INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed common persistent organic chemicals, the major sources of which include natural and anthropogenic combustion processes, and they drained into natural waters through both point and non-point source discharges (Manoli and Samara, 1999). Global discharges of PAHs into aquatic environment have been estimated over 80,000 tons per year (Wright and Welbourn, 2002). PAHs are hydrophobic chemicals, and tend to be associated with fine particles in environment, and known to be genotoxic and carcinogenic. They exist abundantly not only in sediment but also in the surface water and suspended charges.

ABSTRACT — We estimated acute toxicity of benzo[a]pyrene (B[a]P) using two cladoceran species, Ceriodaphnia reticulata and Daphnia magna, and also analyzed its impact on zooplankton community throughout an exposure experiment using small-scale mesocosms. LC₅₀ of B[a]P for C. reticulata and D. magna was 4.3 and 4.7 μg/l, respectively. However, individuals fed with Chlorella showed higher LC₅₀, 6.1 μg/l for C. reticulata and 8.0 μg/l for D. magna. In the exposure experiment, we examined the impact of B[a]P on zooplankton community using conceivable concentrations in the environment (5 and 10 μg/l) using typical zooplankton community in eutrophicated systems. Despite the residence time of B[a]P in the water column was short as < 4 days, application of B[a]P induced decrease of zooplankton abundance. However, the recovery pattern was different among cladocerans and rotifers. Consequently, B[a]P showed insecticide-like impacts, suppressing cladoceran populations and inducing the dominance of rotifers particularly under high concentration (10 μg/l). Results have suggested that, even such short duration of B[a]P in the water body can have impact on zooplankton abundance and community structure. Since B[a]P easily precipitate to the bottom and rapidly disappears from the water body, careful monitoring and further assessment of the potential toxicity of polycyclic aromatic hydrocarbons are necessary.

Key words: Mesocosm, Cladocera, Rotifer, Principle response curves

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particulate matters in the water column (Guo et al., 2007; Zhou and Maskaoui, 2003). Thus, PAHs can be an important pollution source having potential hazardous impact on aquatic organisms.

As the toxicity of PAHs, characteristic suite of abnormalities and gene expression in fish and bivalve species were reported (Incardona et al., 2004; Pariseau et al., 2011), and its possible negative impact on aquatic ecosystem has been suggested. However, there have been few studies on toxic effect of PAHs on zooplankton community that plays a central role in lake food web. Since zooplankton community is composed of species having different sensitivities to toxic chemicals and has different roles in the ecosystem, toxic chemicals often modify the structure and function of plankton community through their selective effects (Hanazato, 2001). In particular, information on the responses of zooplankton abundance and community structure to PAHs exposure is still insufficient. Thus, it is necessary to evaluate the response of zooplankton to PAHs in community and individual level to understand the hazardous impacts of PAHs on aquatic ecosystems.

In the present study, we tested the toxicity of one of the most toxic PAHs, benzo[a]pyrene (B[a]P), which is specified as a persistent toxic substance (PTS), and its negative impact on lake organisms has been of great concern due to its high toxicity and lipophilic characteristics (Miller and Ramos, 2001). We carried out community-level experiments by exposing common freshwater species, small cladocerans and rotifers, to B[a]P in small-scale mesocosms where we could easily control water temperature, food availability and the concentrations of toxic substances.

**MATERIALS AND METHODS**

The acute toxicity tests were followed by OECD standard procedure (OECD, 2004). Less than 24-hr-old Ceriodaphnia reticulata which originated from Lake Suwa and Daphnia magna strain (NIES clone) were used as test organisms. Genus Ceriodaphnia is widely distributed species having medium body size among cladoceran genera, and often dominates zooplankton community in natural lakes. The stock solution of B[a]P was diluted with MeOH to six concentrations with dilution rates of 2.0 (1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 μg/l), and the solvent control (10 ppm, MeOH) was prepared as well. In this study, we add the Chlorella as feed in mesocosms experiment. Thus, we have to know about the effects of Chlorella to the tolerance of B[a]P to lake organisms. Accordingly, we also carried out the acute toxicity test adding 5 × 10^4 cells/ml of Chlorella. At 48 hr after the start of the exposure, mortality was analyzed, and 48-hr-LC50 values with 95% confidence intervals were determined by Probit analysis with program EcoToc-Statics Version 2.5 (Oita Univ., Japan). The EC50/LC50 values for the other plankton species were surveyed by using the US Environmental Protection Agency’s ECOTOX database (http://www.epa.gov/ecotox).

The community-level mesocosm experiment was carried out for 39 days in indoor culture experiments space in Institute of Mountain Science, Shinshu University. Six 20-l cylindrical polyethylene tanks (diameter, 30 cm; high, 31 cm) were used as the mesocosms. To establish the zooplankton communities, 1 kg of bottom mud collected from the eutrophic Lake Suwa (36 2 N, 138 5 E), Japan, containing resting stages of zooplankton was placed in each mesocosm. All the mesocosms were kept in a temperature-controlled room (20°C) with a photoperiod of 16 hr light and 8 hr dark. Throughout the experimental periods, all mesocosms were weakly aerated to maintain the enough concentration of dissolved oxygen. The green alga Chlorella (Chlorella Industry Co. Ltd, Fukuoka, Japan) was added to the mesocosms tanks to final density of approximately 1.0 × 10^5 cells/ml on day 10 and every three days thereafter to feed zooplankton constantly. On day 25, B[a]P were added to the mesocosm, and mesocosms were divided into three groups: high (10 μg/l) and low (5 μg/l) B[a]P concentration mesocosms and control mesocosms with two replicates, respectively. B[a]P was well mixed with Chlorella, and introduced to the mesocosms to prevent B[a]P from absorbing to the wall of mesocosm tank. Concentration levels of B[a]P were determined by acute toxicity test using D. magna and C. reticulata.

Zooplankton were sampled from each mesocosm on days 14, 19, and 24 (before B[a]P application), and 25, 28, 30, 33, 36, and 39 (after B[a]P application). One liter of mesocosm water was collected and filtered through a 40-μm mesh net and preserved with sugar-formalin at a final concentration of 4%. The fixed samples were concentrated by settling for 24 hr and zooplankton abundance and species composition were analyzed.

Total of 30 ml of the mesocosm water was sampled from one representative mesocosm tank of each treatment on day 24, 25, 28, and 30, and the sampled water was mixed with 30 ml of 1 M KOH to dissolved the organic particles. Then, the samples were poured into separation funnel and mixed with 10 ml of hexane. After shaking the separation funnel carefully, B[a]P were extracted into the hexane layer. The extracted operation was repeated 3 times. After extraction, hexane layer were added and concentr-
ed by rotary evaporator. Then, extracts were redissolved into 1 ml MeOH to analyze B[a]P concentration. HPLC with fluorescence detector (Shimadzu, Kyoto, Japan, RF-10AXL), equipped with ODS column (Kanto Kagaku Reagent, Mighty sil RP-18 GP 150-2.0 (5 μm)) were used to analyze B[a]P. Conditions of analysis were isocratic mode of 100% MeOH at a flow rate of 0.2 ml/min with excitation and emission wavelength as 295 nm and 405 nm.

The Principal Response Curves (PRC) method, which was specially designed for micro- and mesocosm studies (Van den Brink and Ter Braak, 1999), was used for the interpretation of zooplankton community responses to B[a]P application. The PRC method is based on the ordination technique partial redundancy analysis, which is a constrained form of principal component analysis. The PRC technique uses dimension reduction to summarize all information on the investigated populations simultaneously, so as to elucidate effects of treatments at the community level. The PRC method plots the first principal component of the treatment effects (Cdt: canonical coefficients) against time, expressing the treatment effects as deviations from the control. As a result, the vertical axis of a PRC diagram contrasts each treatment with the control. When the coefficients (Cdt) are plotted against the sampling date, curves are obtained, one for each treatment, that can be interpreted as the principal response curves of the community. A set of species weights is shown on the right side, and the species weights can be interpreted as the affinity of each species with the diagram (for detail information about the PRC technique, see Van den Brink and Ter Braak, 1999). The higher the weight, the more the actual response pattern of the species is likely to follow the pattern in the PRC. Taxa with a high negative weight are inferred to show the opposite pattern, whereas taxa with near zero weight either show no response or a response that is unrelated to the pattern shown by the PRC. The significance of the PRC diagram can be tested by performing a Monte Carlo permutation of the mesocosms, i.e., by permuting whole time series, in the partial RDA from which PRC is obtained, using an F-type test statistic based on the eigenvalue of the component. For the analysis of plankton populations, the abundances were log (x+1) transformed, centered over time and standardized before the analysis. The analysis was performed using CANOCO 4.5 for Windows (Biometris, Netherlands).

**RESULTS AND DISCUSSION**

The 48-hr-LC50 values of *C. reticulata* and *D. magna* were 4.3 and 4.7 μg/l, respectively (Table 1). On the other hand, after the supply of food, Chlorella in the experiment, 48-hr-LC50 values were 6.1 and 8.0 μg/l for *C. reticulata* and *D. magna*, respectively, indicating that 48-hr-LC50 values of lake organisms increase under favorable food condition. From these results, we set up the experimental concentration of B[a]P as 10 μg/l and 5 μg/l for high and low concentration mesocosms, respectively.

Before the zooplankton communities in the mesocosms were exposed to B[a]P (day 24), concentration of B[a]P in

| Table 1. Acute toxicity data for tested species in the present study, and other plankton species from previous studies |
|-------------|-------------|-------------|-------------|-------------|
| Species | Procedure | Food condition | Reference |
| | Endpoint | Exposure period | Without food | With food (Chlorella) |
| Zooplankton<sup>a</sup> | | | |
| *Ceriodaphnia reticulate* | LC<sub>50</sub> | 48 hr | 4.3 μg/l | 6.1 μg/l | Present study |
| *Daphnia magna* | LC<sub>50</sub> | 48 hr | 4.7 μg/l | 8.0 μg/l | Trucco et al. (1987) |
| *Daphnia pulex* | LC<sub>50</sub> | 4 d | 5 μg/l | - | Forget-Leray et al. (2005) |
| *Eurytemora affinis* | LC<sub>50</sub> | 4 d | 58 μg/l | - | |
| Algae and Cyanobacteria<sup>b</sup> | | | |
| *Anabaena flosaquae* | EC<sub>50</sub> | 3 d | 4000 μg/l | - | Schoeny et al. (1988) |
| *Chlamydomonas reinhardtii* | EC<sub>50</sub> | 3 d | 4000 μg/l | - | |
| *Pseudokirchneriella subcapitata* | EC<sub>50</sub> | 3 d | 15 μg/l | - | |
| *Scenedesmus acutus* | EC<sub>50</sub> | 3 d | 5 μg/l | - | |

<sup>a</sup> observation on individual behavior or survival.<br/><sup>b</sup> observation on population growth.
all the mesocosms were below detection limit. After exposure (day 25), concentration of B[a]P was 4.1 μg/l for high concentration and 1.1 μg/l for low concentration mesocosms, and below detection level in control mesocosms. On day 28, the concentration of B[a]P was 0.1 μg/l for high concentration mesocosms and below detection level for low concentration and control mesocosms. On day 30, B[a]P were not detected in all mesocosms. The concentration of B[a]P was lower than the established points and disappeared immediately from the water body. Since B[a]P is hydrophobic substances (log Kow = 6.44), and was introduced to the mesocosms mixed with *Chlorella*, it can be presumed that the introduced B[a]P that were adsorbed to food particles was consumed by zooplankton and precipitated to the bottom sediment.

Although the residence time of B[a]P in water column was short, the abundance and species composition of zooplankton community showed the differences between control and treated mesocosms. Application of B[a]P decreased the densities of rotifers and cladocerans in both low and high concentrations even during the short period of our experiment. Unlike the control, where cladoceran species (mainly *Bosminopsis deitersi* and *Diaphanosoma brachyurum*) increased and completely dominated zooplankton community as was normally found in zooplankton succession pattern in natural condition of mesocosms (Hanazato, 2001), the increase of cladocerans was delayed and their abundance was quite lower in both low and high concentration of B[a]P (Fig. 1). On the other hand, opposite pattern was observed in rotifer populations. Since we prepared only two replicate mesocosms for each treatment, statistical comparison on the difference in their abundances was not possible. However, the result of PRC analysis for the zooplankton species composition data set showed a clear concentration-dependent deviation in the low and high concentration treatments from that in the controls (Fig. 2). Of the total variance, 44.3% could be attributed to B[a]P treatment by observing the significance of canonical axes obtained by the permutation test ($P = 0.0020$). Small rotifer species, *Lepadel-
la spp. and Monostyla spp. showed negative weights in accordance with negative values in canonical coefficient in the diagram, indicating increased abundance of the taxon in the B[a]P-treated mesocosms. However, cladoceran species including B. deitersi, D. barchyurum and Bosmina fatalis with high positive weights showed a decrease in number in higher-concentration treatments compared with the control. On the other hand, other rotifer species showed small positive or negative weights, indicating that they had less impact by B[a]P treatment.

Due to the difficulty in handling, we could not obtain the acute toxicity data for small cladoceran species dominant in the mesocosms. However, mesocosm experiment has shown that B[a]P treatment induces delay and suppression of population growth of small cladoceran species particularly under high concentration treatment. Observed effect of B[a]P on zooplankton community is similar to that of some insecticides such as diazinon and carbaryl (Giddings et al., 1996; Hanazato 2001). It has been suggested that insecticides often induce suppression of cladocerans while inducing increase of rotifers that are less sensitive to chemical disturbances (Hanazato, 1991). Our results suggest that B[a]P has potential effects on aquatic ecosystems by modifying the food web structure and its overall function.

B[a]P is widely distributed in various aquatic ecosystems often at high concentrations, particularly in the sediments (Li et al., 2006; Guo et al., 2007). As it was found in our experiment, B[a]P in a water body might easily and rapidly precipitate to the bottom absorbing to particles. In our experiment, we used conceivable concentrations of B[a]P, 5 μg/l and 10 μg/l, the concentrations often detected from the polluted water associated with various pollution sources such as sewage discharges (Zhou and Maskaoui, 2003; Zhang et al., 2004). Particularly, it has been reported that the concentration of B[a]P reaches at 10 μg/l in surface water of rivers and lakes from the pollution survey carried out in surface water of Hangzhou, China (Zhu et al., 2004). Therefore, our results suggest that its short duration in the water column can cause changes in zooplankton community structure in natural rivers and lakes.
It is not clearly understood how short exposure of B[a]P induces suppression of cladoceran populations in the mesocosm. It is possible that consumption and accumulation of B[a]P by cladocerans induced high mortality and inhibition of their reproduction at their early stage of population build-up. However, further investigations are needed to estimate the toxic impacts of PAHs on aquatic ecosystems, particularly focusing on the species-specific toxic impacts and physiological and molecular analyses.

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REFERENCES