 53, 395–407.

Richardson, G.M., Currie, D.J., 1995. Using empirical methods to assess the risk of mercury accumulation in fish from lakes receiving acid rain. Human and Ecological Risk Assessment 1 (3), 306–322.

Richman, L.A., Wren, C.D., Stokes, P.M., 1988. Facts and fallacies concerning mercury uptake by fish in acid stressed lakes. Water, Air and Soil Pollution 37, 465–473.

Sokal, R.R., Rohlf, F.J., 1995. Biometry, third ed.. W.H. Freeman and Co, NY.

Somers, K.M., Jackson, D.A., 1993. Adjusting mercury concentration for fish-size covariation: a multivariate alternative to bivariate regression. Canadian Journal of Fisheries and Aquatic Sciences 50, 2388–2396.

Sonesten, L., 1989. Sammanställning och utvärdering av syrgasdata från ett hundratal sjöar i Uppsala län (Compilation and evaluation of oxygen measurements from some hundred lakes in the county of Uppsala, Sweden. In Swedish). Available from: The Uppland Foundation, SE-751 48 Uppsala, Sweden.

Sonesten, L., 2001. Mercury content in roach (*Rutilus rutilus* L.) in circumneutral lakes—effects of catchment area and water chemistry. Environmental Pollution 112 (3), 471–481.

Sonesten, L., 2003. Catchment area composition and water chemistry heavily affects mercury levels in perch (*Perca fluviatilis* L.) in circumneutral lakes. Water, Air, and Soil Pollution 44, 1–4.

Tremblay, G., Legendre, P., Doyon, J.-F., Verdon, R., Schetagne, R., 1998. The use of polynomial regression analysis with indicator variables for interpretation of mercury in fish data. Biogeochemistry 40, 189–201.

Verta, M., Rekolainen, S., Mannio, J., Surma-Aho, K., 1986. The origin and levels of mercury in Finnish forest lakes. Publications of the Water Research Institute, National Board of Waters, Finland 65, 21–31.

Watras, C.J., Back, R.C., Halvorsen, S., Hudson, R.J.M., Morrison, K.A., Wente, S.P., 1998. Bioaccumulation of mercury in pelagic freshwater food webs. The Science of the Total Environment 219, 183–208.

Winfrey, M.R., Rudd, J.W.M., 1990. Environmental factors affecting the formation of methylmercury in low pH lakes. Environmental Toxicology and Chemistry 9, 853–869.