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Stable isotope-guided analysis of congener-specific PCB concentrations in a Japanese coastal food web

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ABSTRACT

Organisms collected from a coastal ecosystem in Japan were analyzed for concentrations of 205 polychlorinated biphenyls (PCBs) congeners; analyses were guided by $\delta^{13}C$ and $\delta^{15}N$ measurements. The regression slopes of log PCB concentration on $\delta^{15}N$ value are regarded as indices of biomagnification in food webs. The slope (wet weight basis) of ΣPCBs was +0.104; the slope (lipid weight basis) was close to zero. Lipid content increased from 0.06% in a primary producer to 8.32% in the highest trophic level consumer. Hence, biomagnification of ΣPCBs (wet weight basis) can be attributed to increase of lipid content through the food web. For most of the congeners, the slopes (wet weight basis) exceeded those (lipid weight basis) by ca. 0.10. Slopes increased with increasing PCB chlorination levels between chlorine numbers 1–6; slopes decreased at higher chlorination levels. This decrease is likely caused by a decrease in membrane permeability with increasing molecular weight.

1. Introduction

Persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane and metabolites (DDTs), and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/DFs) have accumulated to hazardous levels in living organisms. The threats of POPs to environmental and human health led to the establishment of the Stockholm Convention on Persistent Organic Pollutants in 2004. Although the production and use of PCBs has been prohibited in Japan, USA and Europe for >20 yr, relatively high concentrations have been detected continually in various species of fish; contaminated fish represent one of the main human dietary intake routes for PCBs (e.g., Domingo and Bocio, 2007). Consumption of farmed Atlantic salmon is reported to pose health risks through exposure to a variety of POPs including PCBs (Hites et al., 2004).

In the last two decades, the study of biomagnification profiles of POPs including PCBs, organotins and trace elements through aquatic food webs has been guided through stable isotope ratio analysis of bioelements, such as carbon and nitrogen (e.g., Fisk et al., 2001, 2003; Hop et al., 2002; Ruus et al., 2002, 2006; Hoekstra et al., 2003; Naito et al., 2003; Burreau et al., 2004; Evenset et al., 2004; Mackintosh et al., 2004; Wan et al., 2005; Hu et al., 2006; Kelly et al., 2007; Helm et al., 2008; Ikemoto et al., 2008a,b; Murai et al., 2008; Takeuchi et al., 2009). In general, the stable nitrogen isotope ratio $\delta^{15}N$ in consumers increases by 3.4‰ (on average) per trophic level (Minagawa and Wada, 1984). Thus, the value of $\delta^{15}N$ is suitable for determining the trophic position of each organism in a food web. The stable carbon isotope ratio, $\delta^{13}C$, is enriched slightly by about 1‰ per trophic level and, thus, is used mostly to identify primary carbon sources in a food web (DeNiro and Epstein, 1978; Peterson and Fry, 1987).

Food web magnification factors (FWMFs) have been used as indices of trophic level-dependent POPs accumulation in Northwater Polynia (e.g., Fisk et al., 2001). FWMFs was derived from the slope of the POP-trophic relationship determined using $\delta^{15}N$, and provided an overall magnification factor for the food web. FWMFs increased 6-fold from 1.7 in PCB congener IUPAC # (hereafter represented as "#") 97 to 10.7 in #180 (Fisk et al., 2001). The same trend of biomagnification increase with increase in congener number was reported in studies conducted in the Arctic Sea and adjacent areas (e.g., Hop et al., 2002; Ruus et al., 2002; Hoekstra et al., 2003), the Baltic Sea (Burreau et al., 2004), Bohai Bay (Wan et al., 2005) and Tokyo Bay (Naito et al., 2003; Takeuchi et al., 2009). Recently, PCB concentrations were reported to increase with animal size and trophic level [determined by the stable nitrogen isotope ratio of blue fin tuna *Thinness thymus* (Linnaeus 1758)] in the Mediterranean Sea (Corsolini et al., 2007).

The occurrence of 209 PCBs with 1–10 chlorine substitutions suggests a variety of physico-chemical characteristics. For example, log octanol–water partition coefficients (K_{ow}) of PCBs range

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widely from 4.65 to 8.63 in congeners with 1–10 chlorine substitutions (Jäntschi and Bolboacă, 2006). However, most studies dealing with biomagnificaion profiles of PCBs and/or total PCBs have been based on <100 congeners (e.g., Fisk et al., 2001, 2003; Ruus et al., 2002; Evenset et al., 2004; Carubelli et al., 2007; Corsolini et al., 2007), except in the work of Hoekstra et al. (2003) and Hites et al. (2004). Restricted analysis among 209 congeners might lead to underestimation of total PCB concentration and/or indistinctness of the biomagnification trend with increase in congener number.

Hence, we undertook the present study, which was an analysis of 205 congeners (all 1–8 chlorinated PCBs) in organisms from the coastal food web in the innermost part of Ise Bay, central Japan; this study was guided by a concurrent analysis of stable carbon and nitrogen isotope ratios. The congeners analyzed comprise 98% of all known PCBs.

2. Materials and methods

2.1. Sample collection

A variety of biological samples was collected from April 22 to May 1, 2006 (including particulate organic matters (POM), seaweeds, invertebrates and fishes) from the innermost part of Ise Bay facing a central metropolitan region in Japan (35°05′N 136°53′E) (Fig. 1; Table 1). Fish and crustaceans were collected using traps. Mollusk and seaweed samples were collected directly by hand. One large sized individual of *Lateolabrax japonicus* was collected by angling. POM was collected with vertical tows of a North Pacific standard net (NORPAC net) (45 cm i.d. × 180 cm long, 0.10 mm mesh). POM consisted mostly of diatoms. Seawater temperature in the sampling period was 15.5 °C. All samples were kept frozen at −20 °C until dissection and chemical analysis.

2.2. Stable carbon and nitrogen isotope analysis

Muscle was dissected from fishes and invertebrates, except in the case of *Caprella equilibra* (amphipod Crustacea), which was processed whole, as were samples of POM and seaweeds. All materials for analysis were dried at 60 °C (⩾24 h). The lipid was removed by immersion in a chloroform:methanol (2:1) solution for 24 h. In addition, carbonates in the POM, *Caprella* and seaweeds were removed by immersion in a 1 N HCl solution for 24 h.

Stable carbon and nitrogen isotopes in the samples were measured by gas chromatograph–combustion-isotope ratio mass spectrometry (GC–C-IRMS) (PDZ Europa Ltd., ANCA-SL). Stable isotope ratios were expressed by δ as ‰ by

$$\delta X = \left[\left(R_{\text{sample}}/R_{standard}\right) - 1\right] \times 1000 \ (‰)$$

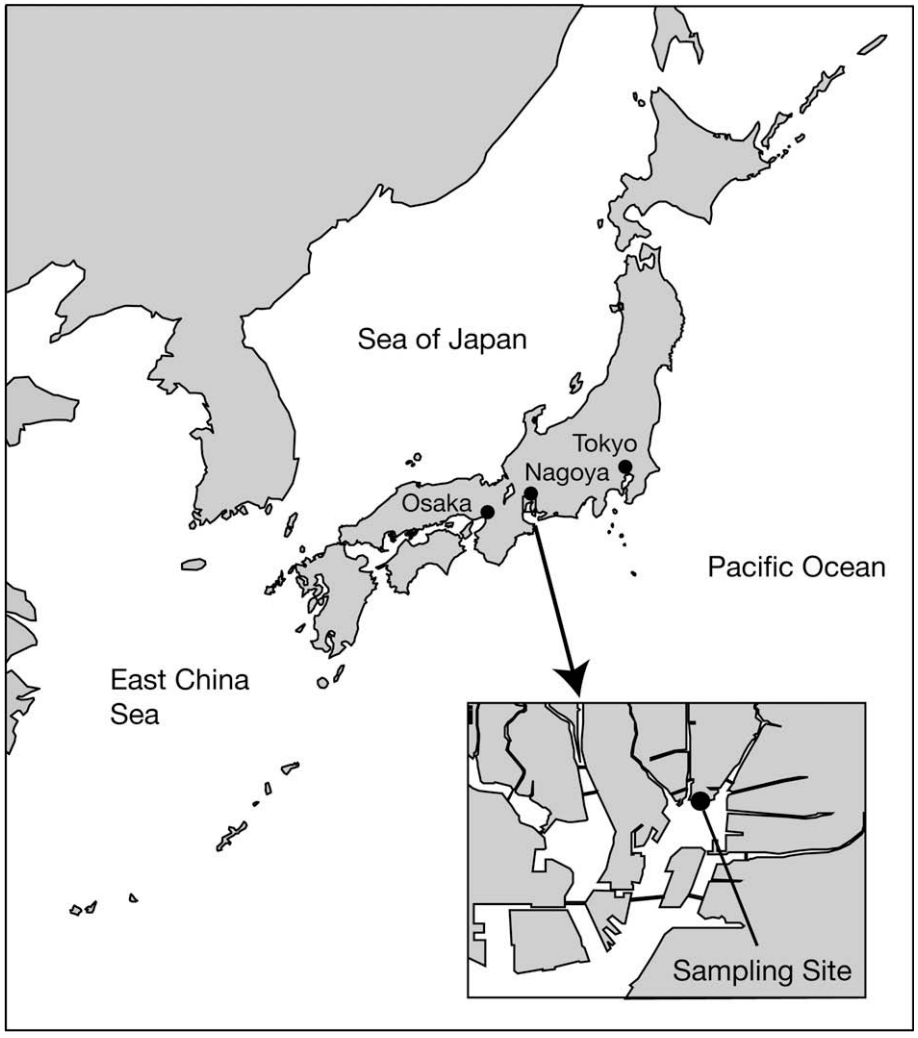

Fig. 1. Sampling location of organisms in the innermost part of Ise Bay, Japan.

Table 1
Biometry of organisms and ΣPCBs concentrations (ng/g wet and lipid weight) from the innermost part of Ise Bay, Japan.

Species	Body length[a] (mm)	Body weight[b] (g)	δ^{15}N ± SD (‰)	δ^{13}C ± SD (‰)	n[c]	Lipid content (%)	Water content (%)	ΣPCBs (ng/g wet weight)	ΣPCBs (ng/g lipid weight)
POM (Particulate Organic Matter)	–	–	−1.4 ± 0.4	−20.6 ± 0.1	6	0.28	85.9	32.81	11716.1
Macro-algae									
Cladophora sp.	–	–	−3.6 ± 2.5	−20.9 ± 0.8	6	0.06	80.8	1.25	2085.0
Ulva sp.	–	–	−0.3 ± 1.7	−20.3 ± 1.8	6	0.06	86.9	0.72	1195.0
Mollusks									
Crassostrea gigas	65.0 ± 13.6	17.2 ± 9.9	1.1 ± 1.1	−19.9 ± 0.3	8	1.27	76.0	112.40	8850.0
Mytilus galloprovincialis	37.9 ± 16.8	4.4 ± 4.9	−1.2 ± 1.2	−20.2 ± 0.4	6	1.32	73.7	61.05	4625.2
Xenostrobus securis	24.2 ± 3.3	0.5 ± 0.2	4.8 ± 1.3	−17.6 ± 0.6	6	0.67	70.5	52.78	7877.2
Crustaceans									
Caprella equilibra	–	–	−2.7 ± 0.2	−17.9 ± 0.3	6	0.56	79.3	32.22	5753.0
Carcines mediterraneus	41.3 ± 10.9	26.0 ± 12.5	8.6 ± 0.4	−16.9 ± 0.9	8	2.58	60.2	78.57	3045.5
Ascidians									
Ciona savignyi	54.0 ± 17.7	5.4 ± 3.9	0.0 ± 1.0	−21.4 ± 0.8	6	0.24	83.0	8.10	3376.7
Fish									
Lateolabrax japonicus large	392.0	570.0	13.5 ± 0.1	−16.1 ± 0.2	6	8.32	70.3	415.67	4996.0
Lateolabrax japonicus juvenile	29.7 ± 2.6	0.3 ± 0.1	8.5 ± 0.9	−17.6 ± 0.3	6	2.48	76.8	127.20	5129.2
Pleuronectes yokohamae	49.0 ± 7.1	1.4 ± 0.6	6.5 ± 1.0	−18.1 ± 0.4	6	1.09	69.3	99.35	9114.8

[a] Fish total length, and mollusk length of shell (mean ± SD).
[b] Mollusk soft issue (mean ± SD).
[c] Number of samples for stable isotope analysis.

where X is ^{15}N or ^{13}C, R_{sample} is the corresponding ratio ^{15}N/^{14}N or ^{13}C/^{12}C of the sample. PeeDee Belemnite (PDB) and atmospheric nitrogen were used as $R_{standard}$ for carbon and nitrogen, respectively. L-Histidine (δ^{13}C–PDB (‰) = −10.18; δ^{15}N–Air (‰) = −7.81; Shoko Co., Ltd., Minato-ku, Tokyo) was included as an internal standard every 10th sample to check analytical accuracy. The precisions of the δ^{15}N and δ^{13}C measurements were ±0.1‰ and ±0.2‰, respectively.

2.3. Measurement of lipid content

Approximately 10 g of each whole homogenized sample (or dissected soft tissue for bivalves) were mixed with 100 g anhydrous sodium sulfate; lipids were extracted into 150 ml of dichloromethane from 100 g anhydrous sodium sulfate. One extraction took 2 h. This extraction was repeated three times for each sample of anhydrous sodium sulfate. The dichloromethane extract was filtered through a glass filter and dried under a nitrogen gas current. The extracted lipids in the dichloromethane extract were measured gravimetrically.

2.4. Congener-specific analysis of PCBs

Concentrations of each PCB congener were determined using the procedure provided by the Environmental Agency of Japan (1998), with some modifications. Approximately 10 g of each whole homogenized sample (or soft tissue for bivalves) were dissolved in 1 N KOH/ethanol, and 500 pg of each ^{13}C-labelled internal standard (#3, #15, #28, #54, #118, #153, #180, #194, #208 and #209) were added to them. After digestion at room temperature, pure water (50 ml) was added to digests, and PCBs were extracted with hexane (50 ml). This extraction was repeated three times. The extracted solution was mixed with sulfuric acid and the separated hexane layer was rinsed with pure water, then dried by passing it through anhydrous sodium sulfate in a glass funnel. The solution was concentrated to about 2 ml. The extract was cleaned with activated silica gel (2 g) in a column. PCBs were eluted with 5% dichloromethane (DCM)/hexane (100 ml). The solution was again concentrated to about 2 ml. Further interfering substances in the extract were removed by passage through a column of activated alumina (9 g). PCBs were eluted with 50% DCM/hexane (100 ml). The solution was concentrated to about 2 ml. ^{13}C-labelled internal standard (#138) in decane (100 μl) was added to the solution as a syringe spike to correct for injection error. The solution was then concentrated to 100 μl with nitrogen gas. Identification and quantification of each congener were performed with a high-resolution gas chromatograph (HRGC; HP6890 series, Agilent Technologies, Inc., Santa Clara, USA) equipped with a high-resolution mass spectrometer (HRMS; JMS-800D, -700D, JEOL Ltd., Akishima, Tokyo, Japan) at resolution more than 10,000. A HT8-PCB capillary column (Kanto Chemical Co., Inc., Chuo-ku, Tokyo, Japan) was used for gas chromatographic separation of target congeners.

Procedural blanks were analyzed simultaneously with every batch of three or four samples to check for interference or contamination from solvents and glassware. The recovery rate was 96.3 ± 9.0%. The detection limit was 0.002 ng/g wet for each congener of PCB.

2.5. Statistical analysis

One-half of the detection limit was substituted for those values below the limit of detection; these half-detection limit values were used in obtaining ΣPCBs and for statistical analyses of each congener. When >50% of the observations were below the detection limit, further statistical analyses were not conducted. The linear regressions of log-transformed concentrations of PCBs (wet or lipid weight bases) on δ^{15}N were calculated as follows:

$$\mathrm{Log}_{10}(\Sigma\mathrm{PCBs\ or\ each\ congener\ concentration}) = a(\delta^{15}\mathrm{N}) + b$$

The slope of the each regression, a, was regarded as an index of biomagnification for total PCBs and for each conger. These analyses were done with InStat software (version 3.0, Graphpad Software, Inc., San Diego, California, USA). The slopes of the regressions on wet and lipid weight bases were compared against log K_{ow}, chlorination number and molecular weight.

3. Results

3.1. Food web structure

Samples of the fish, *L. japonicus*, were divided into a large individual and juveniles based on body size. The values of δ^{15}N and δ^{13}C in

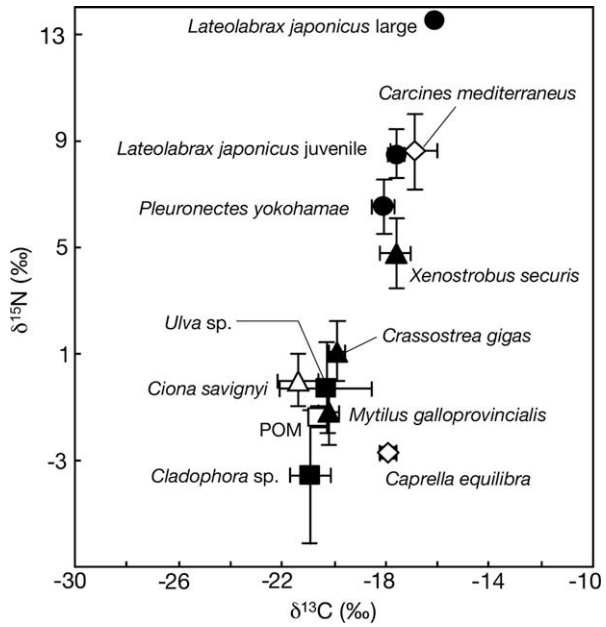

Fig. 2. Stable isotope diagram of the aquatic food web in the innermost part of Ise Bay, Japan: δ^{15}N (‰; mean ± SD) versus δ^{13}C (‰; mean ± SD).

the organisms ranged from −3.6 ± 2.5‰ to 13.5 ± 0.1‰ and from −21.4 ± 0.8‰ to −16.1 ± 0.2‰, respectively (Table 1, Fig. 2). The "map" resulting from the plot of δ^{15}N against δ^{13}C indicated that the primary producers (POM, *Cladophora* sp. and *Ulva* sp.) fell within a narrow range of coordinate space on the "map".

As indicated in the introduction, δ^{15}N in consumers increases 3.4‰ on average relative to prey eaten (Minagawa and Wada, 1984). The primary producer range was −3.6‰ to −0.3‰, with an average of −1.8‰. The primary consumers, viz. the two species of mollusks (*Crassostrea gigas* and *Mytilus galloprovincialis*), *Caprella equlibra* (amphipod crustacean) and *Ciona savignyi* (ascidian) were in the range −2.7‰ to 1.1‰, with an average of −0.7‰. Juveniles of *L. japonicus*, *Pleuronectes yokohamae*, *Carcinus mediterraneus* (decapod crustacean) and *Xenostrobus securis* (mollusk) were secondary and tertiary consumers (4.8–8.6‰), while a large *L. japonicus* was at the highest trophic level (13.5‰). Thus, the distributions of δ^{15}N values revealed a food web comprising 4–5 trophic levels.

3.2. Lipid content

Lipid contents as a proportion of wet weight (%) increased >100-fold from 0.06% in seaweed (primary producer trophic level) to 8.32% in a large *L. japonicus* (highest trophic level consumer) (Table 1). Lipid content increased exponentially with δ^{15}N (Fig. 3a).

3.3. Biomagnification profiles of ΣPCBs

Among 205 congeners of 1–8 chlorinated biphenyls, we detected 160 that were above the detection limit in at least one biological sample. One-half of the respective detection limit was substituted for those values below the limit of detection and was used in the sum of PCBs and in statistical analyses. The concentration of ΣPCBs (sum of 205 congeners) in organisms ranged from 0.72 ng/g wet weight in *Ulva* sp. to 415.67 ng/g wet weight in *L. japonicus* (Table 1). On a lipid weight basis, the concentration of ΣPCBs in organisms ranged from 1195.0 ng/g lipid weight in *Ulva* sp. to 11716.1 ng/g lipid weight in POM, indicating that the lipid based biomagnification profile differed from that based on wet weight (Table 2).

ΣPCBs on a wet weight basis increased significantly with increasing δ^{15}N (slope = 0.104, $p = 0.015$); on lipid weight basis,

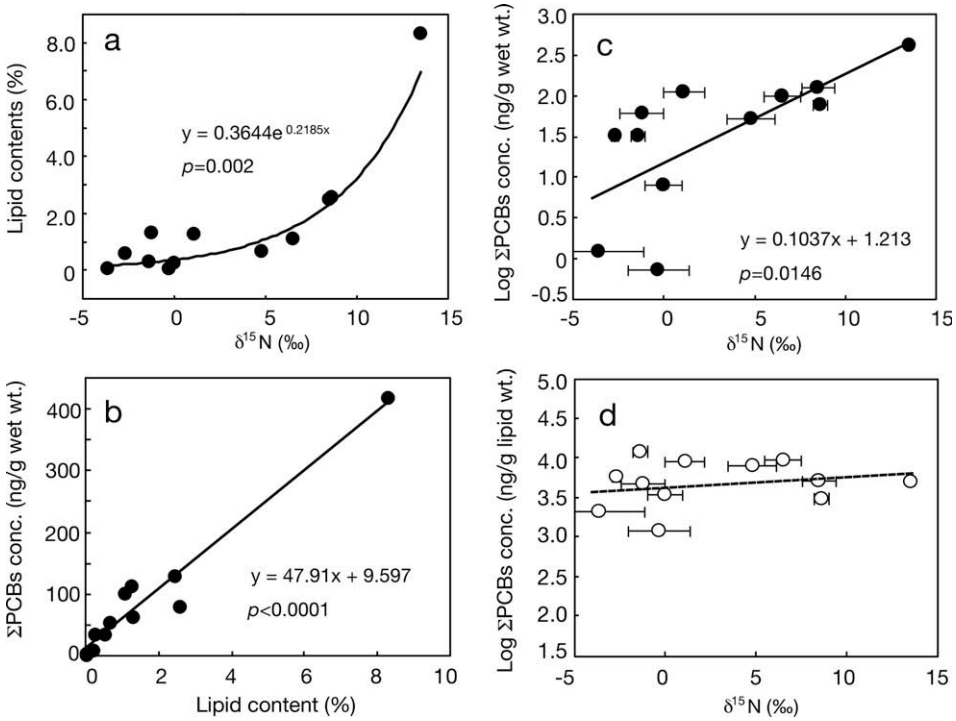

Fig. 3. Relationships of δ^{15}N (‰), lipid contents (%) and log ΣPCBs concentrations in organisms collected from the innermost part of Ise Bay, Japan; (a) relationships between δ^{15}N and lipid contents; (b) relationships between lipid contents and log ΣPCBs concentrations (ng/g wet weight); (c) relationships between δ^{15}N and log ΣPCBs concentrations (ng/g wet weight); (d) relationships between δ^{15}N and log ΣPCBs concentrations (ng/g lipid weight).

Table 2
Statistics for the regression between $\delta^{15}N$ values and $\log_{10}$ PCB concentration (ng/g wet or lipid weight bases).

IUPAC, No	Wet weight basis		Lipid weight basis		IUPAC, No	Wet weight basis		Lipid weight basis	
	Slope	*p*-value	Slope	*p*-value		Slope	*p*-value	Slope	*p*-value
ΣPCBs[a]	0.104	0.015	0.009	0.608	#71, 72[b]	0.092	0.055	−0.003	0.896
1 chlorinated congener (mono-chlorinated congener)					#74	0.114	0.015	0.019	0.345
#1	0.032	0.159	−0.063	0.013	#77	0.076	0.100	−0.019	0.417
#2	−0.023	0.305	−0.118	<0.001	5 chlorinated congener (penta-chlorinated congener)				
#3	−0.005	0.808	−0.100	<0.001	#82	0.095	0.039	0.001	0.983
2 chlorinated congener (di-chlorinated congener)					#83	0.110	0.015	0.015	0.456
#4	0.092	0.073	−0.003	0.938	#85	0.122	0.007	0.027	0.133
#5	−0.022	0.659	−0.117	0.012	#86, 97, 116, 117, 125[b]	0.055	0.034	−0.040	0.088
#6	0.031	0.548	−0.064	0.100	#87, 111, 115[b]	0.111	0.015	0.016	0.436
#7	0.031	0.269	−0.064	0.025	#90, 101[b]	0.141	0.003	0.047	0.077
#8	0.060	0.150	−0.035	0.134	#91	0.112	0.017	0.017	0.439
#9	0.044	0.154	−0.051	0.074	#92	0.122	0.008	0.027	0.187
#10	0.038	0.163	−0.057	0.029	#93, 95, 98, 102[b]	0.111	0.016	0.016	0.454
#11	−0.040	0.468	−0.134	0.018	#94	0.098	0.004	0.003	0.871
#12, 13[b]	−0.026	0.547	−0.121	0.003	#96	0.066	0.111	−0.029	0.343
#15	0.007	0.856	−0.088	0.010	#99	0.130	0.005	0.035	0.090
3 chlorinated congener (tri-chlorinated congener)					#100	0.130	0.001	0.035	0.189
#16	0.049	0.289	−0.046	0.197	#103	0.146	0.001	0.051	0.055
#17	0.065	0.106	−0.030	0.200	#105	0.121	0.007	0.026	0.152
#18	0.066	0.103	−0.029	0.235	#109	0.127	0.007	0.032	0.120
#19	0.039	0.334	−0.056	0.093	#110, 120[b]	0.112	0.016	0.017	0.427
#20, 33[b]	0.017	0.765	−0.078	0.101	#112, 119[b]	0.123	0.003	0.028	0.088
#22	0.068	0.092	−0.027	0.182	#114	0.120	0.006	0.026	0.126
#25	0.050	0.202	−0.045	0.032	#118	0.124	0.007	0.029	0.137
#26	0.069	0.088	−0.026	0.184	#122	0.019	0.621	−0.076	0.040
#27	0.056	0.254	−0.038	0.273	#123	0.111	0.005	0.016	0.218
#28	0.096	0.021	0.001	0.956	#124	0.089	0.025	−0.005	0.746
#31	0.056	0.151	−0.040	0.052	#126	0.081	0.019	−0.014	0.473
#32	0.066	0.120	−0.029	0.267	6 chlorinated congener (hexa-chlorinated congener)				
#36	0.076	0.121	−0.019	0.478	#128	0.136	0.007	0.041	0.121
#37	0.041	0.420	−0.054	0.150	#129	0.119	0.001	0.024	0.184
4 chlorinated congener (tetra-chlorinated congener)					#130	0.150	0.002	0.055	0.079
#40	0.053	0.363	−0.042	0.384	#131	0.088	0.005	−0.007	0.718
#42	0.101	0.032	0.007	0.755	#132	0.125	0.020	0.030	0.326
#44	0.095	0.044	0.000	0.995	#133	0.130	<0.001	0.035	0.097
#45	0.068	0.124	−0.027	0.318	#134	0.102	0.015	0.007	0.767
#46	0.061	0.241	−0.034	0.384	#135	0.131	0.003	0.036	0.110
#47, 48, 75[b]	0.105	0.018	0.010	0.600	#136	0.111	0.020	0.016	0.594
#49	0.101	0.022	0.006	0.746	#137	0.131	0.001	0.036	0.055
#51	0.078	0.111	−0.017	0.587	#138	0.134	0.003	0.039	0.059
#52, 69[b]	0.100	0.016	0.005	0.749	#141	0.146	<0.001	0.051	0.056
#53	0.072	0.128	−0.023	0.462	#144	0.147	0.002	0.052	0.053
#55	0.081	0.145	−0.014	0.745	#146	0.125	0.002	0.030	0.063
#56	0.062	0.154	−0.033	0.165	#149	0.168	0.007	0.073	0.149
#58	0.072	0.197	−0.023	0.590	#151	0.152	0.002	0.057	0.068
#59	0.091	0.064	−0.004	0.889	#153	0.178	0.004	0.083	0.104
#60	0.120	0.011	0.025	0.210	#154	0.132	0.001	0.037	0.095
#63	0.115	0.013	0.020	0.312	#156	0.131	0.003	0.036	0.086
#64	0.098	0.035	0.003	0.907	#157	0.115	0.001	0.020	0.154
#66	0.110	0.018	0.015	0.457	#158	0.134	0.002	0.039	0.052
#67	0.066	0.114	−0.029	0.158	#163, 164[b]	0.137	0.003	0.042	0.073
#68	0.122	0.010	0.027	0.220	#167	0.134	0.002	0.039	0.080
#70	0.071	0.096	−0.023	0.269	7 chlorinated congener (hepta-chlorinated congener)				

(*continued on next page*)

Table 2 (*continued*)

IUPAC, No	Wet weight basis		Lipid weight basis		IUPAC, No	Wet weight basis		Lipid weight basis	
	Slope	*p*-value	Slope	*p*-value		Slope	*p*-value	Slope	*p*-value
#170	0.141	0.001	0.046	0.090	#190	0.135	0.001	0.040	0.221
#171	0.131	0.002	0.036	0.081	#191	0.109	<0.001	0.014	0.503
#172	0.139	<0.001	0.044	0.180	#193	0.163	<0.001	0.068	0.070
#174	0.155	0.001	0.060	0.126	8 chlorinated congener (octa-chlorinated congener)				
#175	0.105	<0.001	0.010	0.591	#194	0.110	0.018	0.015	0.691
#176	0.113	0.005	0.018	0.485	#195	0.069	0.200	-0.026	0.608
#177	0.139	0.004	0.044	0.087	#196	0.126	0.001	0.031	0.354
#178	0.139	0.001	0.044	0.125	#198	0.151	0.001	0.056	0.177
#179	0.133	0.005	0.038	0.161	#199	0.157	0.001	0.062	0.134
#180	0.176	0.001	0.081	0.040	#200	0.050	0.249	-0.045	0.280
#182, 187[b]	0.159	0.003	0.064	0.093	#201	0.094	0.001	-0.001	0.979
#183	0.152	0.003	0.057	0.116	#202	0.106	0.001	0.011	0.599
#185	0.128	<0.001	0.033	0.292	#203	0.136	0.001	0.041	0.242
#189	0.097	<0.001	0.002	0.943					

[a] Sum of 205 congeners, N.D, were regarded as a half of detection limit.
[b] The congeners were came in succession the peak.

ΣPCBs concentrations changed little with increasing $\delta^{15}N$ (slope = 0.009, p = 0.608) (Fig. 3c and d). Thus, it is clear that ΣPCBs in the lipids did not biomagnify through the food web and the high biomagnification of ΣPCBs on the wet weight basis is attributable to an increase in lipid content with increasing trophic level (Fig. 3b).

3.4. Biomagnification profiles of single PCB congeners

Among the 160 congeners, 144 were detected in >50% of the biological samples and were used in regressions analysis between $\delta^{15}N$ and log-transformed PCB concentrations (wet or lipid weight bases). On a wet weight basis, 138 congeners had regression slopes >0 (Table 2, Fig. 4). Slopes of the regressions < 0 were restricted to 1 and 2 chlorinated congeners (#2, #3, #5, #11, #12 and #13). On a lipid weight basis, slopes > 0 were restricted to 90 congeners. Slopes of the regressions >0 were restricted to mostly >5 chlorinated congeners (Table 2, Fig. 4). The regression slopes on a lipid weight basis decreased 0.096 ± 0.009 on average (±S.D.) from those on wet weight bases.

Relationships between the slopes of individual congeners plotted against the number of chlorine substitutions revealed peaks in both wet and lipid weight based plots (Fig. 4a). Relationships were as follows:

- Congeners with 1 to 6 chlorine substitutions;

Wet weight basis; $Y = 0.0267\,X_2 - 0.0260$ ($r^2 = 0.65; p < 0.001$).

Lipid weight basis; $Y = 0.0267\,X_2 - 0.1209$ ($r^2 = 0.65; p < 0.001$).

- Congeners with 7 to 8 chlorine substitutions;

Wet weight basis; $Y = -0.0264\,X_2 + 0.3221$ ($r^2 = 0.19; p = 0.025$).

Lipid weight basis; $Y = -0.0264\,X_2 + 0.2271$ ($r^2 = 0.19; p = 0.025$)

where X_2 is the number of chlorines.

Relationships between the slopes of individual congeners against molecular weight (Mackay et al., 1992) and log K_{ow} (Jäntschi and Bolboacă, 2006) showed the same trend, with the peak of molecular weight at 360.9 and a log K_{ow} of 7.5 (Fig. 4b,c).

- Molecular weight ≦360.9

Wet weight basis; $Y = 0.0008\,X_3 - 0.1455$ ($r^2 = 0.65; p < 0.001$).

Lipid weight basis; $Y = 0.0008\,X_3 - 0.2405$ ($r^2 = 0.65; p < 0.001$).

- Molecular weight > 360.9

Wet weight basis; $Y = -0.0008\,X_3 + 0.4398$ ($r^2 = 0.19; p = 0.025$).

Lipid weight basis; $Y = -0.0008\,X_3 + 0.3448$ ($r^2 = 0.19; p = 0.025$).

- log $K_{ow} \leqq 7.5$

Wet weight basis; $Y = 0.0495\,X_4 - 0.2157$ ($r^2 = 0.62; p < 0.001$).

Lipid weight basis; $Y = 0.0495\,X_4 - 0.3106$ ($r^2 = 0.62; p < 0.001$).

- log K_{ow}>7.5

Wet weight basis; $Y = -0.023\,X_4 + 0.2963$ ($r^2 = 0.02; p = 0.658$).

Lipid weight basis; $Y = -0.023\,X_4 + 0.2013$ ($r^2 = 0.02; p = 0.658$).

where X_3 and X_4 are molecular weight and log K_{ow}, respectively.

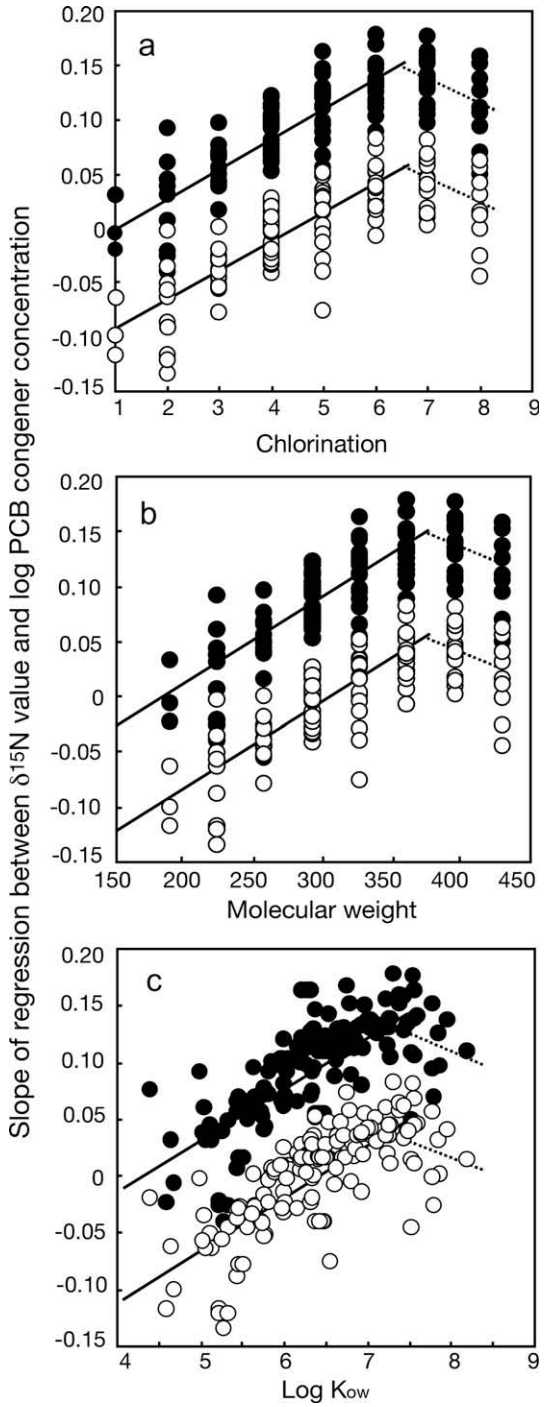

Fig. 4. Relationship between the slope of the regression of log PCB concentration on physico-chemical parameters; (a) chlorination; (b) molecular weight; (c) log K_{ow}. (black symbol, wet weight basis. White symbol, lipid weight basis).

3.5. Composition of PCBs by chlorine number

Since 205 congeners of PCBs were analyzed in the present study, compositions of individual congeners were summarized as the number of chlorine substituted PCBs.

The compositions of PCB congeners in the organisms at higher trophic levels were dominated by 4–6 chlorinated congeners, while in those of organisms situated at lower trophic levels, ratio of more than 4 chlorinated congeners decreased (Fig. 5). For example, 1–3 chlorinated congeners decreased from 33.4% in *Cladophora* sp. to 8.1% in a large *L. japonicus*, while 4–8 chlorinated congeners increased from 66.1% in seaweed to 91.9% in a large *L. japonicus*.

4. Discussion

The present study indicates that the ΣPCBs expressed on a lipid weight basis (the sum of 205 congeners of 1–8 chlorinated biphenyls) did not biomagnify through the coastal food web, while the food web biomagnification of PCBs based on wet weight was attributable to the increase in organism lipid content with increasing trophic level. In "Our Stolen Future", Colborn et al. (1996) summarized biomagnifications profiles of PCBs through the Lake Ontario food web. They suggested that PCB concentrations in a bird (herring gull) and a large fish (lake trout) increased ca. 100,000 and 10,000 times above those in phytoplankton (lipid weight basis), respectively. Recently, the Stockholm Convention on Persistent Organic Pollutants Statement noted that "POPs including PCBs and DDTs are chemicals that remain intact in the environment for long periods, become widely distributed geographically, accumulate in the fatty tissue of living organisms and are toxic to humans and wildlife". However, the present study shows that, for this coastal food web in central Japan, biomagnification profiles for ΣPCBs are not concordant with this view.

Why did we find no biomagnification of total PCBs based on lipid weight? One of the fundamental reasons is that there are no standards on the number of congeners to be included in estimates of the total PCB concentration. Furthermore, ca. 40% of the PCB congeners (especially 1–3 chlorinated congeners) had regression slopes < 0 in plots of log PCB concentrations (on a lipid weight basis) against δ^{15}N (see Fig. 4a, Table 2). The decrease of higher chlorinated congeners might be due to their larger molecular size. A final reason was that there were no air-breathing animals in the food web we studied.

Although PCBs comprise 209 congeners, most published studies, even those appearing after 2000, deal with <100 (e.g., Fisk et al., 2001, 2003; Ruus et al., 2002; Evenset et al., 2004; Carubelli et al., 2007; Corsolini et al., 2007). Notable exceptions are the works of Hoekstra et al. (2003) [126 congeners] and Hites et al. (2004) who included all congeners. The ratio of total PCBs based on congeners analyzed in the above studies to those of the present study was estimated by inserting the congeners analyzed in earlier works into our data base, and then comparing results with those from 205 congeners analyzed in the present study. This comparison shows that the concentration of total PCBs in previous work has been underestimated by 16.8% to >60% (Table 3). Thus, variation in the number of PCB congeners analyzed obscures the concept of "Total PCBs".

In general, concentration of PCBs has been reported to gradually increase with increase in trophic level (due to high log K_{ow}) (e.g., Fisk et al., 2001, 2003; Hop et al., 2002; Naito et al., 2003; Burreau et al., 2004; Mackintosh et al., 2004). Most of these studies indicate that the increase in congener concentrations with increasing trophic level is dependent on the PCB congener number (#). For example, Naito et al. (2003) working on the food web in the northern part of Tokyo Bay reported a slope of <2 for the regression of log-transformed PCB concentrations on δ^{15}N for #81 and #77, and >2 for congeners #105 to #189. The mechanisms by which chemical substances accumulate after transmission through the food web were studied in a series of modeling exercises and laboratory experiments by Gobas (e.g., Gobas et al., 1988, 1993, 1999). Gobas et al. (1993) presented fish food intake efficiencies of hydrophobic and organic chemicals. They showed that intake efficiency increased with increasing log K_{ow}, and the equations derived provided explanations for accumulation of several hydrophobic halogenated aromatic hydrocarbons through the food chain. The

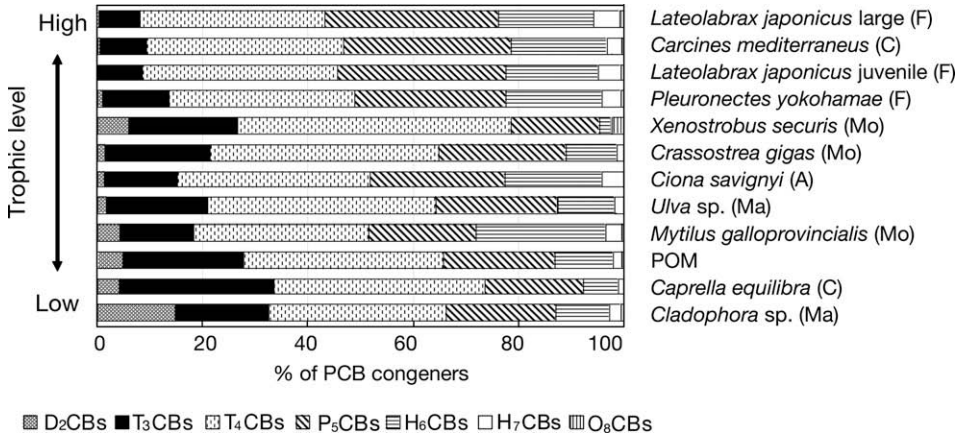

Fig. 5. Composition of PCB congeners (%) in organisms collected from the innermost part of Ise Bay, Japan. Upper case letters in parentheses indicate: A, Ascidian; C, Crustacean; F, Fish; Mo, Mollusk; Ma, Macro-alga (D_2CBs, 2 chlorinated congeners; T_3CBs, 3 chlorinated congeners; T_4CBs, 4 chlorinated congeners; P_5CBs, 5 chlorinated congeners; H_6CBs, 6 chlorinated congeners; H_7CBs, 7 chlorinated congeners; O_8CBs, 8 chlorinated congeners).

Table 3
The ratio of the total PCBs based on congeners analyzed in representative previous studies as a proportion of total PCBs based on the 205 congeners (our study).

Number of congeners analyzed	Ranges of congeners analyzed	Ratios[a]	Reference	Remarks
209	1–10CBs	100	Hites et al. (2004)	Salmoms collected from various countries
126	2–10CBs	83.6	Hoekstra et al. (2003)	Southern Beaufort-Chukchi Seas
89	1–9CBs	76.4	Fisk et al. (2003)	Canadian and Alaskan Arctic Seas
59	1–10CBs	62.2	Carubelli et al. (2007)	Sea-bass from central Italian coast
43	3–9CBs	61.7	Fisk et al. (2001)	Northwater Polynya
34	3–10CBs	49.2	Ruus et al. (2002)	Southeastern Norway
43	4–9CBs	35.2	Corsolini et al. (2007)	Bluefin tuna from the Mediterranean Sea
7	5–7CBs	16.8	Evenset et al. (2004)	Bjørnøya (Bear Island), Norway

[a] The ratio was estimated by inserting the congeners analyzed by each study into the data base of our present study, and compared with the total PCBs based on the analysis of 205 congeners.

increase in chemical substance concentrations with increasing trophic level is attributed to an increase in the chemical's fugacity through food digestion in the gastrointestinal tract, and this fugacity increase enables simple passive transmission across the intestinal wall, leading to a concentration in the predator that exceeds the concentrations of corresponding chemicals in its prey.

In the present study, however, the slope of the log PCB concentrations against δ^{15}N increased in 1–6 chlorinated congeners (following the trend in previous studies), while there was a decrease in the slopes of more highly chlorinated congeners.

Kannan et al. (1998) reported that in bioaccumulation studies of highly chlorinated PCBs in organisms from the coastal area of southeastern Georgia, USA 8–10 chlorinated PCBs did not reach concentrations that could be predicted from log K_{ow}. Kannan et al. (1998) hypothesized that the steric factors of superhydrophobic chemicals are major factors inhibiting biomagnification in organisms situated at high trophic levels. Most polychlorinated naphthalones (PCN), depending on their hydrophobicity, accumulate rapidly in fish, whereas for the two congeners of hepta- and octa-chloronaphthalenes, there are no detectable concentrations in the fishes (Opperhuizen et al., 1985). The degree of ΣPCNs biomagnification was significantly lower than that of the total *n*/*m*-*o* PCBs through the food web of Lake Ontario (Helm et al., 2008). These studies suggested that a loss of membrane permeation for hydrophobic molecules with diameters >0.95 nm reduces accumulation (Opperhuizen et al., 1985) and less bioavailability of ΣPCNs in fish than PCBs (Helm et al., 2008).

The present study, which analyzed biomagnification profiles of 205 congeners, clearly showed increasing biomagnification inhibition with increasing congener chlorination number (>6) even though log K_{ow} increased for congener chlorination numbers above 6. This indicates that biomagnification inhibition increases with increasing membrane permeation (due to larger molecular size). This trend is also apparent with increasing chlorination levels of PCB congeners in organisms at higher trophic levels in the food web we studied. Thus, on a lipid weight basis, slopes >0 were restricted to 90 congeners of 144 analyzed so that there was no biomagnification through the coastal food web.

Published biomagnification profiles of highly chlorinated PCB congeners (>8 chlorines) are rare. In addition to Kannan et al. (1998), Mackintosh et al. (2004) mentioned that the slopes of the regressions of log-transformed concentrations of congeners #194 and #209 on δ^{15}N were lower than the slope of congener #180. However, neither Kannan et al. (1998) nor Mackintosh et al. (2004) included critical congeners that would have reduced the biomagnification predicted by log K_{ow}.

Recently, Kelly et al. (2007) showed that there is a difference in the biomagnification profiles of POPs between an aquatic piscivorous food web (water-respiring organisms only) and a combined marine mammalian food web (including water-respiring and air-breathing organisms) in northern Canada. In the aquatic piscivorous food web, no biomagnification occurred for less hydrophobic chemicals with a log $K_{ow} < 5$ and superhydrophobic substances with log $K_{ow} > 8$. However, in the marine mammalian food web, chemicals with log K_{ow} between 3 and 8 biomagnified to a high degree because of their high octanol–air partition coefficient (K_{oa}) and correspondingly low rate of respiratory elimination to air. We included no marine mammals in our study. Hence, we were dealing with an aquatic piscivorous food web for which K_{oa} is not relevant, and the regression slope trends of log PCB concentrations against δ^{15}N are well represented by increasing log K_{ow}. Thus, on a lipid weight basis, slopes >0 were restricted to 90 congeners of 144

analyzed so that there was no biomagnification through the coastal food web.

In conclusion, the total concentrations of PCBs on a lipid weight basis did not biomagnify through a coastal food web which lacks air breading animals. There was an increase of biomagnification for 1–6 chlorinated congeners, but there was a decrease in 7–8 chlorinated congeners, which may be connected to increase in molecule size with chlorination level. Moreover, most recent studies, including those published since 2000, have underestimated concentrations of ΣPCBs. In order to measure ΣPCB concentrations and/or biomagification profiles, an analysis of more than 200 congeners (including highly chlorinated species) is necessary.

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