# Trend Analysis of Mercury in Fish Using Nonparametric Regression

Mohamed Hussian and Anders Grimvall

Department of Mathematics, Linköping University, SE-58183 Linköping, Sweden

## Abstract

The International Council for the Exploration of the Sea (ICES) has long compiled extensive data on contaminants in biota. We investigated how trend assessment of mercury in muscle tissue from fish (flounder and Atlantic cod) might be facilitated by using nonparametric regression to normalise observed levels of this contaminant with respect to body length and weight. Specifically, we examined response surfaces and annual normalised means obtained by employing purely additive models (AM), thin plate splines (TPS), and monotonic regression (MR) to model mercury levels as functions of sampling year and one or two covariates. Our analysis showed that TPS and MR models can be more satisfactory than purely additive models, because the former techniques enable estimation of time-dependent relationships between the mercury concentration and the covariates. However, the major obstacle for trend assessment of the collected mercury data was a substantial interannual variation that was related to factors other than body length and weight. Nevertheless, several time series of flounder data that started in the 1970s and 1980s showed obvious downward trends, and these trends were particularly strong in the Elbe estuary. When the analysis was limited to data collected after 1990, an overall Mann-Kendall test for all sampling sites revealed a statistically significant downward trend for flounder, whereas it was not significant for cod.

**Key words**: additive models, thin plate splines, monotonic regression, trend assessment, normalisation, mercury, fish.

## Introduction

The concentrations of contaminants in biota are often correlated with other characteristics of the analysed samples. An example of this is that levels of heavy metals in fish muscle can be strongly associated with body length (Phillips, 1980; MacCrimmon *et al*., 1983; ICES, 1989; Dixon and Jones, 1994), and, consequently, assessments of temporal trends in such contaminant data are often based on length-adjusted concentrations (ICES, 1989; Jensen and Cheng, 1987; Evans *et al*., 1993; Jørgensen and Pedersen, 1994). Similarly, the variation in collected data can be reduced by adjusting for other covariates, such as body weight or size of the analysed organ (Braune, 1987; Rees and Nicholson, 1989; ICES, 1990; Nicholson *et al*., 1991).

From a statistical point of view, the cited adjustment or normalisation methods are rather simple. First, it is usually assumed that the impact of the covariate on the response variable under consideration is constant over time. Secondly, the current practice is dominated by linear regression models. In the present study, we examined the performance of three nonparametric methods for simultaneous adjustment and trend analysis of mercury in fish muscle. The underlying regression models, which described the mercury levels as functions of sampling year and one or two covariates, included two generalised additive models (GAMs). More precisely, we used one purely additive model (AM) and one thin plate spline (TPS) model that allowed complex interaction effects. In addition, we employed monotonic regression (MR), that is models in which the expected response increases or decreases in relation to one or more explanatory variables. Detailed descriptions of GAM models can be found in textbooks on nonparametric regression (e.g., Hastie and Tibshirani, 1990; Hastie *et al.*, 2001), whereas MR in two or more explanatory variables has been addressed in recent reports from our research group (Burdakov *et al*., 2004, 2005, 2006).

Empirical data for our study were acquired from the International Council for the Exploration of the Sea (ICES), which has long collected data on contaminants in fish caught in the North Atlantic. Our analysis focused on mercury levels in muscle from Atlantic cod (*Gadu morhua*) and flounder (*Platichthys flesus*) caught in the North Sea and along the coast of Norway.

The main objectives of the current study were as follows:

(i) to examine the performance of simple additive models for time-independent normalisation as compared to TPS and MR models that allow time-dependent adjustments for covariates;

(ii) to assess temporal trends in the levels of mercury in cod and flounder.

## Study area and data sets

From the ICES database for mercury in fish, we selected all sampling sites for which a minimum of five years of data were available. This resulted in 10 time series of data for mercury in Atlantic cod and 24 for mercury in flounder

(Table 1). The quality of the data submitted to ICES has been assured by the countries responsible for the monitoring. We also screened all data by examining time series plots and scatter charts of mercury levels, body lengths and body weights. This approach revealed that, for a few sites, there were subsets of data with strongly deviating combinations of records on body length and body weight. The data on mercury levels in such subsets were excluded from the statistical analyses, whereas all other data were retained.

**Table 1a.** Sampling sites for the analysis of mercury in muscle tissue from Atlantic cod (*Gadu morhua*)

| Sampling site | | Periods | No. of samples |
|---|---|---|---|
| Latitude | Longitude | | |
| 51$^o$ 20′ 0″ N | 2$^o$ 50′ 0″ E | 1978-98, 2000-03 | 531 |
| 53$^o$ 10′ 0″ N | 2$^o$ 5′ 0″ E | 1982-83, 1986-91, 1993-94, 1996 | 280 |
| 56$^o$ 52′ 12″ N | 18$^o$ 38′ 12″ E | 1979, 1981-96 | 340 |
| 57$^o$ 13′ 14″ N | 11$^o$ 50′ 0″ E | 1979-96 | 417 |
| 58$^o$ 3′ 0″ N | 6$^o$ 43′ 0″ E | 1990-2003 | 343 |
| 59$^o$ 2′ 0″ N | 10$^o$ 32′ 0″ E | 1981-85, 1990-2003 | 477 |
| 60$^o$ 10′ 0″ N | 6$^o$ 34′ 0″ E | 1990-93, 1995-2003 | 357 |
| 60$^o$ 16′ 0″ N | 6$^o$ 2′ 0″ E | 1987, 1990-2003 | 324 |
| 68$^o$ 12′ 0″ N | 14$^o$ 48′ 0″ E | 1992-2003 | 293 |
| 69$^o$ 56′ 0″ N | 29$^o$ 40′ 0″ E | 1994-2003 | 240 |

**Table 1b.** Sampling sites for the analysis of mercury in muscle tissue from flounder (*Platichthys flesus*)

| Sampling site | | Periods | No. of samples |
|---|---|---|---|
| Latitude | Longitude | | |
| 49° 25′ 59″ N | 0° 50′ 0″ E | 1986-91 | 148 |
| 51° 20′ 0″ N | 2° 50′ 0″ E | 1978-2003 | 612 |
| 51° 25′ 0″ N | 3° 35′ 0″ E | 1986-90 | 140 |
| 51° 25′ 0″ N | 3° 31′ 0″ E | 1979-85, 1992-93 | 140 |
| 51° 26′ 0″ N | 3° 57′ 0″ E | 1997-2001 | 123 |
| 52° 57′ 0″ N | 5° 0′ 0″ E | 1997-2001 | 124 |
| 53° 0′ 0″ N | 5° 1′ 0″ E | 1986-90 | 122 |
| 53° 4′ 0″ N | 5° 4′ 0″ E | 1979-85 | 99 |
| 53° 23′ 0″ N | 6° 54′ 0″ E | 1997-2001 | 121 |
| 53° 24′ 6″ N | 6° 53′ 1″ E | 1986-90 | 87 |
| 53° 38′ 0″ N | 8° 7′ 0″ E | 1990-93, 1996-97, 2001 | 229 |
| 53° 38′ 9″ N | 8° 22′ 9″ E | 1986-87, 1989-93, 1995-97 | 240 |
| 53° 42′ 0″ N | 6° 51′ 0″ E | 1989, 1991-95, 1997 | 122 |
| 53° 45′ 0″ N | 8° 21′ 0″ E | 1986, 1988-89, 1991-93, 1995-97, 2001 | 308 |
| 53° 52′ 0″ N | 8° 52′ 0″ E | 1986-95 | 361 |
| 53° 53′ 0″ N | 9° 11′ 0″ E | 1996-2001 | 142 |
| 53° 56′ 0″ N | 8° 38′ 0″ E | 1986-95 | 326 |
| 53° 57′ 11″ N | 8° 30′ 0″ E | 1996-2001 | 126 |
| 55° 15′ 4″ N | 11° 10′ 29″ E | 1979-1980, 1982-84, 1986-87 | 99 |
| 55° 18′ 0″ N | 10° 56′ 0″ E | 1998-2003 | 268 |
| 55° 55′ 0″ N | 12° 32′ 0″ E | 1979-1980, 1982-1991, 1993-96 | 248 |
| 58° 31′ 0″ N | 10° 54′ 0″ E | 1981-94 | 307 |
| 59° 31′ 25″ N | 10° 21′ 0″ E | 1983, 1985, 1990-2003 | 148 |
| 60° 10′ 0″ N | 6° 34′ 0″ E | 1984, 1990-93, 1995-2003 | 99 |

## Statistical procedures

### Estimation of response surfaces using generalised additive models

GAMs allow very flexible modelling of the relationship between a response variable $Y$ and a set of predictors or explanatory variables $X_1, \ldots, X_p$. In a nonparametric setting, the conditional expectation

$$\mu = E(Y \mid X_1, \ldots, X_p)$$

is assumed to have the general form

$$g(\mu) = \alpha + f_1(X_1) + \ldots + f_p(X_p) \tag{1}$$

where $g$ is a so-called link function, and $f_1, \ldots, f_p$ are unspecified smooth functions that average zero over the data and are estimated using algorithms involving scatter-plot smoothers (Hastie and Tibshirani, 1990; Hastie *et al.*, 2001).

An important class of GAMs is obtained by letting $g(\mu) = \mu$ in equation (1). We refer to these models as additive models (AMs), and in the present study, the variables

$$X_1 = \text{sampling year}$$
$$X_2 = \text{body length}$$
$$X_3 = \text{body weight}$$

were used as predictors of the mercury concentration $Y$ in fish muscle.

Furthermore, it can be noted that the algorithms developed for GAMs also can estimate surface smoothers with second or higher order interaction. We used two thin plate spline (TPS) models that were obtained by setting

$$\mu = \alpha + h(X_1, X_2) \qquad (2)$$

and

$$\mu = \alpha + f_1(X_1) + h(X_2, X_3) \qquad (3)$$

respectively. All the cited GAMs were estimated using ‘proc GAM’ in SAS® software.

## Estimation of response surfaces using monotonic regression

MR is a statistical method for estimating models of the form

$$E(Y \mid X_1, \ldots, X_p) = f(X_1, \ldots, X_p) \qquad (4)$$

where $f$ is increasing (or decreasing) in each of the coordinates. We used MR to estimate the expected mercury concentration in relation to sampling year and one or both of the variables body length and body weight. A detailed description of the algorithm employed can be found in the previously cited reports from our research group (Burdakov *et al.*, 2004, 2005, 2006).

## Normalisation with respect to body length or weight

There was substantial variation in the observed mercury concentrations, and part of this variability could be attributed to variation in body length or weight. Therefore, we normalised the observed concentrations with respect to one or both of these variables. This entailed normalising the observed concentrations by computing

$$Y - \sum_{j=2}^{p} \left( \hat{f}_j(X_j) - \hat{f}_j(\overline{X}_j) \right)$$

for the additive models with one or two covariates ( $p$ = 2 or 3),

$$Y - \left( \hat{h}(X_1, X_2) - \hat{h}(X_1, \overline{X}_2) \right)$$

$$Y - \left( \hat{h}(X_2, X_3) - \hat{h}(\overline{X}_2, \overline{X}_3) \right)$$

for the TSP models (2 and 3), and

$$Y - \left( \hat{f}(X_1, X_2) - \hat{f}(X_1, \overline{X}_2) \right)$$

$$Y - \left( \hat{f}(X_1, X_2, X_3) - \hat{f}(X_1, \overline{X}_2, \overline{X}_3) \right)$$

for the MR models (4). In the third case, it should be noted that the algorithm for MR provides fitted values $\hat{f}$ only for the observed values of the $X$ variables. Hence, the calculation of

$$\hat{f}(X_1, \overline{X}_2)$$

and

$$\hat{f}(X_1, \overline{X}_2, \overline{X}_3)$$

may require extrapolation of these fitted values to new points, which has been described in detail in a recently published article (Hussian *et al.*, 2005).

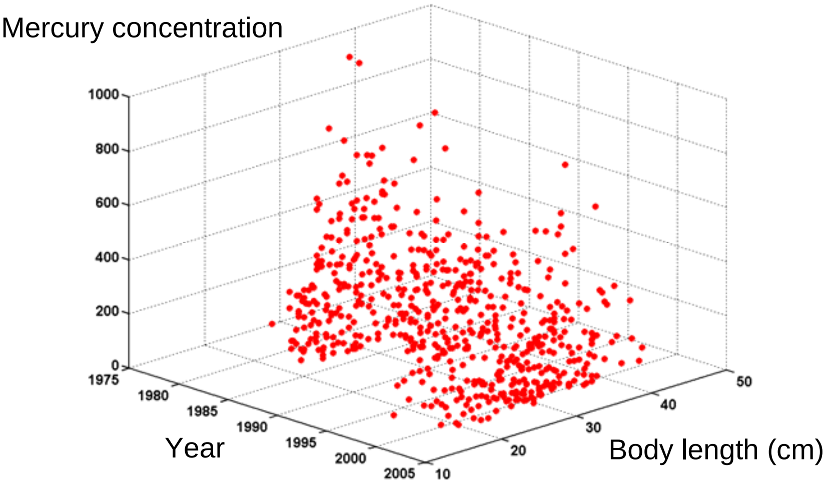
## Comparison of response surface methodologies

All regression models that were fitted to the collected data showed that the mercury levels in flounder and cod increased in relation to body length and weight. However, for several of the data sets, the different statistical methodologies resulted in rather diverse response surfaces. This is illustrated in Figures 1 and 2, which show that the response surface for the additive model differed markedly from the response surfaces for the TSP and MR models. Closer examination TSP and MR models revealed that the former, which has a single smoothing parameter, tended to suppress differences in trend slopes for different body lengths, whereas the MR-based response surfaces allowed the temporal trend to be much steeper for large fish than for small. Figures 3 and 4 primarily demonstrate that the effective dimension (degrees of freedom) of MR models can be much larger than for AMs or TSP models in which the smoothing parameter is selected by cross-validation.

## Normalisation of mercury levels

Due to the strong relationship between mercury levels in muscle tissue and the body length or weight of the analysed fish, a substantial part of the variability in observed concentrations could be removed by normalisation (Figure 5). For several of the analysed data sets, normalisation also resulted in a substantial decrease in the year-to-year variation of the annual means (Figure 6). Notwithstanding, the normalisation was less successful for some of the data sets, regardless of which response surface methodology we used (Figure 7).

a)




b)

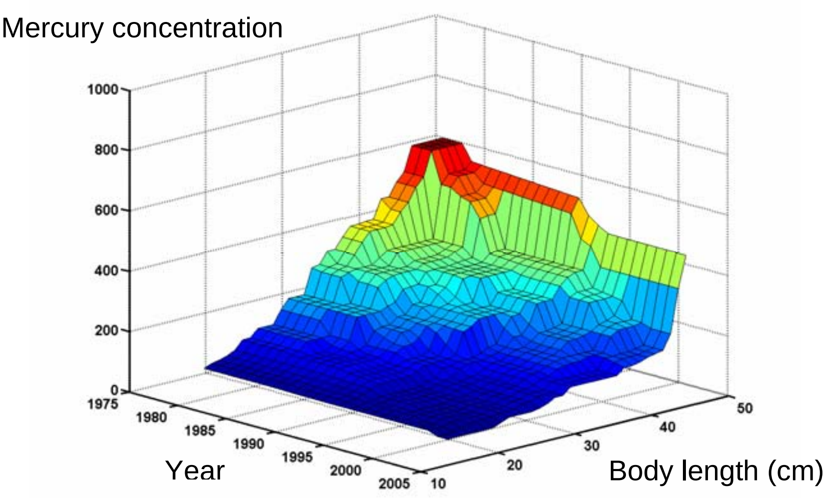
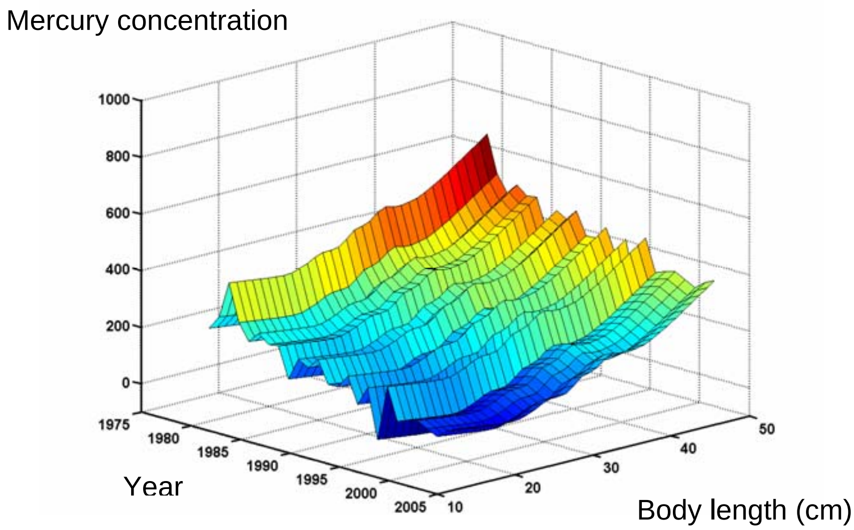



**Figure 1.** Concentrations of mercury in muscle tissue from flounder (*Platichthys flesus*) caught in the North Sea (51° 19′ 59″ N, 2° 10′ 0″ E) in relation to sampling year and body length. The diagrams illustrate observed concentrations (a) and an MR-based response surface (b).

a)




b)

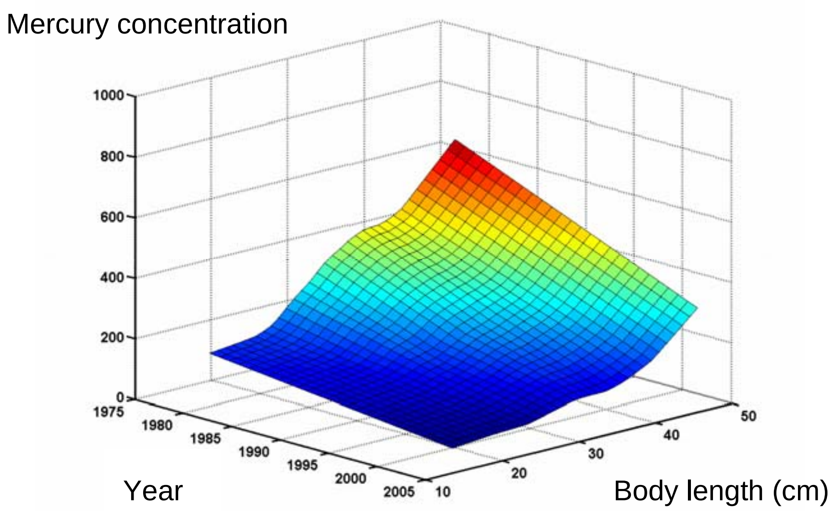
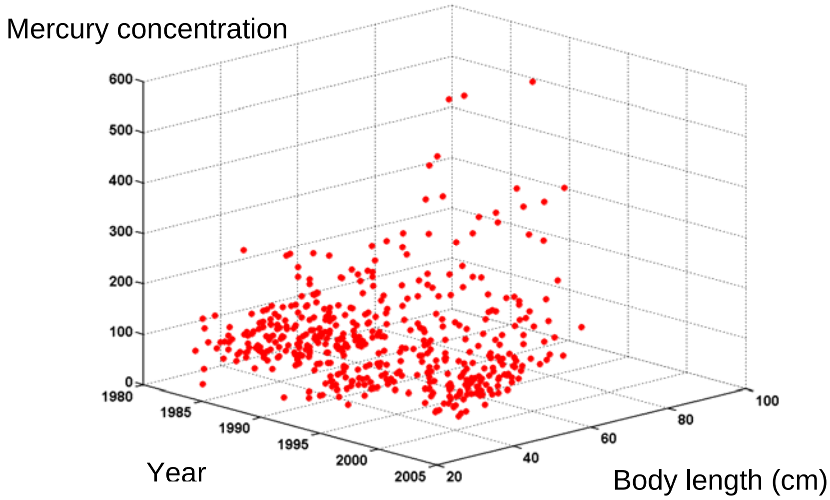



**Figure 2**. Response surfaces for the concentration of mercury in muscle tissue from flounder (*Platichthys flesus*) caught in the North Sea (51° 19′ 59″ N, 2° 10′ 0″ E) in relation to sampling year and body length . The two graphs were obtained using an AM (a) and a TSP model (b).

a)




b)

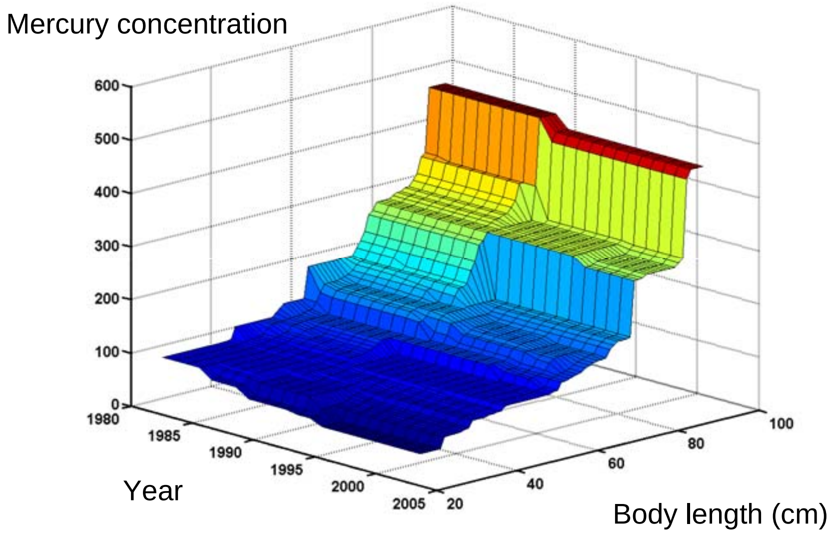
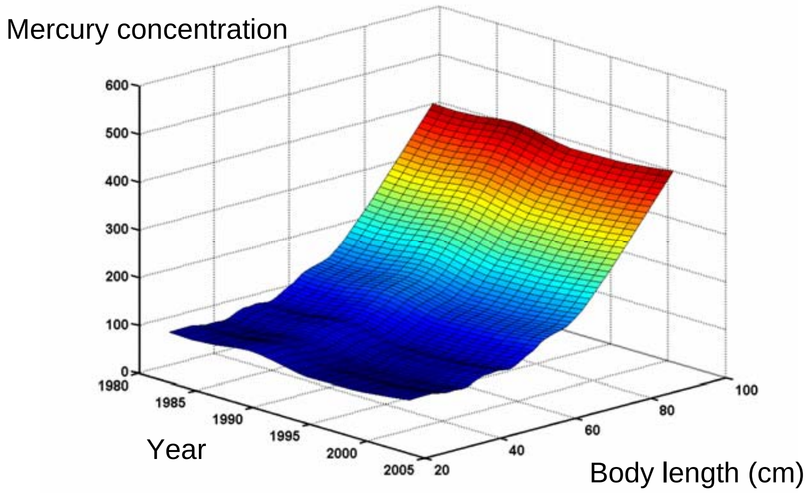



**Figure 3**. Concentrations of mercury in muscle tissue from Atlantic cod (*Gadu morhua*) caught in the North Sea (51$^{o}$ 19′ 59″ N, 2$^{o}$ 10′ 0″ E) in relation to sampling year and body length. The two diagrams illustrate observed concentrations (a) and an MR-based response surface (b).

a)




b)

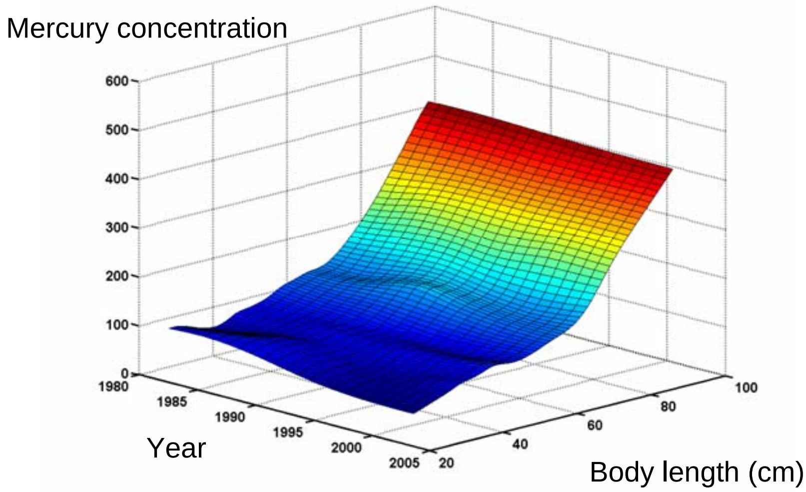



**Figure 4**. Response surfaces for the concentration of mercury in muscle tissue from Atlantic cod (*Gadu morhua*) caught in the North Sea (51$^{o}$ 19′ 59″ N, 2$^{o}$ 10′ 0″ E) in relation to sampling year and body length. The two graphs were obtained using an AM (a) and a TSP model (b).

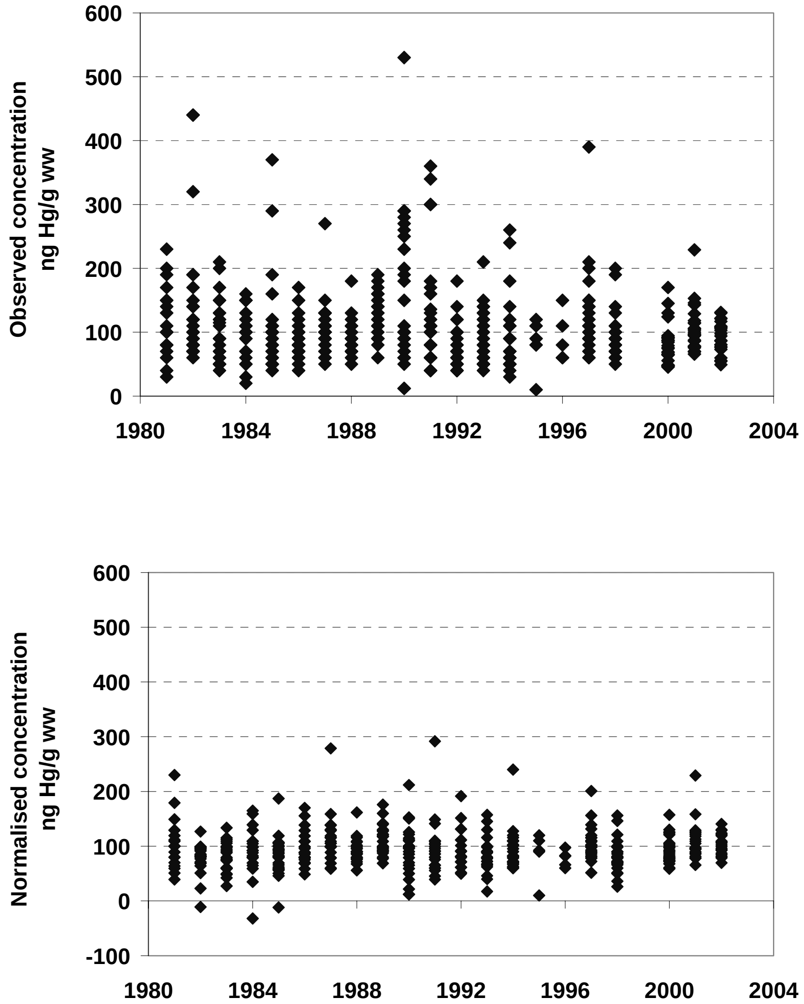



**Figure 5.** Observed and normalised concentrations of mercury in muscle tissue from Atlantic cod (*Gadu morhua*) caught in the North Sea (51$^{o}$ 19′ 59″ N, 2$^{o}$ 10′ 0″ E). The normalisation to a body length of 47.3 cm was achieved using an MR model.

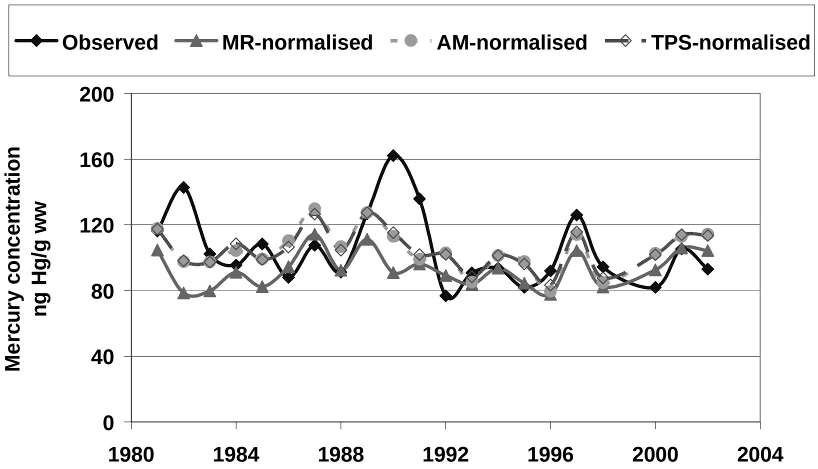



**Figure 6**. Annual means of observed and normalised (to a body length of 47.3 cm) mercury concentrations in Atlantic cod (*Gadu morhua*) caught in the North Sea (51° 19′ 59″ N, 2° 10′ 0″ E).

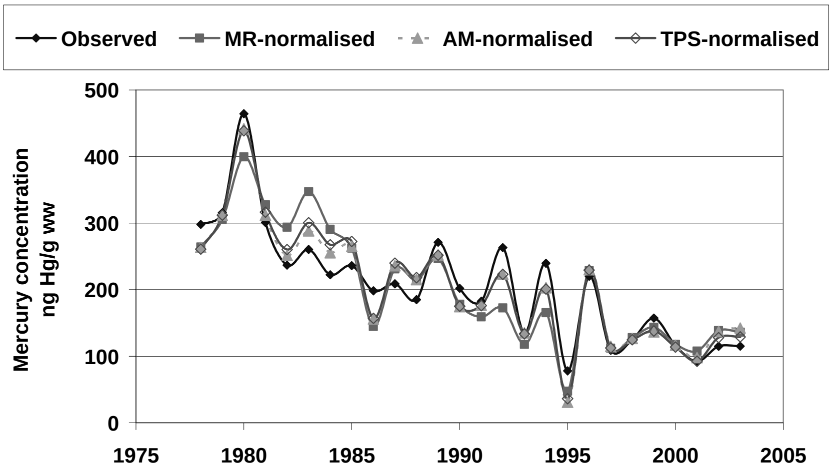



**Figure 7**. Annual means of observed and normalised (to a body length of 31.5 cm) mercury concentrations in muscle tissue from flounder (*Platichthys flesus*) caught in the North Sea (51° 19′ 59″ N, 2° 10′ 0″ E).

In many contexts, it is reasonable to assume that the impact of humans on the quality of the environment under consideration can be represented by a function that varies smoothly with time. Accordingly, it seems logical to regard the smoothness of the curve of annual mean normalised values as an indicator of how successful the normalisation has been. We computed

$$\sum_{i=2}^{n-1} (\tilde{Y}_i - \frac{\tilde{Y}_{i-1} + \tilde{Y}_{i+1}}{2})^2$$

for all time series $\tilde{Y}_i,\ i = 1, ..., n,$ of annual mean normalised or observed values that comprised a minimum of ten consecutive years of data. The results of this analysis showed that, on average, the curves of normalised values were smoother than the curves of observed values. However, the performance of the different normalisation methods varied strongly from data set to data set, and Friedman's nonparametric test indicated that none of the differences between normalisation methods were statistically significant.

## Temporal trends in mercury levels

All time series of observed and normalised annual means of mercury in flounder and cod were analysed for monotonic trends using two-sided multivariate Mann-Kendall tests (Loftis *et al*., 1991). These tests provided strong evidence of downward trends in the longest time series of mercury data for flounder, but considerably weaker evidence of trends in the cod data (Table 2). In addition, it can be noted that there was a step change from 1990 to 1991 in the flounder data reported from stations influenced by the Elbe River, and that this change coincided in time with decreasing mercury emissions to that river (Figure 8).

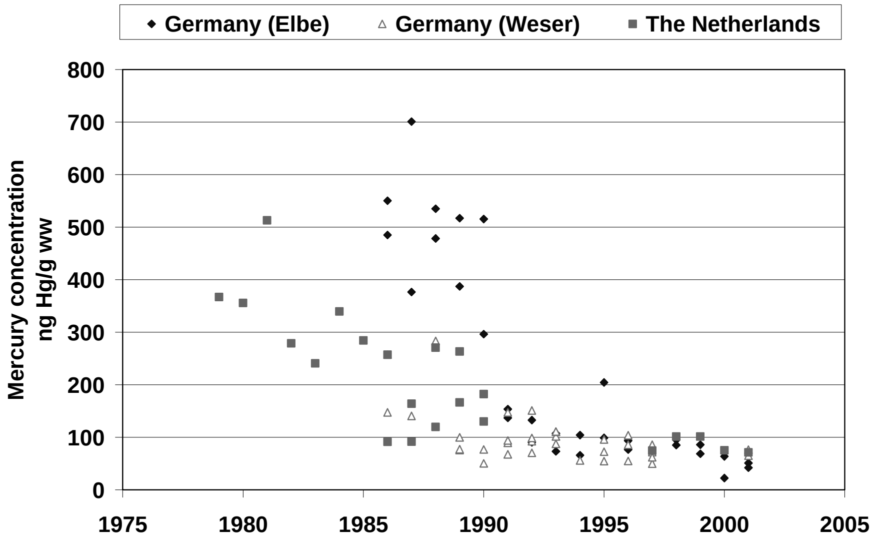



**Figure 8**. Annual means of observed mercury concentrations in muscle tissue from flounder (*Platichthys flesus*) caught in the North Sea at stations between 53 and 54$^{o}$ N.

When the analysis of all time series was limited to data collected from 1991 and onwards, there was still a statistically significant downward trend in the mercury levels for flounder, whereas the temporal change for cod was not significant (Table 2). It is also notable that, although normalisation did increase the precision of the annual mean values, this treatment of observed data did not result in substantially lower significance levels for the detected trends. Closer examination of the data revealed that this was due primarily to the presence of a substantial interannual variation that was not related to the variation in body length and weight. Moreover, the normalisation suppressed the spurious trends caused by high mercury levels for the unusually large cod specimens caught at the beginning of the study period.

**Table 2.** Significance levels (*p*-values) achieved in two-sided multivariate Mann-Kendall tests for monotonic trends in observed and normalised annual mean mercury levels.

| Normalisation method | Covariates | Flounder | | Cod | |
|---|---|---|---|---|---|
| | | 1978–2003 | 1991–2003 | 1978–2003 | 1991–2003 |
| Unadjusted | | 5.56E-07 | 0.031 | 0.011 | 0.167 |
| AM | Length | 2.49E-06 | 0.031 | 0.057 | 0.686 |
| AM | Length, weight | 1.05E-06 | 0.023 | 0.079 | 0.717 |
| TPS | Length | 1.17E-05 | 0.054 | 0.451 | 0.133 |
| TPS | Length, weight | 2.07E-06 | 0.050 | 0.008 | 0.381 |
| MR | Length | 1.35E-05 | 0.061 | 0.084 | 0.924 |
| MR | Length, weight | 2.77E-05 | 0.139 | 0.027 | 0.653 |

## Discussion

Our results show that the observed mercury levels were strongly related to body length and weight, and, accordingly, normalisation with respect to such characteristics of the analysed fish improved the precision of the annual means. In addition, some of the data sets confirmed previously reported findings concerning temporal trends that varied with the length or weight of the analysed fish (Nicholson *et al.*, 1991). This problem calls for regression methods such as TPS and MR, which enable time-dependent adjustment of observed data. TPS is preferable if the expected mercury level is a smooth function of time, and the slope of the temporal trend does not vary excessively with the body length or weight. MR may be a better choice if the temporal trend is much steeper for large fish than for small. However, MR is obviously limited to applications in which the expected mercury level is monotonic with regard to both time and covariate(s). Also, it should be kept in mind that MR can result in over-fitting, because the effective dimension of such models can be very high (Burdakov *et al*., 2005).

Although the modelling of mercury in relation to body length and weight was usually successful, normalisation had only a minor effect on the statistical evidence of temporal trends. We found that this was due primarily to the presence of a substantial interannual variation in mercury that was caused by factors other than body length or weight (e.g., calibration errors or unknown changes in the sampled fish populations), and identification of such factors should therefore be prioritized. Furthermore, it should be noted that some of the highest mercury concentrations were observed for unusually large specimens of fish caught at the beginning of the study period, and the impact of these observations on the estimated trends was reduced when data were normalised using a regression method that allows for time-dependent relationships between the response variable and the covariate(s).

The statistical evidence of downward trends in the older parts of the analysed time series of data confirms earlier reports (Jensen and Cheng, 1987; ICES, 1989, 1990; Misra *et al.*, 1990; Jørgensen and Pedersen, 1994), but the step change that occurred from 1990 to 1991 at the stations influenced by the Elbe River now appears much more clearly. The evidence of decreasing trends after 1990 was much weaker but statistically significant for flounder, whereas it was not significant for cod. Closer examination of the data revealed that only a few sites contributed to the more recent trends. Furthermore, the strongest decreases were found at coastal sites that had previously been exposed to heavy pollution.

## Acknowledgement

The authors are grateful for financial support from the Swedish Environmental Protection Agency.

## References

Braune, B.M. 1987. Mercury accumulations in relation to size and age of Atlantic herring (*Clupeus harengus harengus*) from the south-western Bay of Fundy, Canada. *Archives of Environmental Contamination and Toxicology*, **16**, 311–320.

Burdakov, O., Grimvall, A., and Hussian, M. 2004. A generalised PAV algorithm for monotonic regression in several variables. In: Antoch, J. (ed.) *COMPSTAT, Proceedings of the 16th Symposium in Computational Statistics held in Prague*. Heidelberg, New York: Physica-Verlag (Springer).

Burdakov, O., Grimvall, A., Hussian, M., and Sysoev, O. 2005. Hasse diagrams and the generalized PAV algorithm for monotonic regression in several explanatory variables. Submitted to *Computational Statistics and Data Analysis*.

Burdakov O., Sysoev O., Grimvall A., and Hussian M. 2006. An $O(n^2)$ algorithm for isotonic regression. In: Di Pillo, G. and Roma, M. (eds) *Large Scale Nonlinear Optimization. Series: Nonconvex Optimization and Its Applications*, Springer-Verlag, **83**, pp. 25-33.

Dixon, R. and Jones, B. 1994. Mercury concentrations in stomach contents and muscle of five fish species from the north east coast of England. *Marine Pollution Bulletin*, **28**, 741–745.

Evans, D.W., Dodoo, D.K., and Hanson, P.J. 1993. Trace element concentrations in fish livers: Implications of variations with fish size in pollution monitoring. *Marine Pollution Bulletin*, **26**, 329–334.

Hastie, T. and Tibshirani, R. 1990. *Generalized Additive Models.* London: Chapman and Hall.

Hastie, T., Tibshirani, R., and Friedman, J. 2001. *The Elements of Statistical Learning: Data Mining, Inference and Prediction*. New York: Springer-Verlag.

Hussian, M., Grimvall, A., Burdakov, O., and Sysoev, O. 2005. Monotonic regression for the detection of temporal trends in environmental quality data. *MATCH Commun. Math. Comput. Chem*., **54**, 535-550.

ICES. 1989. Statistical analysis of the ICES co-operative monitoring programme data on contaminants in fish muscle tissue (1975–1985) for determination of temporal trends. *ICES Cooperative Research Report No*. 162.

ICES. 1990. Statistical analysis of the ICES co-operative monitoring programme data on contaminants in fish liver tissue and *Mytilus edulis* (1978–1988) for determination of temporal trends. *ICES Cooperative Research Report No. 176.*

Jensen, A. and Cheng, Z. 1987. Statistical analysis of trend monitoring data of heavy metals in flounder *(Platichthys flesus). Marine Pollution Bulletin*, **18**, 230–238.

Jørgensen, L.A. and Pedersen, B. 1994. Trace metals in fish used for time trend analysis and as environmental indicators. *Marine Pollution Bulletin*, **28**, 24–32.

Loftis, J.C., Taylor, C.H., and Chapman, P.L. 1991. Multivariate tests for trend in water quality. *Water Resources Research*, **27**, 1419-1429.

MacCrimmon, H.R., Wren, C.R., and Gots, B.L. 1983. Mercury uptake by lake trout, *Salvelinus namaycush*, relative to age, growth, and diet in Tadenac Lake with comparative data from other PreCambrian Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **40**, 114–120.

Misra, R.K., Uthe, J.K., and Vyncke, W. 1990. A weighted procedure of analysing for time trends in contaminant levels in CMP data: applications to cod and flounder data of the Belgian coast (1978–1995). *ICES Journal of Marine Science*, **47**, 65–75.

Nicholson, M.D., Green, N.W., and Wilson, S.J. 1991. Regression models for assessing trends in cadmium and PCBs in cod livers from the Oslofjord. *Marine Pollution Bulletin*, **22**, 77–81.

Phillips, D.J.H. 1980. *Quantitative aquatic biological indicators*. London: Applied Science.

Rees, H.L. and Nicholson, M.D. 1989. Trends in lead levels in the horse-mussel from the western North Sea. *Marine Pollution Bulletin*, **20**, 86–89.