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Differences in contamination load between pelagic and sympagic invertebrates in the Arctic marginal ice zone: influence of habitat, diet and geography

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ABSTRACT: Concentration and transport of organic pollutants by Arctic sea ice may expose ice-associated fauna to high contaminant concentrations relative to pelagic organisms. Zooplankton and ice-associated amphipods were collected in the marginal ice zone near Svalbard to investigate whether habitat, diet and geographic sampling site influenced their organochlorine burden. Organochlorine concentrations were low in both zooplankton (*Calanus hyperboreus*, *Thysanoessa inermis*, *Parathemisto libellula*, Chaetognatha) and ice-associated amphipods (*Apherusa glacialis*, *Gammarus wilkitzkii*, *Onisimus* spp.), from 0.3 ng g^{-1} lipid weight *trans*-chlordane in *A. glacialis* to 36.9 ng g^{-1} lipid weight hexachlorobenzene (HCB) in *G. wilkitzkii*. Diet accounted for most of the explained organochlorine variance, followed by habitat and geographic sampling site. The concentrations were higher in carnivores (*P. libellula*, Chaetognatha, *G. wilkitzkii*, *Onisimus* spp.) than in herbivores (*C. hyperboreus*, *T. inermis*, *A. glacialis*). In comparison with zooplankton, ice fauna had high levels of organochlorines with high residence time in surface layers (hexachlorocyclohexane [HCH] and HCB), whereas compounds with higher particle affinity did not differ between habitats. This was attributed to the sea ice serving as a habitat that keeps ice fauna in the surface layer, rather than to the release of contaminants from the sea ice itself. HCHs were the only compounds that differed geographically. Higher α-/γ-HCH ratios in *C. hyperboreus* and *A. glacialis* from the Greenland Sea relative to North of Svalbard, are consistent with the geographic patterns in HCH levels reported for air, water, ringed seals *Phoca hispida* and polar bears *Ursus maritimus*.

KEY WORDS: Zooplankton · Ice-associated · Svalbard · Greenland Sea · Crustaceans · Organochlorines · γ-HCH · α-HCH · Redundancy analysis

INTRODUCTION

Sea ice may be important in the concentration and transport of organic pollutants within the Arctic (Pfirman et al. 1995). Organochlorines originating from industrial and agricultural areas in North America, Europe and Asia are transported to the Arctic by atmospheric and oceanic currents, and river discharge, of which the most important transport route is atmospheric currents (AMAP 1998). Annual ice mainly carries contaminants from atmospheric deposition, whereas multi-year ice may accumulate contaminants from atmospheric deposition and from the entrainment of contaminated sediments at the surrounding shelf seas (Weeks 1994). Due to the potential contaminant accumulation by sea ice, organisms living in association with the drifting sea ice may be exposed to high concentrations when the contaminants are released in the melting areas (Alexander 1995, Pfirman et al. 1995).

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The Arctic marginal ice zone has a gradient in ice cover from open water to consolidated pack ice (NSKV 1990). The receding ice edge of the marginal ice zone has high biological productivity relative to open water areas and to areas further into the pack ice (Smith 1988, Smith & Niebauer 1993, Ramseier et al. 1999). The high primary production in the marginal ice zone is a combination of nutrients available under the ice after winter mixing of water masses, increased light intensity as the ice breaks and a marked stratification caused by melt water, resulting in the retention of phytoplankton in the euphotic zone (Smith 1988, Sakshaug 1997).

Some animals live their whole life cycle in association with sea ice (autochthonous sympagic fauna), with amphipods (*Apherusa glacialis*, *Gammarus wilkitzkii*, *Onisimus nanseni*, *O. glacialis*) being the dominant taxon (Melnikov 1997). In the pelagic system of the marginal ice zone, calanoid copepods (*Calanus* spp.) and chaetognaths (*Sagitta* spp., *Eukrohnia hamata*) are abundant among the zooplankton (Hirche & Kwasniewski 1997). Both sympagic amphipods and zooplankton such as copepods, euphausiids (*Thysanoessa* spp.) and amphipods (*Parathemisto libellula*) are important in the transfer of energy from primary producers and consumers to predators, such as other zooplankton (Falkenhaug 1991, Scott et al. 1999), fish (Bradstreet & Cross 1982, Lønne & Gulliksen 1989, Scott et al. 1999), seabirds (Lønne & Gabrielsen 1992, Mehlum & Gabrielsen 1993) and seals (Lydersen et al. 1991, Lindstrøm et al. 1998, Siegstad et al. 1998). Of the dominating sympagic fauna and zooplankton, *A. glacialis*, *Calanus* spp. and *Thysanoessa* spp. are considered as being primarily herbivores although their diet also consists of bacteria, detritus, protozoa and small zooplankton (Werner 1997, Levinsen et al. 2000, Poltermann 2001), whereas *P. libellula* and Chaetognatha mainly feed on other animals, including calanoid copepods (Falkenhaug 1991, Scott et al. 1999). *G. wilkitzkii* and *Onisimus* spp. have a mixed diet dominated by other animals like copepods and *A. glacialis*, but also feed on ice algae and detritus (Werner 1997, Scott et al. 1999, Poltermann 2001).

If transport of pollutants with sea ice is important for contaminant accumulation in sympagic organisms, the contaminant levels could be expected to be higher in sympagic fauna than in zooplankton. The marginal ice zone is a dynamic system with large variability and interactions between the sympagic and pelagic communities (Sakshaug 1997, Scott et al. 1999). Nevertheless, habitat related differences in contaminant levels could be caused by a general increased dilution with distance from melting sea ice and/or different food chains being exploited, with either predominantly phytoplankton or ice algae as first trophic level. As a result of contaminant release from sea ice, elevated contamination in sympagic fauna would to be important for the accumulation of persistent organic pollutants in the Arctic food web (Pfirman et al. 1995) and may help explain regional differences in organochlorine levels in the polar bear *Ursus maritimus*, a marine top predator (Norstrom et al. 1998).

Organochlorines have been found in sympagic amphipods and zooplankton both in the Canadian Arctic (Hargrave et al. 1992, Fisk et al. 2001a) and in the European Arctic (Borgå et al. 2001, 2002a). Due to different processes such as dietary organochlorine uptake, direct partitioning with water and age-related accumulation, an organism's diet, size and age may influence the organochlorine accumulation in aquatic invertebrates (Landrum 1988, Borgå et al. 2002b); however, more field data are needed to clarify which factors may be important for the accumulation in Arctic zooplankton and ice fauna.

The ratios of α-/γ-hexachlorocyclohexane (HCH) in air and water are often used to trace the sources of technical HCH (mixture of different isomers) and Lindane (100% γ-HCH), because of the increasing ratio with distance from the source of Lindane (e.g. Iwata et al. 1993, Jantunen & Bidleman 1996). Spatial differences are found in the α-/γ-HCH ratios in sympagic amphipods (Borgå et al. 2002a) and similar patterns are seen in air, water, ringed seals *Phoca hispida* and polar bear (Jantunen & Bidleman 1996, Harner et al. 1999, Muir et al. 2000, Lie et al. in press). Whether this geographical difference in α-/γ-HCH ratio is also found in zooplankton is presently unknown.

In light of the above-mentioned habitat, dietary and geographic sources of variation, zooplankton (*Calanus hyperboreus*, *Thysanoessa inermis*, *Parathemisto libellula*, Chaetognatha) was collected together with sympagic amphipods (*Apherusa glacialis*, *Gammarus wilkitzkii*, *Onisimus* spp.) in the marginal ice zone north of Svalbard and in the Greenland Sea to investigate whether: (1) the organochlorine levels are higher in ice-associated relative to pelagic organisms; (2) the organochlorine levels are higher in more carnivorous species; and (3) there is a geographic difference in α-/γ-HCH ratios in zooplankton.

MATERIALS AND METHODS

Collection of organisms. Zooplankton and sympagic amphipods were collected in the marginal ice zone north of Svalbard and in the Greenland Sea from 25 September to 5 October 1999 (Fig. 1). Calanoid copepods (*Calanus hyperboreus*) and macrozooplankton-like euphausiids (*Thysanoessa inermis*), amphipods (*Parathemisto libellula*) and chaetognaths (*Sagitta ele-*

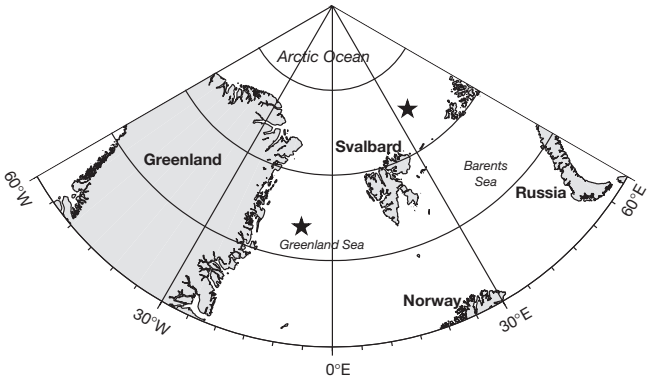


Fig. 1. Sampling positions (★) in the marginal ice zone north of Svalbard and in the Greenland Sea from September 25 to October 5, 1999

gans, *Eukrohnia hamata*) were collected with a WP-3 net (1000 µm mesh), MEGA net (1550 µm mesh) and Tucker trawl (1000 µm mesh) in the upper water masses. North of Svalbard, zooplankton was sampled with nets from 50, 100, 200, 300, 500 and 800 m depth to the surface. In the Greenland Sea, zooplankton (except *P. libellula*) was sampled from 50, 100 and 150 m depth to the surface. In the Greenland Sea, *P. libellula* was collected with a bottom trawl (309 to 319 m depth). Sympagic amphipods were collected from the sea ice underside by SCUBA divers, using an electric suction pump (Lønne 1988). After sampling, the organisms were transferred to polypropylene buckets (10 l) with seawater and stored in a cool room (+2°C) for less than 1 d before they were sorted to species, using clean stainless steel utensils. *Calanus hyperboreus* was separated into copepodite stages V (CV) and VI (CVI, only adult female).

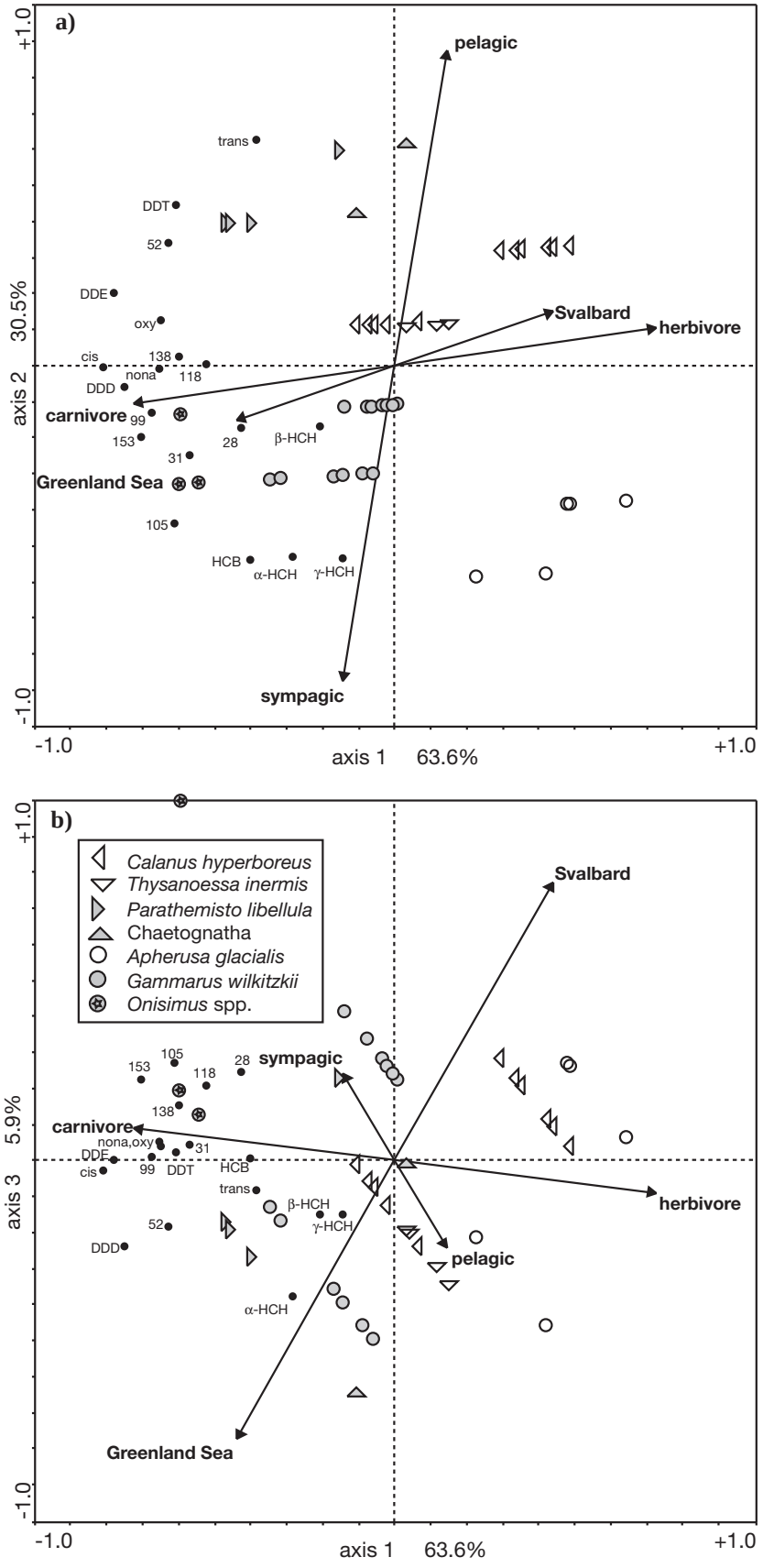
Due to low organochlorine concentrations in Arctic organisms occupying low trophic levels, each sample consisted of pooled individuals. We aimed at a minimum of 5 g wet weight (wet wt) for each sample, based on the organisms' lipid content. The samples consisted of 650 *Calanus hyperboreus* individuals (CV, total body length 5 to 8 mm, Hirche et al. 1994) and 350 *C. hyperboreus* individuals (CVI, 7 to 10 mm, Brodskii et al. 1983); 100 *Thysanoessa inermis* individuals (21 to 30 mm); 35 *Parathemisto libellula* individuals north of Svalbard (11 to 35 mm) and 25 *P. libellula* individuals from the Greenland Sea (31 to 49 mm); 200 to 600 Chaetognatha individuals, depending on occurrence (all sizes); 100 to 400 *Apherusa glacialis* individuals, depending on occurrence (5 to 13 mm); 10 to 350 *Gammarus wilkitzkii* individuals, depending on size (3 to 44 mm); and 30 to 200 *Onisimus* spp. individuals, depending on occurrence (3 to 21 mm).

After sorting into species and measuring their size, the organisms were stored frozen (–20°C) in polypropylene containers.

Station description. The organisms were collected in areas covered by annual ice (ice that has not yet survived its first summer, NSKV 1990) mixed with multiyear ice (ice that survived at least 2 summers of melting, NSKV 1990). The stations' starting position was chosen after steaming the research vessel into the pack ice as far as the ice conditions allowed. North of Svalbard, the vessel was anchored to a multi-year ice floe with position 82° 27′ N, 33° 14′ E at start, and position 82° 40′ N, 30° 35′ E when the station was left after 3 d. In the Greenland Sea, the vessel was anchored to a multi-year ice floe with position 76° 46′ N, 08° 07′ W at start, and position 75° 13′ N, 09° 53′ W when the station was left after 2 d. The total depth at the stations north of Svalbard and in the Greenland Sea was >3000 m and ~350 m, respectively. The upper water masses, where the animals were collected (except *Parathemisto libellula* in the Greenland Sea and some individuals of *Calanus hyperboreus* from north of Svalbard), consisted of polar surface water (Borgå et al. 2002a). The water masses were determined by comparing conductivity, temperature and density measurements from each station to water mass characteristics (Loeng 1991).

Chemical analysis. Organochlorine content was determined as described in detail by Borgå et al. (2002b), modified from Bernhoft & Skaare (1994). In short, pooled samples of each species were coarsely homogenised and an internal standard (PCB-29, -112 and -207) was added before further homogenisation, and extraction of lipids and organochlorines with cyclohexane and acetone (3:2, v/v). The lipid extracts were concentrated in a water bath (40°C) under a nitrogen flow and the final volume was adjusted with cyclohexane to 5 ml. A portion of the extract (1 ml) was used to gravimetrically determine the lipid content. To remove lipids, the extracts (3 × 1 ml) were cleaned up with a surplus of concentrated sulphuric acid. Due to expected low organochlorine concentrations, the lipid-free extracts were concentrated 3 to 5 times before separation of organochlorine compounds by high resolution gas chromatography (Agilent 6890 Plus GC system, Agilent Technologies). The gas chromatograph was equipped with 2 fused silica capillary columns of different polarity (SPB-5 and -1701; 60 m length, 0.25 mm ID, 0.25 µm film; Supelco) and ^{63}Ni-micro-electron capture detector (Agilent Technologies).

The samples were analysed for contents of α-, β- and γ-HCH, hexachlorobenzene (HCB), *cis*-chlordane, *trans*-chlordane, oxychlordane and *trans*-nonachlor, the dichlorodiphenyltrichloroethane compounds *p,p′*-DDE, *p,p′*-DDD, and *p,p′*-DDT, and polychlorinated



biphenyl (PCB) congener numbers 28, 31, 52, 99, 105, 118, 138, 153 and 180. The selection of organochlorines was based on a previous study in which the selected compounds contributed >1% to the sum of analysed organochlorines in sympagic amphipods (Borgå et al. 2002b). In the analysis of sympagic fauna, the recoveries of all compounds ranged from 70 to 127% (mean 101%) and the mean coefficient of variance of repeatability of the in-house reference sample was 13.7%. The detection limit ranged from 0.003 to 0.078 ng g^{-1} wet wt. In the analysis of zooplankton, the recoveries of all compounds ranged from 72 to 123% (mean 101%) and the mean coefficient of variance of repeatability of the in-house reference sample was 8.9%. The detection limit ranged from 0.003 to 0.157 ng g^{-1} wet wt.

Due to low concentrations, especially in calanoid copepods, some of the compounds were quantified below the quantification limit (3× detection limit). A few compounds were quantified below the detection limit (3× background noise). However, since the calibration curves were extended through the origin, all compounds' concentrations are included without any adjustment.

Fig. 2. Ordination diagram based on redundancy analysis (RDA) of wet wt organochlorine data in zooplankton and ice-associated amphipods with lipid content (%) as the covariable, and diet, habitat and station as nominal environmental variables. Triplot of nominal environmental variables (arrows), organochlorines' scores on the ordination axes (black dots) and samples' scores on the ordination axes (white symbols: primarily herbivore, grey symbols: primarily carnivore; all circles: sympagic fauna, all triangles: zooplankton). Polychlorinated biphenyl (PCB) congener numbers are given by their IUPAC numbers (Ballschmiter & Zell 1980), DDE, DDT and DDD are all *p,p'*-isomers, *trans*, *cis*, oxy and nona refer to *trans*-chlordane, *cis*-chlordane, oxychlordane and *trans*-nonachlor, respectively. Of the total variance in the samples' organochlorine content explained in the RDA (54.2%), 48% was accounted for by the environmental variables and the fraction of the total explained variance displayed by each ordination axis is given

The laboratory's accredited analytical quality was approved in international inter-calibration tests, and the present analyses' precision, linearity and sensitivity were within the laboratory's accredited requirements.

Statistical analyses. To analyse the amount of total variance in the organisms' organochlorine concentrations (ng g^{-1} wet wt) explained by habitat, diet and sampling station, the data were subjected to a multivariate direct ordination analysis (redundancy analysis, RDA; CANOCO 4.0 for Windows, Ter Braak 1995, Ter Braak & Simlauer 1998). Before the analysis, the wet wt concentrations were ln-transformed to normalise the distribution and to reduce variance heterogeneity. Each explanatory variable had 2 levels: pelagic or sympagic habitat, herbivorous or carnivorous diet and sampling station north of Svalbard or the Greenland Sea. Although many of the species are omnivorous, they were grouped by the dominance of their diet as herbivore (*Calanus hyperboreus*, *Thysanoessa inermis*, *Apherusa glacialis*) or carnivore (*Parathemisto libellula*, Chaetognatha, *Onisimus* spp., *Gammarus wilkitzkii*). The organisms' diets were derived from the literature (e.g. Falkenhaug 1991, Scott et al. 1999, Levinsen et al. 2000, Poltermann 2001). Since the organochlorines are highly lipid soluble, the sample's lipid content was entered as a covariable to adjust for its influence on variation in organochlorine concentrations. Thus, the organisms' organochlorine data were constrained by habitat, diet and sampling station to minimise the residual sum of squares after accounting for variation due to lipids. In the RDA (performed on the correlation matrix), the samples (organisms) and organochlorines were assigned scores on ordination axes presented in a diagram, and arrows represent the environmental variables pointing in the direction of the maximum variance accounted for by the variable (Fig. 2). Ordination techniques and rules of interpretation of the redundancy diagram are summarised and reviewed in detail elsewhere (Gower 1987, Ter Braak 1995, Van Wijngaarden et al. 1995, Van den Brink & Ter Braak 1999). The RDA was followed by a Monte-Carlo permutation test to analyse whether the organochlorine levels in the investigated organisms were significantly related to habitat, diet and station (stronger relation than expected by chance). The Monte-Carlo permutation test was run with 199 unrestricted permutations and an overall significance of $p \leq 0.005$.

Generalised linear models with analysis of variance (ANOVA type III sum of squares) were used to analyse the influence of the different predictor variables (habitat, diet, station) on the individual organochlorine concentrations in more in detail; $\alpha = 0.05$, using SAS V8 for Windows (SAS Institute 1989). In addition, spatial variation in α- and γ-HCH concentrations and α/γ-HCH ratios was analysed in *Calanus hyperboreus* females (pelagic) and *Apherusa glacialis* (sympagic). These 2 herbivores were selected for comparison as sufficient numbers of samples were collected from both stations. Since the lipid content differed between the species (ANOVA, $F_{6,32} = 18.2$, $p < 0.0001$, adjusted $R^2 = 0.71$), wet wt organochlorine concentrations were lipid adjusted (ng g^{-1} lipid weight) before the analysis and then $\log_{10}$-transformed.

RESULTS

The organochlorine concentrations in both sympagic and pelagic fauna were low, with mean lipid-adjusted concentrations ranging from 0.3 ng g^{-1} for *trans*-chlordane in *Apherusa glacialis* to 36.9 ng g^{-1} for HCB in *Gammarus wilkitzkii* (Table 1). In the pelagic herbivorous *Calanus hyperboreus*, α-HCH followed by *cis*-chlordane, *trans*-nonachlor and γ-HCH had highest concentrations (Table 1). The herbivorous *Thysanoessa inermis* had highest concentrations of HCB followed by α-HCH. The pelagic predator *Parathemisto libellula* had highest concentrations of *cis*-chlordane and *p,p'*-DDE, followed by *p,p'*-DDT and *trans*-nonachlor, whereas the organochlorine profile in the predator Chaetognatha was dominated by *cis*-chlordane, PCB-138, *trans*-nonachlor, *p,p'*-DDT and *p,p'*-DDE (Table 1). In all species of sympagic amphipods, α-HCH and HCB had highest concentrations, followed by *trans*-nonachlor and γ-HCH in the herbivorous *A. glacialis*, *cis*-chlordane and *trans*-nonachlor in the more carnivorous *G. wilkitzkii* and *Onisimus* spp. (Table 1). Although the organochlorine profile differed between the species, the relative composition of each compound class was generally similar (Table 1).

Structure in organochlorine data

The RDA accounted for 54.2% of the total variance in organochlorine concentrations. The environmental variables explained 48% of the variance accounted for by the RDA. The environmental variables' correlation on the RDA axes showed that diet was most correlated to Axis 1, habitat to Axis 2 and geographic location to Axis 3 (Table 2, Fig. 2). The fraction of explained variance accounted for by each axis in the diagram was 63.6, 30.5 and 5.9% for Axes 1, 2 and 3, respectively. The RDA diagram shows that predominantly carnivores had higher organochlorine concentrations than herbivores, especially of *cis*-chlordane, *p,p'*-DDE, *p,p'*-DDD, PCB-153, PCB-99, *trans*-nonachlor and oxychlordane (separation along Axis 1, Fig. 2a,b). Sympagic amphipods had higher concentrations than zooplank-

Table 1. Mean organochlorine concentrations (ng g^{-1} lipid wt) ± SE in organisms from the Arctic marginal ice zone north of Svalbard (NOS) and the Greenland Sea (GS) collected from 25 September to 5 October 1999

Species	Zooplankton				Ice fauna		
	Calanus hyperboreus	*Thysanoessa inermis*	*Parathemisto libellula*	Chaetognatha[a]	*Apherusa glacialis*	*Gammarus wilkitzkii*	*Onisimus* spp.[b]
NOS n[c]	6	0	1	1	3	8	1
GS n[c]	5	4	3	1	2	6	2
Lipid (%)	9.5 ± 0.8	10.7 ± 0.4	5.8 ± 0.8	1.4 ± 0.5	7.7 ± 0.8	3.9 ± 0.3	10.1 ± 0.4
Organochlorines							
HCB	1.4 ± 0.3	19.7 ± 0.2	24.9 ± 2.1	17.4 ± 4.2	19.9 ± 0.9	36.9 ± 3.2	28.6 ± 2.8
α-HCH	12.0 ± 1.7	17.2 ± 0.7	15.6 ± 1.7	11.2 ± 2.1	26.7 ± 9.4	36.0 ± 9.5	35.1 ± 11.1
β-HCH	2.2 ± 0.4	4.2 ± 0.2	4.9 ± 0.7	2.7 ± 0.3	2.9 ± 0.7	4.9 ± 1.1	4.8 ± 1.3
γ-HCH	4.3 ± 0.7	4.6 ± 0.1	4.7 ± 0.6	3.7 ± 0.0	6.9 ± 1.2	9.5 ± 1.7	8.2 ± 1.9
Oxychlordane	1.8 ± 0.4	2.3 ± 0.2	5.7 ± 0.6	4.7 ± 2.4	0.8 ± 0.2	4.8 ± 0.5	5.6 ± 1.3
Trans-chlordane	2.1 ± 0.2	2.9 ± 0.3	7.9 ± 1.4	12.4 ± 6.4	0.3 ± 0.0	1.8 ± 0.3	3.0 ± 1.3
Cis-chlordane	7.1 ± 0.7	7.6 ± 0.1	31.2 ± 3.3	23.3 ± 8.0	4.4 ± 0.4	21.7 ± 2.1	23.7 ± 8.1
Trans-nonachlor	5.2 ± 0.5	5.9 ± 0.1	25.6 ± 2.8	19.8 ± 8.1	6.9 ± 3.4	13.2 ± 1.1	22.3 ± 11.3
p,p'-DDE	2.6 ± 0.3	4.6 ± 0.2	30.2 ± 4.6	18.0 ± 8.7	1.2 ± 0.3	11.9 ± 1.5	17.9 ± 10.4
p,p'-DDD	0.9 ± 0.1	2.0 ± 0.1	8.6 ± 1.4	7.7 ± 4.0	1.1 ± 0.4	6.1 ± 0.6	2.7 ± 0.8
p,p'-DDT	4.3 ± 0.5	4.3 ± 0.3	24.8 ± 3.6	19.5 ± 8.6	1.3 ± 0.2	5.9 ± 0.7	14.9 ± 8.9
PCB-31	1.0 ± 0.2	1.6 ± 0.1	4.7 ± 0.6	4.1 ± 0.4	1.8 ± 0.3	3.4 ± 0.3	3.9 ± 0.9
PCB-28	0.8 ± 0.1	0.8 ± 0.1	2.7 ± 0.7	3.3 ± 1.0	1.2 ± 0.2	2.3 ± 0.2	2.3 ± 0.7
PCB-52	1.0 ± 0.1	1.9 ± 0.3	6.0 ± 0.3	4.7 ± 1.8	0.5 ± 0.2	3.2 ± 0.3	1.3 ± 0.1
PCB-99	1.4 ± 0.1	3.3 ± 0.3	9.9 ± 0.6	8.4 ± 4.0	2.4 ± 0.3	6.0 ± 0.5	5.7 ± 1.3
PCB-118	3.3 ± 0.4	2.3 ± 0.5	6.9 ± 0.6	7.7 ± 3.0	2.1 ± 0.3	6.5 ± 0.8	8.5 ± 1.2
PCB-153	1.9 ± 0.2	2.8 ± 0.2	12.8 ± 0.8	10.6 ± 6.1	3.2 ± 0.8	9.0 ± 0.9	20.8 ± 11.7
PCB-105	0.6 ± 0.1	0.7 ± 0.0	2.0 ± 0.3	2.5 ± 1.0	1.2 ± 0.2	3.4 ± 0.8	4.1 ± 1.7
PCB-138	3.3 ± 0.2	5.3 ± 0.9	20.2 ± 1.8	20.9 ± 10.8	4.7 ± 0.5	11.4 ± 1.6	13.6 ± 5.0
PCB-180	2.8 ± 0.4	1.1 ± 0.2	2.3 ± 0.3	4.3 ± 1.4	2.6 ± 0.8	4.4 ± 0.5	10.7 ± 6.3

[a] *Eukrohnia hamata* and *Sagitta elegans*
[b] *Onisimus glacialis* and *Onisimus nanseni*
[c] Number of pooled samples analysed

Table 2. Summary of redundancy analysis (RDA) where zooplankton and ice-associated amphipods' organochlorine concentrations (ln[ng g^{-1} wet wt]) were constrained by habitat, diet and station to minimise the residual sum of squares after accounting for variation due to lipids

		Ordination axis		
		1	2	3
Canonical eigenvalues[a]		0.305	0.146	0.028
Cumulative percentage variance[b]		63.6	94.1	100
Correlation by environmental variables on ordination axes:				
Habitat	pelagic/sympagic	0.13/–0.13	0.87/–0.87	–0.20/0.20
Diet	herbivore/carnivore	0.80/–0.80	0.13/–0.13	–0.09/0.09
Station	north of Svalbard/Greenland Sea	0.39/–0.39	0.02/–0.02	0.64/–0.64

[a] Eigenvalues constrained by environmental variables after fitting lipid content (%) as a covariable
[b] Cumulative percentage of the explained variance (48%) in the organisms' organochlorine concentrations accounted for by the different ordination axes

ton of HCB, γ- and α-HCH, and PCB-105, and lower concentrations of *trans*-chlordane and *p,p'*-DDT (separation along Axis 2, Fig. 2a). Organisms sampled in the Greenland Sea had higher α-HCH concentrations than organisms collected north of Svalbard (separation along Axis 3, Fig. 2b).

The structure in the organochlorine data was significantly described by variables that correlated to Axis 1 (diet; $F = 19.97$, $p < 0.005$). Additionally, the full model of all axes significantly described the observed pattern in the organochlorine concentrations ($F = 14.99$, $p < 0.005$).

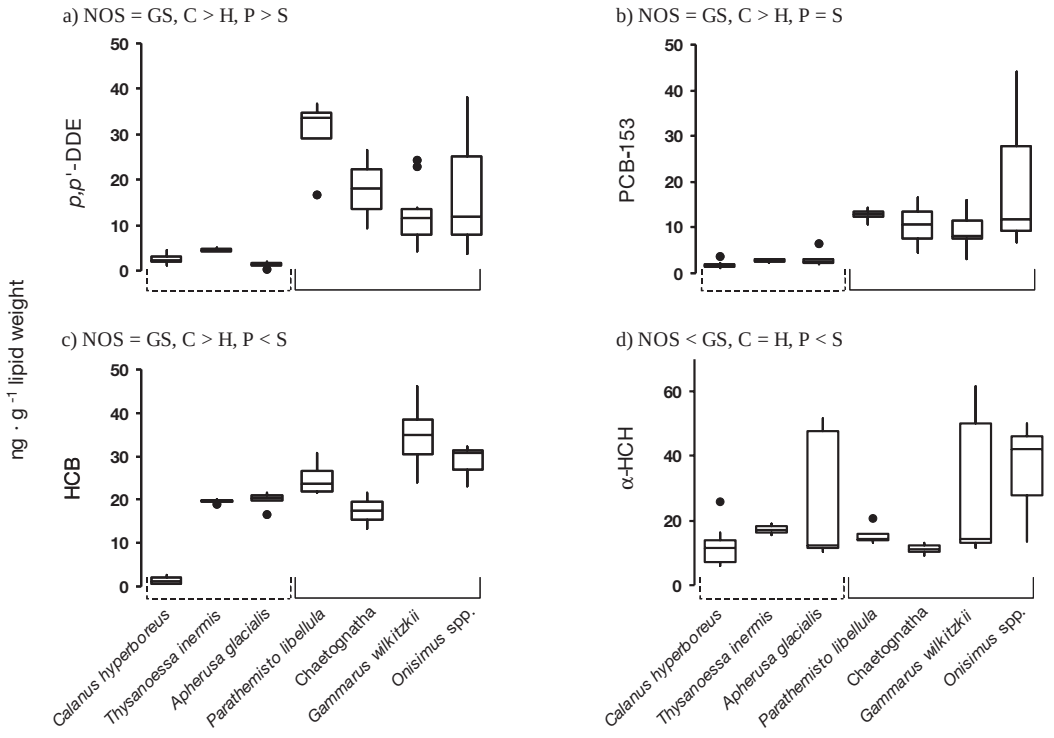


Fig. 3. Box plots of selected organochlorines in herbivorous (---) and carnivorous (—) zooplankton (*Calanus hyperboreus*, *Thysanoessa inermis*, *Parathemisto libellula*, Chaetognatha) and ice-associated amphipods (*Apherusa glacialis*, *Gammarus wilkitzkii*, *Onisimus* spp.) from the Arctic marginal ice zone. The compounds represent categories: (a,b,c) no difference between organisms collected north of Svalbard (NOS) and in the Greenland Sea (GS), and higher levels in carnivores (C) than in herbivores (H); (a) higher levels in pelagic (P) than in sympagic (S) animals; (b) no difference between S and P; (c,d) lower levels in P than in S; and (d) higher levels in GS than NOS, and no difference between H and C. Filled circles: outliers. One outlier in *Gammarus wilkitzkii* is not shown in (d), but is included in the statistical analysis referred to in the text since the exclusion of the outlier did not affect the results of the statistical test

Specific influence of habitat, diet and sampling site

When the effect of habitat and sampling site was adjusted for, the concentrations of all compounds except α- and γ-HCH were higher in the carnivores compared to the herbivores ($F_{1,40} > 6.16$, $p < 0.0173$ for all compounds; Fig. 3a–c). No difference was found between herbivores and carnivores in α- and γ-HCH concentrations ($F_{1,40} < 0.5$, $p > 0.4920$ for both; Fig. 3d).

When the effect of diet and sampling site was accounted for, the levels of *trans*-nonachlor, *p,p'*-DDD, PCB-28, 31, 99, 118, 138, 153 and 180 did not differ between pelagic and sympagic species ($F_{1,40} < 3.4$, $p > 0.0709$ for all compounds), whereas the levels of oxychlordane, *trans*-chlordane, *cis*-chlordane, *p,p'*-DDE and *p,p'*-DDT, and PCB-52 were higher in pelagic than sympagic organisms ($F_{1,40} > 7.5$, $p < 0.0092$ for all compounds; Fig. 3a,b). HCB, PCB-105 and the HCHs' concentrations were higher in sympagic than pelagic organisms ($F_{1,40} > 9.5$, $p < 0.0036$ for all compounds).

The sampling site (station) was only significant for α- and γ-HCH ($F_{1,40} > 17.6$, $p < 0.0002$ for both), with higher concentrations in samples from the Greenland Sea than north of Svalbard.

Spatial variation

From the RDA, only Axis 3 showed a significant influence of sampling station (Table 2), and the compound influencing Axis 3 mostly was α-HCH (Fig. 2b). With all species in the ANOVA, sampling station was only significant for α-HCH and γ-HCH ($F_{1,40} > 17.6$, $p < 0.0002$ for both). In the ANOVA with only *Calanus hyperboreus* and *Apherusa glacialis*, both α- and γ-HCH concentrations were higher in the sympagic *A. glacialis* than in the pelagic *C. hyperboreus* ($F_{1,10} = 45.7$, $p < 0.0001$; $F_{1,10} = 13.9$, $p < 0.0039$, respectively, Table 3); whereas the α-/γ-HCH ratios did not differ between the 2 species and thereby not between the 2 habitats ($F_{1,10} = 3.57$, $p = 0.0880$; Table 3, Fig. 4). However, in both species the α-/γ-HCH ratios were lower in samples collected north of Svalbard than from the Greenland Sea ($F_{1,10} = 171.7$, $p < 0.0001$, Table 3, Fig. 4). The difference was due to higher α-HCH concentrations in animals collected in the Greenland Sea than north of Svalbard ($F_{1,10} = 70.3$, $p < 0.0001$), since no spatial difference was found in the γ-HCH concentration when only *C. hyperboreus* and *A. glacialis* were included in the ANOVA ($F_{1,10} = 0.9$, $p = 0.3541$).

Table 3. Mean ± SE of α- and γ-hexachlorocyclohexane (HCH) lipid adjusted concentrations (ng g^{-1}) and their ratios in *Calanus hyperboreus* (copepodit stage VI, females) and *Apherusa glacialis*

Station	n[a]	α-HCH[b,c]	γ-HCH[b]	α-/γ-HCH[c]
Calanus hyperboreus				
North of Svalbard	4	6.8 ± 0.4	3.8 ± 0.9	2.0 ± 0.3
Greenland Sea	4	14.3 ± 0.6	3.1 ± 0.1	4.6 ± 0.1
Apherusa glacialis				
North of Svalbard	3	11.4 ± 0.5	5.0 ± 0.4	2.3 ± 0.1
Greenland Sea	2	49.7 ± 1.9	9.7 ± 0.5	5.1 ± 0.1

[a]Number of samples of pooled individuals
[b]Higher concentrations in *Apherusa glacialis* than *Calanus hyperboreus* ($p < 0.05$)
[c]Higher concentrations and ratios in organisms from the Greenland Sea than in the north of Svalbard ($p < 0.05$)

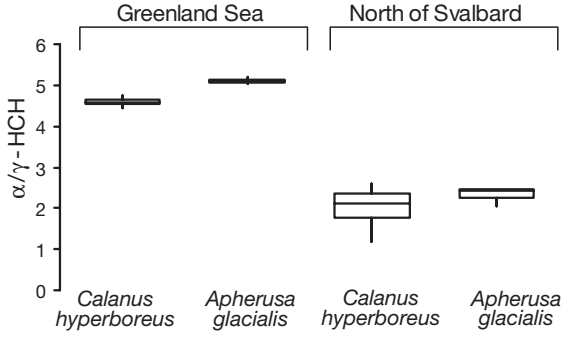


Fig. 4. Spatial difference in α- to γ-hexachlorocyclohexane (HCH) ratios (unitless) in the herbivorous zooplankton *Calanus hyperboreus* and the herbivorous ice-associated amphipods *Apherusa glacialis*

DISCUSSION

Even though the organochlorine concentrations were low and comparable among the species, the explained variation in the organisms' organochlorine load was primarily related to the species' diet, followed by habitat and geographic sampling site.

Diet

Although classification of organisms as either herbivorous or carnivorous eliminates variation in feeding preferences within species and within trophic guilds (Vander Zanden et al. 1997), this classification was sufficient for investigating whether organisms that differ in diets have different contamination loads.

As expected, the organochlorine load was higher in carnivores than in herbivores, with diet correlating strongest to ordination Axis 1 which accounted for most of the explained variation in the organisms' organochlorine concentrations. However, when higher organochlorine concentrations were found in the carnivorous *Parathemisto libellula* relative to the herbivorous *Calanus hyperboreus* in the Northwater Polynya in the Canadian Arctic, the organisms' size was suggested to be more important than trophic position in determining bioaccumulation, since *P. libellula* was larger than *C. hyperboreus* (Fisk et al. 2001a). The elimination rate of organochlorines across the body surface in aquatic invertebrates decreases with increasing body size due to reduced surface-to-volume ratio and lower respiration rate (Landrum 1988, Thomann 1989). Thus, increasing body size would increase the organochlorines' retention time in the body lipids, and lead to increasing concentrations. Since zooplankton feed low in the food web, the direct partitioning process clearing contaminants from the body would not be overshadowed by dietary organochlorine uptake (Fisk et al. 2001a,b). Samples of mixed zooplankton species separated into different size-groups (ranging from 60 to 125 µm to >2035 µm), both from the Canadian Arctic (Hargrave et al. 1992, 2000) and elsewhere (Harding et al. 1997 and references therein), did not show increasing organochlorine concentrations with size group. However, the size groups may have consisted of species with different diets. In the present study, the zooplankton and ice amphipods were sorted into species or genus, and represent organisms of overlapping sizes. Predominantly carnivores with different size did not differ in the concentrations of lipid soluble compounds (e.g. PCB-153 in *Gammarus wilkitzkii* and *Onisimus* spp.), but had higher concentrations than herbivores of similar size (e.g. *Gammarus wilkitzkii* and *Thysanoessa inermis*). Less lipid soluble compounds like HCHs did not differ due to diet. The increase of lipid soluble and persistent compounds with trophic level and comparable levels of more water soluble compounds, is in accordance with earlier studies of bioaccumulation in aquatic organisms with low trophic positions (e.g. Joaquim-Justo et al. 1995, Kaag et al. 1997, Zaranko et al. 1997, Russell et al. 1999).

Additionally, larger individuals within a species are usually older and may have higher concentrations since the organochlorines have been accumulated over a longer period of time. Age-related organochlorine accumulation in Arctic invertebrates does not, however, seem to be of importance, since 3 to 5 yr old *Gammarus wilkitzkii* had comparable concentrations to 2 to 3 yr old *Onisimus* spp. (Borgå et al. 2002a,b) and the

predominately herbivorous 3 to 5 yr old *Calanus hyperboreus* has similar or lower concentrations as the similar sized 1 to 2 yr old *Apherusa glacialis* (present study). Higher organochlorine concentrations in species classified as carnivores relative to organisms classified as herbivores are therefore likely due to feeding higher in the food web.

There is still a lot of unexplained variance in the zooplankton and ice fauna organochlorine data. This may be caused by factors that were not addressed in this study, such as physiological aspects like respiration pathway across gills or the integument, and differences in metabolic rates reflecting activity levels.

Habitat

When analysing all data simultaneously in the RDA, habitat accounted for a smaller part of the explained variance than diet. HCB, γ- and α-HCH correlated strongest with the habitat variable due to higher concentrations of these compounds in sympagic amphipods than in zooplankton. If the release of contaminants from the melting sea ice was important for the accumulation in ice-associated organisms, the contamination load in ice fauna could be higher than in zooplankton, eventually resulting in higher concentrations in ice fauna predators relative to zooplankton predators (Norstrom et al. 1998). The exposure of contaminants due to sea ice concentration, transport and release would especially be high for compounds like DDTs, PCBs and chlordanes, which are entrained into the sea ice adhered to sediments (Aagaard 1994, Schlosser et al. 1995). The ice fauna's concentrations would be higher due to higher exposure directly from the water and/or indirectly from their diet. In the redundancy analysis, however, DDTs, PCBs and chlordanes did not differ between the habitats. HCHs and HCB, which are found in the dissolved phase in surface water under the sea ice, were higher in sympagic amphipods than zooplankton. This difference between the compounds corresponds to differing compounds' vertical distribution in the water column (Tanabe & Tatsukawa 1983, Jantunen & Bidleman 1998, Harner et al. 1999). Less water soluble compounds like DDTs and high chlorinated PCBs have high particle affinity and tend to adsorb to particles and sink to deeper waters, resulting in a relatively homogenous vertical distribution in the water column (Tanabe & Tatsukawa 1983). HCHs and HCB are more dissolved in the water (Jantunen & Bidleman 1998, Lakaschus et al. 2002), have a higher residence time in surface waters and have diminishing concentrations with depth both in ice-covered and open waters (Tanabe & Tatsukawa 1983, Jantunen & Bidleman 1998, Harner et al. 1999). In addition, most of the particles transported by the sea ice will sediment in the marginal ice zone when the ice melts (Hebbeln & Wefer 1991, Ramseier et al. 1999, Hebbeln 2000), making contaminants, adhered to particles and released in the marginal ice zone, unavailable for uptake by ice-associated biota. This pattern, with higher HCH levels and no difference or lower levels of DDTs and PCBs in ice fauna relative to zooplankton, correspond in general to findings from the Canadian Arctic (Hargrave et al. 1992), and also to the comparison of deep-sea fish to surface water fish in the Arctic and elsewhere (Fischer & Ballschmiter 1987, Berg et al. 1997).

The comparable concentrations between zooplankton and sympagic fauna of most compounds, except HCHs and HCB, could also have been caused by interactions between the sympagic and pelagic food webs. Ice algae are considered an important energy source for Arctic zooplankton both in the Greenland Sea (Hobson et al. 1995, Hirche & Kwasniewski 1997, Werner 2000) and the Canadian Arctic (Michel et al. 1996, France et al. 1998). In addition, ice fauna may feed on calanoid copepods (Scott et al. 1999). If interactions between the pelagic and the sympagic food chain were important in determining the organisms' organochlorine content, all contaminants would be expected to be similar between the 2 habitats. However, the organochlorines that dominate sympagic amphipods and herbivorous zooplankton are higher in the former group. Thus, a relatively homogenous vertical distribution of PCBs, DDTs and chlordanes, rather than interaction between pelagic and sympagic food chains, seems to be of importance in organochlorine accumulation.

Therefore, higher HCB and HCH levels in ice-associated amphipods relative to zooplankton were probably caused by the diminishing HCH and HCB levels with depth, which is also found in open waters, rather than an influence of sea ice contamination itself. For the higher trophic levels like seabirds and seals, a diet of ice fauna will increase the exposure to HCHs and HCB, whereas for compounds like DDTs and PCBs, the prey's trophic level is more important than habitat to determine the predator's exposure to contaminants.

Spatial variation

Since γ-HCH concentrations in *Calanus hyperboreus* and *Apherusa glacialis* did not differ between the Greenland Sea and north of Svalbard, the inter-area difference in α-/γ-HCH ratios was not due to different distances to recent use of Lindane. Higher α-/γ-HCH ratios in *C. hyperboreus* and *A. glacialis* sampled in the Greenland Sea relative to north of Svalbard were caused by elevated α-HCH concentrations. Sympagic

amphipods are transported along with their drifting habitat and the spatial variation in α-HCH concentrations could be related to the sea ice's drift path to the sampling stations (Borgå et al. 2002a). *C. hyperboreus* are advected by Polar Surface Water from the core area in the Arctic Ocean to the Greenland Sea (Smith 1988), which may help explain the elevated α-HCH levels in *C. hyperboreus* collected in the Greenland Sea. Due to extensive ice cover in the central Arctic Ocean, exchange of α-HCH from the ocean to the atmosphere is prevented and results in high α-HCH levels relative to adjacent shelf seas with less consolidated ice cover (Jantunen & Bidleman 1996, AMAP 1998, Harner et al. 1999). Thus, the HCH concentrations in organisms drifted with ice or advected with water from the Arctic Ocean reflect the geographic pattern reported for air, water, ringed seals and polar bears with higher α-HCH levels in the central Arctic Ocean and towards the Canadian Arctic relative to the adjacent seas (Jantunen & Bidleman 1996, Harner et al. 1999, Muir et al. 2000, Lie et al. in press).

α-HCH concentrations in water taken in 1994 and 1996 were higher north of Svalbard relative to the Greenland Sea (Jantunen & Bidleman 1996, 1998, Harner et al. 1999). This was contrary to the present results in zooplankton and ice fauna. However, since HCH concentrations vary seasonally and annually (Hargrave et al. 2000), comparison should preferentially be made between water and biota collected simultaneously. Nevertheless, high α-HCH concentrations in biota drifted or advected from the Arctic Ocean to the Greenland Sea may result from zooplankton and sympagic fauna that only slowly reach equilibrium with water, as was suggested for *Calanus hyperboreus* (Fisk et al. 2001b). Based on similar isomeric ratios in zooplankton and water, organochlorine concentration equilibrium between zooplankton and seawater may be reached within 3 to 4 wk (Harding et al. 1997, Hargrave et al. 2000). However, during the 4 wk before sampling, sea ice at the Greenland Sea station had drifted south at least 360 km with the East Greenland Current (from 79° 72′ N, 01° 55′ W; O. Pavlova pers. comm., based on back-trajectory estimation of sea ice drift from satellite data). The ice had drifted from an area of consolidated drift ice (7/10–10/10 ice cover) to an area with open drift ice (4/10–7/10 ice cover; based on routinely produced ice maps by the Norwegian Meteorological Institute [DNMI]). Thus, a change in α-HCH concentrations in water within the Greenland Sea marginal ice zone, due to exchange from water to the atmosphere, may not have been reflected in the biota. During the 4 wk before sampling, sea ice at the station north of Svalbard had drifted west at least 80 km (from 82° 51′ N, 41°89′ E) in an area of open drift ice (4/10–7/10 ice cover). The biota north of Svalbard had therefore been influenced by less consolidated ice cover for a longer period of time and the lower α-HCH levels may reflect the leaking of α-HCH from water to the atmosphere.

Although the α-/γ-HCH ratios were similar in the pelagic *Calanus hyperboreus* and the sympagic *Apherusa glacialis*, both α- and γ-HCH concentrations were higher in *A. glacialis* than in *C. hyperboreus*, corresponding to diminishing HCH concentrations with depth. Similar α-/γ-HCH ratios between *C. hyperboreus* and *A. glacialis* due to feeding by *C. hyperboreus* on ice algae or being present in surface layers was not considered important, since the HCH concentrations in *C. hyperboreus* were lower than in *A. glacialis*. In addition to vertical distribution of HCHs, factors such as size, age and uptake differences may also explain the concentration difference in *C. hyperboreus* and *A. glacialis*. Differences in concentrations due to size-related accumulation (both uptake and clearance) can be excluded since the size was comparable between the species (*C. hyperboreus* 7 to 10 mm, *A. glacialis* 5 to 13 mm). The effect of age-related accumulation leading to increased concentrations with age can also be excluded since female *C. hyperboreus* in the Greenland Sea are 3 to 5 yr old (e.g. Cairns 1967, Hirche 1997) and *A. glacialis* 1 to 2 yr (Poltermann 2000). The absence of gills in *C. hyperboreus* was not considered to result in different HCHs between *C. hyperboreus* and *A. glacialis*, since the HCH levels in *C. hyperboreus* and the gilled pelagic herbivore *Thysanoessa inermis* were similar. Thus, the lower HCH levels in *C. hyperboreus* relative to *A. glacialis* were mainly due to the vertical distribution of both α- and γ-HCH even though the α-/γ-HCH ratios in water changes with depth (Harner et al. 1999). The difference in α-/γ-HCH ratio with depth between water and biota may be due to reduced ability of polar zooplankton to reach equilibrium between body organochlorine concentrations and water.

Organochlorines previously reported in Arctic zooplankton and ice fauna

Due to the laborious work collecting, identifying and sorting zooplankton and sympagic amphipods, and due to the need for a high number of individuals per sample for chemical analysis, few studies have been conducted on the contamination in 'pure' species-samples of Arctic zooplankton and ice fauna (e.g. Fisk et al. 2001b, Moisey et al. 2001, Hoekstra et al. 2002, present study). Some studies of organochlorine load have been carried out on samples of mixed zooplankton (e.g. Hargrave et al. 1992, Borgå et al. 2001). The mean organochlorine levels in Arctic zooplankton and

ice fauna are low, with considerable associated variation around the estimates even within each study (e.g. Fisk et al. 2001b, Moisey et al. 2001). Due to differences between studies such as sample composition (different species, size and age), sampling season, year and the laboratories' organochlorine quantification techniques, comparing levels between studies should be done with caution. Nevertheless, the organochlorine levels were generally within the same range or same order of magnitude as reported in the sympagic amphipod *Gammarus wilktizkii* and mixed zooplankton samples from the Canadian Arctic in the mid-1980s and early 1990s (Bidleman et al. 1989, Hargrave et al. 1992, 2000), from the Barents Sea in the mid-1990s (Borgå et al. 2001, Hop et al. in press), and in single-species samples from the Canadian Arctic in the late 1990s (Moisey et al. 2001, Hoekstra et al. 2002) and the Alaskan Arctic in 2000 (Hoekstra et al. 2002).

CONCLUSION

The organochlorine levels in Arctic zooplankton and sympagic fauna were low, and much of the variance remains unexplained. Nevertheless, the structure of the organochlorine concentrations in zooplankton and sympagic fauna was primarily explained by the species' diet, followed by habitat and geographic sampling site.

The organochlorine concentrations were higher in predominantly carnivores (*Parathemisto libellula*, Chaetognatha, *Gammarus wilkitzkii*, *Onisimus* spp.) than in predominantly herbivores (*Calanus hyperboreus*, *Thysanoessa inermis*, *Apherusa glacialis*), especially the more lipid soluble compounds that are known to biomagnify in the food web.

Habitat accounted for a smaller part of the explained variance than diet, and only a few compounds were found to differ between sympagic and pelagic species. HCB, γ- and α-HCH concentrations were higher in ice-associated amphipods relative to zooplankton, in accordance with the vertical distribution of HCHs and HCB in the water column rather than being exposed to contaminants released from the sea ice.

Higher α-/γ-HCH ratio in samples from the Greenland Sea than North of Svalbard, found in both *Calanus hyperboreus* and *Apherusa glacialis*, reflects the geographic trend reported for air, water, ringed seals and polar bears of high α-HCH concentrations towards the Canadian Arctic and the Arctic Ocean, and lower levels when moving east towards the Barents Sea and the Siberian shelf seas.

Altogether, the small variation in concentrations of highly biomagnifying organochlorines between sympagic and pelagic fauna suggests that occasional selection of sympagic fauna over zooplankton prey by vertebrate predators does not alter or influence the predator's pollutant load to any significant extent. The trophic level a predator feeds upon as well as geographic differences in contaminant distribution seem to be more important for the accumulation of most contaminants.

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