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Comparative Study of Pesticide Effects (Herbicide and Fungicide) on Zooplankton Community

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Abstract—We tested the response of zooplankton community against the application of methylthiotriazine herbicide, simetryn, and organophosphorus fungicide, iprobenfos, using natural zooplankton community in microcosm tanks (50 l). Total of 15 microcosm tanks (each 3 replicates for control and 4 treatments; low and high concentrations of simetryn and iprobenfos) having plankton community which transported from natural lake, were prepared. Water temperature of the tanks was kept at approximately 15°C during the experiment. The experiment was conducted during 12 days, and the chemicals were applied to the treatment tanks on day 2. Although zooplankton abundance decreased soon after the application of iprobenfos with high concentration, no significant differences in zooplankton abundances were observed among the treatments. For the changes of species composition, application of simetryn induced more diverged composition among the treatments. The results showed that both herbicide and fungicide have less apparent direct impact on zooplankton abundance within short period. However, changes in diversity and species composition induced by simetryn suggest the possibility that structure of zooplankton can be altered by the herbicide application.

Keywords: zooplankton, herbicide, fungicide, microcosm

INTRODUCTION

Usage of pesticide in agriculture has potential hazardrous impact on aquatic organisms since they are often concentrated in lakes and ponds through the agriculture runoff during rainfall (Richards and Baker, 1993). In particular, insecticide is known to have serious impact on certain zooplankton genera, and consequently affect zooplankton community by the modification of biological

interactions throughout planktonic food web (Hanazato, 2001; Chang *et al.*, 2005). However, compared with insecticide, there is only limited information about the impacts of herbicide and fungicide on zooplankton community.

Plankton food web consists of not only "phytoplankton-zooplankton interaction" but also "microbial food web" which involves mainly bacteria and protozoa which linked to phyto-zooplankton interactions through complex biological interactions (Nakano *et al.*, 1998; Kent *et al.*, 2006). Thus, it can be expected that herbicide and fungicide can have not only direct impact on zooplankton populations but also indirect impact through the changes of phytoplankton and bacterial communities (Kasai and Hanazato, 1995; Willis *et al.*, 2004; Mohr *et al.*, 2008). In the present study, we tested the response of plankton community to the application of methylthiotriazine herbicide, simetryn and organophosphorus fungicide, iprobenfos, using microcosm tanks (50 l) and natural zooplankton community. Simetryn is highly soluble, and one of popular herbicides, often detected from various freshwater ecosystems in Japan (Kibe *et al.*, 2000; Watanabe *et al.*, 2006). Iprobenfos is also one of common fungicide in Japan, and often detected from Japanese freshwaters (Nohara and Iwakuma, 1996). We exposed zooplankton community to these two different pesticides at the same time, and compared their impacts on the abundance and species composition of zooplankton community.

MATERIAL AND METHODS

Lake water containing natural plankton was collected from 0.5 m depth of Lake Suwa (Nagano Prefecture, Japan) using Van Dorn sampler, and transported to the outdoor microcosm tanks set up at Division of Science for Inland Water Environment, Institute of Mountain Science, Shinshu University. Total of 15 microcosm tanks (each 3 replicates for control and 4 treatments; low and high concentrations of simetryn and iprobenfos) were prepared. Water temperature of the tanks was kept at approximately 15°C during the experiment using thermostat heaters. The experiment was conducted from November 15 (day 1) to November 26 (day 12), and the chemicals (final concentrations of 20 and 80 μg l^{-1} for simetryn and 100 and 600 μg l^{-1} for iprobenfos) were applied to the treatment tanks on day 2. Symetrin and iprobenfos concentrations in the tanks were measured using Liquid Chromatography-Mass Spectrometry (LC-MS).

Total of 2 l water was collected from the tanks, and filtered through plankton net (40 μm mesh size). They were preserved with sugar contained formalin (final concentration of 4%). Total of 500 ml water was preserved with acid Lugol's solution at a final concentration of 1% for phytoplankton analysis. Zooplankton and phytoplankton were identified and counted under microscope. The water of 100 ml was collected from each tank, and preserved with glutaraldehyde for the enumeration of bacteria and hetero-nanoflagellates. The cell numbers of bacteria and nanoflagellates were counted using the DAPI (Porter and Feig, 1980) and primulin (Caron, 1983) staining methods, respectively.

At final day, 200 ml of water was collected from each tank and filtered through a Whatman GF/C filter, and then phytoplankton biomass (Chl. *a*

Table 1. Results of repeated-measures ANOVA on the differences in the abundances of microbial components (bacteria, heterotrophic nanoflagellates, ciliates and zooplankton).

	Simetryn		Iprobenfos	
	F value	*p*-value	*F* value	*p*-value
Bacteria	0.112	0.894	0.327	0.723
Heterotrophic nanoflagellates	0.068	0.934	0.097	0.908
Ciliates	0.910	0.411	6.456	0.004
Zooplankton				
Rotifers	0.485	0.619	3.218	0.054
Cladocerans	1.339	0.273	0.011	0.989

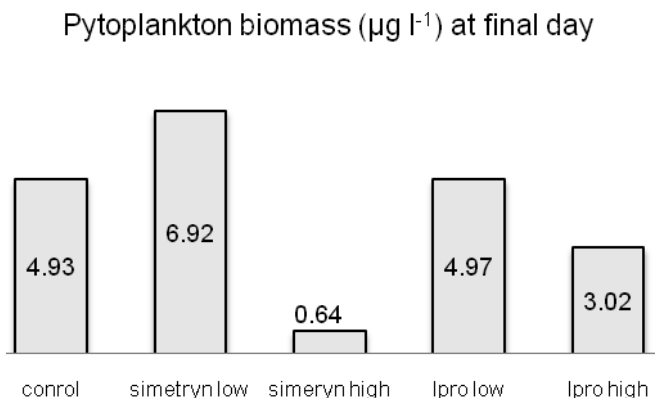

Fig. 1. Mean phytoplankton biomass (chlorophyll *a* concentration) of each treatment tanks at the final day of experiment.

concentration) was measured according to Marker *et al*. (1980). All the statistics and multidimensional scaling ordinations (MDS) were performed by StatView version 5 (SAS Institute Inc.) and PRIMER 5 (PRIMER-E Ltd), respectively.

RESULTS

During the experiment, both pesticides did not show apparent impact on plankton community (Table 1). In particular, the application of simetryn induced no significant differences in the plankton abundances. Bacterial densities in the tanks continued to decrease regardless of pesticide application, while heterotrophic nanoflagellates and ciliates showed rather increasing patterns. The application of low concentration of iprobenfos induced short-term increase of ciliates abundance, and consequently their abundances differed among tanks. Dominant zooplankton groups, rotifers and cladocerans, also showed no marked differences among treatments, but rotifer abundances increased after the application of low

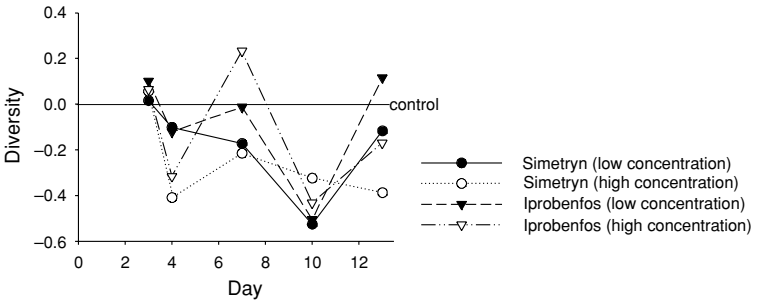

Fig. 2. Changes in mean diversity (*H′*, Shannon's diversity index) of zooplankton species, expressed as differences from average diversity of control tanks with no pesticide application.

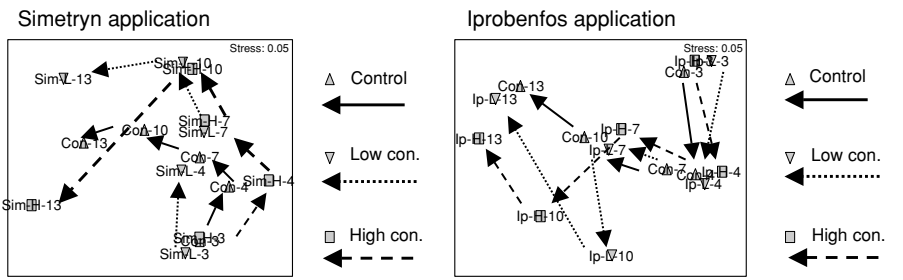

Fig. 3. MDS ordination of Bray-Curtis similarity matrix of zooplankton community data during the experiments (Sim, simetryn applied; Ip, iprobenfos applied; L, low concentration applied; H, high concentration applied; numbers indicate sampling days).

concentrations of both pesticides. Low concentration of iprobenfos induced higher abundance of cladocerans at the end period of the experiment. Marked difference of plankton abundance was observed in the phytoplankton abundance. High concentration of simetryn induced apparently lower phytoplankton biomass (Fig. 1).

Despite these subtle changes in abundance, zooplankton community structure showed marked response to the both simetryn and iprobenfos. Both pesticide induced the decrease of species diversity of zooplankton. Compared with the controls, the diversity index (*H′*, Shannon's diversity index) of zooplankton community maintained lower in the tanks treated with both simetryn and iprobenfos (Fig. 2). For the changes of zooplankton species composition, simetryn modified species composition more seriously than iprobenfos. Multidimensional scaling ordinations (MDS) representing the similarities of zooplankton species composition among the sampling points showed that zooplankton community continued to diverge particularly after the application of simetryn (Fig. 3).

DISCUSSION

Simetryn is one commonly used herbicide, and is often identified as the most toxic compound causing algal growth inhibition in river water discharged from the agricultural area (Okamura *et al*., 2002). In general, its direct toxic effects such as lethal effect on zooplankton are not so marked, but it is known that simetryn can affect zooplankton populations through the modification of reproduction and feeding behavior of zooplankton as well as reduction of algal productivity (Kasai and Hanazato, 1995; Villarroel *et al*., 2003). In the present experiment, the application of simetryn suppressed the phytoplankton abundance. Although we did not show the changes in the species composition of phytoplankton, collapse of *Aulacoseira* spp. and *Cryptomonas* spp. populations might be main reason for the decrease of phytoplankton abundances in the simetryn-applied tanks. However, under the low concentration of simetryn, the collapsed phytoplankton populations were recovered at the end period of the experiment, and consequently, significant difference of their abundance with the control was not observed. It suggests that the impact of simetryn lasts for a certain period when it exists with high concentration. However, despite such decrease of phytoplankton abundance, we could not observe the changes of other plankton components such as bacteria and zooplankton. Kasai and Hanazato (1995) showed that application of simetryn induce the decrease in zooplankton density through the indirect effects resulting from reduced food supply. The discrepancy with our results might be due to the fact that dominant zooplankton species in the present experiment was smaller species which less depend on phytoplankton than larger *Daphnia*, as well as the fact that their experiment was conducted for longer duration (2 months) with higher concentrations of simetryn (0.1 and 1.0 mg l^{-1}). However, our results suggest that even lower concentration (80 μg l^{-1}) can also cause serious reduction in phytoplankton abundance. In addition, the application of simetryn induced lower diversity and different species composition of zooplankton along with time. Thus, the further effects of low concentration of simetryn on zooplankton community can be expected if they are exposed to simetryn for longer period.

The toxic effects of fungicide on zooplankton have been reported through mesocosm experiment as well as acute and chronic toxicity tests (Willis *et al*., 2004; Mangas-Ramirez *et al*., 2007). But, there is still insufficient information about the impact of fungicide on zooplankton community compared with other pesticides. Same as herbicide, simetryn, it has been suggested that fungicide also have rather indirect effects on zooplankton than direct lethal impacts (Willis *et al*., 2004). However, in the present experiment, the application of iprobenfos induced neither decrease in the zooplankton density nor changes in species composition, indicating that fungicide, iprobenfos, has less impact on plankton community.

In conclusion, both pesticides have less marked impact than insecticide on plankton community, particularly on zooplankton community in terms of abundance. However, decrease of diversity and changes in species composition of zooplankton indicate that they can be possible threats affecting the structure

and function of plankton community in freshwater ecosystems. Further examination of pesticides impacts on plankton community is necessary with special emphasis on the long term effects under low pesticides concentrations.

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