Summary

"Effects of pesticides on aquatic macrofauna in the field" by Oleksandra Ieromina

Agricultural activities in the Netherlands are typically performed close to ditches, which inevitably leads to contamination of surface and ground waters with pesticides. The current PhD thesis addressed the effects of pesticides on aquatic macrofauna in the field. The study aimed to identify the extent of pesticide effects on aquatic macrofauna, given that aquatic ecosystems are influenced by abiotic and biotic factors that affect the performance of aquatic biota. Field research was performed in the flower bulb growing area of the Netherlands where pesticides are used intensively.

To address the study aim, the following research questions were formulated:

1. Did pesticide levels and aquatic macrofauna diversity in ditches of the flower growing region of the Netherlands change over the previous decades?
2. What proportion of the total variance in the community composition of aquatic macrofauna can be explained by pesticides, environmental factors (nitrate, nitrite, phosphate, dissolved organic carbon, dissolved oxygen, temperature, macrophyte coverage), presence of other biota, and time (seasonal and annual variation)? What is the predictive power of species trait approach in quantifying pesticide effects on aquatic macrofauna in the field?
3. What are the effects of pesticides combined with environmental factors on aquatic invertebrates exposed in situ in ditches bordering flower bulb fields?
4. Does food quality affect the responses of *Daphnia magna* to the insecticide imidacloprid?

Research question 1

To study temporal variation in pesticide levels and macrofauna diversity in ditches next to flower bulb fields, a dataset obtained from the Water Management Board Rijnland was analyzed (Chapter 2). The dataset consisted of pesticide concentrations measured at various locations over the years 1975 - 2010, and the species composition of aquatic macrofauna collected over the years 1983 - 2010 in ditches adjacent to flower bulb fields and in watersheds in a nature reserve. Pesticide levels in surface waters were expressed as toxic units (TU). In addition, TU were normalized by the number of pesticides measured per sample. Macrofauna diversity was estimated based on the Shannon diversity index. Normalized TU did not change in 1974 - 1998, followed by a decrease in 2000 - 2010. Concentrations of the most frequently measured pesticides (for instance, tolclophos-methyl, imidacloprid, chloridazon, isoproturon, chlorpropham, diuron, simazine, metoxuron) decreased over time. Macrofauna diversity in the flower bulb growing area and the nature reserve tended to

increase over time. Correlative analysis between pesticide levels and macrofauna diversity could not be performed because pesticides and macrofauna data were not consistent over time and location. Further field research is needed to elucidate causal relationships between pesticide levels in surface waters and macrofauna diversity.

Research question 2

To identify the relative contribution of pesticides to the total variance in the community composition of aquatic macrofauna, field work was performed in the flower bulb growing area of the Netherlands (Chapter 3, 4). Sampling and taxonomic identification of macrofauna, measurements of water chemistry and pesticide concentrations in ditches were performed during the years 2011 - 2012. Variance partitioning based on the partial redundancy analysis (pRDA) was applied to divide the total variance in macrofauna community composition into the variance explained by pesticides, environmental factors, the presence of other biota, and time. In addition, macrofauna species were classified into trait modalities of nine species traits. Sensitive insect species (Trichoptera, Diptera) were found at high abundances in watersheds of the nature reserve, while molluscs (Gastropoda) and annelids (Haplotaxida) were favored by eutrophic conditions of ditches adjacent to flower bulb fields. The largest proportion of variance in both taxonomic and species trait composition was explained by environmental factors, followed by pesticides and time.

Research question 3

To study the effects of pesticides in combination with abiotic factors on aquatic invertebrates, in situ exposure experiments with *Daphnia magna*, *Chydorus sphaericus* and *Asellus aquaticus* were deployed in ditches bordering flower bulb fields (Chapter 5). Relationship between survival, reproduction and growth of animals, pesticide concentrations and environmental factors (nitrate, nitrite, temperature, phosphate, dissolved organic carbon) was analyzed with a General Linear Model (GLM). Pesticides did not affect the performance of *D. magna* and *A. aquaticus*. Nutrients explained the largest proportion of variance in growth and reproduction of *D. magna*. Dissolved organic carbon and temperature contributed to the variance in survival and growth of *A. aquaticus*. Environmental factors largely determined the performance of aquatic invertebrates exposed in situ.

Research question 4

To study the combined effects of the insecticide imidacloprid and food quality (expressed as carbon: phosphorus ratio of algae) on the performance of *D. magna,* laboratory experiments were performed (Chapter 6). These experiments involved exposure of *D. magna* juveniles supplied with algae of varying nutritional quality to imidacloprid. A stronger effect of imidacloprid on the survival and growth of *D. magna* was observed at the conditions of food deficiency. It was shown in previous studies that algae grown at the conditions of low phosphorus concentrations tend to increase the width of the cell wall, which is a protective

mechanism against unfavorable conditions. Daphnids in turn cannot fully digest algae with thick walls and assimilate sufficient carbon as needed for growth. When obtaining insufficient amounts of carbon, daphnids spend more energy on filtering then on growth. Imidacloprid possibly acted as an additional stressor to daphnids, and strengthened the negative effects of food deficiency on the performance of animals. It can be concluded that food quality may affect the sensitivity of aquatic filter-feeding invertebrates to pesticides.

Conclusions

Pesticides residual concentrations in ditches of the flower bulb growing region of the Netherlands were found at detectable levels. Pesticides contributed to the total variance in aquatic macrofauna community composition. Environmental factors (amongst all nutrients, DOC, temperature) explained the largest proportion of variance in survival, growth and reproduction of aquatic invertebrates exposed in situ. Similarly, the highest proportion of variance in macrofauna community composition was ascribed to environmental factors. Based on the results it can be concluded that field-relevant factors should be considered in pesticide effect assessment.