

Accumulation of polychlorinated biphenyls by the infaunal brittle stars *Amphiura filiformis* and *A. chiajei*: effects of eutrophication and selective feeding

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ABSTRACT: Polychlorinated biphenyl (PCB) accumulation by the 2 brittle stars *Amphiura filiformis* and *A. chiajei* was studied in a laboratory experiment and in the field. In the laboratory study, the fate of ¹⁴C-2,2',4,4'-tetrachlorobiphenyl (TCB) was determined in benthic microcosms, with and without the addition of phytoplankton (*Phaeodactylum tricornutum*). Added phytoplankton was rapidly mineralised and stimulated an increased dissolved organic carbon content in the water-column and bacterial production on the sediment surface. TCB uptake by the brittle stars was significantly higher in the microcosms enriched with phytoplankton. Differences in TCB concentrations were still significant after normalisation to lipid content, suggesting that selective feeding rather than equilibrium partitioning was the cause of the increased TCB burden. Treatment effects were more apparent in body (disk) tissue, than in the arm fraction of the brittle stars, in agreement with the lipid content of the tissues. No difference in total organic carbon, total nitrogen or TCB concentrations of the sediment surface was detected. In the field, ophiuroids and sediment cores were collected at a coastal urban estuary off the city of Göteborg, Sweden, and at an offshore station in the Kattegat Sea. Sum-PCBs of sediment and brittle stars were ca 3 times higher at the coastal station than at the offshore station. Biota sediment accumulation factors, determined from the laboratory and field exposures, ranged from 1.5 to 5.9. The results from this study suggest that eutrophication processes, such as increased phytoplankton production, may contribute to increasing the accumulation of organic pollutants in benthic sediment-ingesting fauna. The significance of *A. filiformis* in the transfer of PCBs to higher trophic levels is also discussed based on data of sublethal predation by the demersal flat fish *Limanda limanda* and from production estimates of an *A. filiformis* population.

KEY WORDS: Bioaccumulation · Sediment accumulation factors · Trophic transfer · Microcosms · Kattegat · Skagerrak · Echinodermata · Ophiuroidea

INTRODUCTION

Organic matter plays a fundamental role in controlling the bioavailability and distribution of hydrophobic organic contaminants. Due to their hydrophobic character, toxic hydrophobic organic compounds (HOCs) such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) rapidly sorb to

organic matter in the water-column and sediments (Karickhoff et al. 1979). In the water-column, the binding of hydrophobic contaminants to particulate and dissolved organic matter (POM, DOM) may increase their apparent solubility and water retention (Chiou et al. 1987), or on the contrary enhance the sedimentation rate of particle-sorbed contaminants (Millard et al. 1993). Several studies have shown that the bioavailability of organic compounds via aqueous and pore water exposure can be decreased in the presence of DOM (Carlberg et al. 1986, Kukkonen et al. 1991). Likewise, both bioaccumulation and toxicity of sedi-

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ment-bound contaminants were reported to be inversely proportional to the total organic carbon (TOC) content of the sediment (Rubinstein et al. 1983, Nebeker et al. 1989). Inverse relationships between bioavailability and DOM or TOC content are explained by the assumption that contaminants bound to DOM are unavailable for uptake, because the contaminant-DOM complex is too large to pass through biomembranes, and its dissociation too slow to compete with the freely dissolved contaminant fraction (Landrum et al. 1996). This applies to organisms for which respiration in the water-column or interstitial water is the main exposure route (Adams 1987). Sediment-ingesting organisms, however, may show a different response, and on the contrary increase their bioaccumulation of HOCs by selective feeding on TOC-rich contaminated particles (Boese et al. 1990, Gunnarsson et al. 1996). By providing more energy to the infaunal organisms, an organic matter supply may also contribute to increase lipid reserves and/or increase metabolic rates which, in turn, may affect the bioaccumulation and toxicity of contaminants (Niimi & Cho 1981).

Phytoplankton constitutes an important reservoir for organic contaminants (Stange & Swackhamer 1994). Contaminants accumulated in phytoplankton will either be grazed upon and transported into other compartments of the pelagic food chain or carried to the sediment by dead cells and sinking aggregates (Swackhamer & Skoglund 1993). During the last decades, the Baltic Sea, the Kattegat and the southern Skagerrak have experienced an increased anthropogenic input of toxic contaminants and nutrients (Richardson & Heilmann 1995, Rosenberg et al. 1996). Reports of high concentrations of organic contaminants in sediment and biota have been presented concomitantly with studies revealing dramatic effects caused by eutrophication processes (Baden et al. 1990, Magnusson et al. 1996). Since the distribution and bioavailability of organic contaminants are likely to be coupled to the production and turnover of organic matter, it is essential to evaluate how the cycling of contaminants in an aquatic system may be affected by changes in the trophic status of that system (Gunnarsson et al. 1995).

Eutrophication of the Kattegat and Skagerrak may have contributed to increase the abundance and biomass of benthic macrofaunal species in areas where the water mixing is high enough to supply oxygen renewal (Pearson et al. 1985). The dominant infaunal brittle star *Amphiura filiformis* is one of the species which seems to have increased in number and biomass following the increased food supply (Josefson et al. 1993). *A. filiformis* dominates the bottom of vast areas of the Kattegat, Skagerrak and North Sea, with densities from about 250 to 3900 ind. m⁻² (Duineveld et al. 1987). *A. chiajei* is another infaunal brittle star com-

monly found in *Amphiura* communities (Petersen 1915). *A. filiformis* can switch from suspension to deposit feeding, while *A. chiajei* is a strict deposit feeder (Buchanan 1964). These species also provide an important food source for many fish and invertebrate predators, including dab (*Limanda limanda*: Duineveld & Noort 1986), haddock (*Melanogrammus aeglefinus*: Mattson 1992) and Norway lobster (*Nephrops norvegicus*: Baden et al. 1990). These predators do not generally consume the entire brittle star but crop only the arms, which are later regenerated (Sköld et al. 1994). Accumulation of PCBs by infaunal organisms has been shown to be the first step in their transfer to higher trophic levels, including human consumers (Thomann et al. 1986). Loizeau & Menesguen (1993) showed that 8 to 15% of the PCB burden in dab from the Bay of Seine could be explained by ophiuroid consumption. *Amphiura* communities may therefore play an important role in the accumulation, remobilisation and transfer of PCBs and other sediment-associated contaminants to higher trophic levels.

The objectives of the present study were: (1) to observe the effects of phytoplankton addition, simulated in benthic microcosms, on the distribution and bioaccumulation of PCB by *Amphiura filiformis* and *A. chiajei*, (2) to determine *in situ* PCB concentrations of *A. filiformis* and *A. chiajei* from the Kattegat Sea, (3) to determine accumulation factors in the field and in laboratory exposure, and (4) to discuss the importance of predation on *Amphiura* communities for the transfer of PCBs to higher trophic levels.

MATERIAL AND METHODS

Experimental system and contamination. The experiment was run in six 15 l (22 × 29 × 24 cm) whole-glass aquaria (microcosms), for 78 d at the Marine Research Station Solbergstrand (MRSS), Norwegian Institute for Water Research (NIVA). Each microcosm (Fig. 1) received 4.8 l homogenized clay-sediment from the Oslofjord (60 m, total carbon [TC] = 13.5 mg g⁻¹ dry weight [DW], total nitrogen [TN] = 1.2 mg g⁻¹ DW, mean grain size ϕ = 4.80), giving a sediment bottom layer of 8 cm depth, covered by a water-column of 15 cm. The microcosms were sealed with glass lids randomly perforated for sampling, and plugged with silicone corks. Water mixing was enabled by the action of a magnetic stirrer placed upside down on the lid of each aquarium and a Teflon coated magnet-bar on the inside. Temperature was maintained between 6.5 and 8.5°C by placing the microcosms in a water-bath continuously circulated with natural seawater (from 40 m depth). During an initial 'contamination phase' of 8 d all the microcosms were continuously supplied

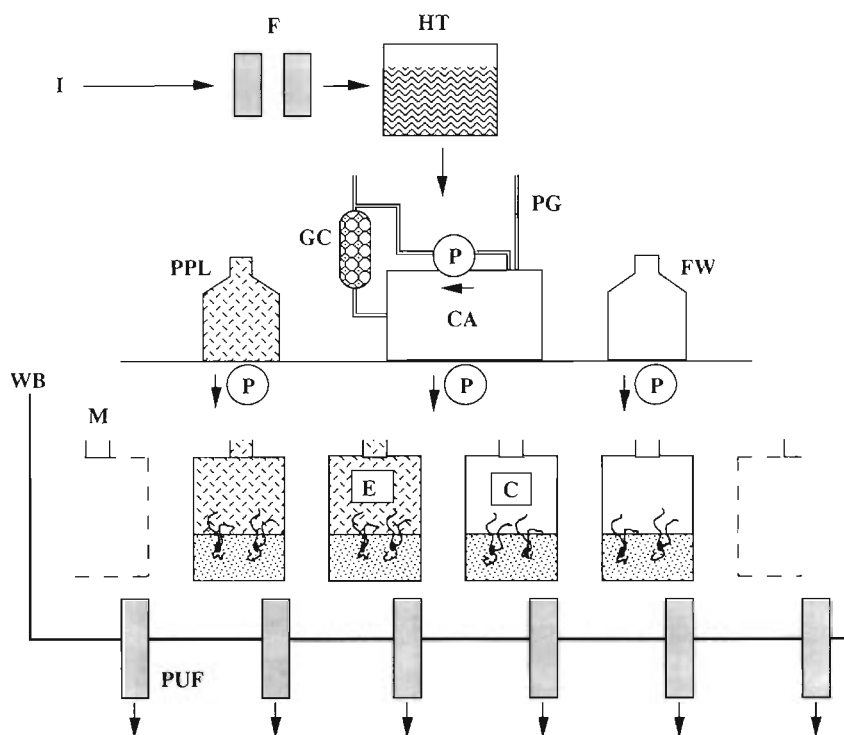


Fig. 1. Experimental system (I: Incoming deep [40 m] seawater; F: filters; HT: head tank; P: peristaltic pump; GC: generator-column charged with ^{14}C -TCB; PG: pressure gauge in equilibrium with the water level of the head tank; CA: contamination aquarium with ^{14}C -TCB-saturated seawater; PPL: batch with phytoplankton [*Phaeodactylum tricornutum*]; FW: batch with filtered seawater; WB: water bath with deep seawater maintaining the microcosms at constant temperature; M: benthic microcosms with sediment and ophiuroids; E: enriched, i.e. with algal addition; C: controls, i.e. without algal addition; PUF: polyurethane filters

with tetrachlorobiphenyl (TCB)-contaminated seawater. During this initial contamination phase half of the microcosms ('eutrophic' or 'enriched') were also continuously supplied with a phytoplankton culture, the other half ('oligotrophic' or 'controls') receiving an equivalent volume of filtered seawater ($0.45\ \mu\text{m}$). TCB-contaminated water and phytoplankton were added to the microcosms through separate inlets of the glass lids. The phytoplankton addition was constituted of 8 l of a chemostat-culture of the marine diatom *Phaeodactylum tricornutum*, containing $41.9\ \text{mg l}^{-1}$ TOC and $8.1\ \text{mg l}^{-1}$ DOC, and corresponding to a C enrichment of $5.6\ \text{g C m}^{-2}$. TCB contamination was done using radiolabelled [$\text{U-}^{14}\text{C}$]-2,2',4,4'-TCB, IUPAC 47, with a specific activity of $13.8\ \text{Ci mol}^{-1}$ and a purity of $>98\%$ (Sigma Chemicals, St. Louis, MO, USA). Owing to their great stability, and hydrophobic properties, radiolabelled PCB congeners are suitable model contaminants for bioaccumulation studies and for modelling the behaviour of other hydrophobic organic pollutants. In order to avoid the presence of an organic carrier, TCB was added to the water using a generator-column

according to the technique of Veith & Comstock (1975). The column consisted of a 1.0 l glass column, filled with 500 mg of glass beads coated with ^{14}C -TCB. Coating was done by mixing the beads with 500 ml of hexane, containing 2.5 mg ^{14}C -TCB ($120\ \mu\text{Ci}$), and evaporating the solvent in a rotary evaporator. The column was connected to a 20 l glass aquarium ('contamination aquarium') mixed by a magnetic stirrer. Filtered seawater ($0.45\ \mu\text{m}$) was recirculated through the generator-column with a peristaltic pump, and TCB concentration of the water was adjusted by regulating the flow rate through the column. Before the experiment, water was circulated through the column for 7 d to allow glass surfaces and water to be saturated with eluted ^{14}C -TCB. At the start of the experiment, TCB concentration of the contamination aquarium was $15.1\ \text{ng TCB ml}^{-1}$. The TCB-contaminated water was dosed to the microcosms using a peristaltic pump and glass tubings. The water volume of the contamination aquarium was maintained constant by the refilling action of a header tank, supplied with serially filtered ($50\ \mu\text{m} + 20\ \mu\text{m}$) natural

seawater (from 40 m depth, 34‰). During the contamination phase, the generator-column maintained a fairly constant elution capacity until the last day of exposure when TCB concentration of the contamination aquarium declined, due to a saturation of the column with organic matter and bacteria of the incoming seawater. An elution capacity of 55% was obtained, corresponding to a total addition of $0.23\ \text{mg }^{14}\text{C}$ -TCB microcosm^{-1} during the contamination phase. Following this initial contamination phase of 8 d, the microcosms were left to equilibrate in a recirculating mode for 38 d before the brittle stars were introduced in the microcosms. A more complete description of the experimental system and the observed partitioning of ^{14}C -TCB between the different pelagic fractions, i.e. TCB associated with particles and phytoplankton (TCB_{PART}), associated with DOC (TCB_{DOC}), or truly dissolved (TCB_{DISS}), and the sediment during the initial phase and the equilibration phase of the experiment is given in Gunnarsson & Rosenberg (1996). The present paper focuses on the accumulation of TCB by the brittle stars.

Exposure of brittle stars. After 38 d of equilibration in a recirculating mode, O_2 concentration was increased from hypoxic ($2.05 \text{ mg } O_2 \text{ l}^{-1}$) to normoxic ($6.86 \text{ mg } O_2 \text{ l}^{-1}$) conditions by establishing a flow-through circulation with natural (from 40 m depth), unfiltered seawater. A water flow of 6.4 ml min^{-1} , giving a residence time of $24 \text{ h microcosm}^{-1}$, was used. The brittle stars *Amphiura filiformis* and *A. chiajei* (Ophiuroidea: Echinodermata) were sampled at the mouth of the Gullmarsfjord, Sweden, from 35 m depth with a box corer (0.09 m^2). The brittle stars were removed cautiously from the sediment to prevent arm amputations. Specimens were acclimated for 3 d in the laboratory before initiating exposure. Eight *A. filiformis* and 7 *A. chiajei* were added per microcosm, corresponding to a density of 133 and 116 ind. m^{-2} , respectively, and exposed to the contaminated sediments for 31 d. Nine sediment cores (height: 1 cm, diameter: 4 mm) were sampled per microcosm at the start and end of the exposure to determine TOC, TC, TN content and TCB concentrations of the sediment. Total TCB concentration of the water-column and release of TCB from the sediment to the water-column were measured by continuously filtering outflowing water from each microcosm through polyurethane filters (PUF). Six foam plugs, each with a height of 5 cm and a diameter of 5.5 cm, were placed in series per filtration column. Activity of infaunal organisms is difficult to observe since they are buried into the sediment. Infaunal brittle stars feed by collecting food particles with 1 to 3 arms protruding from the sediment (Buchanan 1964). Daily counting of visible arms was used as a measure of brittle star activity. Temperature (7.4 to 9.2°C) and salinity (ca 34‰) of the incoming water were registered continuously during the experiment with a data logger. Oxygen concentration of the in- and outflowing water of the microcosms was recorded daily with an oxygen meter. The amount of resuspended particles due to animal activity and to the mechanical mixing was measured by collecting 1 l water samples of outflowing water at 4 different occasions during the experiment. Total particulate matter of the water-column (expressed as g DW l^{-1} and g AFDW l^{-1} [ash-free dry weight]) was determined by filtration on pre-combusted GF/F filters (Whatman). At the end of the experiment, the ophiuroids were recovered from the microcosms by wet-sieving the sediment, rinsed cautiously with filtered ($0.45 \mu\text{m}$) seawater, and frozen (-20°C) in a scintillation vial.

Chemical analyses. TC, TOC and TN content of freeze-dried sediments and ophiuroids was measured with a Carlo Erba analyser. TOC was determined after treating the samples with HCl to remove carbonates

according to Hedges & Stern (1983). DOC content of the plankton culture was analysed on a Dohrman DC-190 C analyser on filtered ($0.45 \mu\text{m}$) samples treated with H_3PO_4 . Radioactivity was determined by liquid scintillation counting on a Beckman LS 5000 TD, with correction made for quenching and background. Duplicate (5 ml) samples were counted for 10 min, and chemoluminescence was kept under 5%. The detection limit was 0.61 ng TCB . Extraction of TCB in the sediment, water and PUFs was done by solvent extraction (acetone/hexane, 1:1), using soxhlet extraction and shaking according to Gunnarsson & Rosenberg (1996). TCB concentrations of pore water samples (TCB_{PW}) were below detection limits and instead estimated from the equation: $\text{TCB}_{\text{PW}} = \text{TCB}_{\text{sed}} / (f_{\text{OC}} K_{\text{OC}})$, where TCB_{sed} is the TCB fraction associated with sediment particles ($\mu\text{g kg}^{-1}$), f_{OC} the organic C fraction of the sediment ($\text{kg orgC kg}^{-1} \text{ DW sed.}$) and K_{OC} the organic carbon/water partition coefficient (Di Toro et al. 1991). $\log K_{\text{OC}} = 4.88$ was determined from the equation: $\log K_{\text{OC}} = 0.007 + 0.834 \log K_{\text{OW}}$ (Chou & Griffin 1986), using $\log K_{\text{OW}}(\text{PCB-47}) = 5.85$, where K_{OW} is the octanol/water partition coefficient (Hawker & Connell 1988). Frozen ophiuroids were carefully dissected in order to separate body (disk) from arms. TCB extraction from the tissues (arms and disks) was done with microwave-assisted extraction in 30 ml acetone/hexane (1:1) (pressure: 100 PSI, power: 100%, temp.: 120°C , ramp time: 8 min, extraction time: 20 min) with an extraction efficiency >90%. Duplicate 5 ml samples of the extract were mixed with 10 ml scintillation cocktail Ultima gold (Packard) and counted for 10 min. Radioisotope activity was expressed on a tissue dry weight basis ($\text{dpm g}^{-1} \text{ DW}$), normalised to lipid, TOC or TN content, and converted into micrograms of TCB using the specific activity of the compound ($1 \text{ dpm} = 9.45 \times 10^{-3} \text{ ng TCB}$). Small sub-samples (2 to 5 mg) of disk and arm tissues were extracted with chloroform/methanol (2:1), and total lipid content was measured according to the micro-gravimetric technique of Gardner et al. (1985).

PCB analyses of *Amphiura filiformis* and *A. chiajei* from the field. Ophiuroids and sediment samples were collected from 2 sites of the Kattegat Sea in order to get *in situ* PCB contamination levels. A coastal station ('BC 64': $57^\circ 40.31' \text{N}$, $11^\circ 41.39' \text{E}$; 21 m) in the Göteborg estuary was chosen since previous sediment toxicity and high contaminant concentrations had been reported from this site (Dave & Dennegård 1994, Magnusson et al. 1996). An offshore station ('BC 69': $57^\circ 51.11' \text{N}$, $11^\circ 18.06' \text{E}$; 70 m) on the slope of a deep trench was chosen as a high biomass of both species of ophiuroids had been reported from it (Rosenberg 1995). Five sediment cores from each station were collected. Overlying water was carefully removed, and the upper 2 cm were scraped off into

acetone-washed glass vials. Three of the sediment samples were pooled for contaminant analyses and the remaining 2 used for TC, TOC, TN and granulometry analyses. Total PCBs and 7 specific congeners of the sediment were analysed and quantified by the Swedish Environmental Research Institute (IVL) using electron-capture gas chromatography (GC-ECD) according to Brorström-Lundén & Mowrer (1992). Three box core samples (0.09 m²) were sieved (1 mm mesh size) and preserved in 70% ethanol to determine macrofaunal abundance and biomass, according to Rosenberg (1995). PCB content of the ophiuroids was measured in 25 *Amphiura filiformis* from the 2 stations and in 25 *A. chiajei* from the offshore station only, since no *A. chiajei* were found at the coastal station. The brittle stars were gently removed from the sediment, rinsed cautiously with filtered (0.45 µm) seawater and frozen (-20°C) in acetone-washed glass vials. Lipid content, total PCB and 31 detectable PCB congeners were measured in triplicate pools of 5 to 10 ind. station⁻¹ by the Environmental Toxicology Laboratory at the Norwegian College of Veterinary Medicine, Oslo, Norway, using GC-ECD according to Bernhoft & Skaare (1994).

Statistics. Data were analysed with factorial analysis of variance (ANOVA). Pooling of nested and treatment terms was done after the preliminary test if the nested factor was not significant ($p > 0.25$), as recommended by Underwood (1997). Data were transformed (root or log) if necessary to meet the assumptions of normality and homogeneity of variances. Homogeneity of variances was evaluated using the Cochran C-test ($\alpha = 0.05$) (Snedecor & Cochran 1989).

Table 1 Total carbon (TC), organic carbon (TOC) and nitrogen (TN) (% of freeze-dried surface sediment wt) (n = 3)

Sampling	Treatment	TC (SD)	TOC (SD)	TN (SD)
Start (Day 46)	Control	1.33 (0.02)	1.27 (0.01)	0.09 (0.01)
	Enriched	1.26 (0.14)	1.22 (0.14)	0.10 (0.01)
End (Day 78)	Control	1.28 (0.02)	1.25 (0.09)	0.09 (0.01)
	Enriched	1.17 (0.16)	1.07 (0.16)	0.10 (0.01)

RESULTS

Laboratory study

Carbon, nitrogen and TCB distribution in sediment and water

C and N content of the sediment surface, at start and end of the exposure, is presented in Table 1. Neither treatment nor time had any significant effects on C, N and TOC values. TCB concentrations measured in sediment, water and brittle stars are presented in Table 2. No treatment effect, i.e. effect of the phytoplankton addition, was observed on TCB concentrations of the sediment surface (TCB_{sed}) (0 to 1 cm). TCB_{sed} decreased significantly from the start to the end of the experiment ($F_{1,8} = 10.8$, $p = 0.011$), indicating that a loss of TCB from the sediment surface occurred during the exposure. A substantial amount of TCB was recovered in the polyurethane filters, showing that a fraction of the TCB lost from the sediment surface was released into the water-column. Average TCB concentrations of the water-column and desorption fluxes (Table 2) were calculated from the total concentrations of the polyurethane filters divided by flow rate and exposure time.

Table 2. Tetrachlorobiphenyl (TCB) concentrations in sediment, water and *Amphiura* spp. TCB concentration in sediment (0 to 1 cm) at start and end of exposure; TCB concentration in water, collected in PUF filters; mean TCB desorption flux, measured from PUF values; TCB concentration in pore water (Pw), predicted from $\log K_{OC} = 4.88$; TCB concentration in tissue fractions and total concentration per animal

TCB concentrations	Control	(SD)	Enriched	(SD)
[TCB] sed, start (Day 46) (µg kg ⁻¹ DW sed)	61.4	(23.4)	61.3	(20.7)
[TCB] sed, end (Day 78) (µg kg ⁻¹ DW sed)	39.1	(21.0)	26.9	(12.3)
[TCB] water at start (Day 46) (ng l ⁻¹)	10.3	(1.6)	9.7	(2.7)
[TCB] water at end (Day 78) (ng l ⁻¹)	10.9	(1.0)	12.0	(1.3)
TCB desorption (µg desorbed d ⁻¹ m ⁻²)	1.6	(0.1)	1.8	(0.2)
[TCB] Pw, start (Day 46) (ng l ⁻¹)	63.8	—	66.1	—
[TCB] Pw, end (Day 78) (ng l ⁻¹)	41.2	—	33.1	—
[TCB] <i>A. filiformis</i> arms (µg kg ⁻¹ DW arm tissue)	81.6	(15.4)	103.1	(29.1)
[TCB] <i>A. filiformis</i> disk (µg kg ⁻¹ DW disk tissue)	217.4	(71.6)	488.3	(101.9)
[TCB] <i>A. filiformis</i> total (µg kg ⁻¹ DW tissue)	117.1	—	213.0	—
[TCB] <i>A. chiajei</i> arms (µg kg ⁻¹ DW arm tissue)	74.9	(48.9)	68.3	(18.1)
[TCB] <i>A. chiajei</i> disk (µg kg ⁻¹ DW disk tissue)	324.7	(147.5)	488.4	(144.2)
[TCB] <i>A. chiajei</i> total (µg kg ⁻¹ DW tissue)	157.9	—	197.8	—

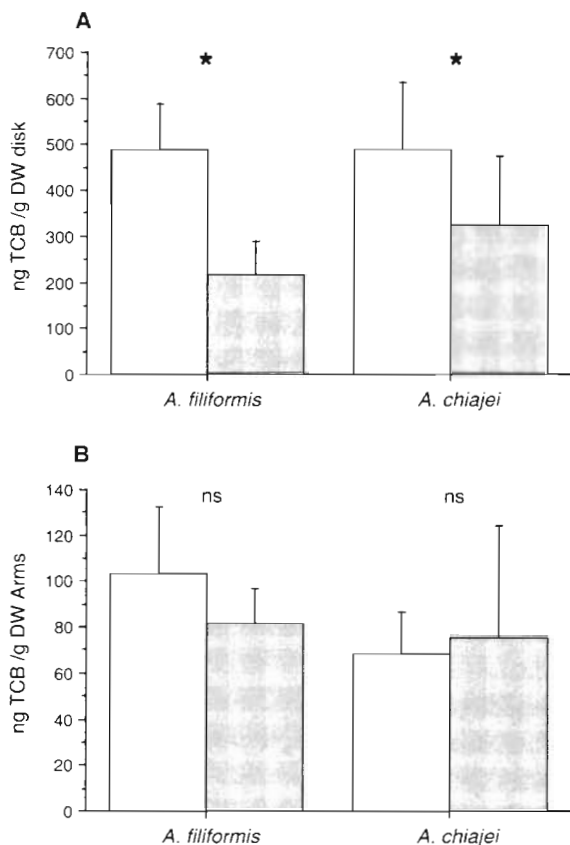


Fig. 2. *Amphiura* spp. TCB accumulation (ng TCB g⁻¹ DW) in (A) disks and (B) arms (open bars: enriched with phytoplankton; filled bars: controls; error bars: standard deviation, *p < 0.05; ns: not significant)

TCB accumulation by brittle stars

TCB accumulation per gram tissue DW by the ophiuroids is presented in Fig. 2. The phytoplankton enrichment induced a significantly higher TCB accumulation in the disks of the 2 species (*Amphiura filiformis*, $F_{1,4} = 288.30$, $p = 0.0001$; *A. chiajei*, $F_{1,4} = 8.12$, $p = 0.04$). No treatment effect was found for the arm fraction (*A. filiformis*, $F_{1,4} = 3.66$, $p = 0.12$; *A. chiajei*, $F_{1,4} = 0.20$, $p = 0.64$). No significant differences between species were found.

DW, lipid, TOC and TN content in disks and arms

Total lipids, TOC and TN content of disk and arms of *Amphiura filiformis* are presented in Table 3. Lipid content of the arms was below detection level. Lipid content of the disks was 2.9% in controls, and 5.0% in enriched aquaria. A treatment effect could, however, not

be verified statistically since the variances of the lipid values within treatments were high and not homogeneous. No significant treatment effect was found for total DW, TN or TOC content of the disk of *A. filiformis*.

Normalisation of accumulated TCB concentrations to percent lipid, TOC and TN

TCB concentrations of *Amphiura filiformis* normalised to percent total lipid reduced the difference between treatments; however, lipid-normalised TCB concentrations were still significantly higher in the enriched aquaria ($F_{1,4} = 22.3$, $p = 0.0092$). Normalisation of disk TCB concentrations to TOC or TN content did not reduce the differences between treatments.

Resuspension, brittle star activity and temperature

Resuspended particulate matter content (g DW l⁻¹ and g AFDW l⁻¹) of filtered water samples showed no differences between treatments. Visual observations of brittle star activity (i.e. counts of active arms) revealed no difference between treatments. Since no significant difference was observed between treatments, arm activity from both treatments was pooled and showed an increase with time during the experiment (Fig. 3). Water temperature was not different between treatments, but decreased during the experiment (Fig. 3). Increasing animal activity and absence of mortality or sublethal stress behaviour (e.g. arm autotomy or emergence out of the sediment) indicated that the specimens were in good health during the entire exposure.

Field study

Accumulation of PCBs by *Amphiura filiformis* and *A. chiajei* in the field

Table 4A presents total PCB concentrations, sediment characteristics, total biomass, abundance and lipid content of the ophiuroids from the coastal and offshore stations. Total PCB concentrations of sediment and animals were higher at the coastal than at the offshore station, as

Table 3. *Amphiura filiformis*. Dry weight (DW; g), total lipids (% g DW), total organic carbon (TOC; % g DW) and total nitrogen (TN; % g DW) of disk and arms (ND: below detection limit)

Treatment	Tissue	DW (SD)	Lipid (SD)	TOC (SD)	TN (SD)
Control	Disk	0.020 (0.004)	2.9 (1.2)	20.93 (1.40)	3.31 (0.32)
	Arms	0.056 (0.017)	ND –	17.02 (0.41)	2.68 (0.18)
Enriched	Disk	0.022 (0.004)	5.0 (4.3)	21.93 (1.39)	3.55 (0.46)
	Arms	0.055 (0.012)	ND –	18.08 (0.88)	3.05 (0.28)

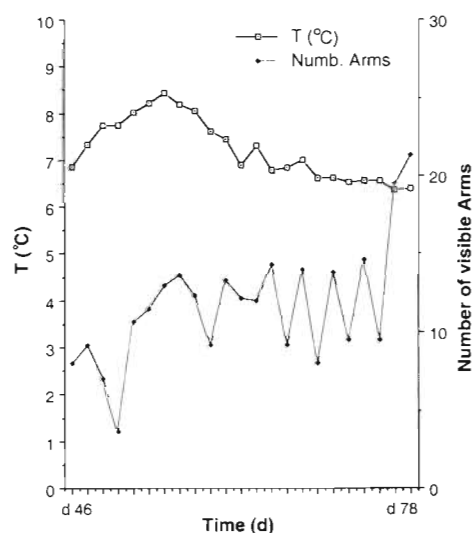


Fig. 3. Temperature (°C) and brittle star activity (number of arms) (*Amphiura filiformis* and *A. chiajei*, pooled data)

expected from the location of the stations. The coastal station, situated at the mouth of the Göta Älv River, is exposed to anthropogenic loading from industries along the river and the harbour of Göteborg. Total PCB concentration of the sediment at the coastal station was approximately 3.6 times higher, on a DW basis, than at the offshore station, and 2.1 times higher, when normalised to sediment TOC content. Total PCB accumulated by *Amphiura filiformis* at the coastal station was 2.7 times higher on a DW basis, than at the offshore station, and 5.3 times higher on a lipid basis. Total PCB concentrations of the 2 ophiuroids at the offshore station were similar on a DW basis, and 1.5 times higher in *A. chiajei* when normalised to lipid content. No *A. chiajei* was found at the coastal station. A list of the analysed PCB congeners is given in Table 4B. The sum of 6 PCB congeners analysed in both sediments and brittle stars, termed 'sum common PCBs' (Table 4A), was used for the determination of bioaccumulation factors (BAFs) and biota sediment accumulation factors (BSAFs). BAFs and BSAFs calculated from the field and from our experiment are given in Table 5. There was a good correspondence between BAFs and BSAFs from the field and from the experiment, especially between

Table 4. PCB concentrations of sediments and *Amphiura* spp. in the field. NA: not analysed; 0.00: below detection level

	Coastal station			Offshore station		
	Sediment	<i>A. filiformis</i>	<i>A. chiajei</i>	Sediment	<i>A. filiformis</i>	<i>A. chiajei</i>
(A) General biological and chemical characteristics						
TOC (% g DW)	3.24	—	—	1.88	—	—
Md ϕ (Md grain size)	22.00	—	—	16.00	—	—
Lipid (% g DW)	—	4.40	—	—	8.10	4.80
Biomass (g WW m ⁻²)	—	75.45	—	—	340.20	84.00
Abundance (ind. m ⁻²)	—	1373	—	—	1917	247
Total PCB ($\mu\text{g kg}^{-1}$ DW)	30.00	80.24	—	8.40	27.90	25.94
Sum common PCBs ^a ($\mu\text{g kg}^{-1}$ DW)	5.62	42.73	—	2.14	13.61	13.03
(B) PCB congeners ($\mu\text{g kg}^{-1}$ DW) (n = 3 pooled samples)						
<u>PCB-28</u>	0.17	1.37	—	0.27	1.39	1.55
PCB-31	NA	3.17	—	NA	1.69	2.07
PCB-47 ^b	NA	0.10	—	NA	0.00	0.00
PCB-52	0.23	NA	—	0.11	NA	NA
PCB-56	NA	0.71	—	NA	0.22	0.54
PCB-66	NA	1.17	—	NA	0.00	0.00
PCB-74	NA	1.13	—	NA	0.61	0.41
PCB-87	NA	0.00	—	NA	0.00	0.00
PCB-99	NA	1.49	—	NA	0.73	0.51
<u>PCB-101</u>	0.79	3.55	—	0.30	3.23	4.25
PCB-105	NA	1.20	—	NA	0.54	0.45
PCB-110	NA	2.98	—	NA	2.15	1.13
PCB-114	NA	0.00	—	NA	0.00	0.00
<u>PCB-118</u>	0.87	4.09	—	0.39	1.44	0.92
PCB-128	NA	1.69	—	NA	0.42	0.33
PCB-132	NA	2.67	—	NA	0.82	0.77
PCB-136	NA	0.00	—	NA	0.00	0.00
PCB-137	NA	0.26	—	NA	0.00	0.00
<u>PCB-138</u>	1.70	13.04	—	0.57	3.08	2.66
PCB-141	NA	1.20	—	NA	0.31	0.29
PCB-149	NA	5.44	—	NA	1.69	1.09
PCB-151	NA	1.40	—	NA	0.82	0.81
<u>PCB-153</u>	1.40	13.25	—	0.46	3.46	2.73
PCB-156	NA	1.68	—	NA	0.45	0.22
PCB-157	NA	0.61	—	NA	0.30	0.20
PCB-170	NA	3.84	—	NA	0.42	0.41
<u>PCB-180</u>	0.69	7.43	—	0.15	1.01	0.92
PCB-183	NA	0.00	—	NA	0.00	0.00
PCB-187	NA	3.42	—	NA	1.17	1.02
PCB-189	NA	0.12	—	NA	0.20	0.27
PCB-194	NA	1.95	—	NA	0.26	0.27
PCB-196	NA	0.89	—	NA	0.21	0.22
PCB-199	NA	0.00	—	NA	0.00	0.00
PCB-206	NA	0.55	—	NA	0.25	0.24
PCB-209	NA	0.84	—	NA	1.06	1.66

^aSum of 6 PCB congeners analysed both in sediments and animals (underlined), used for the calculation of bioaccumulation factors

^bCongener used in the experiment

the coastal station and the enriched microcosms. BAFs ranged from 3.0 to 7.9, showing that ophiuroids could accumulate between 3 and 8 times higher PCB concentrations than bulk sediment concentrations. BSAFs

Table 5. *Amphiura* spp. Bioaccumulation factors in the field and in microcosms (n = 3). Values in parentheses are biota sediment accumulation factors (BSAFs) calculated for disk tissue only. Compounds: 'sum of common PCBs', sum of 6 PCB congeners analysed both at the coastal and at the offshore stations, 'PCB-47', PCB congener used in the microcosms. BAF: bioaccumulation factor ($\mu\text{g TCB g}^{-1}$ DW tissue)/($\mu\text{g TCB g}^{-1}$ DW sediment); BSAF: [($\mu\text{g TCB g}^{-1}$ DW tissue)/(g lipid g^{-1} DW tissue)]/[($\mu\text{g TCB g}^{-1}$ DW sediment)/(g TOC g^{-1} DW sediment)]

	Species	Station		Microcosm	
		Coastal	Offshore	Control	Enriched
BAF	<i>A. filiformis</i>	7.6	6.4	3.0	7.9
	<i>A. chiajei</i>	–	6.1	4.0	7.3
BSAF	<i>A. filiformis</i>	5.6	1.5	4.3 (2.4)	5.9 (3.9)
	<i>A. chiajei</i>	–	2.4	–	–

ranged between 1.5 and 5.9. As for the BAFs, highest values were found in the enriched microcosms. Slightly lower values were obtained when only the disk concentrations were used.

DISCUSSION

Phytoplankton decomposition

Phaeodactylum tricornutum is a common marine diatom in coastal waters, able to form sinking aggregates of algal cells (Kjørboe et al. 1990). In the present study, despite a phytoplankton pulse of 5.6 g cm^{-2} , or 335 mg TOC , into the water-column of the eutrophic microcosms, no significant differences in sediment C, TOC or TN could be detected between treatments during the entire experiment. Marine phytoplankton constitute a highly attractive organic C source for microbial decomposers (Azam et al. 1983). Diatoms can be decomposed within days to a few weeks in the water-column (Chen & Wangersky 1996) and even more rapidly at the sediment surface, where they stimulate benthic bacterial production (Meyer-Reil 1983). Decomposing algal cells release substantial amounts of DOC (Holmer 1996). Degradation kinetics of this DOC has been described as a 2-step process, with an initial rapid bacterial consumption of highly labile DOC, followed by a slower mineralisation of more refractory DOC (Westrich & Berner 1984). In this experiment, following the phytoplankton and TCB additions, the microcosms were left for 1 mo in a recirculating mode before the brittle stars were added. Hence, at the start of the exposure phase, most of the phytoplankton C is likely to have been processed by microbial consumers. This is consistent with a yellow bacterial film observed on the sediment surface of the enriched microcosms.

Loss of TCB from the sediment surface

A significant amount of TCB was lost from the sediment surface during the experiment. Since the biodegradation of TCBs is extremely slow in natural benthic light and temperature conditions (Pignatello & Chapa 1994), most of the TCB lost from the sediment surface had either been released into the water-column or been buried in the sediment and accumulated in the brittle stars. Several studies have shown that hydrophobic contaminants can be mobilised from the sediment as a result of physico-chemical desorption processes (Di Toro & Horzempa 1982) and mechanical or biological resuspension of contaminated particles (Karickhoff & Morris 1985). By dividing the total amount of TCB collected by the polyurethane filters per day and square meter, desorption of TCB from the sediment surface can be expressed as release rates, thus giving $1.64 \mu\text{g TCB d}^{-1} \text{ m}^{-2}$ and $1.80 \mu\text{g TCB d}^{-1} \text{ m}^{-2}$ from the control and enriched sediments, respectively. Comparing the total TCB loss from the sediment surface during the experiment to the amount collected in the PUFs, and assuming that all TCB leaving the microcosms, as a truly dissolved fraction or associated with particles and colloids, was trapped by the PUFs, we can estimate that ca 5% of the total TCB pulse initially deposited on the sediment surface, was remobilised into the water-column, and ca 95% of it was buried into the sediment or accumulated by the animals. The effects of brittle star activity on the distribution and remobilisation of TCB could, however, not be quantified *per se*, since no aquaria without brittle stars were used in this study.

Accumulation of TCB by the brittle stars

The phytoplankton enrichment increased the accumulation of TCB by the 2 brittle star species, although no differences in sediment C, N or TCB content could be measured between the treatments. Bioaccumulation of organic contaminants may be influenced by differences in exposure pathways and metabolic characteristics (Connel 1988). Infaunal brittle stars may accumulate contaminants either by direct contact with contaminated sediment particles, or via respiration in interstitial and overlying water, or by ingestion of contaminated food. Several possible mechanisms could explain the increased bioaccumulation of TCB following the phytoplankton addition: (1) increased animal activity, (2) simple partitioning to a higher lipid pool, and (3) selective feeding on contaminated particles.

Greater brittle star activity could contribute to increase the encounter rate of individuals with contaminated particles. *Amphiura filiformis* and *A. chiajei* have

been shown to rapidly increase their feeding activity in response to added phytoplankton, and to be able to convert this energy into increased somatic and germinal growth (Sköld & Gunnarsson 1996). Even though no difference in arm activity between treatments could be observed in the present study, the influence of increased animal activity on TCB accumulation should not be excluded.

According to the equilibrium partitioning theory, the bioaccumulation of hydrophobic contaminants can be explained by a simple partitioning between the organic C pool of the sediment and the lipid pool of the organisms (Lake et al. 1990). Increased TCB uptake could result from a new partitioning equilibrium with a higher lipid content of the brittle stars, caused by the phytoplankton enrichment. Mean lipid content was slightly higher in the eutrophic microcosms. A normalisation of accumulated TCB concentrations to the lipid content of the ophiuroids reduced the difference in TCB uptake between treatments; however, the treatment effect remained significant. Passive equilibration of TCB into the lipid pool of the animals was thus not sufficient to explain their higher body burdens. Instead, the increased accumulation may be the result of an active uptake process due to selective feeding on contaminated particles. Selective feeding on TOC-rich particles may explain bioaccumulation levels above equilibrium, because these particles are also the preferential sorption site for hydrophobic organic contaminants (Boese et al. 1990). In other words, selective feeding maintains a thermodynamic disequilibrium, since the animals are accumulating against a fugacity gradient.

The importance of sediment ingestion and selective feeding for the accumulation of organic contaminants has been reported for other deposit feeders (Wyman & O'Connors 1980, Landrum et al. 1992, Harkey et al. 1994). Most benthic deposit feeders selectively ingest fine, nutritious particles, such as algal cells, bacteria and different types of DOC (Lopez & Levinton 1987). Amphiuroids may feed on bacteria and take up DOC (Clements et al. 1988). Both species used in our study actively select food particles with their arms and tube feet and process a mucus-coated food bolus towards their mouth below the sediment surface (Buchanan 1964). Several studies have shown that deposit feeders may accumulate more contaminants than suspension feeders exposed to the same sediment (Roesijadi et al. 1978, Hickey et al. 1995, Meador et al. 1995). In our experiment, no difference in TCB accumulation was found between the 2 species. The lack of difference in TCB accumulation between the species may be attributed to the mixed feeding mode of *Amphiura filiformis*. *A. filiformis* is not a strict suspension feeder but can switch from suspension to deposit feeding, and thus

like *A. chiajei* feed on sediment-associated food items. In our field study no difference between species could be shown.

TCB content of the brittle stars could be separated into disk and arm fractions, and the proportions of TCB accumulated in the 2 body compartments could be compared. The difference between the 2 body compartments was increased in the eutrophic treatment with a shift towards disk tissue. This is in accordance with our lipid analyses, which revealed that lipid content of the arms was below the detection level, and that nearly all the lipid reserves were present in the disk. The lipid content of the disk is probably related to gonad size and development and indicates that the disks of *Amphiura* species are an important target site for hydrophobic contaminants.

PCB accumulation in the field

PCB contamination in fish and seal has been a major cause of concern in the seas surrounding Sweden (Olsson et al. 1994). Sediments are a main sink for PCBs, and substantial sediment surface concentrations from the Kattegat, Skagerrak, Baltic Sea and the Danish Sounds have recently been reported (Broman et al. 1993, Magnusson et al. 1996). However, although uptake by benthic infaunal organisms may be one of the most important vectors of sediment-associated contaminants to demersal fish and higher trophic levels, including human consumers, there are almost no available data on the contamination of natural benthic invertebrate populations of the Kattegat and the Skagerrak. This is to our knowledge the first study which presents concentrations of PCBs in amphiuroids. Brittle stars were sampled from a typical contaminated urban estuary and from an offshore station on the slope of a 90 m deep trench in the Kattegat. The most prominent congeners in biota and sediments were PCB-138, PCB-153 and PCB-180, all 3 commonly found in marine mammals and humans (Safe et al. 1987, Skaare et al. 1990). PCB-47, the congener used in the laboratory experiment, was found just above detection level in *Amphiura filiformis* at the coastal station only. Mean TOC and total PCB concentrations were higher at the coastal station, which is in accordance with the study of Dave & Dennegård (1994) and is probably caused by the high anthropogenic loading from the Göta Älv River and the harbour of Göteborg. Biota and sediment total PCB concentrations at the coastal station were ca 3.5 times higher than at the offshore site, on a DW basis. Normalisations to organic carbon or lipid content are presented in the results and discussed below along with the bioaccumulation factors. The biomass and abundance of brittle stars were considerably higher at

the offshore than at the coastal station. The distribution and abundance of *Amphiura* populations have been related to food availability, hydrodynamic processes such as bottom currents and lateral advection, and deposition of organic matter (Creutzberg et al. 1984, Rosenberg 1995). No *A. chiajei* was found at the coastal station. Although the 2 species are known to coexist in typical *Echinocardium filiformis* communities (Petersen 1915), *A. chiajei* is commonly found in silty sediments at depths over 70 m (Buchanan 1964). The shallow and sandy character of the coastal site is the most likely explanation for the absence of *A. chiajei* from it, although toxic effects caused by the higher concentrations of contaminants cannot be ruled out.

Accumulation factors

In the field study, BAFs (sum common PCBs in tissue/sum common PCBs in sediment) showed that brittle stars accumulated ca 7 times higher PCB concentrations compared to the sediment. BAFs were remarkably similar between the 2 species and the 2 stations (Table 5), and higher than the empirical $\log K_{OC} = 4.88$ value. In the laboratory study, BAFs of the controls were closer to the empirical value; in the enriched aquaria, however, BAFs were higher and closer to the field values. BSAFs (sum common PCBs in lipid-normalised tissue/sum common PCBs in TOC-normalised sediment) are based on the equilibrium partitioning theory and predict that if concentrations are at equilibrium between the lipid pool of the organisms and the carbon pool of the sediment a maximal theoretical value of 1.7 should be reached, regardless of lipid or carbon composition (McFarland & Clarke 1988). In the present study BSAFs ranged from 1.5 to 5.9 and were within the range of values reported from other studies (Bierman 1990, Ferraro et al. 1991). However, our values were often higher than the predicted value of 1.7, especially at the coastal station and in the enriched aquaria, indicating that a greater uptake of PCBs occurred than can be explained by total lipids and TOC content, and supporting this observation accumulation of PCBs by the brittle stars may be strongly related to selective feeding on organically richer, contaminated particles. These results suggest that the equilibrium partitioning approach may underestimate the bioaccumulation of hydrophobic contaminants by selective benthic deposit feeders.

Trophic transfer

Several food chain models of aquatic systems have shown that predation on benthic invertebrates is a

highly contributing factor to the transfer of sediment-associated contaminants to higher trophic levels (e.g. Thomann et al. 1986, Connolly 1991). Since *Amphiura filiformis* populations dominate the benthic macrofauna of vast areas of North European seas (O'Connor et al. 1983, Duineveld & Noort 1986), it is of primary concern to evaluate their potential role in the transfer of pollutants from contaminated sediments to higher trophic levels. *A. filiformis* populations are exposed to extensive predation by some fish species and crustaceans (Baden et al. 1990). Predation on infaunal brittle stars occurs by consumption of the whole animal, but more commonly by cropping exposed arms. Arm loss and subsequent regeneration may occur on average in 50% of the population and account for a substantial part of its secondary production (Bowmer & Keegan 1983, Sköld et al. 1994). Duineveld & Noort (1986) reported that *Amphiura* spp. arms accounted on average for about 60% of the stomach content, on a DW basis, of the dab *Limanda limanda*. They also estimated that the annual food consumption of *Amphiura* spp. arms by an average standing stock of dab in the North Sea was $0.84 \text{ g arms m}^{-2} \text{ yr}^{-1}$. Using the yearly arm consumption obtained by Duineveld & Noort (1986), and data from the present study (Table 4), we can estimate a possible yearly trophic transfer rate for PCBs, from *A. filiformis* to dab. By applying the PCB concentration of *A. filiformis* at the coastal station ($80.24 \text{ } \mu\text{g PCB kg}^{-1}$), and considering that ca 70% of the PCB concentration may be found in the arms, calculated from Table 1 (whole animal: $117.1 \text{ } \mu\text{g TCB kg}^{-1}$ DW = 100%; arm fraction: $81.6 \text{ } \mu\text{g TCB kg}^{-1}$ DW = 70%; disk fraction: $217.4 \text{ } \mu\text{g TCB kg}^{-1}$ DW = 186% of the TCB concentration), we obtain a trophic transfer of ($0.84 \text{ g arms m}^{-2} \text{ yr}^{-1} \times 80.24 \text{ ng PCB g}^{-1} \times 0.70 =$) $47.2 \text{ ng PCBs m}^{-2} \text{ yr}^{-1}$ to the dab by sublethal predation on *A. filiformis* arms.

Alternatively the trophic transfer of PCBs can be estimated from the available production data of *Amphiura filiformis*. Sköld et al. (1994) reported that *A. filiformis* populations have a production biomass ratio of 0.46 yr^{-1} , and that 13.3% of the production is due to arm regeneration. Using the PCB concentration of *A. filiformis* arms at the coastal station (70% of $80.24 \text{ ng PCB g}^{-1}$), and recalculating the biomass data from this station of $75.5 \text{ g WW (wet weight) m}^{-2}$ (Table 4) into DW by a conversion factor of 0.32 according to Sköld et al. (1994), we obtain a trophic transfer of: ($0.46 \times 0.133 \times 0.32 \times 75.5 \times 0.70 \times 80.24 =$) $83.02 \text{ ng PCBs m}^{-2} \text{ yr}^{-1}$. Both estimates of the trophic transfer rates are in the same order of magnitude and show that between 47 and $83 \text{ ng PCBs m}^{-2} \text{ yr}^{-1}$ may be transferred to the dab and other predators by sublethal predation on *A. filiformis* populations. The present study has shown that highest PCB concentrations were found in the disks

and that their PCB burden could be significantly increased by organic enrichment. Hence, one should keep in mind that these estimates are based on yearly production estimates and that the actual trophic transfer will vary significantly during the year due to the build up and shedding of gonads and depending on available food resources. The redistribution of PCB by the shedding of gonadal products can be estimated by the latter approach, again using the estimates of Sköld et al. (1994) for gonad (disk) production being 68.9% of total biomass production in an adult *A. filiformis* population. An analogous calculation, but with the higher PCB concentration found in the disk tissue, gives $(0.46 \times 0.689 \times 0.32 \times 75.5 \times 1.86 \times 80.24 =) 1142.82 \text{ ng PCBs m}^{-3} \text{ yr}^{-1}$. Thus in total $1225.8 \text{ ng PCBs m}^{-3} \text{ yr}^{-1}$ may be redistributed from the sediment due to sublethal predation and shedding of gonads by an *A. filiformis* population.

Conclusions

The accumulation of TCB by the 2 infaunal brittle stars *Amphiura filiformis* and *A. chiajei* increased following an addition of the marine diatom *Phaeodactylum tricornutum*, and was suggested to be caused by selective feeding on TOC-rich, TCB-contaminated food items. PCB accumulation measured in brittle stars collected from 2 field sites revealed ca 3 times higher total PCBs and individual congeners at the urban coastal estuarine site compared to the offshore site, both in brittle stars and sediment. The results from this study suggest that eutrophication processes, such as increased phytoplankton production, may contribute to increase the accumulation of hydrophobic contaminants in infaunal brittle stars and their transfer to higher trophic levels.

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