ICES Journal of Marine Science
JUL 2007; 64 (5) 929-938
http://dx.doi.org/10.1093/icesjms/fsm031

Cadmium and mercury in Seine Estuary flounders and mussels: the results of two decades of monitoring

K. Nakhlé[1], D. Cossa[1], D. Claisse[1,], B. Beliaeff[1] and S. Simon[2,]

[1] IFREMER, Centre de Nantes, BP 21105, F.44311 Nantes Cedex 03, France
[2] Cellule de Suivi du Littoral Haut-Normand, 16, quai C. de la Vigne, F. 76000 Le Havre, France

*: Corresponding author : tel: +33 240 374176; fax: +33 240 374075; e-mail: dcossa@ifremer.fr

Abstract:

The flounder (*Platichthys flesus*) is a flatfish that inhabits marine coastal environments, especially estuaries. It is an alternative quantitative biological indicator to the common marine mussel (*Mytilus* spp.), which is currently used as a sentinel species to monitor chemical contamination in numerous monitoring programmes. Findings from two decades of monitoring cadmium (Cd) and mercury (Hg) using both sentinel species in the Seine Estuary (France) are reported. For comparison, time-series of water concentrations for the same two metals at the mouth of the River Seine are given. Cd concentrations in the liver of the fish and in the soft tissue of mussels show similar temporal trends, consistent with the major temporal variations of Cd concentrations recorded in river water and with changes in industrial discharge of Cd (phosphogypsum waste) within the Seine Estuary. On the other hand, Hg concentrations in the muscles of flounders show temporal variations with no link to that observed in mussels or fluvial Hg contributions, which are in fact nearly covariant. It is concluded that optimization of the use of flounders as sentinel organisms for monitoring temporal trends of metal contamination in estuarine environments requires in-depth knowledge of its ecology within the area studied. An adapted sampling strategy based on this knowledge should provide results that are easier to interpret.

Keywords: cadmium, estuary, flounder, mercury, monitoring, mussel watch, *Platichthys flesus*

Introduction

Despite the complexity of the processes included in the "bioaccumulation" of a substance or element in a living organism, the use of quantitative bio-indicators appears to be a reliable method for both evaluating the bio-availability of contaminants and for monitoring the contamination of the environment (e.g., Phillips and Rainbow, 1993). The organisms used for such bio-monitoring must meet certain conditions, amongst which not only feature their capacity to bio-concentrate the substance being studied, but also their abundance and their sedentarity, respectively allowing good coverage and geographic representation (e.g., Cossa, 1989). For these reasons, the French Mussel Watch, the "Réseau National d'Observation" (RNO), uses the soft tissue of mussels and oysters for monitoring chemical contaminants along the French coasts (Claisse, 1989; www.ifremer.fr/envlit). However, certain international programs also recommend the use of certain fish, especially flat fishes. This is the case of the flounder (*Platichthys flesus*), a fish common to coastal regions and estuaries in the monitoring zones of the Eastern North Atlantic covered by the Oslo-Paris Convention (known as OSPAR (http://www.ospar.org) (Masson, 1987). The life of the *Platichthys flesus (*L.) is characterized by three stages. Born offshore, the juveniles develop in brackish and freshwater environments until they become sexually mature (Wheeler, 1969). Once reproduction has been achieved, genitors disperse around the coast or return into the estuary. In spite of these migrations, this flat fish has demonstrated its ability to perceive contamination in its habitat both by metallic and organic contaminants (e.g., Jensen and Cheng, 1987; Cossa et al., 1992; Leah et al., 1992; Voight, 1999 and 2002). Authors working on the organotropism of metals in this animal have shown that mercury (Hg) tends to accumulate in muscles, whereas cadmium (Cd) is preferentially accumulated in the liver (e.g., Julshamn and Grahl-Nielsen, 1996). Other studies have targeted allometric relations between the size of fish and the concentration of contaminants. In the case of Hg in the muscle of the flounder, positive correlations with the size or the age of the fish have been documented (Luten et al., 1987; Jensen, 1982; Marthinsen and Staveland, 1990; Collings et al., 1996). However, no clear trend has been ascertained for Cd in liver.

In the present work we describe the findings of 18 years of data acquisition relating to the concentration of Hg in the muscle and Cd in the liver of flounders from the Seine Bay (adjacent to the English Channel) collected between 1986 and 2003. Flounders in the Seine Bay and

Estuary are abundant and their ecology has been succinctly documented (Cellule de suivi du littoral Haut-Normand, 1997; Miramand et al., 1998; Minier et al., 2000). Two specific objectives have been pursued: (i) the exploration of variations in concentrations of Cd and Hg in the flesh of flounders depending on their size, and (ii) an exploration of possible temporal patterns in concentrations and their attempted interpretation. Achieving the first objective should maximize the possibility of reaching the second. Indeed, finding a way to minimize the effect of size on metal concentrations in the fish tissue will favor the possibility of indicating potential temporal tendencies in the metal concentration. The results are compared to the temporal trends in Cd and Hg concentrations in mussels and water measured in the Seine Estuary. This survey is part of the RNO, which provides the data required to monitor the quality of coastal water to the Working Group on Concentrations Trends and Effects of Substances in the Marine Environment (SIME) of the OSPAR Convention (Anonymous, 1994; www.ospar.org).

Survey site

The bay of the Seine (Fig. 1) covers a total surface area of approximately 4 000 km^2. Widely open to the English Channel, its depths do not exceed 30 m and the tidal currents are very strong. The bay receives freshwater from the Seine River with an average flow of 490 $m^3 s^{-1}$. The Seine basin, approximately 75 000 km^2, is home to 15 million inhabitants and drains areas that are very much subjected to anthropogenic agricultural, industrial, and urban activities. The contamination of the estuary and the bay by mercury have been recently described by Coquery et al. (1997) and Cossa et al. (2002b), and the cadmium contamination pointed out by Claisse (1989) and described by Chiffoleau et al. (2001). In short, cadmium contamination in the Seine Bay is largely due to local inputs of phosphogypsum, a byproduct of the phosphoric acid industry. Three phosphoric acid plants were present in the estuarine region. Two located near Rouen were discharging their wastes in the Seine Bay during the period 1974-1987. The last one located at Le Havre, which discharged its waste through a pipe (Fig. 1), definitively closed in 1992. Diffuse sources of Cd from the drainage basin have also been revealed by analyzing the Seine River waters upstream fromRouen (Idlafski et al., 1995; Thévenot et al., 1998). For Hg the diffuse inputs from the drainage basin dominate. According to Cossa et al. (2002b) while the annual Hg riverine inputs varied between 500 and 1500 tons, the local discharge within the estuary does not exceed 150 tons.

Material and methods

Sampling and pre-treatment

Flounder: Flounders were caught with bottom trawlers in the Seine Estuary between Le Havre and Honfleur. The smallest individuals were found close to the Channel opposite the river mouth on clay bottoms whereas larger fish were caught in water to the south in areas with sandy bottoms (Fig. 1). Both these sites were trawled during the eighteen-year monitoring period (1986 to 2003).

Sampling followed the guidelines of the OSPAR monitoring program (Anonymous, 1994). Three annual trawls were carried out within two days in November or December and annually at the same locations (Fig. 1). Twenty-five (25) individual fish were selected from the catch in order to obtain a homogenous spread of individuals within the widest size range possible and, providing a size-dependent stratified sampling. In order to avoid contaminating samples, each fish was individually placed in a polyethylene bag inside an insulated box, and transported at +4°C. In the laboratory, each individual was measured and weighed. Samples were then frozen and kept at-20°C until dissection. Under these conditions, the time lapse between the date of sampling and analysis was not a critical factor (Lafleur, 1973). To fully minimize the risk of contamination, dissection was carried out under a laminar airflow hood fitted with an HEPA filter (0.2µm). During the operation the fish was laid onto a polyethylene plate that is rinsed between each sample with demineralized water (Milli-Q®). The fish were defrosted just before dissection. The dissection of the fish was carried out with titanium, stainless steel, or polyethylene utensils, rinsed with Milli-Q® water. Subsamples were stocked in glass vials that had been washed in diluted hydrochloric acid beforehand (1:10 ; v:v) and rinsed with Milli-Q® water. The dorsal fillet and the liver were separated and placed in an identified vial. All the samples taken were freeze-dried, kept at +4°C and sheltered from light until analysis.

Mussels: Mussel (*Mytilus edulis*) are collected in the Seine Estuary four time a year since 20 years within the RNO program at three stations (Villerville, Le Havre, La Hève, Fig. 1) (Claisse, 1989). At each site, the soft tissues of 50 individuals from 30 to 60 mm in shell length were pooled and analyzed. The samplings were is carried out according to the guidelines of the OSPAR monitoring program, described in details by Claisse (1989).

Water: Surface water was samples twice a month at the entrance of the Seine Estuary using clean procedures (Chiffoleau et al., 2001). The water samples were collected in acid cleaned polyethylene bottles, then filtered in the laboratory (0.45 µm, LCR® or

Nuclepore® membranes) under a laminar flow hood. The filtrate was stored acidified (0.4% v:v, HCl Suprapur®) in acid clean Teflon (FEP) bottles until the analyses.

Analyses

Biota

The Hg determination in biota was carried out on an aliquot section of the dried muscle by atomic absorption spectrophotometry using an automatic mercury analyzer (AMA-254, Altec) after dry digestion according to the technique described by Cossa et al. (2002a). The accuracy and the reproducibility of the method were established using a certified fish muscle reference material (DORM-1, National Research Council of Canada). The certified values (0.80 ± 0.07 mg kg^{-1}) were reproduced (0.85 ± 0.01 mg kg^{-1}) within the confidence limits. Repeatability varied from 1 to 7% depending on the concentration of the sample. The detection limit was 0.007 mg kg^{-1} (dry weight). The Cd determination was based on measurement by graphite furnace atomic absorption spectrophotometry with Zeeman correction (SpectrAA600, Varian) after a wet mineralization of tissues by concentrated HNO_3 following the method described by Cossa and Bourget (1980). The analytical conditions were those published by Chiffoleau et al. (2003). The accuracy and reproducibility were determined on certified fish liver reference tissue (DOLT-1, National Research Council of Canada). The coefficient of variation (CV = standard deviation X 100 /mean) on 19 replications was 5%, and reproduced value: average obtained 4.2 ± 0.2 mg kg^{-1} for a certified value of 4.18 ± 0.28 mg kg^{-1}. The detection limit was 0.02 mg kg^{-1} (dry weight).

Water

Metal determinations in water and particulate material were performed according to the analytical procedures described by Chiffoleau et al (2001) and Cossa et al. (2003) for Cd and Hg respectively. Detection limits, defined as three time the standard deviation of the blanks, were 1 ng/L for Cd and 0.1 ng/L for Hg, and both repetabilities better than 12%. The accuracies of the dissolved metal determinations were successively verified using successively SLRS-2, 3 and 4 and ORMS-3 certified reference materials (National Research Council of Canada) for Cd and Hg respectively. For the particulate phase certified sediment samples (MESS-2, National Research Council of Canada) were used. For Cd (using the current SLRS-4) the reproduced value averaged 14 ± 2 ng L^{-1} for a certified value of 12 ± 2 ng L^{-1}. For Hg using the ORMS-3 the average value for replicate analyses was 12.8 ± 1.5 ng L^{-1} for a certified value of 12.6 ± 1.1 ng L^{-1}. Using MESS-2 for the particulate phase the repetabilities were 8 and 7% for Cd and Hg respectively. The calculated average of the determinations obtained for Cd on MESS-2 was 0.28 ± 0.02 mg kg^{-}

[1] for a certified value of 0.25 ± 0.04 mg kg^{-1}, and, in the case of Hg 0.089 ± 0.002 mg kg^{-1} for a certified value of 0.092 ± 0.009 mg kg^{-1}. The detection limits in the particulate matter were 0.01 mg kg^{-1} and 0.007 mg kg^{-1} for Cd and Hg respectively.

Results

Flounder

Between 1986 and 2003, i.e., over an 18 year period, 354 flounders were sampled, processed and analyzed in the course of 14 annual samplings. No fishing took place in 1997, 1998, 2000, nor in 2001.

***Size and weight*:** The fish being studied measured between 170 mm and 430 mm in length. The coefficient of variation of average sizes of fish sampled was approximately 5%. The lowest annual average was 237 mm (1993), the highest 297 mm (1987) and the general average was 272 mm (Table 1). The annual series were differentiated by their maximum size. As an example, the largest flounder in 1993 measured 310 mm, whilst in 1994 it measured 430 mm.

The relation commonly used to describe the allometric variation length (L) *versus* weight (P) is a function of the power of type $P = a\ L^b$. The regression coefficients (*b*) of the allometric relationship for male (2.85, Fig. 2) was similar to the *b* value of the relationship obtain for female (2.96, Fig. 2). Both were close to 3, the value determined by Deniel (1981).

***Mercury*:** The concentrations of mercury in the dorsal muscle of the flounder ranged from 0.01 to 3.27 $mg \cdot kg^{-1}$ in dry tissue weight (d.w.). This concentration range for flounder muscles from the same site is larger than the ranges previously published for the same species (Table 2). The maximum permitted mercury concentration for the sale of this type of fish was set by European Directives 466/2001 and 93/351/EEC at 0.5 mg kg^{-1} (wet weight), i.e., approximately 2.5 $mg \cdot kg^{-1}$ (d.w.). Only 4 of the 362 samples studied surpassed this standard, and these abnormal amounts corresponded to large flounders (>347 mm) (Fig. 3).

The distribution frequency of mercury concentrations was asymmetric, with a “Skewness” asymmetry coefficient of 2.94. A $\log_{10}$ change of variable induced a “Normal shape” in the Hg data frequency distribution. Consequently, calculations were carried out on concentration logarithms. Figure 3 illustrates the statistically significant correlation that existed between mercury concentrations and the length of flounders ($R^2 = 0.28$, n = 346). The concentrations in Hg remained stable up to a size of 300 mm, and above this, there occurred a considerable increase

in concentrations in individuals, but no difference in this relationship with the sex of the fish (Fig. 3). In order to circumvent Hg concentration *vs* length relationship for studying temporal patterns we used only data from individuals smaller than 300 mm in the geometric averages (m_g) calculation. Figure 4 indicates the temporal variations of annual m_g of mercury concentrations in the muscle of flounders (L <300 mm) between 1986 and 2003, with 95% confidence interval. The temporal evolution shows relative stability of the concentrations, with minimum concentrations in 1991 and 1992.

***Cadmium*:** The minimum concentration observed in the liver of the flounders was 0.01 mg kg^{-1} (d.w.) while the maximum attained 9.56 mg kg^{-1} (d.w.). There is no quality standard for Cd in the liver of fish in the European Directive covering cadmium and mercury (466/2001). If we take the value established for shellfish (1 mg kg^{-1} of wet weight), taking into account the percentage of water within the samples of 75 %, (average calculated from all of the liver samples after freeze-drying), this "guide value" expressed in dry weight will be 4 mg kg^{-1} (d.w.). Four (4) samples out of a total of 327 went over this value (Fig. 5), and they corresponded to large specimens. However, big fish did not systematically have high Cd concentrations (Fig. 5). In addition, concentrations of cadmium in the liver and the size of flounders (Fig. 5) were not statistically related ($R^2 = 0.09$, n = 327).

Comparisons with similar measurements performed in flounder livers from of other European regions monitored in the program of the International Council for the Exploration of the Sea (Jensen and Bro-Rasmussen, 1992) indicate that the flounders from the Seine estuary were more contaminated. This statement is consistent with what is known about the Cd contamination of this estuary (e.g., Chiffoleau et al., 2001). In a similar manner to Hg, we proceeded with two series of data processing in order to try to detect temporal trends in the Cd concentration of fish livers. Firstly, we selected individuals smaller than 300 mm given that large size fish were not homogeneously represented in yearly sample sets. Secondly, since Cd data were not normally distributed ("Skewness" = 5.11), a $\log_{10}$ transformation was applied to the Cd results. Figure 6 illustrates the temporal variations of annual averages of concentrations of cadmium in the liver of the flounder (L<300 mm) between 1986 and 2003, accompanied by their confidence interval at 95%. A regular increase in concentrations between 1986 and 1990, a reduction between 1990 and 1995 and a certain stability in concentrations from 1995 onwards are visible (Fig. 6).

Mussel and Water

The historical tendencies of the contamination of the estuary and the bay of the Seine by Cd and Hg are well documented both by monitoring the River Seine inputs which is performed upstream

of the tidal influence at the Poses Dam (upstream Rouen) and the RNO Mussel Watch Program (www.ifremer.fr/envlit).

Figures 7 and 8 illustrate the temporal variations of the Cd and Hg concentrations in the freshwater of the River Seine from monthly sampling at its mouth. For Cd the most striking features is its serious decrease by a factor of 5 within 17 years (Fig. 7). Particulate Cd ranged from around 10 mg kg^{-1} in 1986 to 2 mg kg^{-1} in 2003 and the dissolved Cd decreased from 0.1 to 0.02 µg/l during the same period. The Hg concentrations on particles and dissolved mercury show parallel variations, but with a distinct pattern compared to Cd. There is an increase in concentrations from the outset of the observations between 1990 to 1993, up to 10 mg kg^{-1} and 10 ng/L for particulate and dissolved Hg respectively, followed by a rapid decrease.

The monitoring of Cd and Hg in mussel soft tissue (Fig. 9 and 10) from three stations of the Seine estuary (Villerville, Le Havre, and La Hève, Fig. 1) showed high frequency variations superimposed to smoother long term variations. The high frequency signal has been already noticed for Hg (Laurier et al., 2006) and other metals (Claisse, 1989), and was partially attributed to physiological changes occurring in mussel during seasons. The long-term variations peaked between 1990 and 1993 for Cd and between 1992 and 1994 for Hg. The amplitude of the reduction in Cd concentrations is Factor 10 between the maximum values and the current minimum, whilst it is closer to Factor 5 for Hg.

Discussion

On comparing the pattern of variability in Hg concentrations in the River Seine water (Fig. 8) and mussels sampled from the estuary (Fig. 10) it seems likely that the two are causally linked. On the contrary, the chronological series of Cd in mussels is not simply reflecting the concentration changes in river inputs. The reduction in inputs from the Seine River is continuous (Fig. 7), whereas the distribution of Cd in mussels exhibits an increase between 1988 and 1990 (Fig. 9). As indicated in the “Survey site” section, in addition to the Cd borne by Seine River waters, various direct Cd discharges into the Seine Estuary occurred from 1974 to 1992 (Chiffoleau et al., 2001). According to these authors the Cd contamination of the Seine Estuary was largely due to the discharge of phosphogypsum wastes from three phosphoric acid plants, which definitively ceased their activities in 1992. At the beginning of our sampling period, the phosphogysum waste discharge within the estuary, 200 000 tons yr^{-1} according to Chiffoleau et al. (2001), was made through a pipe placed on the bottom, southeast of Le Havre (Fig. 1). In addition, according to the

same authors, the phosphogypsum wastes were Cd-enriched during the 1987-1992 period. Thus, with a coincidental maximum of Cd concentration, the mussels obviously trace the predominance of this occurrence in the Cd historical discharge within the Seine Estuary.

Within this context, how can the variations in Cd and Hg in flounders be interpreted? In the case of Cd, an increase followed by a decline can be observed in the concentrations in the livers of flounder in the Seine Estuary and Bay (Fig. 6), which looks similar to the temporal changes in Cd concentrations in mussel tissue (Fig. 9). However, the significant increase in concentrations in the liver of the flounder had been perceptible since 1988, i.e., one year before that in the mussels, and the decline also appears to occur earlier (Fig. 9). Does this time lag reflect ecological differences between the two species (habitat, diet, growth, etc.) or, more simply, the gap between the sampling periods? Indeed, the flounders were sampled in November and December, while the mussels were collected four times a year, from February to November. Another small dissimilarity in the temporal trend of Cd concentrations in flounder, compared to the mussel, is its roughly stable values after 1993, while Cd in mussels continues to decrease until 1998. While the internal Cd discharges onto the sediments had stopped since 1992, the Cd concentrations in the Seine River waters continued to decline (Fig. 7). This suggests that the flounder, contrary to the mussel, is more reactive to Cd changes in the sediments than in the water column.

In the case of Hg in the muscles of the flounder (Fig. 4), we do not observe any temporal variation linked to that of mussels or fluvial Hg contributions (Fig. 8 and 10). During the period characterized by high concentrations of Hg in mussels and in the waters of the Seine, large variations may be observed in flounder, with the lowest average Hg concentrations in 1992 and the highest in 1993 (Fig. 4). If we assume that the sediment is the main source of metal contamination for the flounder, the migrations by flounder within the Estuary and the Seine Bay can be seen as one possible explanation for the high variability of mercury concentrations in the fish. Indeed, the spatial heterogeneity of Hg contamination in the Seine Estuary is huge: while in the Seine Bay, concentrations average 0.25 µg g^{-1}, they attain 10 µg g^{-1} upstream near Rouen (Cossa et al., 2002b). However, in the absence of more in-depth knowledge of the migrations and the feeding habits of the flounder, this interpretation will remain speculative.

In summary, the monitoring of Cd using flounder livers since 1986 displays a temporal pattern that can easily be related to the temporal changes in the Cd-rich waste inputs to the estuarine sediments, while the monitoring in mussel tissue also recorded these internal estuarine input variations but superimposed with the continuous decrease trends in Cd concentrations in the Seine River. For Hg, while the temporal trends for mussel tissue and river concentration are similar, the

Hg tendency recorded using flounder muscle do not follow the same pattern. The discrepancy between the responses of the two bioindicators may be due to their different niches. Thus, the optimization of the use of the flounder as a sentinel organism for monitoring Cd and Hg contamination temporal trends in estuarine environments requires a profound knowledge of their ecology within the studied site. An adapted sampling strategy based on this knowledge should provide results easier to interpret.

Acknowledgements

We would like to thank Gerald Leroy and Marielle Olivier from the Seine Navigation Service (Rouen) for supplying data on the monitoring of cadmium in the River Seine, and to Andrian Girard for analyses. Thanks also to William Delor and Heidi Pethybridge for editing the transcript. We are grateful to an anonymous referee for his suggestions, which help to improve the discussion of the results.

References

Anonymous, 1994. Guidelines for the sampling and analysis of organisms and the reporting of results under the Joint Monitoring Programme (JMP). A11/94-E : 12 p.

Cellule de suivi du littoral Haut-Normand. 1997. Recensement des poissons et analyse des régimes alimentaires du bar *Dicentrarchus labrax* et du flet *Platychthys flesus* de l'estuaire de Seine. In : Programme scientifique Seine aval, état d'avancement des travaux du programme coordonné de recherche sur l'estuaire de la Seine. Département de biologie, Université de Rouen, Rapport 1996/FIN-4. 262p.

Chiffoleau, J-F, Auger, D., Chartier, E. Michel, P., Truquet, I., Ficht, A., Gonzalez, J.L., and Romana, L.A. 2001. Spatiotemporal changes in cadmium contamination in the Seine estuary (France). Estuaries, 24: 1029-1040.

Chiffoleau, J. F., D. Auger, E. Chartier and A. Grouhel. 2003. Dosage de certains métaux (Ag, Cd, Cu, Cr, Ni, Pb, V, Zn) dans les organismes marins par absorption atomique. Méthodes d'analyses en milieu marin. Editions Ifremer, Plouzané, France: 43pp. ISBN 2-84433-114-9

Claisse, D., 1989. Chemical contamination of French coasts, the result of a ten years mussel watch. Mar. Pollut. Bull., 20 : 523-528.

Collings, S.E., Johnson, M.S. and Leah, R.T., 1996. Metal contamination of angler-caught fish from the Mersey estuary. Mar. Environ. Res., 41 :281-297.

Coquery, M., Cossa, D. and Sanjuan, J., 1997. Speciation and absorption of mercury in two macro-tidal estuaries. Mar. Chem., 58: 213-227.

Cossa, D. 1989. A Review of the Use of *Mytilus Spp*. as a Quantitative Indicators of Cadmium and Mercury Contamination in Coastal Waters. Oceanol. Acta., 12: 417-432.

Cossa D. and Bourget E., 1980. Trace elements in *Mytilus edulis* L. from the estuary end gulf of St. Lawrence, Canada: lead and cadmium concentrations. Environ. Pollut., Ser. A., 23 : 1-8.

Cossa D., Auger D, Averty B., Lucon M., Masselin P. and Noel J. 1992. Flounder (*Platichthys flesus*) muscle as an indicator of metal and organochlorine contamination of French Atlantic coastal waters. Ambio, 21 : 176-182.

Cossa, D., Coquery, M., Nahklé, K., and Claisse, D. 2002a. Dosage du mercure total and du monométhylmercury dans les organismes et les sédiments marins. Méthodes d'analyse en milieu marin. Editions Ifremer, Plouzané, France. ISBN 2-84433-105-X, 26p.

Cossa, D., Laurier, F. and Ficht, A. 2002b. Mercury Contamination in the Seine Estuary, France: An Overview. Chap. 20. P; 298-320. In: Biogeochemistry of Environmentally important Elements", Y. Cai and O.C. Braids Publishers. ACS Symposium Series 835, American Chemical Society, Washington, DC, 436 pp.

Cossa, D., B. Averty, J.Bretaudeau and A.-S. Senard. 2003. Spéciation du mercure dissous dans les eaux marines. Méthodes d'analyse en milieu marin. Co-édition Ifremer et Ministère de l'Ecologie et du Développement Durable. 27 pp. ISBN 2-84433-125-4

Deniel, C., 1981. Les poissons plats (Téléostéens, Pleuronectiformes) en baie de Douarnenez, reproduction, croissance and migration. Doctorate thesis presented to the University of Western Brittany: 463 p.

Franklin, A. 1987. The concentrations of metals, organochlorine pesticides and PCB residues in marine fish and shellfish: results from MAFF fish and shellfish monitoring programmes, 1977-1984. Aquatic Environment Monitoring Report N° 16, Ministry of Agriculture, Fisheries and Food. Lowestoft, U.K.

Hutchison, S. and Hawkins, L.E., 1990. The influence of salinity in water balance in 0-group flounders, *Platichthys flesus* L. J. Fish Biol., 36: 751-764.

ICES 1989. Statistical analysis of the ICES cooperative monitoring programme data on contaminants in fish muscle tissue (1978-1985) for determination of temporal trends. Coop. Res. Report Council Explor. Sea N° 162, Copenhagen, DK.

ICES 1984 The ICES coordinated monitoring programme for contaminants in fish and shellfish. ICES Coop. Res. Reports 126:1-100

Idlafkih, Z., D. Cossa and M. Meybeck. 1995. Comportement des contaminants en trace dissous et particulaires (As, Cd, Cu, Hg, Pb, Zn) dans la Seine. Hydroécol. Appl., 7: 125-150.

Jensen, A., 1982. Trend monitoring. Statistical analusis of factors affecting the concentrations of mercury in flounder. International Council for the Exploration of the Sea, Copenhagen, 12p.

Jensen, A and Cheng, Z., 1987. Statistical analysis of trend monitoring data of heavy metals in flounder (*Platichthys flesus*). Mar. Pollut. Bull., 18: 230-238.

Jensen, A. and Bro-Rasmussen, F., 1992. Environmental cadmium in Europe. Rev. Environ. Contam. Toxicol., 125: 101-181.

Julshamn, K. and Grahl-Nielson, O., 1996. Distribution of trace elements from industrial discharges in the Hardangerfjord, Norway: a multivariate data analysis of saithe, flounder and blue mussels. Mar. Pollut. Bull., 32 : 564-571.

Lafleur, P.D., 1973. Retention of mercury when freeze-drying biological materials. Anal. Chem., 45 : 1534-1537.

Laurier, F., D. Cossa, C., Beucher, and E. Brévière. 2006. The impact of groundwater discharges on mercury partitioning, speciation and bioavailablity to mussels in a coastal zone. Mar. Chem., doi : 10.1016/j.marchem.2006.10.010.

Leah, R.T., J.J. Evans, and M.S. Johnson. 1992. Mercury in flounder (*Platichthys flesus* L.) from estuaries and coastal waters of the North East Irish Sea. Environ. Pollut., 75: 317-322.

Luten,, J.B., Bouquet, W., Riekel-Booy, G., Rauchbaar, A.B. and Scholte, M.W.M., 1987. Mercury in flounder, *Platichthys flesus*, cod, *Gadus morhua*, and perch, *Perca fluviatilis*, in relation to their length and environment. Bull. Environ. Contam. Toxicol., 38 : 318-323.

Marthinsen, I. and Staveland, G., 1990. Levels of mercury in flounder (*Platichthys flesus* L.) and cod (*Gadus morhua* L.) caught during the year 1988 in the Hvaler Archipelago, Southern Norway. Meeting of the International Council for the Exploration of the Sea, Copenhagen, 8p.

Masson G., 1987. Biologie and écologie d'un poisson plat amphihalin, le flet (*Platichthys flesus flesus*, Linné, 1758) dans l'environnement ligérien : distribution, démographie, place au sein des réseaux trophiques. Doctorate thesis presented to the University of Western Brittany 344p.

Minier, C., Levy, F., Rabel, D., Bocquené, G., Godefroy, D., Burgeot, T., Leboulenger, F. 2000. Flounder health status in Seine Bay. A multibiomarker study. Mar. Environ. Res., 50 : 69-73.

Miramand, P., Guyot, T., Rybarczyk, H. and Bessineton, C., 1998. Cd, Cu, Pb, Zn dans les tissus and in the réseau trophique benthique du bar et du flet de l'estuaire de la Seine. Programme Scientifique Seine Aval, Thème Edifices Biologiques, Rapport 1997/Fin – 4, Rouen 1998, pp171-181.

Phillips, D.J.H. and Rainbow D.W., 1993. Biomonitoring of Trace Aquatic Contaminants. Environmental managment series, Alden Press Ltd, Oxford : 371 p.

Riisgard, H.U. and Hansen, S., 1990. Biomagnification of mercury in a marine grazing food-chain : algal cells *Phaeodactylum triconutum*, mussels *Mytilus edu*lis and flounders *Platichthys flesus* studied by means of a stepwise-reduction-cvaa method. Mar. Ecol. Prog. Ser., 62: 259-270.

Thévenot, D., M. Meybeck, A. Chesterikov and M. Chevreuil. 1998. Métaux: sources multiples et accumulation. pp 391-437; Chap 9. In: La Seine en son bassin: Fonctionnement écologique d'un système fluvial anthropisé. M. Meybeck, G. de Marsily and E. Fustec "editors, Elsevier, ISBN: 2-84299-058-7. 749p.

Voight H.-R., 1999. Concentration of heavy metals in fish from coastal waters around the Baltic Sea. ICES Journal of Marine Science, 56 Suppl.: 140-141.

Voight, H.-R., 2002. Umweltwissenschaften und Schandstoff-Forschung, 15: 234-239.

Wheeler, A. 1969. The fishes in the British Isles and the Northwest Europe. MacMillan Ltd, London; 613pp.

Figure captions

Figure 1. The Seine Estuary and Bay. The black arrows indicate the location of the trawls for collecting the flounders. (★) Location of the outlet of the submarine phosphogypsum discharge pipe.

Figure 2. Relationships between mass and total length of the collected flounders.

Figure 3. Mercury concentrations in muscle (Hg) *versus* fish length (TL). Concentrations are expressed per unit of dry weight (dw). A linear model gives the best correlation coefficient ($Hg = 0.004TL - 0.604$; $R^2 = 0.28$, n=346).

Figure 4. Temporal trend of mercury ($log_{10}Hg$) concentration (annual average ± standard deviation) in the muscle for flounder with total length smaller than 300 mm.

Figure 5. Cadmium concentrations in liver (Cd) *versus* total fish length (TL). Concentrations are expressed per unit of dry weight (dw). The correlation coefficient is statistically unsignificant ($R^2 = 0.09$, n=346).

Figure 6. Temporal trend (1986-2004) of cadmium ($log_{10}Cd$) concentration (annual average ± standard deviation) in the liver for flounder with total length smaller than 300 mm.

Figure 7. Temporal trend (1986-2004) in cadmium (Cd) concentrations in the freshwater of the River Seine near the mouth. Dissolved and particulate Cd refer to fractions smaller and particulate larger than 0.45 µm respectively.

Figure 8. Temporal trend (1986-2004) in mercury (Hg) concentrations in the freshwater of the River Seine near the mouth. Dissolved and particulate Hg refer to fractions smaller and particulate larger than 0.45 µm respectively.

Figure 9. Temporal trend (1986-2004) in cadmium (Cd) concentrations in the soft tissue of mussel (*Mytilus edulis*) from the Seine estuary. Concentrations are expressed per unit of dry weight (dw). Annual average concentrations (± standard deviation) are expressed on a log scale.

F**igure 10.** Temporal trend (1986-2004) in mercury (Hg) concentrations in the soft tissue of mussel (*Mytilus edulis*) from the Seine estuary. Concentrations are expressed per unit of dry weight (dw). Annual average concentrations (± standard deviation) are expressed on a log scale.

Table 1. Average ± standard deviation of total length and metal concentration (per unit of dry weight) in flounders (*Platichthys flesus*) caught in the Seine estuary during the period 1986-2003. In brackets are the minimum and maximum values.

Year	Length (mm)	Cd in liver (mg kg^{-1}, dw)	Hg in muscle (mg kg^{-1}, dw)
1986	284 ± 64 (185 – 410)	0.50 ± 0.46 (0.07 – 1.83)	0.64 ± 0.59 (0.15 –3.15)
1987	297 ± 71 (185 – 405)	1.15 ± 1.82 (0.15 – 8.11)	0.60 ± 0.49 (0.16 – 2.41)
1988	283 ± 67 (185 – 415)	1.08 ± 0.74 (0.22 – 3.02)	0.93 ± 0.73 (0.10 – 2.54)
1989	282 ± 68 (180 – 395)	0.93 ± 0.86 (0.13 – 3.73)	0.61 ± 0.52 (0.17 – 2.41)
1990	254 ± 57 (175 – 355)	1.42 ± 1.98 (0.30 – 9.56)	0.54 ± 0.53 (0.18 – 2.67)
1991	275 ± 54 (190 – 370)	0.82 ± 0.69 (0.11 – 3.62))	0.41 ± 0.63 (0.10 – 3.27)
1992	272 ± 62 (180 – 395)	0.58 ± 0.32 (0.13 – 1.46)	0.26 ±0.22 (0.06 – 0.93)
1993	237 ± 35 (190 – 310)	0.33 ± 0.29 (0.01 – 1.16)	0.55 ± 0.25 (0.07 – 0.99)
1994	273 ± 67 (170 – 430)	0.63 ± 0.85 (0.04 – 2.44)	0.35 ± 0.36 (0.01 – 1.20)
1995	259 ± 51 (195 – 375)	0.29 ± 0.33 (0.09 – 1.75)	0.33 ± 0.17 (0.10 – 0.95)
1996	249 ± 35 (195 – 305)	0.34 ± 0.26 (0.09 – 1.18)	0.42 ± 0.17 (0.13 – 1.00)
1999	275 ± 58 (185 - 380)	0.52 ± 0.76 (0.06 – 3.03)	0.56 ± 0.41 (0.24 – 2.16)
2002	268 ± 52 (186 – 359)	0.26 ± 0.19 (0.08 – 0.81)	0.47 ± 0.23 (0.16 – 1.34)
2003	276 ± 60 (182 – 405)	0.32 ± 0.20 (0.11 – 0.90)	0.55 ± 0.34 (0.22 – 1.61)
1986-2003	*272 ± 59* *(170 – 430)*	*0.66 ± 0.95* *(0.01 – 9.56)*	*0.52 ± 0.46* *(0.01 – 3.27)*

Table 2. Range of Cd and Hg concentrations in flounder from various regions of the North Atlantic. All data have been converted in mg kg^{-1} dry weight (dry weight / wet weight = 5).

Location	Hg in muscle	Cd in liver	Reference
North Atlantic	< 0.01 – 2.60	-	ICES (1989)
North Atlantic	0.10 – 2.00	-	Franklin (1987)
Baltic Sea	-	0.04 – 3.39	Voigt (1999)
English Channel and Bay of Biscay	0.12 – 2.18	-	Cossa et al. (1992)
Seine Estuary and Bay	0.01 – 3.27	0.01 – 9.56	Present results

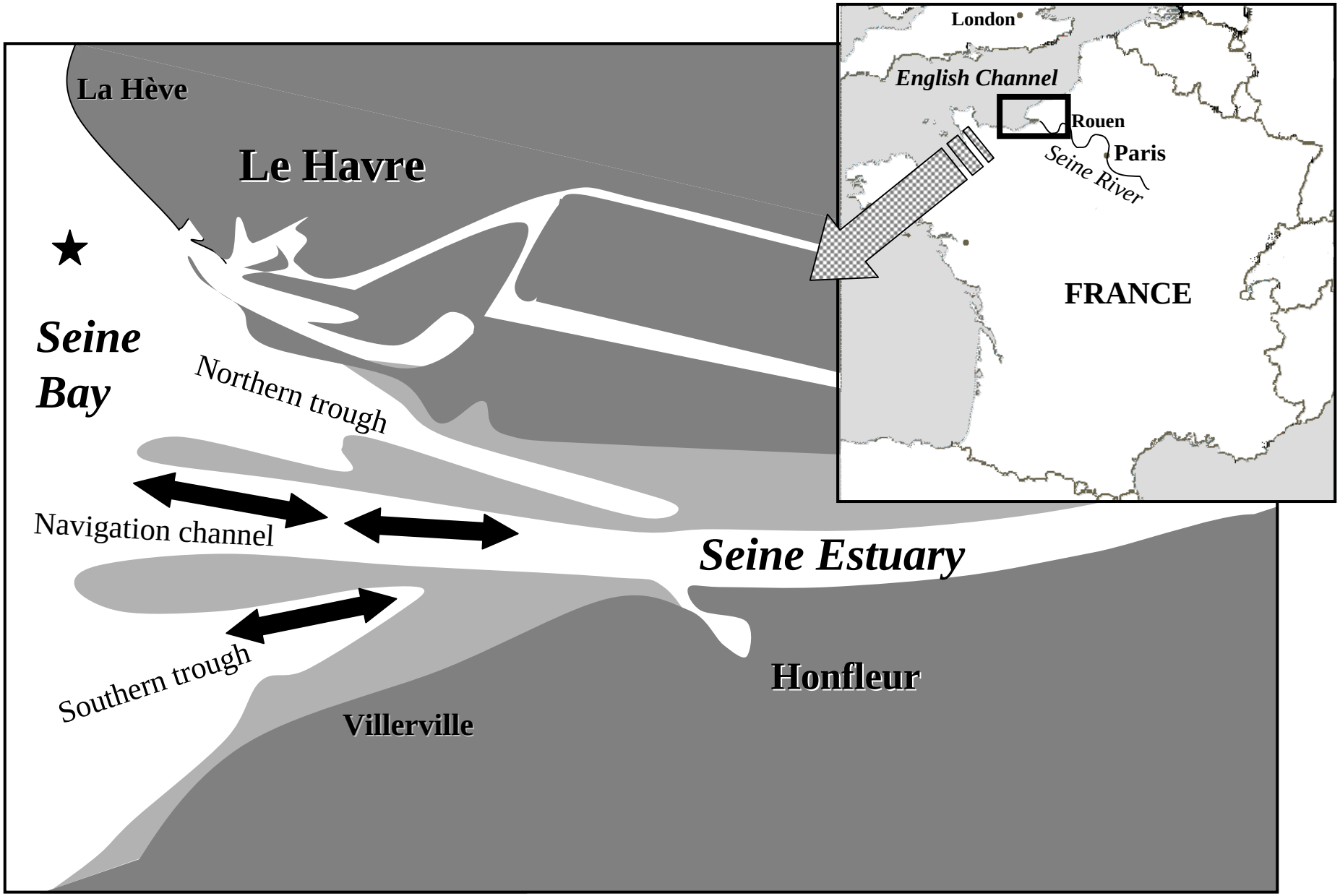
La Hève
Le Havre
Seine Bay
Northern trough
Navigation channel
Seine Estuary
Southern trough
Honfleur
Villerville
London
English Channel
Rouen
Paris
Seine River
FRANCE

Figure 1

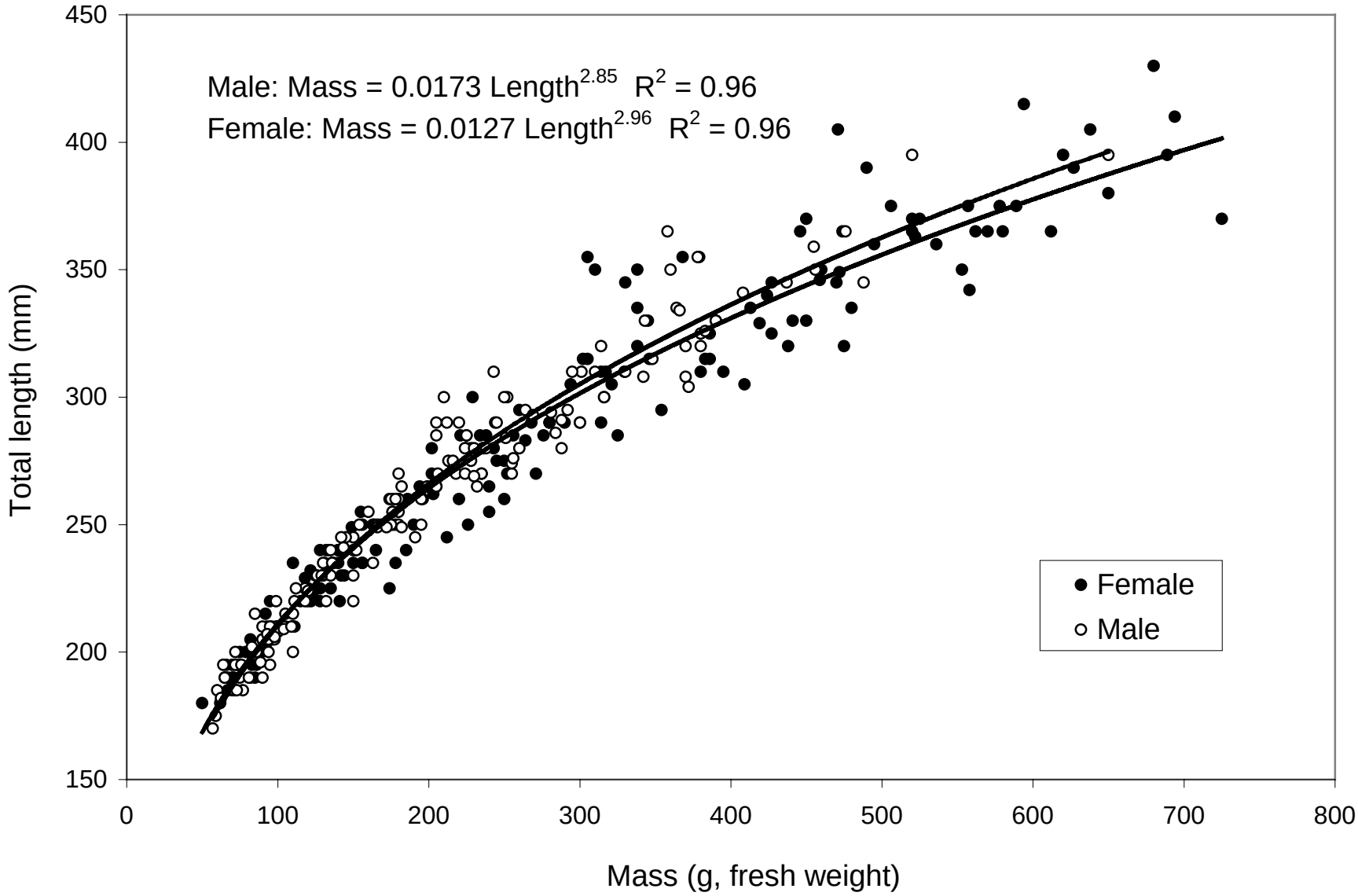
Male: Mass = 0.0173 Length^2.85 R^2 = 0.96
Female: Mass = 0.0127 Length^2.96 R^2 = 0.96
Female
Male
Total length (mm)
Mass (g, fresh weight)
450
400
350
300
250
200
150
0
100
200
300
400
500
600
700
800

Figure 2

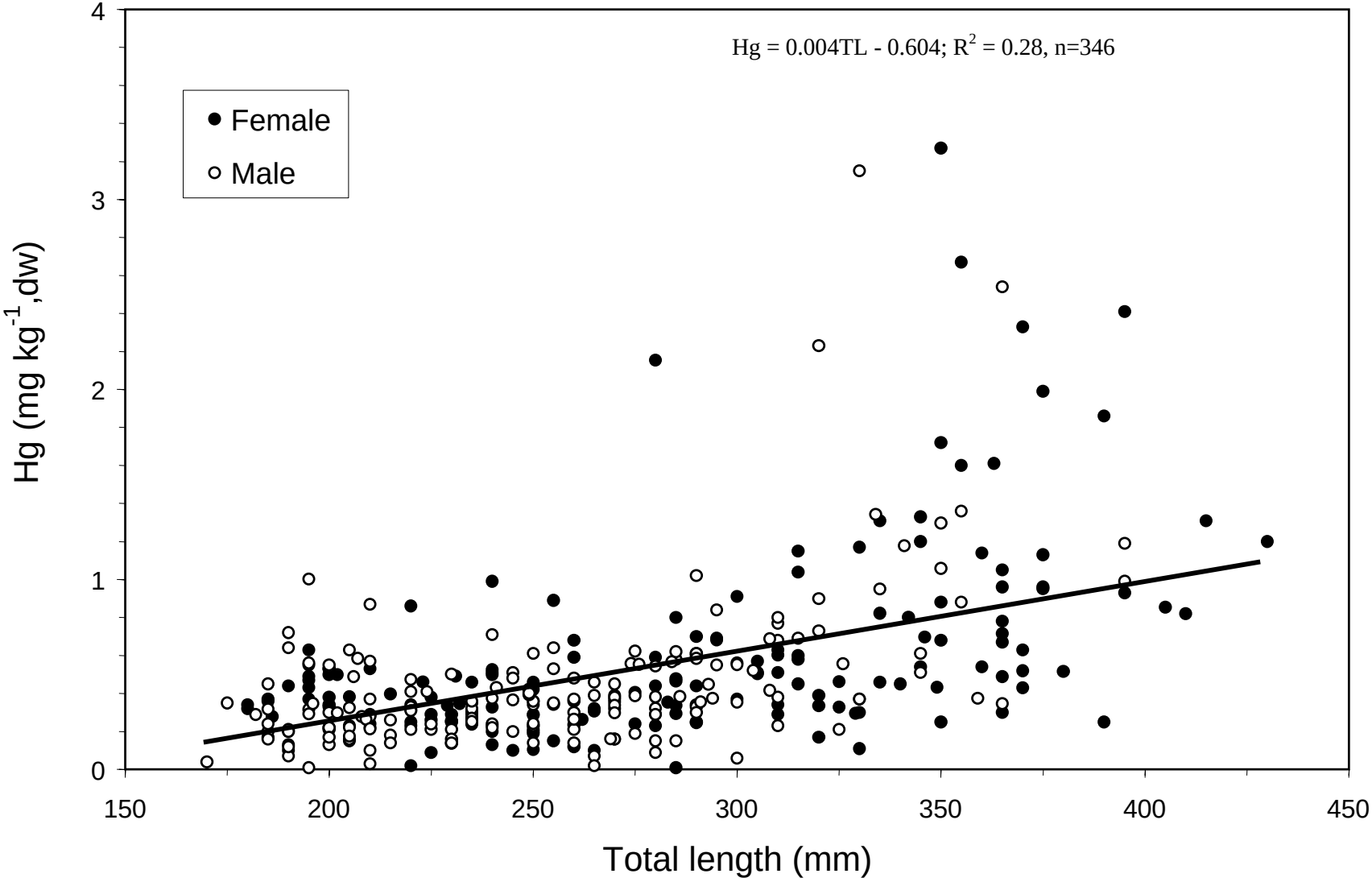
Hg = 0.004TL - 0.604; R2 = 0.28, n=346
Female
Male
Hg (mg kg-1,dw)
Total length (mm)
0
1
2
3
4
150
200
250
300
350
400
450

Figure 3

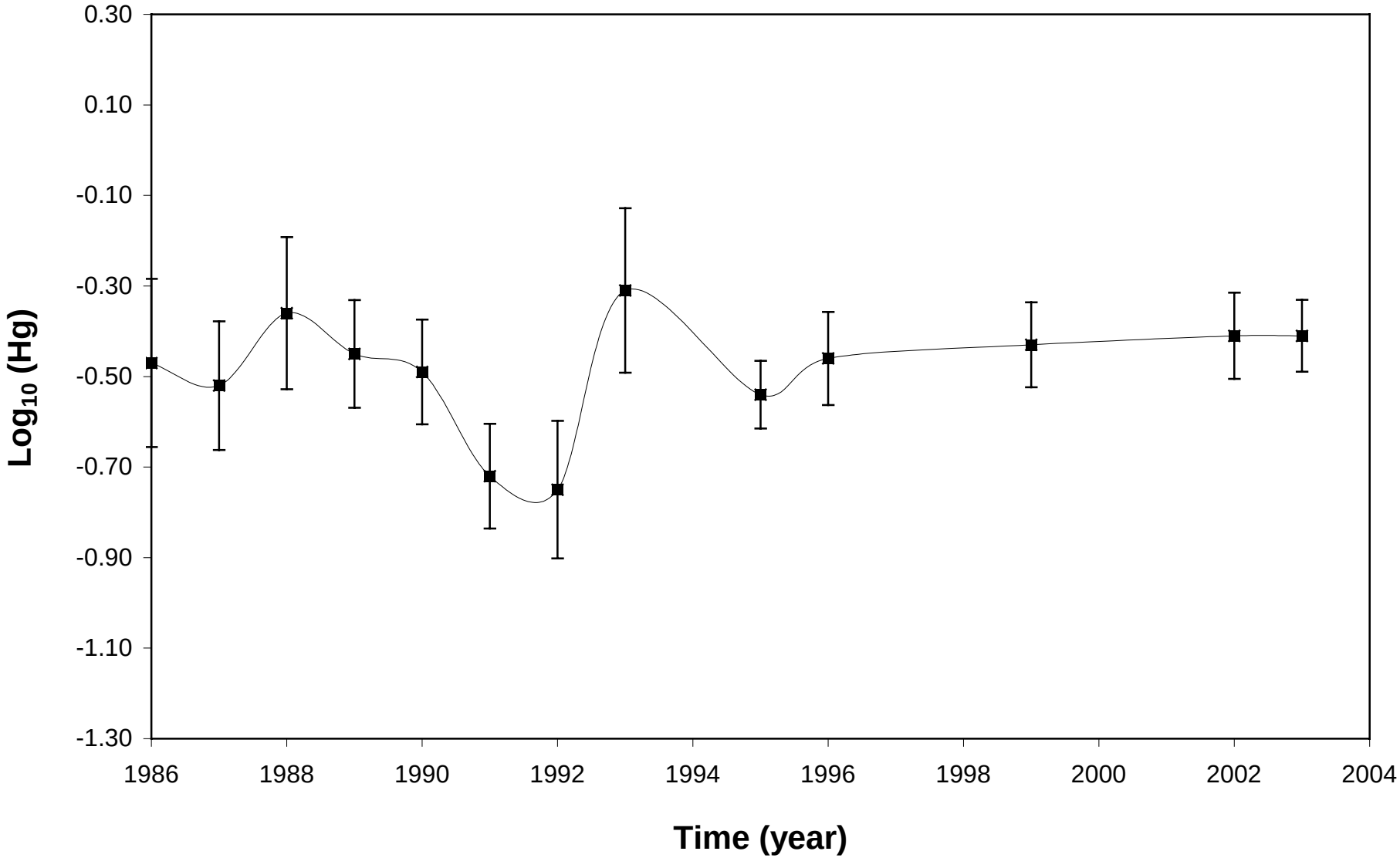
0.30
0.10
-0.10
-0.30
-0.50
-0.70
-0.90
-1.10
-1.30
Log10 (Hg)
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
Time (year)

Figure 4

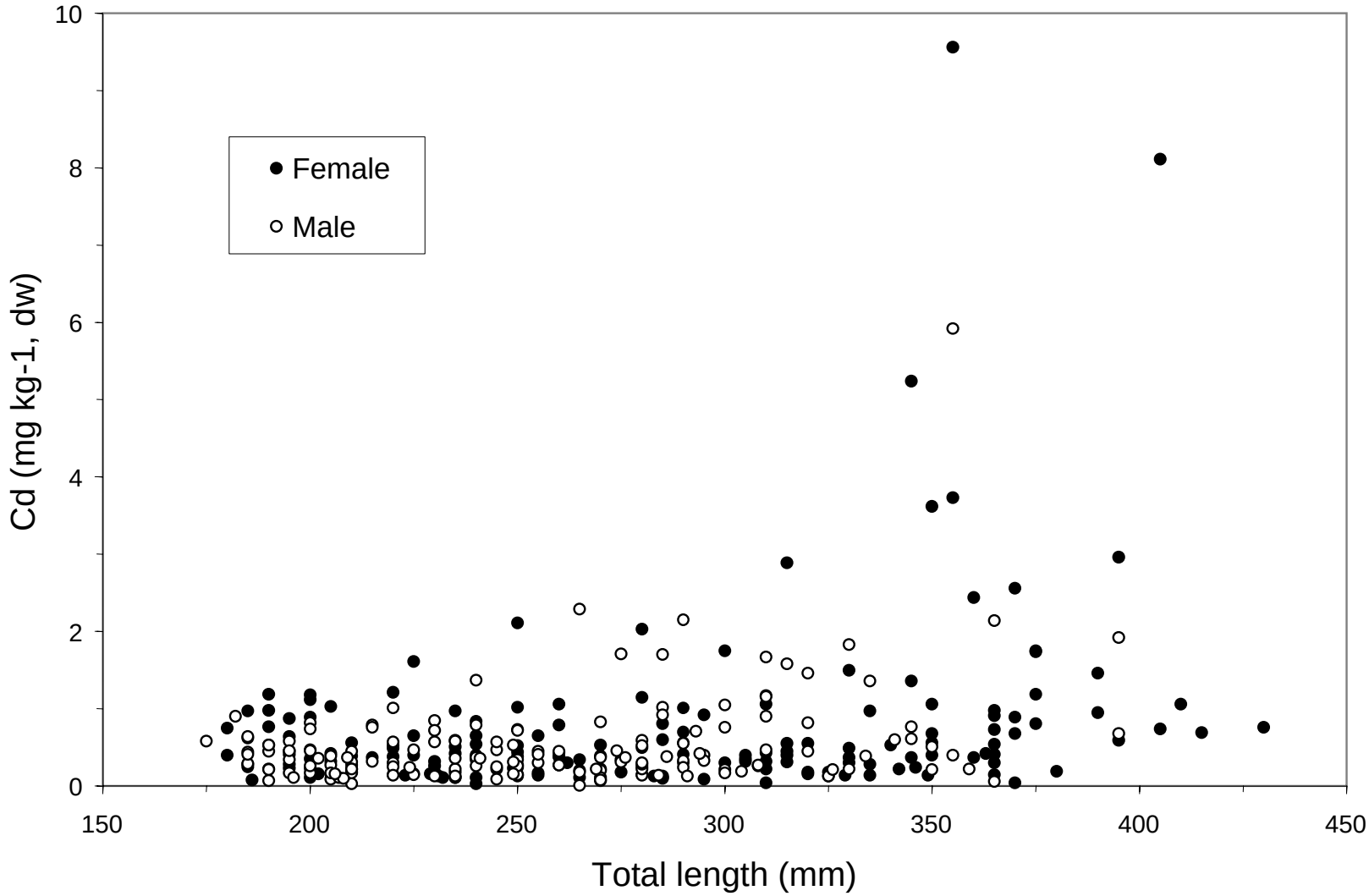
Female
Male
Cd (mg kg-1, dw)
Total length (mm)
0
2
4
6
8
10
150
200
250
300
350
400
450

Figure 5

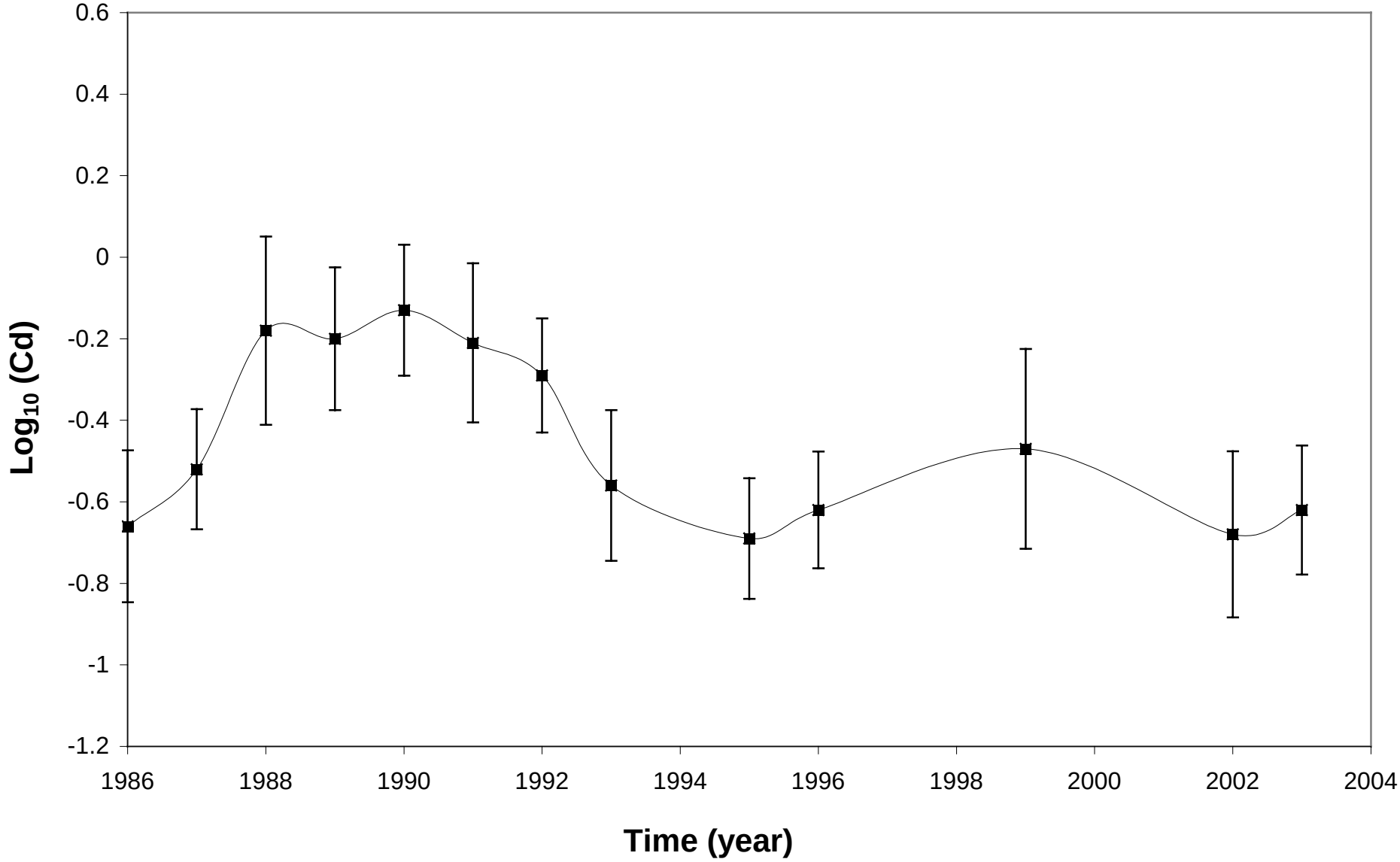
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
Log10 (Cd)
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
Time (year)

Figure 6

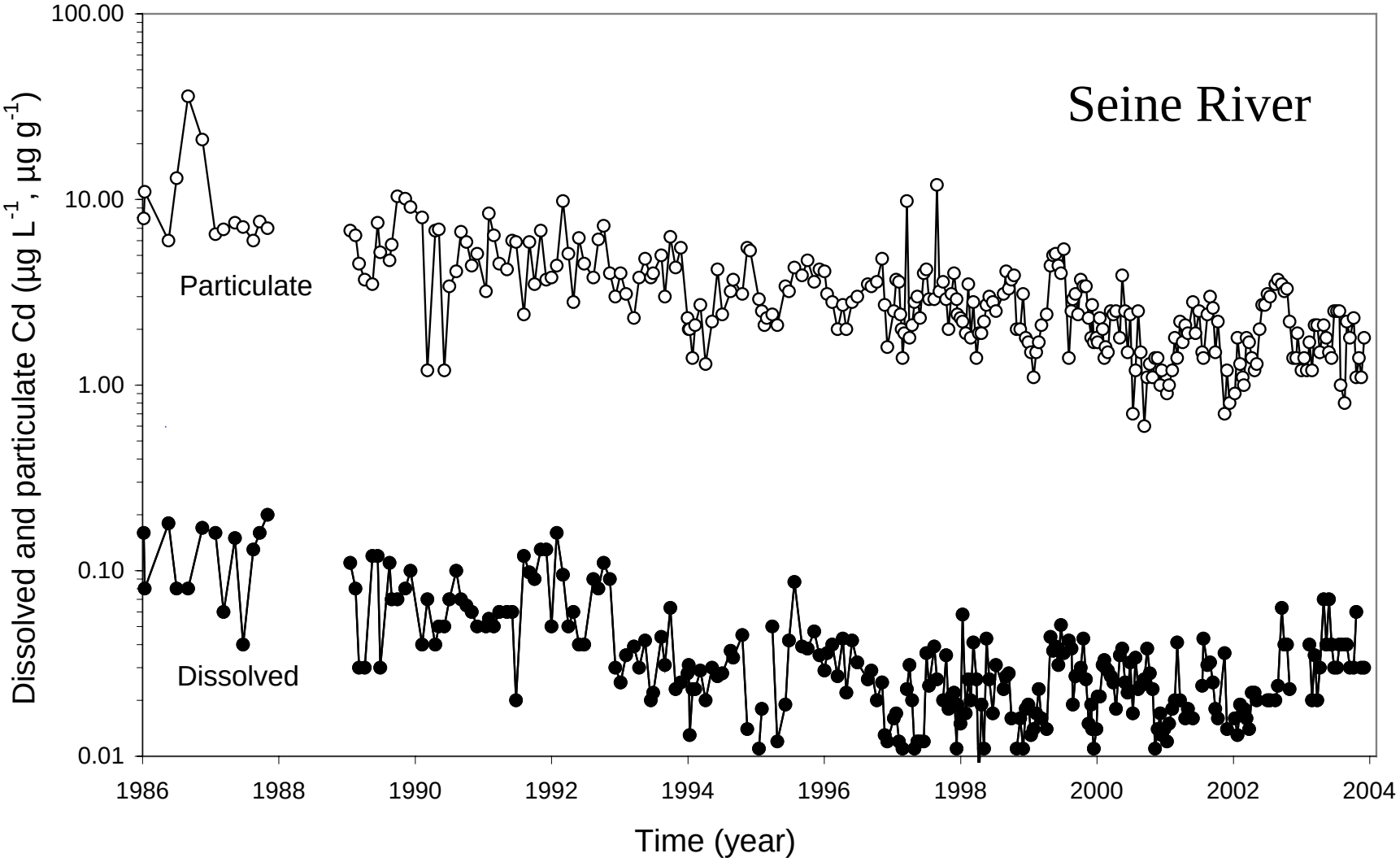
Seine River
Particulate
Dissolved
Dissolved and particulate Cd (µg L-1, µg g-1)
Time (year)
100.00
10.00
1.00
0.10
0.01
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004

Figure 7

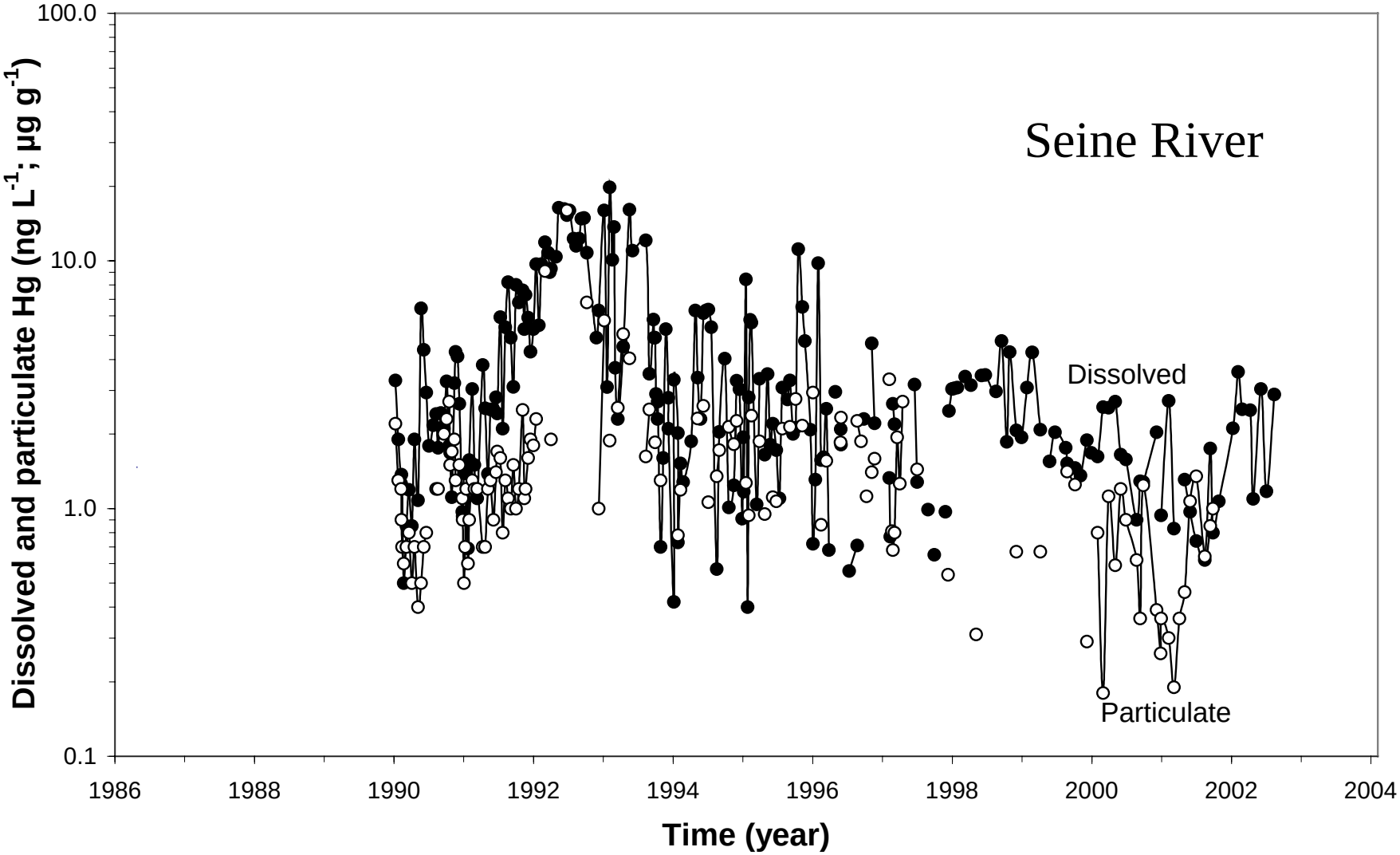
Seine River
Dissolved
Particulate
Dissolved and particulate Hg (ng L-1; µg g-1)
100.0
10.0
1.0
0.1
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
Time (year)

Figure 8

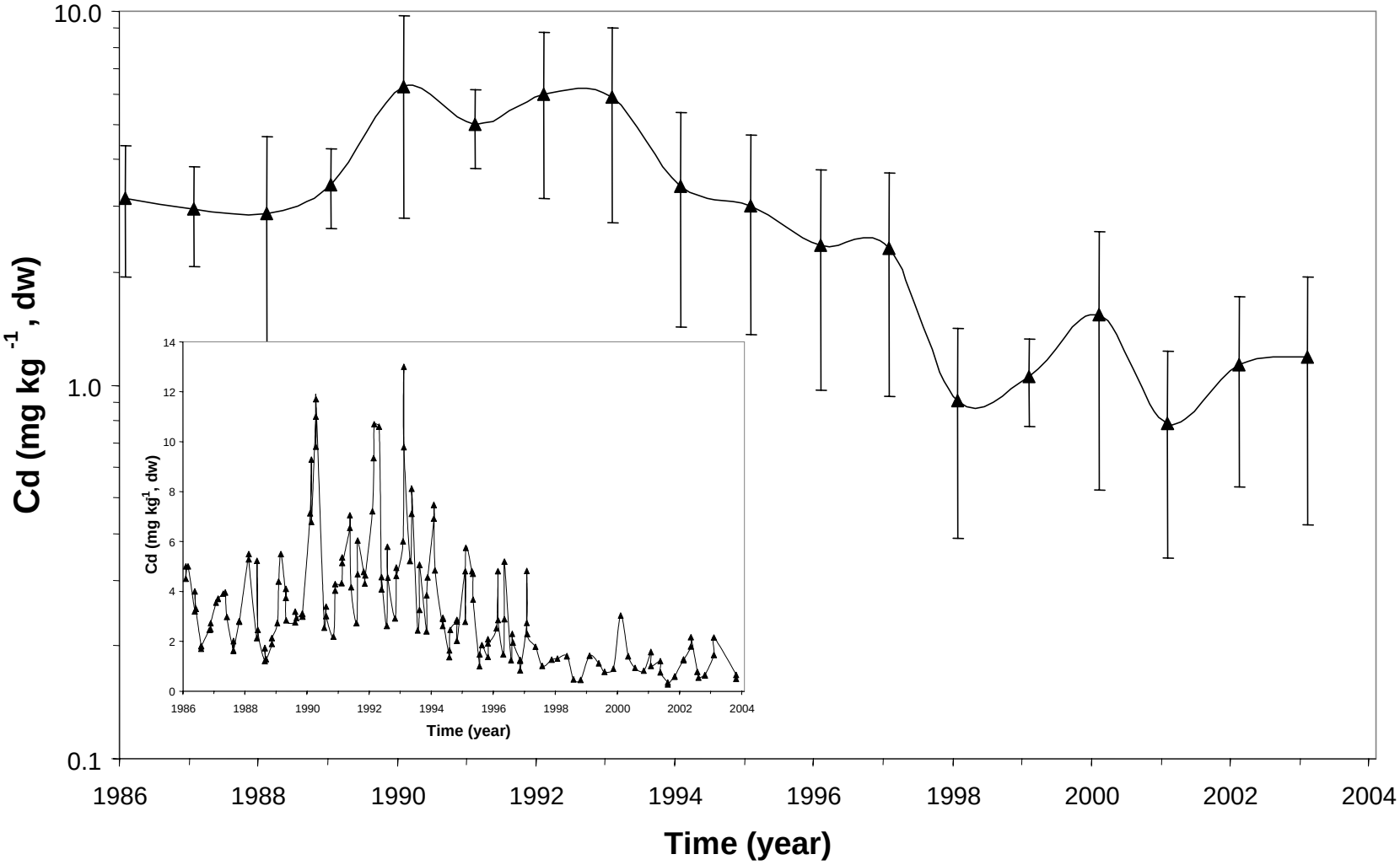
10.0
1.0
0.1
Cd (mg kg -1 , dw)
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
Time (year)
14
12
10
8
6
4
2
0
Cd (mg kg1, dw)
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
Time (year)

Fig. 9

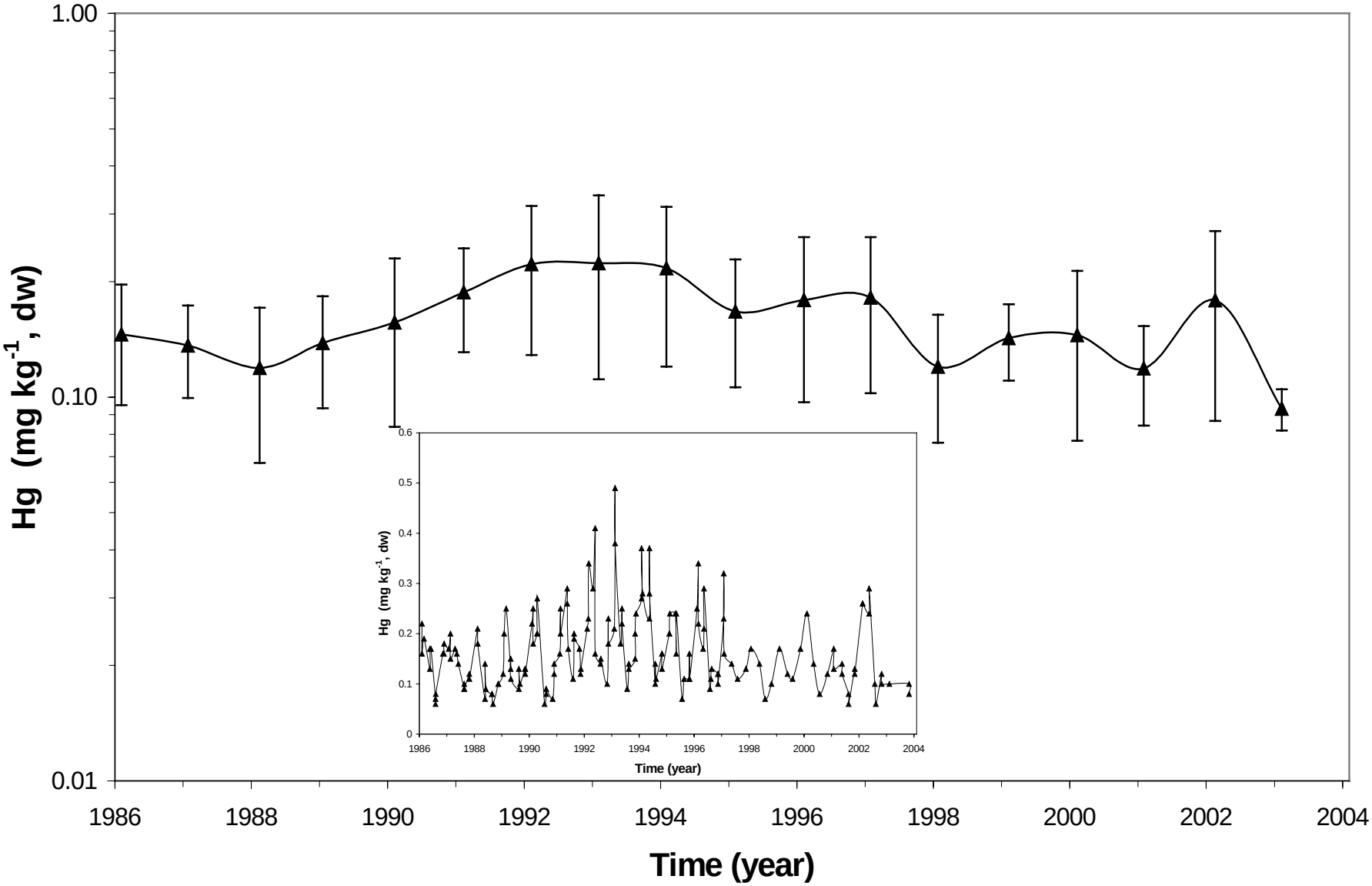
1.00
0.10
0.01
Hg (mg kg-1, dw)
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
Time (year)
0.6
0.5
0.4
0.3
0.2
0.1
0
Hg (mg kg-1, dw)
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
Time (year)

Fig.10