VARIATIONS IN ORGANOCHLORINE CONCENTRATIONS AND PATTERNS WITH GROWTH AND TROPHIC STATUS OF TIDAL FLAT AND COASTAL SPECIES FROM THE ARIAKE SEA, JAPAN

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Introduction

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) are endocrine disrupters which are known to resist chemical and biological degradation in the environment. Because of their lipophilic nature, they tend to accumulate in higher trophic animals through food chains. The features and kinetics of OCs accumulation in gill-breathing fishes have been studied under laboratory and field conditions[1,2]. The uptake and elimination of OCs in fishes is explained by equilibrium partitioning between lipid in organisms and ambient water[3]. In addition, growth rate, sex and physiological functions (fat content, spawning, migration etc.) can also determine the residue levels of OCs. However, little information is available on the accumulation patterns of OCs in tidal flat organisms.

The ecology of tidal flat fishes differs from other marine fishes in a number of ways. Due to their amphibian-like behavior, they have capillary vessels in their skin that enable them to breathe in the terrestrial tidal environment. Their food habit ranges from benthic seaweed and zoo benthos with sediment particles in intertidal flats. These facts suggest the possibility that tidal flat fishes may have a specific mechanism of OCs accumulation.

In this study, we analyzed PCBs and organochlorine pesticides, such as DDTs, chlordanes (CHLs), HCB and HCHs in herbivore mudskippers (*Boleophthalmus pectinirostris*) collected from tidal flat of the Ariake Sea, Japan. This species is suitable to examine growth-dependent accumulation of OCs because their age could be measured by counting the number of marks on the second pectoral radius. We also analyzed OCs in sediments, other tidal flat species (mussels, oysters, worms, omnivore mudskippers, tidal flat fishes and crabs) and coastal animals (mallards, black-headed gulls, finless porpoises and hammerhead sharks) collected from the same region to understand the variations in their concentrations and congener patterns with trophic levels.

Materials and Methods

The herbivore mudskippers (*B. pectinirostris*) were collected from the Ariake Sea, western Japan in 1999. Twenty-eight individuals of fishes were selected to measure their age by counting the number of marks on the pectoral bone by the method of Washio *et al.*[4]. Sediments and tidal flat species (mussels, oysters, worms, herbivore and omnivore mudskipperes, tidal flat fishes and crabs) and coastal animals (mallards, black-headed gulls, finless porpoises and hammerhead sharks) were collected from the Ariake Sea during 1999 and 2001. In this study, we analyzed the whole bodies of tidal flat species and liver tissues for coastal animals, respectively.

Chemical analysis of OCs followed the method previously described Nakata *et al.*[5]. Several to twenty grams of tissues were ground with sodium sulfate and extracted with mixed solvents of dichloromethane and hexane (8:1) using a Soxhlet apparatus. Lipid contents were measured from the concentrated aliquots of these extracts. Seven ^{13}C labeled CB mixtures (CB-28, 52, 101, 153, 138, 180 and 209) were spiked to the extracts as the surrogates, and it was transferred to a glass column packed with Florisil (Wako Pure Chemical, Japan), followed by elution with acetonitrile containing 20% hexane-washed water. The eluates were collected in a separatory funnel and transferred to hexane. The hexane extracts were passed through a florisil-packed glass column for fractionation of OCs. The first fraction eluted with hexane contained HCB, PCB congeners, *p,p'*-DDE and *trans*-nonachlor, while the second fraction eluted with 20% dichloromethane in hexane contained chlordane compounds (*trans*-, *cis*-nonachlors and chlordanes and oxychlordane), *p,p'*-DDD, *p,p'*-DDT and HCHs (α-, β- and γ- isomers). These fractions were injected into GC-MSD (Hewlett Packard 6890 GC coupled with a 5973 MSD) and GC-ECD (Hewlett Packard 6890 GC coupled with a ^{63}Ni ECD) for quantification. A capillary column, DB-1 (J&W Scientific Inc. USA) fused silica capillary column (30 m, 0.25 mm id.) was used in this study. Recovery percentages of PCB congeners were ranged from 80 to 100 %. In case of organochlorine pesticides, the recoveries of target analytes through this method ranged from 90-110 %, and their concentrations were not corrected for recovery percentages. A procedural blank was analyzed with every set of six samples to confirm interfering peaks in chromatograms and to correct sample values, if necessary. The detection limit was 0.04 ng/g for DDTs, 0.03 ng/g for HCHs, 0.02 ng/g for CHLs, 0.01 ng/g for HCB and PCBs.

Results and Discussion

<u>Growth-dependent accumulation of OCs in tidal flat mudskippers</u>

OCs were detected from all mudskipper samples analyzed in this study. A significant positive relationship between body length and PCB concentrations was observed in mudskippers (Fig. 1). Similar results were also observed for the relationships between body length and the concentrations of *p,p'*-DDE (r=0.81, p<0.01), *trans*-nonachlor (r=0.77, p<0.01) and *cis*-nonachlor (r=0.76, p<0.05).

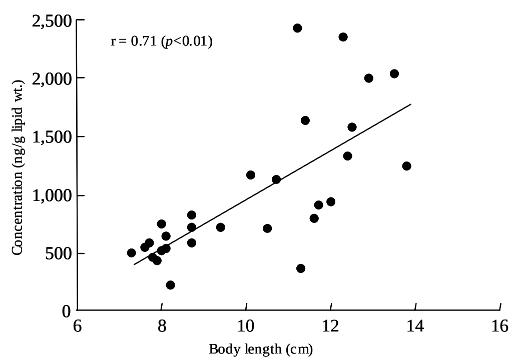

Fig. 1 Relationship between PCBs concentrations and body length of tidal flat mudskippers (*B. pectinitrostris*) collected from the Ariake Sea, Japan (n=28)

These observations suggest that mudskipper fishes accumulate persistent organoclorines with growth, which is similar to that reported for male aquatic mammals[6]. In contrast, a negative correlation between body length and HCB concentrations was found in mudskippers ($r=-0.76$, $p<0.01$), which may due to biodegradation of HCB in this species. Age-dependent accumulations of OCs were also observed in herbivorous mudskippers, but high correlations have been found between concentrations and body length rather than age. This may indicate that the growth rate, which is strongly influenced by the feeding rate, seems to be the predominant factor in determining OCs accumulation. These results also suggest the possibility that most of the OCs were derived from the diet with less contribution from the uptake through ambient water.

Concentrations and compositions of OCs in sediments and biota

OCs were detected in sediments and all tidal flat and coastal organisms analyzed in this study. The average concentrations are shown in Table 1. In general, greater concentrations of OCs were found in coastal species than tidal flat organisms, suggesting the bioaccumulation of these contaminants through the food chains. It is interesting to note that hammerhead sharks showed the highest residue levels of PCBs (15,000 ng/g), DDTs (8,700 ng/g) and CHLs. (2,400 ng/g). While there is a general lack of data of OCs in shark species, relatively greater concentrations and ranges of PCBs were also found in adipose tissues and livers of other sharks from different locations [7,8].

Table 1 Average concentrations of PCBs and organochlorine pesticides (ng/g lipid or carbon wt. basis) in sediments, tidal flat and coastal species from the Ariake Sea, Japan

	n	Fat (%)	PCBs	DDTs	CHLs	HCB	HCHs
TIDAL FLAT							
Sediment*	2	2.0#	NA	160	64	9.4	24
Worm	2	0.8	NA	970	220	12	15
Mussel	2	0.9	300	400	140	1.4	2.0
Oyster	2	0.8	250	1,000	220	1.9	10
Crab	2	0.4	480	570	260	12	23
Mudskipper (herbivore)	5	3.8	360	780	190	6.2	25
Mudskipper (omnivore)	2	3.8	540	430	170	4.8	12
Tidal flat fish	2	2.1	670	900	240	6.1	7.1
COASTAL $							
Mallard	2	4.8	63	85	71	3.0	12
Black-headed gull	2	7.0	2,200	1,200	180	210	90
Finless porpoise	2	4.0	2,800	1,300	910	160	100
Hammerhead shark	2	48	15,000	8,700	2,400	140	7.4

*: Concentrations were presented by carbon wt basis. #: Carbon %. $: All of coastal water species were analyzed for liver samples. NA: Not analyzed.

The PCB profiles of tidal flat and coastal water organisms were compared by principal component analysis. Samples were separated into four groups according to their trophic levels (Fig. 2). While there is a wide range of plots in group A, lower chlorinated tri-, tetra- and penta- CB (IUPAC 28+31, 70, 66, 101 etc.) were dominant congeners in tidal flat organisms (Group A). In contrast, CB-153 and 138 were dominant in all coastal species. Additionally, mono-*ortho* congeners, CB-118 and 115, hexa-CB (#128) occupied greater compositions in mallards (Group B). The congener patterns in black-headed gulls were almost comparable with those of hammerhead sharks (Group C). In finless porpoises, hepta- and octa-CBs were the major components of PCBs. There could be several factors responsible for the differences in the PCB congeners among species, such as differences in their habitat, diet and metabolism. Based on the structure-activity relationship of PCB congeners with H atoms in the molecule and their positions[9], hammerhead sharks seemed to have greater metabolic capacities of PB type enzymes, which was comparable to those of terrestrial mammals.

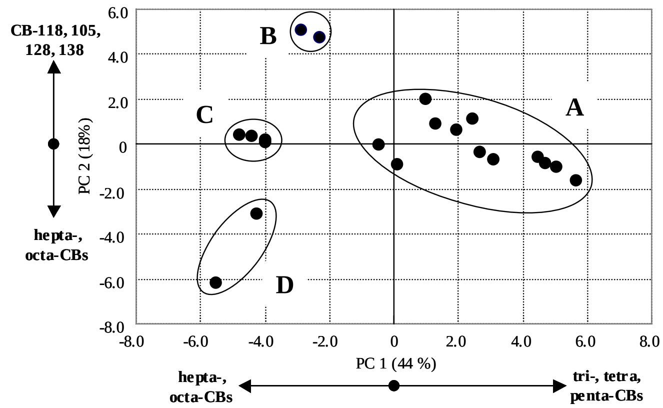

Fig. 2 Score plot of PCA applied to PCB congener compositions in tidal flat and coastal water organisms. Group A: Tidal flat organisms. B: Mallards. C: Black-headed gulls and Hammerhead sharks. D: Finless porpoises.

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