Effects of pesticides on aquatic macrofauna in the field

Ieromina, O.

Author: Ieromina, Oleksandra
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CHAPTER 7

DISCUSSION

Scientific scope and research questions

This PhD thesis provides an insight on the effects of pesticides on aquatic biota in the field. The community composition of aquatic biota inhabiting aquatic ecosystems is determined by different abiotic factors, such as hydrological and environmental factors, intrinsic to aquatic ecosystems. Additional to these abiotic factors, biotic interactions describe a high degree of fluctuation among communities (Clements et al., 2012).

Human activities related to agriculture modify natural aquatic ecosystems. In this respect, pesticide and nutrient pollution of freshwater ecosystems represents one of the central environmental issues worldwide. A substantial amount of research has been dedicated to understanding and characterizing the effects of chemicals on different levels of biological organization: organisms, populations, communities, and ecosystems. The assessment of chemical effects on high organizational levels is challenging due to the high variability in abiotic and biotic factors found in natural ecosystems, which can interfere with chemicals in their effects on aquatic biota. The field relevance of pesticide effects on aquatic biota receives an increasing attention in ecotoxicology.

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The research aims of this PhD project were:

A. To determine which role is assigned to pesticides within the complex field setting in shaping the community composition of aquatic macrofauna
B. To determine the magnitude of impact of field-relevant factors on aquatic macrofauna communities in water systems located in close proximity to agricultural fields and to identify the combined effects of field-relevant factors and pesticides on aquatic biota.

To address these aims, the following research questions were formulated:

1. Did pesticide levels and macrofauna diversity in ditches next to flower bulb fields 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 and macrophyte coverage), the presence of other biota and time (seasonal and annual variation)? What is the predictive power of the taxonomic approach in quantifying pesticide effects on aquatic macrofauna in the field?
3. What are the effects of pesticides combined with environmental factors (nitrate, nitrite, phosphate, dissolved organic carbon, dissolved oxygen and temperature) on aquatic invertebrates exposed in situ in ditches adjacent to flower bulb fields?
4. Does food quality (expressed as the carbon: phosphorus ratio of algae) affect the responses of *Daphnia magna* to the insecticide imidacloprid?

Field research was based in the flower bulb growing area of the Netherlands. Various approaches were used in research, ranging from field monitoring of aquatic macrofauna and in situ bioassays with aquatic invertebrates, to laboratory toxicity experiments. In addition, a database containing pesticide, water chemistry and macrofauna data collected in the flower growing area of the Netherlands over the previous decades, was analyzed.

Answers to the research questions

RQ1. Pesticide levels in ditches bordering flower bulb fields (expressed as TU normalized by the number of pesticides measured per sample) were stable between 1980 - 1998, followed by a decrease in 1998 - 2010. The lowest TU were observed in the years 2000 - 2004. The concentrations of the most frequently measured individual pesticides (for instance, carbendazim, pirimiphos-methyl, imidacloprid, chloridazon, isoproturon and chlorpropham) decreased over time. The Shannon diversity index expressing macrofauna diversity in ditches of the flower bulb growing area and in the watersheds of nature reserve tended to increase over time. Pesticide and macrofauna data were not collected consistently over time and sampling site. Therefore, causal relationships between pesticide levels in surface waters and macrofauna diversity could not be elucidated. It was concluded that further research based in the field is required to infer causal relationships between pesticide levels in ditches and macrofauna diversity.

RQ2. The largest proportion of variance in both the taxonomic and trait composition of macrofauna community was explained by environmental factors, followed by pesticides and by time, as quantified by a variance partitioning procedure based on the partial redundancy analysis (pRDA). Water chemistry parameters were highly variable in ditches of the study area. Concentrations of nutrients fluctuated in a range significantly exceeding the water quality standards. For example, the concentration of phosphate in ditches ranged between 0.8±1.0 mg/L and 4.1±3.2 mg/L, with a concentration of phosphate above 1 mg/L being considered a sign of ”poor” water quality (UKTAG, 2012). Environmental factors varying outside the optimal range for species largely explained the variance in the community composition of aquatic macrofauna (Chapters 3, 4).

RQ3. The responses of aquatic invertebrates exposed in situ in ditches bordering flower bulb fields were mainly determined by environmental factors, as identified by the General Linear Model (GLM). The performance of *Daphnia magna* and *Asellus aquaticus* was not affected by pesticides (expressed as Toxic Units). The reproduction of *C.sphaericus* was negatively correlated to pesticides which explained ~ 6% of variance in the reproduction output of *C.sphaericus*. Nutrients contributed largely to variance in the growth and reproduction of *D. magna*, whilst the survival and growth of *A.aquaticus* was dependent on dissolved organic carbon and temperature. Environmental factors largely affected the performance of aquatic invertebrates exposed in situ (Chapter 5).

RQ4. Imidacloprid induced a stronger effect on *Daphnia magna* supplied with phosphorus-deficient algae. According to previous studies, algae grown at low phosphorus conditions increase the width of the cell wall, as a mechanism of defense against poor external phosphorus conditions (Van Donk et al. 1997; DeMott & Van Donk, 2013). *D. magna* in turn cannot digest such algae with thick cell walls and incorporate carbon needed for growth and development (DeMott & Van Donk, 2013). When not receiving enough food, daphnids allocate energy resources to filtering and not to growth and reproduction (Plath & Boersma, 2001). This possibly resulted in the reduced performance of daphnids supplied with nutrient-deficient algae. Food deficiency enforced effects of imidacloprid on *D. magna*. It can be concluded that food quality (in terms of the carbon: phosphorus ratio of algae) may affect the sensitivity of aquatic filter-feeding invertebrates to pesticides.

What do answers to the research questions mean for aquatic biota?

Are there effects of pesticides on aquatic biota in ditches around agricultural fields?

As found in the results of field work, the concentrations of pesticides (carbendazim, pirimiphos-methyl, imidacloprid, isoproturon, tolclophos-methyl and chlorprofam) in ditches bordering flower bulb fields were above the limits of detection. The concentration of pirimiphos-methyl exceeded NOEC (No Effect Concentration, 21 day with *D. magna*) at several sampling sites (Chapters 3, 5). Pirimiphos-methyl also contributed to the TU (toxic units) exceedings in the flower growing area in the previous decades (Chapter 2). Pesticides explained ~ 5% of the total variance in the total macrofauna community composition, ranging between ~ 1% and ~ 17% for different macrofauna groups (Chapter 3). Relative to the total variance in macrofauna community assumed to be 100 %, and ~25 % of explained variance, such contribution of pesticides to the total variance can be interpreted as substantial. Studies identifying the proportion of variance in the community composition of aquatic biota explained by toxicants area scarce. Setting procedure for such analysis would help to make results of the field studies comparable and enhance our understanding of the effects of toxicants on aquatic communities in the field.

What are the responses of different macrofauna taxonomic groups to pesticides?

Figure 1 shows the RDA triplot visualizing the relationships between the abundances of most common macrofauna species, concentrations of pesticides and environmental factors. Abundances of sensitive insects *Cloeon dipterum* (Ephemeroptera), *Ischnura elegans*, *Pyrrhosoma nymphula* (Odonata), *Chaoborus sp.* and *Chironomus sp.* (Diptera) were negatively correlated to nutrients. At the same time, abundances of species tolerant to pollution, such as molluscs *Physa fontinalis Valvata cristata* and *Valvata piscinalis* (Gastropoda), annelids *Stylaria lacustris* (Haplotaxida) and *Erpobdella octoculata* (Rhynchobdellida) and insects *Corixa punctate, Sigara striata, Plea minutissima, Notonecta glauca* (Hemiptera) were higher in ditches of the agricultural area (Figure 1). Abundances of these species were positively correlated to nutrients and pesticides.

Are there patterns in species trait composition of aquatic macrofauna in response to pesticides?

The trait modality distribution of several traits was also influenced by pesticides and environmental factors (Chapter 4). The biomass of predators (e.g., Odonata, Coleoptera,

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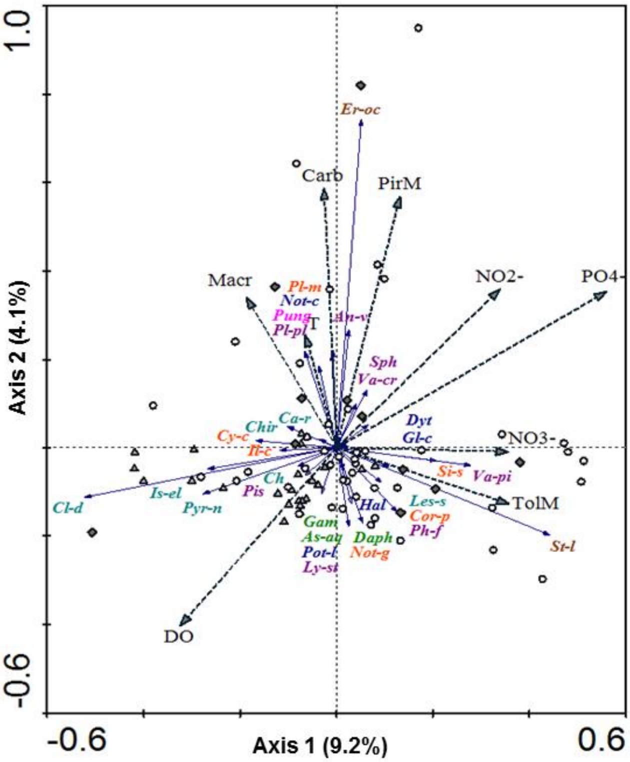

Figure 1. Redundancy analysis triplot showing relationships between Hellinger-transformed species abundances of the most common macrofauna species, environmental factors, and pesticides (the month and the year of sampling were included in the analysis as covariables). Explanatory variables having a correlation coefficient with the first two ordination axis above 0.2 are shown. The significance of the first canonical axis and the significance of all canonical axes (according to Monte Carlo test based on 999 permutations) are $p = 0.004$ and $p = 0.084$, respectively. Dashed lines represent pesticides and environmental factors, solid lines represent species abundances. PirM = pirimiphos-methyl, TolcM = tolclophos-methyl, Carb = carbendazim. Triangular = sampling sites in watersheds of nature reserve, circles = sampling sites in ditches next to flower fields, diamonds = sampling sites in ditches next to pastures. Abbreviations for the species names can be found in Table 1.

Rhynchobdellida, Gasterosteiformes) was higher in the watersheds of nature reserve than in agricultural ditches. The biomass of animals breathing through plastron (e.g., Diptera), i.e. exchanging oxygen and carbon dioxide in a thin air layer around the body, was negatively correlated to nutrients and pesticides. While the biomass of animals breathing through gills (e.g., Gasterosteiformes, Gastropoda, Bivalvia, Crustacea, Odonata), hydrostatic vesicle (e.g., Hemiptera, Coleoptera) and tegument (e.g., Rhynchobdellida, Haplotaxida) was not dependent on the water chemistry composition. Macrofauna on the sensitive pupa (e.g., Diptera) and larvae (e.g. Diptera, Odonata, Trichoptera and Coleoptera) life stages was found at higher biomass in watersheds of nature reserves than in agricultural ditches. Semivoltine species (e.g., Gastropoda) were more abundant in the highly disturbed conditions of agricultural ditches.

Table 1. Abbreviations for the species names shown in Figure 1

Abbreviation	Species	Order	Class
Cor-p	*Corixa punctata*	Hemiptera	Insecta
Il-c	*Ilyocordis cimicoides*	Hemiptera	Insecta
Not-g	*Notonecta glauca*	Hemiptera	Insecta
Pl-m	*Plea minutissima*	Hemiptera	Insecta
Si-s	*Sigara striata*	Hemiptera	Insecta
Cy-c	*Cymatia coleoptrata*	Hemiptera	Insecta
Ch	*Chaoborus sp.*	Diptera	Insecta
Chir	*Chironomus sp.*	Diptera	Insecta
Ca-r	*Caenis robusta*	Ephemeroptera	Insecta
Cl-d	*Cloeon dipterum*	Ephemeroptera	Insecta
Is-el	*Ischnura elegans*	Odonata	Insecta
Les-s	*Lestes sponsa*	Odonata	Insecta
Pyr-n	*Pyrrhosoma nymphula*	Odonata	Insecta
Dyt	*Dytiscus sp.*	Coleoptera	Insecta
Hal	*Haliplus sp.*	Coleoptera	Insecta
Not-c	*Noterus clavicornis*	Coleoptera	Insecta
Pot-l	*Potamanthus luteus*	Coleoptera	Insecta
Pung	*Pungitus pungitus*	Gasterosteiformes	Actinopterygii
Er-oc	*Erpobdella octoculata*	Rhynchobdellida	Hyrudinea
St-l	*Stylaria lacustris*	Haplotaxida	Olygochaeta
As-aq	*Asellus aquaticus*	Isopoda	Malacostraca
Daph	*Daphnia sp.*	Diplostraca	Branchiopoda
Gam	*Gammarus sp.*	Amphipoda	Malacostraca
An-v	*Anisus vortex*	Basommatophora	Gastropoda
Ly-st	*Lymnea stagnalis*	Basommatophora	Gastropoda
Ph-f	*Physa fontinalis*	Basommatophora	Gastropoda
Pl-pl	*Planobris planobris*	Basommatophora	Gastropoda
Va-ma	*Valvata macrostata*	Heterostropha	Gastropoda
Va-cr	*Valvata cristata*	Heterostropha	Gastropoda
Va-pi	*Valvata piscinalis*	Heterostropha	Gastropoda
Pis	*Pisidium sp.*	Veneroida	Bivalvia
Sph	*Sphaerium sp.*	Veneroida	Bivalvia

Taxonomic and species trait composition of aquatic macrofauna in agricultural ditches and watersheds of nature reserve differed (Chapters 3, 4). Eutrophic conditions of agricultural ditches were unfavorable for sensitive macrofauna taxa. Other macrofauna taxa characterized by tolerance to pollution and species traits helping organisms to adapt to disturbances were found in high amounts in ditches of agricultural area.

What do answers to the research questions mean for water management?

The goal of the Water Framework Directive (WFD) is to protect the quality of surface and ground water in Europe (The EU Water Framework Directive, 2015). The WFD sets environmental quality standards for different substances and types of water bodies (amongst all, large drainage ditches, lakes, rivers, coastal waters and ground waters). Remarkably, the WFD is targeted at the protection of large water bodies (lakes larger than 50 ha and rivers larger than 10 km^2), while smaller water bodies (and connected waters) are not protected by the WFD. This lack of regulatory legislation makes control and management of ditches difficult and dependent on the regional water management authorities. Yet, aquatic biota in small waters is relatively rich in biodiversity. These small waters also contain high natural habitat diversity. Rare invertebrate species, not occurring in rivers, can be found in ditches. Aquatic macrophytes also maintain the water purifying function in ditches. Hence, the value of biodiversity in ditches is high. Small waters are also important with regard to ecosystem services: in addition to the main function of water level control, ditches represent an indispensable part of the landscape in the Netherlands and have high value for passive recreation.

As can be seen in the online tool (www.bestrijdingsmiddelenatlas.nl), high pesticide residue concentrations, often exceeding the water quality targets, are often found in surface waters around the flower bulb fields in the Netherlands. As seen in the results of the current study, pesticides in combination with environmental factors did affect community composition of aquatic biota (Chapters 3, 4). Hence, environmental management plan should be developed to reduce pesticide emissions in the study area. First, the emission routes of pesticides to surface waters should be identified. An inventory in the area can be done to determine possible sources of pesticide emissions (for instance, whether emissions occur due to the spray drift, runoff from the fields, or originate from point sources). As a next step, possible solutions to reduce emissions can be developed. The approaches to address the issue of the water quality in the area can be the following: 1) bottom – up approach, in which all actors in the field of agriculture, including water managers and regional stakeholders, combine their efforts in developing measures to reduce pesticide emissions or 2) top-down approach, in which initiatives aiming to reduce chemical emissions and impacts on the watersheds are taken by regulatory authorities. These types of approaches are discussed in

De Snoo & Vijver, 2012 (Chapter 14). As an example of a bottom-up approach, first, emission sources (point and non-point) can be identified, followed by the evaluation of existing policy regulations and setting agreements between the different actors so as to perform agricultural activities in a sustainable manner (Oommen et al., 2004).

As described in the literature, spray drift represents the main route of pesticide emissions in the Netherlands (Chapter 1, Van Linden et al., 2008). An example of a top-down approach to minimize spray drift in a very efficient practical way is to set buffer zones between crops and ditches (De Snoo & Vijver, 2012). A buffer zone is defined as an area typically located between the sensitive area (the ditch) and the crop where no spraying is done. The buffer zone can be covered with vegetation (grass, shrubs or trees) that creates a barrier between the crop and the ditch (Department of Environment and Primary Industries, 2014). Introducing buffer zones was shown to reduce the spray drift of pesticides, as applied on the fields, to ditches (De Snoo & De Wit, 1998).

The width of the buffer zone in the flower bulb growing area as currently prescribed by the policy guideline of Rijkswaterstaat Water (Article 3.8) should be a minimum of 150 cm. In addition, sprayers should be equipped with low drift nozzles, which should be located not higher than 30 cm above the plants during the spraying process. Such regulatory measures aiming to minimize spray drift should be followed. Such regulatory measures will benefit water quality in ditches, as well as aquatic biodiversity.

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