

## BIOREMEDIATION POTENTIAL OF OYSTERS IN WATER AND SEDIMENT CONTAMINATED BY PERSISTENT ORGANIC POLLUTANTS: A PILOT FIELD STUDY

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### Abstract

Uptake and depuration of polychlorinated biphenyls (PCBs) were examined in oysters to evaluate their potential for bioremediation of water and sediment contaminated by persistent organic pollutants. Oysters collected from unpolluted areas in the Ariake Sea, Japan were transplanted into a polluted site, and cultured for 4 weeks. The samples were then re-transplanted to water tanks containing either unpolluted natural seawater or artificial seawater to depurate PCBs in oyster. The concentrations of PCBs in transplanted oysters increased from 4.3 ng/g (wet wt.) to 16 ng/g during a four-week field exposures. On the other hand, PCB concentrations in re-transplanted oysters declined dramatically from 16 ng/g to 1.1 ng/g during a four-week experiment in the unpolluted seawater tank. The decline of PCB residues was also observed in oysters kept in an artificial seawater tank (from 16 ng/g to 3.2 ng/g). Based on the data, it is estimated that oyster have the ability to remove PCBs in water and sediment at a rate of 1.0 to 1.2 mg per 8 weeks in this study. While the concept of using animals for bioremediation is not widely recognized, these results imply that oysters may be effective bioremediators of persistent pollutants in the aquatic environment.

### Introduction

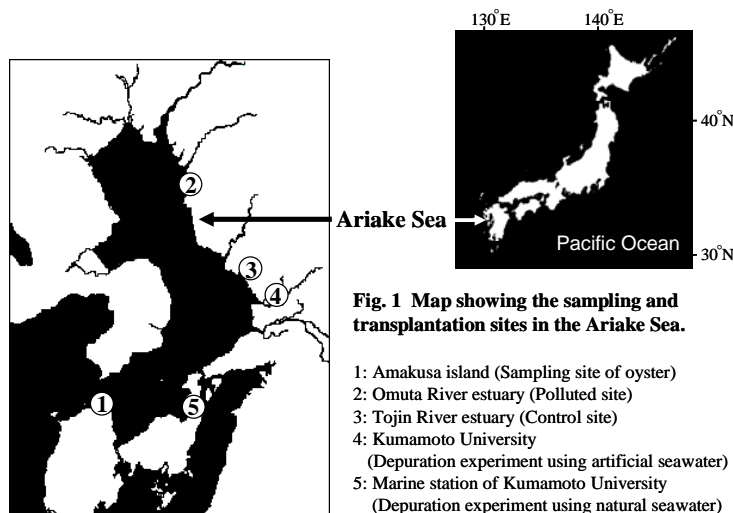
Bivalves are useful indicators to monitor the contamination by persistent organic pollutants in the aquatic environment<sup>1,2</sup>. A large number of 'Mussel Watch' studies have reported the concentrations and distribution of halogenated contaminants in the United States<sup>3</sup> and Asian coastal waters<sup>4,5</sup>. As bivalves are filter feeders, they accumulate persistent organochlorines, such as PCBs, and the concentrations in mussel and oyster reach equilibrium with the surrounding water within a few to several ten days<sup>6-8</sup>. Further, contaminant concentrations in bivalves decrease when sample is transplanted to uncontaminated site, due to natural depuration of pollutants. This implies that bivalves may play a role of 'removal pumps of pollutants' in the aquatic environment and can be used as effective bioremediators.

Bioremediation is the application of living organisms to remove and detoxify contaminants in the environment. While bacteria are the most popular organisms used in bioremediation, bivalves have also reported to be suitable bioremediators in the marine environment. For example, each ton of pearl oyster harvested resulted in 703g metals and 7,425 g nitrogen being removed from an estuary in the east coast of Australia<sup>9</sup>. However, little investigations have focused on using bivalves as bioremediators of persistent organic contaminants in the environment. This study was designed to examine the uptake and depuration rates of PCBs in oyster during transplantation experiments in both field and laboratory conditions. Information on the uptake-depuration of PCBs

in oyster has allowed us to evaluate the bioremediation potential in water and sediment contaminated by PCBs.

### Materials and Methods

**Samples:** A transplantation experiment was conducted in the Ariake Sea, Japan during October and December, 2006. Oysters collected from Amakusa Islands, an uncontaminated area, (St. 1, Fig. 1), were transplanted to the Omuta River (polluted site; St. 2) and Tojin River (control site; St. 3), respectively. Oysters in both stations were sampled at 1, 2, 3, and 4



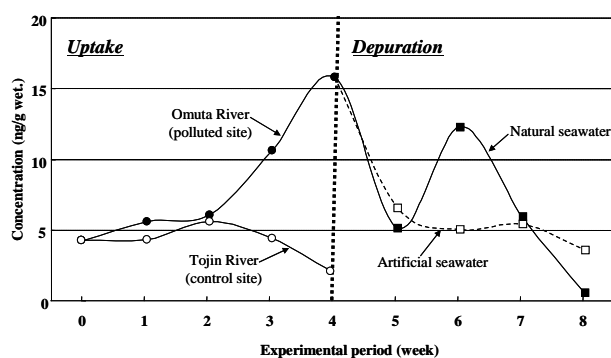
weeks after initial transplantation. The remaining Omuta River oysters were then re-transplanted to water tanks containing either unpolluted natural seawater (at Marine station of Kumamoto University, St. 5) or artificial seawater (at Kumamoto University, St. 4). Samples were also taken during the 1, 2, 3, 4 week of the re-transplantation. The survival rate of oysters was checked during the experiment. All samples were stored at  $-20^{\circ}\text{C}$  until analysis.

**Chemical Analysis:** PCBs were analyzed according to the methods described previously with some modifications<sup>10</sup>. Briefly, approximately 3-5 g of soft tissues of oysters was ground with sodium sulfate and extracted with mixed solvents of dichloromethane and hexane using an accelerated solvent extractor (ASE-100, Dionex, CA, USA). Lipid in the sample extract was removed by gel permeation chromatography using a Bio-beads SX-3 (Bio-Rad Laboratories, Hercules, CA, USA) packed glass column. The eluted solvent was concentrated, and passed through a Florisil packed glass column. Determination of PCBs was performed by GC-MSD (Agilent 6890 and 5973 Series), and the column used was BPX-5 fused silica capillary column (30 m x 0.25 mm i.d., 0.25  $\mu\text{m}$  of film thickness; SGE Co Ltd. Australia). The mean and standard deviation of PCB recovery were  $83 \pm 18\%$  in this study.

### Results and Discussion

#### Uptake and depuration of PCBs:

The survival rate of oyster during the uptake-depuration experiment was 96 % (total number of oysters tested: 160). This implies that oysters were not stressed seriously during both field and laboratory experiments. No significant variation of lipid contents was found in oysters in this study.

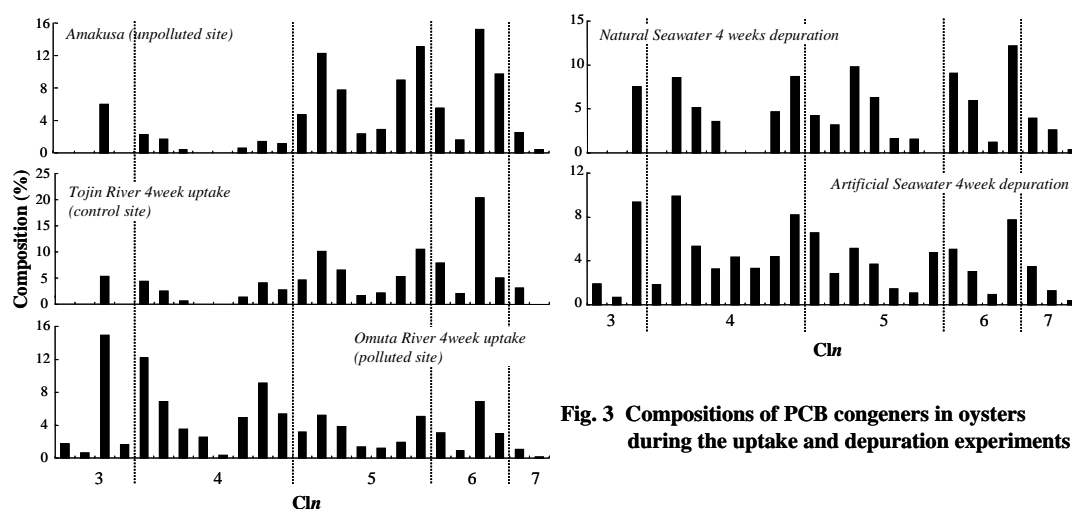


**Fig. 2** Concentrations of PCBs in oysters during the uptake and depuration experiments

PCBs were detected in all samples analyzed in this study. The variations in PCB concentrations in oysters during the experiment are shown in Figure 2. The concentrations of PCBs in Omuta River oysters were constant until 2 week of the transplantation, but the levels increased dramatically at 4<sup>th</sup> week of the experiment. In contrast, PCB concentrations decreased in oysters from Tojin River estuary (control site) during the experiment, suggesting less contamination by PCBs in this river.

Oyster samples in Omuta River estuary were re-transplanted to water tanks containing either less polluted natural seawater or artificial seawater to depurate PCBs in samples. The PCB concentrations in oysters kept in natural seawater declined dramatically from 16 to 1.1 ng/g in 4<sup>th</sup> weeks, although the levels were rather abnormal at the 2<sup>nd</sup> week (Fig. 2). Similarly, PCB concentrations in oysters decreased to 3.2 ng/g during 4 weeks in water tank with circulating artificial seawater. These results indicate that PCB depuration can be achieved in oysters in water tanks containing both natural and artificial seawaters.

**PCB compositions:** The PCB isomer and congener compositions in oysters during the uptake and depuration experiment are shown in Figure 3. Tetra- and hexa-chlorinated congeners were dominant in oysters from Amakusa Islands, and this profile was almost similar to those found in the Tojin River at 4<sup>th</sup> week of the experiment. In contrast, tri- and tetra-chlorobiphenyls were the major congeners in Omuta River oysters after 4 week of the transplantation. The percentages of tri- and tetra chlorobiphenyls in samples were 19 % and 45 % of the total PCB concentrations, respectively. This result suggests the presence of local sources of lower chlorinated PCBs in the Omuta River. Previous studies have reported that PCB concentrations in Omuta River sediments were extremely high (2,200 ng/g dry wt.), and the congener compositions resembled to those of Kanechlor-400<sup>10</sup>. The PCB profiles in oysters after 4 weeks in natural seawater suggested depuration of lower chlorinated congeners in samples.



**Fig. 3** Compositions of PCB congeners in oysters during the uptake and depuration experiments

**Bioremediation potential:** Our results suggest that oyster may be used to remediate water and sediment contaminated by PCBs. In this study, bioremediation potential of oyster to PCBs was quantitatively estimated (Table 1). The soft tissue weight of an oyster was approximately 20g in this study. Assuming that the numbers of oysters contained in a plastic cage and of plastic cages set out in the Omuta River per experiment were 200 and 20, respectively, it was estimated that oysters have the ability to remove 1.0 to 1.2 mg of PCBs in water and sediment in Omuta River estuary within a period of 8 weeks.

This is a preliminary study to investigate bioremediation potential of oyster in aquatic environment contaminated by PCBs. While the concept of using animals for bioremediation is not recognized well, oyster may play a major role as 'removal pumps of contaminants' and as suitable bioremediators in the environment.

**Table 1** Estimation of bioremediation potential of oyster in water and sediment contaminated by PCBs

• Differences of PCB concentrations in oysters of Omuta River during the dupuration phase	
Natural seawater	: $16 - 1.1 = 14.9$ (ng/g wet wt.)
Artificial seawater	: $16 - 3.2 = 12.8$ (ng/g wet wt.)
• Average weight of soft tissue in a oyster : 20 g	
• Number of oysters kept in a plastic cage : 200 individuals	
• Number of plastic cages set in the field : 20 cages	
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<b>Total amount of PCBs removal (natural seawater)</b>	: $14.9 \times 20 \times 200 \times 20 = \mathbf{1.2 \text{ mg}}$
<b>Total amount of PCBs removal (artificial seawater)</b>	: $12.8 \times 20 \times 200 \times 20 = \mathbf{1.0 \text{ mg}}$

#### *Acknowledgements*

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