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Monitoring of Organic Compounds and Trace Metals During a Dredging Episode in the Göta Älv Estuary (SW Sweden) Using Caged Mussels

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Abstract The concentrations of selected trace metals, organotins, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) were determined in caged blue mussels (*Mytilus edulis*) during dredging operations in the Göta Älv Estuary (SW Sweden). Maximum values of pollutants in mussel tissues were found after the dredging started. Our results showed that the dredging caused mobilization of organotins from the sediments to the water column during the experimental period. Multidimensional scaling (MDS) and cluster analysis were applied to compare and establish relationships between levels of pollutants in mussels and sampling sites during the experimental period. In order to evaluate the biological effects of contaminants, genotoxic damage was measured using the Comet assay, and its potential application for environmental monitoring is discussed.

Keywords *Mytilus edulis* · dredging operations · bioaccumulation · metals · organic pollutants · monitoring

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1 Introduction

The great affinity of pollutants for suspended particles causes the enrichment of polluted sediments in the bottom of estuaries and coastal zones close to industrial and urban areas (Beiras, Bellas, Fernández, Lorenzo, & Cobelo-García, 2003a; Beiras, Fernández, Bellas, Besada, González-Quijano, & Nunes, 2003b). Those pollutants do not remain indefinitely attached to sediments, and they can be available to organisms by ingestion of sediment particles and when they are released again to the water column by biological, chemical and/or physical processes (Fichet, Radenac, & Miramand, 1998; Long, Robertson, Wolfe, Hameedi, & Sloane, 1996). On the other hand, effects of pollutants in organisms depend on the bioavailability of chemical compounds, i.e. the fraction of chemical compound present in the environment which can be incorporated by the organism (Adams, 1995; Carr, Long, Windom, Chapman, & Thursby, 1996; Lamberson, DeWitt, & Swartz, 1992). Since not all chemical forms of pollutants are equally bioavailable

and some pollutants can be accumulated in living organisms to a greater extent than others, we need to study the levels of pollutants in the organisms to be able to predict the environmental risk (Connell, Lam, Richardson, & Wu, 1999; Rainbow & Phillips, 1993). Thus, chemical analyses of the tissues of marine organisms are used as a routine approach in studies of marine pollution, providing a temporal integration of the levels of pollutants with biological relevance at higher concentrations than those present in water or sediment, and facilitating their quantification (Rainbow & Phillips, 1993).

Bivalves such as *Mytilus* spp have been successfully used as tools in Mussel watch environmental monitoring programs to reveal geographical patterns and to identify temporal trends in coastal pollution (e.g. Chase et al., 2001; Farrington, Goldberg, Risebrough, Martin, & Bowen, 1983; Sericano et al., 1995; Widdows & Donkin, 1992; Widdows et al., 1995). Also, recent papers show that transplanting bivalves from a reference site to a polluted area can be a feasible strategy for biomonitoring the effects of environmental changes in coastal and estuarine zones where sentinels are not available (Baumard, Budzinski, & Garrigues, 1998; Romeó, Hoarau, Garello, Gnassia-Barelli, & Girard, 2003; M. H. Salazar, & S. M. Salazar, 1995). The caged bivalve approach is a developing technology which appears to be a useful monitoring tool to better understand the potential overall impact of marine pollution (Baumard et al., 1998; Martel, Kovacs, Voss, & Megraw, 2003). On the other hand, we need biological criteria to evaluate the impact of pollutants on organisms and thus determine the levels of pollution with ecotoxicological relevance. The use of biomarkers is becoming increasingly important in the assessment of the effects and bioavailability of pollutants to aquatic organisms (Depledge, Aagaard, & Gyorkos, 1995). For instance, the examination of DNA single-strand breaks in individual cells (Comet assay) is a promising quick, simple and reliable test which has been used as a sensitive biomarker of genotoxicity following in vitro or in vivo exposure to pollutants (Collins, Dobson, Dusinska, Kennedy, & Stetina, 1997; Shugart, 1990; Wilson, Pascoe, Parry, & Dixon, 1998).

Göteborg Harbour (SW Sweden) is the largest harbour of Scandinavia. It is subjected to intense maritime traffic with an annual commercial traffic of more than 30 million tonnes of cargo, and more than 11,000 ship arrivals (Port of Göteborg AB, 2004). The maintenance of this harbour required dredging operations during 2003 to remove 11×10^6 m^3 of sediment to enlarge the harbour channels. During their residence in the harbour system, these sediments adsorb pollutants that originate from industrial and shipping activities in the area. Riverine particulates consisting of sediments from the Göta Älv River are also a source of pollutants that enter the estuary and are deposited in the sediment. The river is an important shipping route to industrial areas around Lake Vänern. Thus, sediment pollution with respect to trace metals, PCBs, PAHs and organotins has been reported in Göteborg Harbour (Brack, 2002 and references therein). For instance, very high concentrations of TBT in sediments from the inner part of Göteborg Harbour have been previously reported (3,250–11,000 ng/g dw; De la Cruz & Molander, 1998). Following the classification introduced by Dowson, Bubb, and Lester (1993) for TBT, those concentrations correspond to "grossly" contaminated sediments (above 500 ng/g dw), and are well above the Ecotoxicological Assessment Criteria (concentrations below which the potential for adverse effects is minimal) of 0.005–0.05 ng/g dw proposed by the OSPAR Commission (2000). Also, moderate pollution has been measured in terms of PCBs and PAHs in sediments (Cato, 2006; SMHI, 2001). Levels of PCBs of 12–100 ng/g dw have been registered, which exceeds the OSPAR background concentrations for Skagerrak of 0.4 ng/g dw (OSPAR Commission, 2000). PAHs concentrations in sediments of 0.4–1.6 μg/g dw have also been measured in the outer part of the Göta Älv Estuary (Cato, 2006).

The aims of the present study were to identify the effects of dredging operations on mobilization of trace metals and organic compounds over a 1-year period in the Göta Älv Estuary, and to evaluate the bioavailability of those compounds with a filter-feeder model, the blue mussel. The results of this study provide information about the extent and time scales of dredging-related mobilization of trace metals, organotins, PAHs and PCBs. The validity of the Comet assay as an ecotoxicological tool for monitoring pollution effects of dredging operations was also investigated.

2 Materials and Methods

2.1 Sampling and caging procedure

Common mussels (*Mytilus edulis*) were provided by a local aquaculture farm situated in an unpolluted area in the Northern part of the Swedish west coast. Once in the laboratory, mussels were sorted by size (45–55 mm) and were then maintained for one week in tanks with a continuous flow of natural sea water. Batches of 50 mussels were placed in cotton net bags (1 cm mesh size, 1 m length, 4 cm width) which were attached to wooden frames (1 m length, 0.5 m width) to permit similar conditions for all individuals (M. H. Salazar & S. M. Salazar, 1995). Caged mussels were deployed at five experimental sites located in the Göta Älv Estuary (SW Sweden) in November 2002 (before start of dredging), and in April and October 2003 (Figure 1). At each site three frames, i.e. three true replicates with 50 mussels each were used. The frames were tied up to leaded ropes and placed at 4–5 m depth. After 30 days the frames were retrieved and the mussels were transported in polyethylene bags to the laboratory. Fifteen mussels were taken from each frame, the shells were removed and the soft tissues were frozen for chemical analysis. In April 2003, seven additional mussels from each site were used to detect DNA single-strand breaks. Weather conditions, trawlers, and the intense traffic of commercial ships at the experimental sites G3 and G4 made the recovery of the frames difficult. Therefore, frames deployed at G3 were only retrieved in April 2003 and those deployed at G4 were only recovered in April and October 2003.

2.2 Chemical analyses

Fifteen mussels from each site were thawed and homogenized for chemical analysis. About 20 g wet weight were used for analysis. The samples were then sent to a certified laboratory (Alcontrol AB, Sweden). Subsamples were taken for determination of fat content and dry weight. For analysis of organic pollutants the samples were thoroughly homogenized

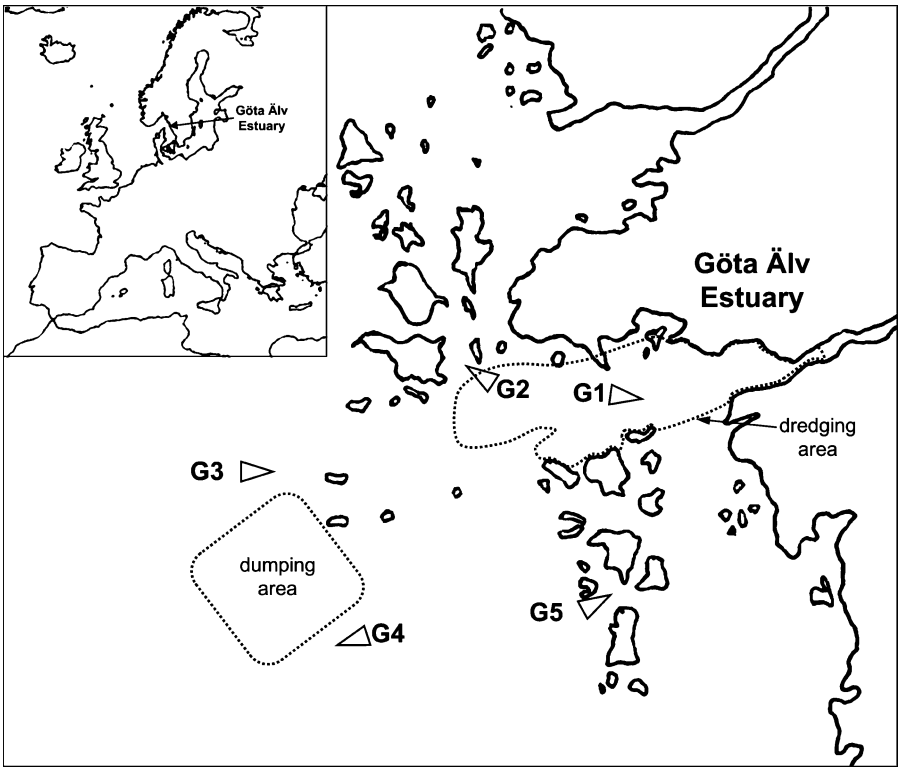

Figure 1 Map of the sampling sites in the Göta Älv Estuary, and location of the estuary in the west coast of Sweden (insert).

and dried for 2–3 h at 105°C. About 5–10 g of the dried samples were then weighted and extracted by Soxhlet for 18 h with toluene. All organic compounds were separated and quantified by high resolution gas chromatography. For determination of PCBs, chemical ionisation with methane was applied (GC-MS-NCI). For PAHs analysis, ionisation with electron impact was used (GC-MS-EI). Organotins were measured using gas chromatography-atomic emission detection (GC-AED) (Hewlett-Packard, Agilent). Identification and quantification were performed by means of internal and external standards. The detection limits were 0.003 μg/g dw for PCBs, 0.002–0.01 μg/g dw for PAHs, and 0.005–0.01 μg/g dw for organotins. All analyses were performed according to SS EN ISO/IEC 17025 and ISO 9001, ISO 9002 and ISO 14001 quality system.

For trace metal analysis mussel samples were digested with a mixture of HNO_3-H_2SO_4 (for mercury) or HNO_3 only (for the other elements). Mercury was determined by cold-vapour atomic absorption spectrometry (CV-AAS), and cadmium, copper, lead, and zinc were determined by inductively coupled plasma-mass spectrometry (ICP-MS).

Lipid content in mussels was determined by hydrolysis with 8 M HCl. The hydrolyzed fat was shaken intensely with a mixture of ethanol, diethyl ether and petroleum ether (1:2.5:2.5 vol.). The petroleum ether layer was evaporated, and the remnants were weighted and expressed as % of total weight. Dry weight was determined by drying according to the Swedish standard (SS 02 81 13).

2.3 Comet assay procedure

Seven mussels (length 46±2 mm) from each site in April 2003 were used for analyses of DNA single-strand breaks in hemocytes. The Comet assay was based on the protocol of Singh, McCoy, Tice, and Schneider (1988) with some modifications. All work was carried out in dim light to avoid UV damage to the DNA. As positive controls, two samples from each site were exposed to 100 μM H_2O_2 for 1 h at 4°C.

The DNA was stained with 50 μl of ethidium bromide (20 μg ethidium bromide/ml distilled water) and examined with a fluorescence microscope (Leitz) coupled to a CCD camera and an image analysis package (Komet 5, Kinetic Imaging Ltd., Liverpool, U.K.). All slides were independently coded prior to examination and 100 randomly selected cell nuclei were scored per slide. The levels of DNA single-strand breaks are expressed as percentage of DNA in the tail (% Tail DNA). Highly damaged nuclei ("clouds") were excluded since they presumably represent dead cells (Hartmann & Speit, 1997).

2.4 Statistical analyses

Data were log transformed to achieve normality and homogeneity of variances. Multidimensional scaling (MDS) (Kruskal & Wish, 1978) and cluster analysis were applied to compare and establish relationships between levels of pollutants in mussels and sampling sites during the experimental period. ANOVA and Tukey test were performed to analyse differences between sites. A difference at $p<0.05$ was considered statistically significant. Correlation analysis was performed according to Zar (1984) between chemical data and Comet assay results. Tests were performed using the SPSS® version 12.0 statistical software.

3 Results

3.1 Metals

In general terms, the concentrations of metals in caged mussels were low, but patterns differed for different metals (Table I). Cadmium levels were similar among the experimental sites, although a significant reduction of cadmium body burdens was observed in April 2003 and in October 2003 (during dredging), compared to the background levels found in November 2002 (before dredging) ($p<0.05$, $F=9.85$). Copper concentrations showed some temporal differences. Copper levels in April 2003 peaked at sites G1 and G2, decreasing in October 2003 below the values found in April 2003 ($p<0.05$, $F=6.21$). Mercury levels in April and October 2003 were significantly elevated at all the experimental sites compared to concentrations measured in November 2002 ($p<0.05$, $F=16.21$). Variations in lead and zinc concentrations during the sampling period were not significant and ranged from 0.7 to 1.2 and from 68 to 94 μg/g dw, respectively.

Table I Trace metal (μg/g dw) and organotin compounds concentrations (ng/g dw) (±standard deviations) in the soft tissues of *Mytilus edulis* mussels caged during 30 days at the sampling sites of the Göta Älv Estuary

		Cd	Cu	Hg	Pb	Zn	TeBT	MPT	DPT	TPT	MOT	DOT	TCyT	TBT/DBT	TBT/MBT
Nov 2002	G1	0.95	7.5	0.047	0.95	83	nd	nd	nd	nd	nd	nd	nd	14.5	–
		±0.064	±1.86	±0.0062	±0.186	±6.4								–	–
	G2	0.93	7.6	0.041	0.73	87	nd	nd	nd	nd	nd	nd	nd	–	–
		±0.075	±0.47	±0.0052	±0.105	±8.4								–	–
	G5	0.99	7.5	0.037	1.1	94	nd	nd	nd	nd	nd	nd	nd	–	–
		±0.101	±0.56	±0.0040	±0.372	±14								–	–
Apr 2003	G1	0.67	8.5	0.066	1.1	78	nd	nd	nd	nd	nd	nd	nd	9.2	25.9
		±0.093	±0.65	±0.0072	nc	±5.1									
	G2	0.73	8.6	0.065	1.0	85	nd	nd	nd	nd	nd	nd	nd	8.6	34.8
		±0.031	±0.21	±0.0056	±0.072	±4.5									
	G3	0.78	7.7	0.061	0.7	90	nd	nd	nd	nd	nd	nd	nd	6.9	22.2
		±0.021	±0.36	±0.0085	±0.064	±1.0									
	G4	0.69	6.6	0.058	0.7	68	nd	nd	nd	nd	nd	nd	nd	7.3	26.7
		nc	nc	nc	nc	nc									
	G5	0.67	7.5	0.059	0.7	73	nd	nd	nd	nd	nd	nd	nd	6.3	21.3
		±0.03	±0.15	±0.0035	±0.167	±9.0									
Oct 2003	G1	0.68	6.4	0.082	1.2	79	nd	4.0	nd	26	nd	nd	nd	3.1	13.1
		±0.014	±0.35	±0.0141	±0.07	±0.7		±5.66		±2.1					
	G2	0.71	7.0	0.068	1.1	93	nd	1.7	1.5	23	nd	nd	nd	3.5	14.1
		±0.072	±0.94	±0.0057	±0.10	±6.0		±2.94	±2.66	±0.6					
	G4	0.77	6.3	0.063	0.82	82	nd	1.9	2.2	27	nd	nd	nd	3.3	11.9
		±0.075	±0.96	±0.0035	±0.015	±6.1		±3.29	±3.87	±4.0					
	G5	0.72	6.7	0.071	0.81	85	nd	5.4	1.6	20	nd	nd	nd	3.1	7.9
		±0.040	±0.51	±0.0083	±0.045	±6.1		±3.12	±2.77	±3.5					

MBT, Monobutyltin; DBT, dibutyltin; TBT, tributyltin; TeBT, tetrabutyltin; MPT, monophenyltin; DPT, diphenyltin; TPT, triphenyltin; MOT, monooctyltin; DOT, dioctyltin; TCyT, tricyclohexyltin. nd. Below detection limits. nc. Standard deviation not calculated.

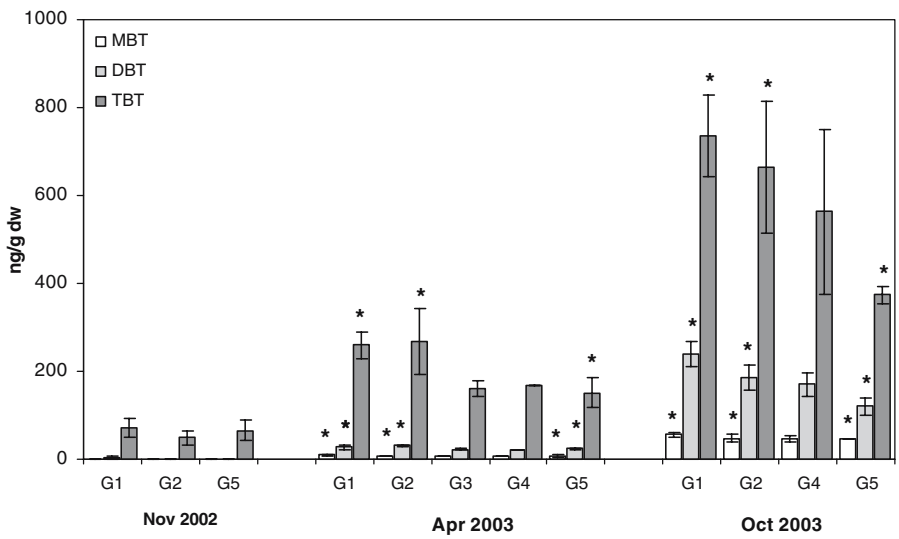

Figure 2 Concentration of MBT, DBT and TBT (ng/g dw) in mussels deployed at the sampling sites during November 2002, April 2003 and October 2003. Bars represent standard deviations. * indicates significant differences at the sampling sites ($p<0.05$) with respect to levels of pollutants in November 2002.

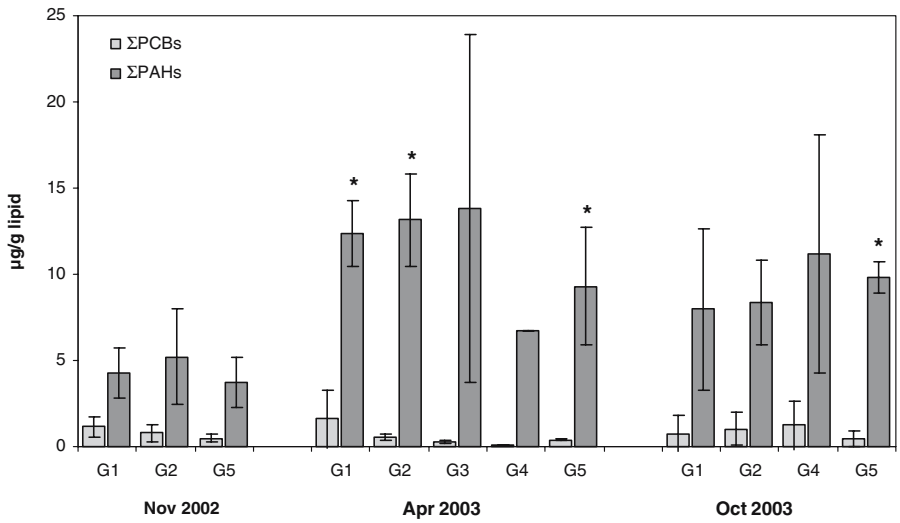

Figure 3 Concentration of the sum of PCBs and PAHs (µg/g lipid) in mussels deployed at the sampling sites during November 2002, April 2003 and October 2003. Bars represent standard deviations. * indicates significant differences at the sampling sites ($p < 0.05$) with respect to levels of pollutants in November 2002.

3.2 Organotin compounds

Temporal trends were detected in the levels of organotin compounds in mussels over the period of exposure, as well as differences among compounds (Figure 2, Table I). MBT levels were below the detection limit (0.005 µg/g dw) in November 2002, increasing significantly in April 2003 and October

Table II Concentration of PCBs (µg/g lipid) (±standard deviations) in the soft tissues of *Mytilus edulis* mussels caged during 30 days at the sampling sites of the Göta Älv Estuary

		PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180
Nov 2002	G1	nd	0.06 ±0.063	0.18 ±0.080	0.16 ±0.077	0.30 ±0.125	0.39 ±0.169	0.047 ±0.0818
	G2	nd	0.026 ±0.0368	0.12 ±0.060	0.066 ±0.0683	0.25 ±0.157	0.28 ±0.161	0.028 ±0.0312
	G5	nd	nd	0.074 ±0.0421	0.066 ±0.0390	0.15 ±0.057	0.19 ±0.120	0.0046 ±0.00790
Apr 2003	G1	nd	0.83 ±1.434	0.16 ±0.061	0.12 ±0.049	0.19 ±0.065	0.24 ±0.072	0.069 ±0.0279
	G2	nd	0.036 ±0.0629	0.11 ±0.041	0.056 ±0.0525	0.14 ±0.047	0.15 ±0.101	0.05 ±0.0481
	G3	nd	nd	0.058 ±0.0117	0.018 ±0.0309	0.076 ±0.0119	0.085 ±0.0014	0.029 ±0.0495
	G4	nd	nd	nd	nd	0.059 nc	0.073 nc	nd
	G5	nd	0.024 ±0.0418	0.045 ±0.0448	0.016 ±0.0279	0.079 ±0.0454	0.083 ±0.0244	0.15 ±0.150
Oct 2003	G1	nd	0.11 ±0.15	0.12 ±0.16	0.091 ±0.129	0.17 ±0.24	0.26 ±0.36	nd
	G2	nd	0.18 ±0.15	0.097 ±0.167	0.13 ±0.117	0.29 ±0.289	0.34 ±0.30	nd
	G4	nd	0.13 ±0.22	0.16 ±0.27	0.12 ±0.21	0.38 ±0.365	0.45 ±0.42	nd
	G5	nd	0.076 ±0.132	nd	nd	0.17 ±0.15	0.21 ±0.19	nd

nd. Below detection limits. nc. Standard deviation not calculated.

Table III Concentration of PAHs (μg/g lipid) (±standard deviations) in the soft tissues of *Mytilus edulis* mussels caged during 30 days at the sampling sites of the Göta Älv Estuary

		A	ANA	Fl	P	ANTH	Fluo	Py	BaA	Chry/Triph	BbF	BkF	BaP	IP	BPer	DahA
Nov 2002	G1	nd	nd	0.49 ±0.164	0.62 ±0.168	nd	0.97 ±0.372	0.86 ±0.311	0.25 ±0.129	0.73 ±0.273	0.36 ±0.141	nd	nd	nd	nd	nd
	G2	nd	nd	1.2 ±0.62	0.93 ±0.412	nd	1.1 ±0.53	0.69 ±0.374	0.22 ±0.175	0.72 ±0.407	0.33 ±0.283	nd	nd	nd	0.11 ±0.074	nd
	G5	nd	nd	1.1 ±0.59	0.61 ±0.247	nd	0.52 ±0.196	0.27 ±0.162	0.15 ±0.107	0.47 ±0.216	0.31 ±0.212	0.09 ±0.060	nd	0.076 ±0.0359	0.11 ±0.094	nd
Apr 2003	G1	0.24 ±0.130	0.64 ±0.332	3.8 ±0.81	2.4 ±0.85	0.24 ±0.154	2.1 ±0.47	0.74 ±0.217	0.34 ±0.031	1.2 ±0.23	0.45 ±0.106	0.13 ±0.021	0.0093 ±0.01611	0.068 ±0.0129	0.077 ±0.0205	0.021 ±0.0362
	G2	0.17 ±0.052	0.53 ±0.154	3.8 ±0.13	2.9 ±0.99	0.17 ±0.043	2.6 ±0.87	0.83 ±0.268	0.26 ±0.031	1.3 ±0.31	0.39 ±0.026	0.076 ±0.0682	nd	0.046 ±0.0426	0.042 ±0.0368	nd
	G3	0.27 ±0.291	4.3 ±6.78	2.3 ±0.36	3.1 ±1.98	0.18 ±0.182	1.7 ±0.50	0.49 ±0.379	0.22 ±0.058	0.88 ±0.069	0.28 ±0.022	0.049 ±0.044	0.027 ±0.0474	0.015 ±0.0268	0.019 ±0.0329	nd
	G4	0.065 nc	0.41 nc	2.1 nc	1.5 nc	0.12 nc	1.1 nc	0.26 nc	0.17 nc	0.78 nc	0.21 nc	nd	nd	nd	nd	nd
	G5	0.11 ±0.051	0.72 ±0.317	3.6 ±2.31	1.8 ±0.27	0.12 ±0.084	1.4 ±0.21	0.36 ±0.076	0.15 ±0.041	0.73 ±0.241	0.22 ±0.077	0.068 ±0.0238	nd	0.016 ±0.0279	0.033 ±0.0298	nd
Oct 2003	G1	0.1 ±0.141	0.059 ±0.083	2.7 ±1.09	0.79 ±0.369	0.67 ±0.214	0.57 ±0.437	0.72 ±0.481	0.11 ±0.149	0.95 ±0.906	0.44 ±0.624	0.2 ±0.282	0.64 ±0.074	nd	nd	nd
	G2	0.037 ±0.0638	0.074 ±0.067	3.1 ±0.75	0.77 ±0.209	0.54 ±0.048	0.63 ±0.324	0.77 ±0.481	0.14 ±0.119	0.81 ±0.317	0.57 ±0.515	0.2 ±0.171	0.57 ±0.060	0.047 ±0.0821	0.053 ±0.0911	0.019 ±0.0334
	G4	nd	0.28 ±0.328	4.2 ±1.72	1.1 ±0.54	0.88 ±0.737	0.97 ±0.905	1.2 ±1.46	0.13 ±0.231	0.9 ±0.703	0.8 ±0.43	0.11 ±0.189	0.6 ±0.37	nd	nd	nd
	G5	0.05 ±0.087	nd	4.2 ±0.14	0.81 ±0.209	0.65 ±0.229	0.64 ±0.143	0.68 ±0.152	0.31 ±0.191	0.84 ±0.116	0.74 ±0.091	0.18 ±0.158	0.69 ±0.099	nd	nd	nd

A, acenaphthylene; ANA, acenaphthene; ANTH, anthracene; Fl, fluorene; P, phenanthrene; Fluo, fluoranthene; Py, pyrene; BaA, benzo-[a]-anthracene; Chry/Triph, chrysene + triphenylene; BbF, benzo-[b]-fluoranthene; BkF, benzo-[k]-fluoranthene; BaP, benzo-[a]-pyrene; IP, indeno-[1,2,3-cd]-pyrene; BPer, benzo-[ghi]-perylene; DahA, dibenzo-[a,h]-anthracene. nd. Below detection limits. nc. Standard deviation not calculated.

Table IV Lipid content (%) in the soft tissues of *Mytilus edulis* mussels caged during 30 days at the sampling sites of the Göta Älv Estuary

		Lipid content (percent)
Nov 2002	G1	1.63±0.513
	G2	1.43±0.289
	G5	1.77±0.115
Apr 2003	G1	3.40±0.794
	G2	2.37±0.153
	G3	2.43±0.351
	G4	2.20 (nc)
	G5	2.90±0.100
Oct 2003	G1	1.55±0.212
	G2	1.50±0.361
	G4	1.23±0.153
	G5	1.57±0.153

Standard deviations are given in brackets.

nc. Standard deviation not calculated.

2003 at all the sites. A similar pattern was observed for DBT. In November 2002 only G1 showed detectable DBT values in mussels, increasing in April 2003 at all sites, and reaching considerably high values in October 2003, particularly at G1. A high increase was also found for TBT concentrations at all experimental sites. TBT levels in November 2002 were similar at all sites; however, higher levels were found in mussels at G1 and G2 compared to the other sites in April 2003 and in October 2003. All the other organotin compounds were below the detection limits except for MPT, DPT and TPT in October 2003.

3.3 PCBs

The concentrations of total PCBs in caged mussels ranged from 0.13 to 1.60 μg/g (on a lipid basis) during the study period (Figure 3). The levels of PCBs did not show significant variations except for congener #180 which concentration decreased in October 2003 below the detection limit (Table II).

3.4 PAHs

The lowest average concentrations of ΣPAHs in mussels were detected in November 2002 and ranged

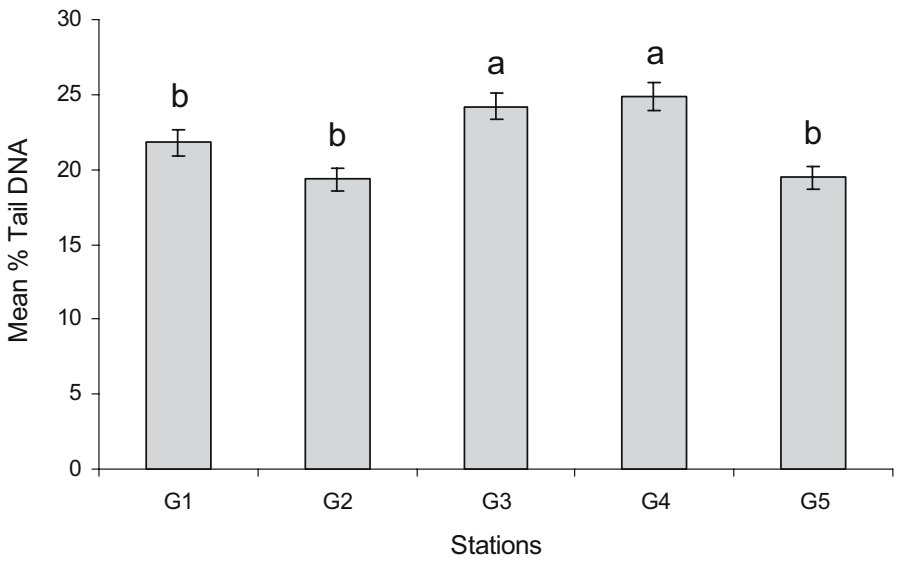

Figure 4 DNA single-strand breaks (mean % Tail DNA±S.E.) in hemocytes of mussels (*Mytilus edulis*) deployed at different sites in Göta Älv estuary. Bars represent standard deviations. Bars with the same letter are not significantly different ($p<0.05$).

from 3.7 to 5.2 μg/g (on a lipid basis). Those levels increased in April 2003 (7–14 μg/g) and in October 2003 (8–11 μg/g) for all sampling sites (Figure 3, Table III). The patterns differed for the different PAHs in mussels, thus, in April 2003 an increase in the levels of low molecular PAHs was recorded whereas in October 2003 the levels decreased, with the exception of fluorene and anthracene.

Since large variations were found in lipid content in mussels (Table IV), PCBs and PAHs concentrations were normalised to lipid.

3.5 Comet assay

The average percentage of tail DNA recorded for the hemocytes showed significant differences among sites (Figure 4). DNA tail values for cells from mussels transplanted at G3 and G4, were significantly higher than those of mussels deployed at G1, G2 and G5.

3.6 Multidimensional scaling

Figure 5 shows the bidimensional configuration of the sampling sites according to multidimensional scaling (MDS) and classification into groups by using cluster analysis, based on pollutant levels in mussels. The MDS configuration (Figure 5; stress=0.012; n=35) discriminated between a group which contained all sampling sites in October 2003 together with G1 and G2 in April 2003; a second group formed with sites G3, G4 and G5 for April 2003, and a third group included sites G1, G2 and G5 for November 2002. This ordination is consistent with the dredging activity pattern, which started in January 2003 and

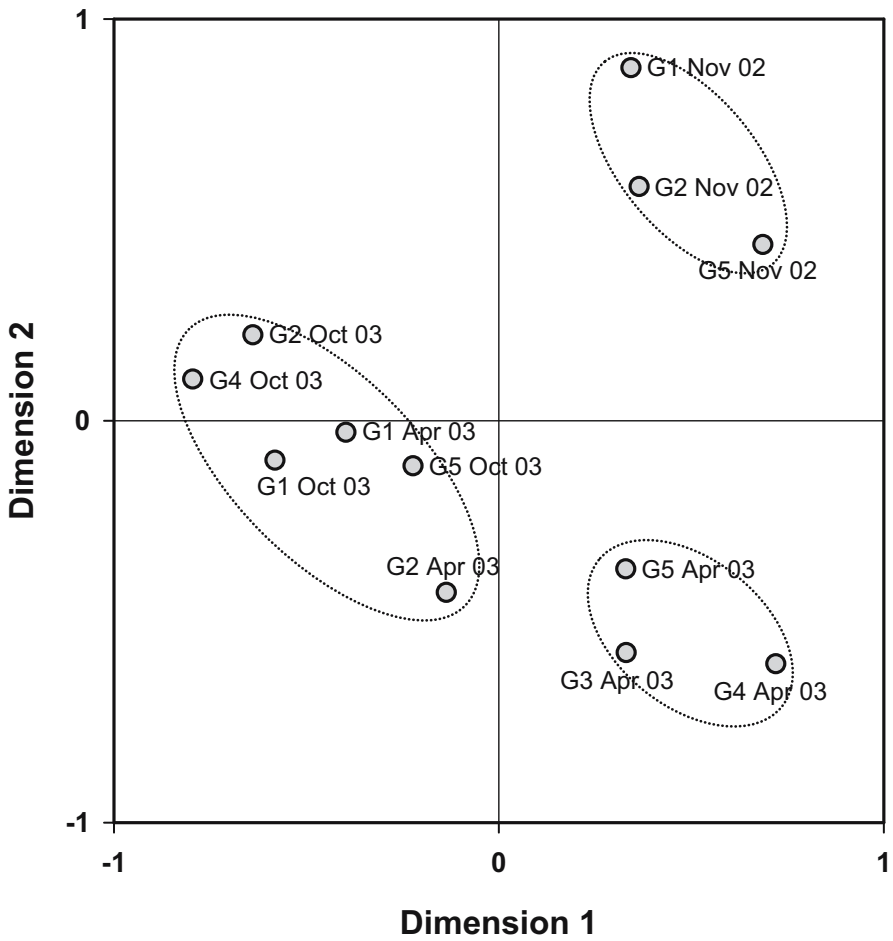

Figure 5 Bidimensional configurations of the sampling sites obtained by multidimensional scaling of pollutant contents in mussels. Groups from the hierarchical cluster analysis are circled on the plot.

was more intense in August–October 2003. Despite temporal variability, sites from different sampling occasions were separated. Moreover, sites G1 and G2 in April 2003 which presented high levels of pollutants in mussel tissues were plotted together with sites in October 2003.

3.7 Correlation between comet assay and chemical results

Although Comet assay results correlated negatively with concentrations of pollutants in mussels (except for acenaphthene, PCB52 and cadmium), significant inverse correlations were only found between Comet assay results and fluorene levels in mussel tissues ($r^2=-0.903$; $p<0.05$; $n=5$). Besides fluorene, the strongest negative correlations were detected between DNA strand breaks and DBT ($r^2=-0.732$), PCB180 ($r^2=-0.684$), copper ($r^2=-0.606$), and fluoranthene ($r^2=-0.538$).

4 Discussion

Most trace metals and organic pollutants such as PAHs and PCBs tend to be distributed at higher concentrations in the particulate phase of sediments (Cheung, Zheng, Lam, & Richardson, 2002; Van den Berg, Meijers, Van der Heijdt, & Zwolsman, 2001). However, mixing of sedimentary matter with the water column may result in an increase of pollutant levels in water during dredging episodes (Goosens & Zwolsman, 1996; Voie, Johnsen, & Rossland, 2002). Thus, the effects of oxidation of reduced sediment phases on mobilization and bioavailability of trace metals have been indicated in several laboratory and field studies (Petersen, Willer, & Williamowski, 1997; Simpson, Apte, & Bately, 1998; Tramontano & Bohlen,1984; Van den Berg et al., 2001). After dredging operations mussels may accumulate pollutants both directly from the dissolved phase, as a result of the equilibrium partitioning process, and also from ingested food particles (Axelman, Næs, Näf, & Broman, 1999). Furthermore, we have to bear in mind that the bioavailability is different according to the speciation of the pollutants (Campbell & Tessier, 1996; Landrum, Harkey, & Kukkonen, 1996). In general, results from the present study only show substantial differences after the dredging episode in organotin concentrations accumulated in mussel tissues. PAHs concentrations showed also a significant increase, but PCBs did not increase in transplanted mussels after dredging.

Regarding metals, little differences were detected during the study period. Data presented here indicate that the dredging operation in Göteborg Harbour may have caused some mobilization of the bioavailable fraction mercury, increasing the levels in mussels after dredging. A slight increase of copper levels in mussels deployed close to the dredging area (G1, G2) was also detected. However, those levels were reduced in October 2003 probably due to removal of the metal polluted material. Moreover, the use of *Mytilus edulis* has been questioned for the study of copper and zinc pollution, because this organism is able to regulate these essential elements at moderately low concentrations, although they cannot be regulated at highly polluted areas where concentrations can increase to 400 μg/g dw (Lorenzo, Aierbe, Mubiana, Blust, & Beiras, 2003; Radenac, Miramand, & Tardy, 1997). In general terms, metal contents in mussels sampled in the present study are close to background levels along the Skagerrak (Cato, 2000; Green & Knutzen, 2003; SMHI, 2001).

TBT levels in mussels exceeded (5–70 times) the Ecotoxicological Assessment Criteria of 0.01 μg/g dw proposed for mussel tissues by OSPAR Commission (2000). For instance, TBT concentrations of 0.2–10 μg/g dw have been reported to uncouple the oxidative phosphorilation in *M. edulis* (Widdows et al., 2002). Also, Page, Dassanayake, and Gilfillan (1996) reported a positive correlation between shell thickening and TBT levels in *M. edulis* tissues. An increase in the shell thickening index was reported at TBT tissue levels similar to the highest concentrations reported here. In contrast, concentrations reported here are below the ca. 2 μg/g dw threshold effect level affecting scope for growth in adult mussels (Page & Widdows, 1991), and below the concentrations reducing growth in field transplanted mussels (4 μg/g dw, M. H. Salazar & S. M. Salazar, 1998), or inducing inhibitory effects on the feeding rate (>4 μg/g dw, Widdows et al., 2002).

Henriksen et al. (2001) found levels of TBT in *M. edulis* between 200–300 ng/g dw in coastal areas of the strait between Denmark and Sweden which is comparable with levels in molluscs from other areas with intense shipping traffic (Strand, Jacobsen,

Pedersen, & Granmo, 2003). Those values are similar to our data for April 2003 in G1 and G2, whilst values obtained in October were 2–4 times higher. Although in some shallow Danish fjords the TBT concentration in mussels can exceed 3,000 ng/g dw (Henriksen et al., 2001). Besides the rise of the TBT level in mussels after the start of dredging, a lowered TBT/DBT ratio in the bivalves indicates a release of organotins from the sediment. Thus, TBT/DBT and TBT/MBT ratios from the present study were higher in November 2002 (>15) than in April (6–9, 21–35) and in October 2003 (3, 8–14) (Table I). Moreover, the ratios in October 2003 were similar to the ratios found for sediments during the whole period (1–3, 1–9, I. Cato, unpublished data), where degradation is the main cause of lower TBT/DBT and TBT/MBT ratios (Brack, 2002). Although a reduction of TBT concentrations in water and mussels has been registered worldwide since 1989, levels of TBT in sediments did not decline due to its refractivity (Dowson et al., 1993; Harino, Fukushima, & Kawai, 1999). Therefore, resuspension of organotins by turbulence or dredging episodes can mobilize TBT accumulated in the sediments which can be problematic at highly polluted localities. Butyltin compounds including TBT and its breakdown products, DBT and MBT, were detected in all the bivalve samples analyzed in the present study. Moreover, a remarkable increase in organotin concentrations in caged mussels was observed after the dredging started, particularly at stations G1 and G2. Calculated average water concentrations derived from levels in mussels were found to be in the range 2–15 ng/l. Those concentrations are high enough to cause shell malformations in bivalves or imposex in gastropods (Bryan & Gibbs, 1991). Thus, the occurrence of imposex in sea snails (*Nassarius reticulatus*) has been found to be widespread in the Göta Älv Estuary (Å. Granmo, unpublished results). Therefore, measured levels of butyltins in mussel tissues reported in the present work indicate that the dredging operations might have contributed to mobilize organotins from sediments at high enough concentrations to pose a threat to the environment.

PCBs concentrations reported here ranged from 0.13 to 1.6 μg/g on a lipid basis (3–52 ng/g dw) which is very similar to the values found by Cato (2000) in the same area between 1992 and 1997, and also to the levels registered by Henriksen et al. (2001) in the strait between Denmark and Sweden. The values found in the present study are below the reference levels suggested by Green and Knutzen (2003) for mussels (after conversion to dry weight units) except for G1 in November 2002 and April 2003, and G2 in October 2003, which are above those levels. The profile of contribution for individual congeners to Σ_7PCB in mussels (with a higher contribution of congeners #153 and #138) is comparable to previous reports (Green & Knutzen, 2003). This finding broadly agrees with the contribution of the individual congeners to Σ_7PCB in sediments from the same area (I. Cato, unpublished results) and it may suggest mobilization of PCBs from the sediment. However, taking into account the reduction of the PCB levels found in April 2003, dredging conditions may have reduced the availability of these compounds in the water column by diluting the more contaminated surface sediment particles with clean particles from pre-industrial deep-sediment.

Values of PAHs for *M. edulis* reported here (4–14 μg/g lipid weight, 0.04–0.42 μg/g dry weight) are within the ranges observed in other studies (Axelman et al., 1999; Baumard et al., 1999; Toro, Palma-Fleming, & Navarro, 2004). In a previous work carried out in the Göta Älv Estuary, Cato (2000) reported concentrations of 0.032 μg/g dw for Σ_6PAHs (phenanthrene, anthracene, fluoranthene, pyrene, benzo-(a)-anthracene, chrysene/triphenylene). The levels found in the present study are above these concentrations (with the single exception of G5 in November 2002). The qualitative pattern of distribution of PAHs was similar to those in other mussels (e.g. *M. edulis*, Burns & Smith, 1981; *Arca zebra*, Widdows, Burns, Menon, Page, & Soria, 1990; *Choromytilus chorus*, Toro et al., 2004) which showed a tendency to accumulate PAHs of low molecular weight (di- and tri- aromatics) that predominate in the dissolved forms, rather than high molecular weight PAHs which predominate in the particulate forms. The low proportion of high molecular weight PAHs accumulated by mussels in the present study after the dredging started (particularly in April 2003; 0–10%) despite the high levels in nearby sediments (I. Cato, unpublished results), where penta- and hexa aromatics were 30–40% of the total PAHs, would indicate that these compounds are not readily bioavailable as has been observed in previous studies (Farrington et al., 1983; Widdows et al., 1990), and therefore it may indicate that mussels appear to absorb PAHs mainly

through the gills. The same conclusion as drawn about PCBs, i.e. lowered availability by dilution with clean deep sediment particles may also be valid for the heavier PAHs. However, an overall increase of ΣPAHs was observed during the studied period.

Multidimensional scaling (MDS) and cluster analyses are employed to explore multivariate set of data, and obtain a global vision of the results in order to identify groups with similarities (Shin & Fong, 1999). In the present study, multivariate analysis performed with data of pollutants in mussels discriminated October 2003 from the other sampling occasions. Thus, the multivariate analyses consistently discriminated sites with the highest contents of pollutants from the remaining, less contaminated locations.

Mussels deployed at those sites located in the vicinity of the dumping area (G3 and G4) showed significantly increased DNA single-strand breaks compared to the other sites ($p<0.05$). Furthermore, sites G1 and G2 (dredging area) did not show elevated DNA damage compared to site G5. Hence, there was not a clear correlation between chemical body burdens in the mussels and DNA single-strand breaks found in their hemocytes. We have found inverse correlations between the Comet assay results and the levels of pollutants in mussels. However, significant correlations were only detected between the DNA strand breaks and the concentration of fluorene. Although this does not necessarily imply that this compound is involved in the toxic effects on the transplanted mussels. Possible explanations for the differences observed between the dredging area and the dumping area may be related to different interactions within, and between the pollutants (complex mixtures) and the mussels. Different conditions in the immediate environment (e.g. food supply), or physiological factors such as hormonal status, that can influence enzymes activity (Mitchelmore & Chipman, 1998), may also be affecting the formation of DNA strand breaks.

The interaction between pollutants and organisms, leading to DNA strand breaks, may be complicated. Genotoxic substances may react directly with DNA, or following biotransformation to reactive oxygen species or other reactive intermediates. Several factors may influence the interaction, such as ambient sunlight, inducing phototoxicity (Mitchelmore, Birmelin, Chipman, & Livingstone, 1998; Steinert, Streib Montee, & Sastre, 1998). For instance, a reduction in the DNA strand breaks in oysters (*Crassostrea virginica*) following field deployment and then allowed to recover in the laboratory for 7–10 days has been reported, indicating the reversibility and non persistence of such damage (Nacci, S. Nelson, W. Nelson, & Jackim, 1992). Thus, due to its reversibility, DNA damage measured by the Comet assay indicates recent pollution episodes.

5 Conclusions

This and other studies showed that organotins are accumulated at moderate to high concentrations in sediments and organisms throughout the Göta Älv Estuary. Moreover, this study only shows substantial differences in organotin concentrations after dredging operations started. Based on the TBT concentrations found in caged mussels as well as on the long half life of TBT in sediments (360–775 days, Dowson et al., 1993), TBT is likely to continue to pose a threat to organisms in the Göta Älv Estuary. Therefore a programme to monitor the levels of organotins periodically should be established. An increase in PAHs concentrations was also detected in mussel tissues after dredging, in contrast with metals and PCBs concentrations.

The Comet assay is a non-specific biomarker of genotoxicity in aquatic species with potential use in environmental monitoring. However, from the data presented here, it seems that more research is needed to validate the Comet assay as an adequate biomarker. Also, there is a need to combine this approach with the use of other biological responses in order to better understand the nature and physiological aspects of the DNA strand breaks and their formation.

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