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CHAPTER 1

GENERAL INTRODUCTION

Biodiversity in European and global context

Biological diversity is essential for the healthy state of our planet. Every species plays an important role in the biosphere and should be protected. Biodiversity adds value to our society by providing various ecosystem services and creating the basis for culture, science, and education (Wall & Nielsen, 2012).

During the last decades, humanity has faced the problem of rising demand for food, energy and fresh water. Because of a growing need for resources, natural ecosystems within the past few years have been modified to a much broader extent than before. Loss of biodiversity is significantly high and often non-reversible in many areas around the world (Millennium Ecosystem Assessment, 2005). High biodiversity loss is observed in developed countries where economic success is associated with a large threat to the natural environment (Millennium Ecosystem Assessment, 2005).

The European Union pays significant attention to issues related to biodiversity and climate change. In 2001, the EU Government started an initiative addressing the problem of nature degradation and set the objective to prevent the decline of biodiversity in Europe by the year 2010 and further (EU Biodiversity Policy, 2015). In 2006, the "*EU Biodiversity Action Plan*", confronting the problem of biodiversity loss in Europe, was introduced. This plan established specific aims towards preventing species extinctions and habitat degradation (European Communities, 2008). The objectives set in the Plan included the preservation of natural habitats, protection of farmland biodiversity, lowering pollution levels, conservation of marine and freshwater life, control over invasive species and climate change (European Communities, 2008). In 2011, the initiative on the reduction of biodiversity degradation in Europe was continued in the form of the new "*EU 2020 Biodiversity Strategy*", setting a target year of 2020 (European Commission, 2011). The new plan continued and extended the goals set in the "EU Biodiversity Action Plan" aiming at "*resource efficient and green economy*" in the European Union (European Commission, 2011).

Much attention in the EU biodiversity policy is given to the protection of freshwater biodiversity (European Communities, 2008). Freshwater occupies 3% of all water on the planet (McMichael, 2014). Accounting for 0.3% of all freshwater, surface water provides a habitat for approximately 6% of all species living on the planet (McMichael, 2014; Dudgeon et al., 2006). The biodiversity of inland waters is an important natural resource of high economic value. At the same time, freshwater biodiversity is subjected to a number of threats. At a global scale, water pollution represents an important negative factor affecting freshwater biodiversity, along with the over-exploitation of water resources, habitat degradation, species invasion, and the modification of flow regimes (Dudgeon et al., 2006). Because various types of disturbances resulting from human activities affect aquatic life, the biodiversity of fresh waters declines at a much higher rate than the biodiversity of terrestrial or marine ecosystems (Ricciardi & Rasmussen, 1999; Strayer & Dudgeon, 2010).

Water quality in the Netherlands

Being the sixth smallest country in Europe by land area (Centraal Bureau voor de Statistiek, 2004), the Netherlands occupies second place in the world by the amount of exported agricultural products (behind the USA in first place) (Netherlands Enterprise Agency, 2013). At the same time, a special feature of the Netherlands is that it is an extremely water-rich country. The total length of ditches in the lower parts of the Netherlands is approximately 300.000 km (Higler, 1989). The necessity of having such intense water coverage is explained by the location of the country: a large area of the Netherlands lies below sea level (Rijkswaterstaat, 2011). A continuously functioning system of ditches and canals connected to pumping stations helps to control water levels and protect the country from floods. In addition, the ditch systems ensure irrigation and drainage of agricultural fields. The first ditch systems were built in the middle of the XIII century, the period corresponding to the beginning of agricultural development in the country (Highler, 1989; Wolff, 1993). Since that time, polder areas represent the most common type of landscape in the Netherlands and drainage ditches are the dominant aquatic ecosystems.

Dense water coverage creates a very special situation in the country in terms of water management. The water is pumped in and out of the ditches constantly, depending on the levels of precipitation and evaporation. This creates a dynamic environment in ditches in terms of hydrology, even though the speed of the water flow in ditches is relatively low. Management activities at the ditch banks (mowing) and removal of aquatic vegetation excess from the ditch (dredging) makes ditches highly disturbed ecosystems. In addition, ditch systems are located in intensively used agricultural areas occupied by pastures and different types of crops. Intense farming close to interconnected ditch systems is often coupled with high pesticide levels in surface waters (Vijver et al., 2008). At the same time, the protection of aquatic biodiversity in inter-connected water systems is very challenging. After all, ditch systems are not isolated bodies of water, and hence environmental managers should control the upstream and downstream reaches, as well as the adjacent land (Dudgeon et al., 2006).

The importance of water quality protection in agricultural areas, where pesticides and fertilizers are used intensively, is highlighted in different environmental policies at the European and national levels. The correct use of plant protection products is considered a very important issue in Europe, starting from the authorization process and ending with the control of pesticide residues in surface waters and - if needed – the development of mitigation strategies. To manage the use of plant protection products accordingly, the EU has developed a joint legislation that applies to all EU Member States: "*Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market*." The aim of this legislation is to promote consistency in Europe with regard to the use of agricultural chemicals. The final goal of the regulation is to minimize the individual differences in risk assessment of chemicals between EU Member States, so that the legislation in one country can also be recognized in another Member State.

The national government of the Netherlands pays significant attention to the quality of surface waters (Government of the Netherlands, 2015). The local water managers monitor water quality and quantity in the Netherlands (Rijkswaterstaat, 2011). The Dutch office, managing the plant protection and biocidal products, is represented by the CTGB – "College voor de toelating van gewasbeschermingsmiddelen en biociden" (CTGB, 2015). To carry out the impact assessments, the CTGB has developed the Evaluation Manuals. These manuals cover various aspects related to the properties of plant protection products, their fate and the effects on the environment and human health. All tests included in the manuals should be performed according to the standard guidelines, and all evaluation criteria should be met before the product enters the market. The procedure of tiered risk assessment is followed, that begins with the toxicity assessment by simple conservative laboratory tests, and only proceeds to a more complex test setting (higher-tiered assessment) if more information on chemical toxicity is needed. Higher-tiered assessment (toxicity tests with wide ranges of species, microcosm and mesocosm experiments, or test settings in the field) is relatively more expensive than laboratory tests, but provides ecologically relevant information on chemical toxicity enabling field-relevant predictions to be made.

Biodiversity of ditches in the Netherlands

One of the important reasons for such strict monitoring and control over pesticide use in the Netherlands is the fact that agro-ecosystems in the country still contain high biodiversity (for instance, plants, pollinator insects, birds), essential not only for the healthy functioning of crops, but also for the overall high value of the area in terms of species diversity. Many aquatic plant and animal species find their habitats in drainage ditches (Verdonschot, 2012a; Herzon & Helenius, 2008). The number of aquatic invertebrate species in ditches is relatively high even though ditches are affected by physical and chemical disturbances: aquatic biodiversity in ditches was shown to be comparable to that in small lakes (Verdonschot, 2012a). Moreover, the structural composition of aquatic biota influences the hydrological functions of the ditches. For instance, aquatic plants in ditches can reduce the water flow, which increases the retention and degradation of pollutants (Beltman et al., 2004). Aquatic invertebrates represent an important component of the aquatic food web and take part in biochemical cycles in aquatic ecosystems (Herzon & Helenius, 2008; Kristensen & Kostka, 2005).

Despite a large contribution of aquatic biota in ditches to the overall biodiversity of agroecosystems in the Netherlands, to our knowledge not many studies focused on macrofauna inhabiting ditch systems. For instance, the earlier research of Scheffer et al. (1984) and Higler & Verdonschot (1989) identified patterns in the invertebrate community composition in ditches in relation to habitat structure (vegetation structure). The study of Canters et al. (1989) addressed aquatic fauna in ditches of the Netherlands. Higler (1989) described the general trends in water chemistry parameters in ditches. A more recent study of Verdonschot et al. (2012a) described the taxonomic composition of fauna and flora of ditches in the Netherlands in comparison to that of small lakes. In the other work of Verdonschot et al. (2012b), the relationship between the invertebrate community composition and the structure of aquatic macrophytes was investigated. Verdonschot (2012c) also performed a comprehensive study of ecological processes and biodiversity in ditches.

Compared to the relatively small amount of research on aquatic fauna in ditches, a much larger number of studies have investigated the ditch bank vegetation in the Netherlands, and the effects of associated management practices on the ditch bank vegetation (for instance, Best et al., 1992; Leng et al., 2011ab; Noordijk et al., 2011; Dijk et al., 2013; Blomqvist et al., 2006; Whatley et al., 2014 and others).

Impact of pesticides on biodiversity in ditches

Being indispensable components of Dutch polders, the ditch systems constantly receive chemical loadings from the surrounding agricultural fields (amongst all, pesticides and nutrients). Pesticides can enter the aquatic environment through different pathways. Pesticides are in most cases applied in agricultural fields by spraying. After spraying, pesticides undergo several processes: volatilization, spray drift and adsorption by the crop and soil (Van Linden et al., 2008). The largest proportion of pesticides sprayed is assumed to end up on soil and crops. Pesticides deposited onto soil can leach through the root zone into the surface and ground water (Van Linden et al., 2008). This leaching, together with the inflow from field drainage systems, leads to the contamination of surface and ground waters (Van Linden et al., 2008). The direct drift of pesticides to water systems during the spraying process accounts for the largest percentage of pesticide emissions to the environment in the Netherlands (up to 70%) (De Zwart, 2003). Elevated concentrations exceeding the environmental quality standards are found in many ditches and larger waterbodies in the Netherlands (Vijver et al., 2008).

Such high chemical input creates unfavorable conditions for ditch fauna because many invertebrate and vertebrate species living in ditches are highly sensitive to pesticides. It was shown in previous research conducted in controlled settings that pesticides may affect population density, reproduction rate, and the birth and mortality rates of invertebrates (Hanazato, 2000). At the ecosystem level, pesticides may produce changes in the structure of aquatic communities. Hanazato (2000) observed such effects of pesticides on aquatic ecosystems, as the lengthening of the food chain accompanied by lowered energy transport between different components of the food web. Several studies focused on the effects of chemicals on aquatic invertbrates in semi-field conditions (for instance, Van den Brink, 1996; Wijngaarden et al., 1996, 2004). In semi-field experiments of Van Wijngaarden et al. (2004) pesticide mixtures (applied at the concentrations of up to 5% of the spray drift emission) did induce negative effects on invertebrate communities in ditches. However, to our knowledge no study other than ours focused on the effects of pesticides on aquatic macrofauna in the field drainage ditches of the Netherlands.

Flower growing in the Netherlands and research area

The flower bulb growing region located in the province South Holland (the Netherlands) that represents a highly productive agricultural area. Flower cultivation makes the country famous around the world for its beautiful flowers and other floricultural products (such as bulbs, potted plants, foliage) (Dinham, 2008). Floricultural production occupies a large part of the agricultural land in the Netherlands (Dinham, 2008).

Flower diseases induced by pests and the growth of weeds are controlled by pesticides, including insecticides, herbicides and fungicides (Jansma et al., 2002). As a result of environmental policy aiming to diminish the use of plant protection products in the Netherlands by the year 2010, the use of chemicals in agriculture has lowered two fold already by the year 2000 when compared to the previous two decades (Van Eerdt, 2007). Consequences of such policy measures were the reduction of chemical emissions and overall improvement of environmental quality (Van Eerdt, 2007). For instance, the percentage of pesticide measurements in surface water exceeding MTR (Maximum Tolerable Risk) decreased by 75% between 1988 and 2009. The percentage of locations at which msPAF (Potentially Affected Fractions of species) was higher than 5%, diminished by 58% during the same period (De Snoo & Vijver, 2012). In the subsequent years, however, an increase in pesticides use was observed again (De Snoo & Vijver, 2012). Even though, as a general trend, the use of chemicals on the national scale reduced during the previous decades, it should be noted that the amounts of chemicals applied varies greatly between the crops. The amount of pesticides applied in bulb crops in 2012 was 54.4 kg/ha (Centraal Bureau voor de Statistiek, data from 2014). This amount was much higher when compared to the other crops. For instance, in 2012 pesticide use in champignon and glass house vegetable growing was lower than in bulb crops: 0.3 kg/ha and 12.1 kg/ha respectively (Centraal Bureau voor de Statistiek, data from 2014). Only in rose, chrysanthemum and lily fields, the amount of pesticides applied in 2012 was higher than in bulb crops (106.2 kg/ha, 70.7 kg/ha and 134.6 kg/ha, respectively) (Centraal Bureau voor de Statistiek, data from 2014).

In addition to pesticides, nitrogen- and phosphorus- containing fertilizers are also applied extensively in flower fields (Jansma, 2002). The total use of nitrogen-containing fertilizers in the Netherlands in 2013 was 8 million kg, compared to 11 million kg used in 2000. The amount of phosphorus-containing fertilizers applied in flower fields was lower: 3 million kg applied in 2013 compared to 4 million kg applied in the year 2000 (Centraal Bureau voor de Statistiek, data from 2015). Even though an overall reduction of fertilizer use has been observed in the country, nutrients applied at the agricultural fields can enter surface waters and cause various effects to aquatic life. Janse & Van Puijenbroek (1998) found that excessive nutrient loads in ditches produced shifts in the structural composition of aquatic macrophytes. Thus, a shift from submerged macrophytes towards floating macrophytes dominated by *Lemna sp.* was observed (Janse & Van Puijenbroek, 1998). Such shifts initiate a chain of consequences to aquatic life: duckweed dominance causes shading and reduces light availability for algae and invertebrates. Excessive algae growth leads to lowered dissolved oxygen concentrations. This results in direct and indirect effects to aquatic faunal organisms. Nutrients therefore constitute an important factor likely to affect aquatic biota in addition to pesticides.

The research area selected for the current study covered polders located in the flower bulb growing area of the Netherlands. The area represents a typical example of agricultural area, where different farming activities are performed throughout the year. All crops in the research area are grown in a close proximity (approximately 25 cm to 2 m) to ditches. In addition, the research area is covered with open crops (while most of flowers, e.g. 69% are grown in glass houses) (Dinham, 2008). Therefore, chemicals applied in the fields can enter water systems directly after spraying through spray drift. In addition, watersheds in the nature reserve next to the polder area were sampled as control sites.

Context dependency concept

As an independent field of science, ecotoxicology aims to identify the effects of toxicants on the environment across different levels of biological organization: sub-organisms, organism, populations, communities, and ecosystems. Understanding the effects of toxicants on the higher levels of biological organization is associated with high uncertainty because toxicants explain only a part of the overall variation in communities, while the remaining variation can be attributed to other natural abiotic and biotic factors, intrinsic to ecosystems. Clements et al. (2012) introduced the theoretical framework of the context-dependency approach in ecotoxicology, which introduces abiotic and biotic factors into the assessment of toxicant effects on communities.

In relation to pesticides, previous studies showed that factors, such as the type of ecosystem, location, weather, and environmental conditions, might all affect the toxicity of pesticides in the aquatic environment (Maund et al., 1999). The importance of the food web structure in shaping responses of aquatic invertebrates to toxicants was also demonstrated by several studies. For instance, the interaction of organisms within one population was found to be an important factor affecting the responses of the Trichoptera populations to the pesticide fenvalerate (Liess, 2002). Beketov & Liess (2006) found that predation pressure intensified the adverse effects of esfenvalerate on the zooplankton species *Artemia sp.* A similar result was observed in the study of Liess (2013), in which the aquatic insect *Culex pipiens* was more sensitive to the insecticide thiacloprid under predating pressure than without the presence of the predator. Species interactions, such as predation or competition, were shown to be important factors affecting the responses of aquatic invertebrates to pesticides in several studies, for instance, in Trekels et al. (2010) and in Foit et al. (2012).

The sensitivity of organisms to pesticides and their potential to recover from toxic stress is largely determined by the species functional characteristics (species traits) (Pof, 1997). The trait-based approach in community ecology was introduced a few decades ago.

The species trait approach was used to study the effects of various types of disturbances (for instance, farming types, metal pollution, cargo ship traffic, eutrophication and climate change, land use, and nutrient pollution) on invertebrate communities (Magbanua et al., 2010; Statzner et al., 2010; Vandewelle et al., 2010; Doledec, 2006). Recently, the species trait approach was recognized as an important tool in ecotoxicology. Baird et al. (2008) introduced the concept of TERA (trait-based ecological risk assessment). Rubach et al. (2011) demonstrated the potential of the species trait approach in ecotoxicological research. With regard to pesticides, the studies incorporating the species trait approach into pesticide effect assessment are scarce. For instance, Liess & Van der Ohe (2005) introduced the SPEAR index to identify the effects of pesticides on invertebrate communities. This index includes the species traits that determine the sensitivity of species to pesticides: generation time, dispersal ability, the presence of aquatic life stage and its sensitivity to toxicants (Liess & Van der Ohe, 2005). In the research of Rubach et al. (2010), the sensitivity of the arthropoda taxa was related to concentrations of organophosphate, carbamate and pyrethroid pesticides using species traits. Even though several initiatives aiming to link pesticides and species traits of aquatic invertebrates were undertaken, more research is needed to understand the effects of pesticides on the species trait composition of aquatic biota in the field, taking into account multi-stressor conditions of natural ecosystems.

Thesis aims

The overall aim of the thesis was to study the effects of pesticides on aquatic macrofauna in the field. There is a large amount of research addressing the effects of pesticides on aquatic species under control or semi-field conditions. Yet, the studies focusing on pesticide effects on aquatic biota in the complex field setting are scarce. Ditch systems located in the agricultural area of the Netherlands represent an example of highly dynamic aquatic ecosystems influenced by chemical and physical disturbances, and provide a good setup to study the effects of pesticides on aquatic biota in the field.

The main aims of research were:

1. To study the temporal variation in pesticide concentrations and macrofauna diversity in ditches of the flower growing region of the Netherlands over the period 1975 - 2010
2. To quantify 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), presence of other biota and time, and to investigate the predictive power of the species trait approach in quantifying pesticide effects on aquatic macrofauna in the field
3. To study the effects of pesticides combined with environmental factors on aquatic invertebrates exposed in situ in ditches bordering flower bulb fields
4. To study the combined effects of the insecticide imidacloprid and nutrient limitation on the aquatic invertebrate *Daphnia magna*

Thesis outline

Chapter I. In this chapter, the major threats to freshwater biodiversity are discussed. An overview of the environmental issues in the Netherlands is given, with respect to water quality and agricultural practices (flower growing in particular). The importance of aquatic biodiversity with regard to nature conservation and ecosystem functioning, and the adverse effects of pesticides on aquatic ecosystems are discussed. An overview of the current challenges in risk assessment of pesticides (context dependency of pesticide effects and species trait considerations) is given.

Chapter II. In this chapter, annual trends in pesticide concentrations in surface waters (expressed as toxic units and concentrations of individual pesticides) and aquatic macrofauna diversity (expressed as Shannon diversity index) in ditches of the flower growing area of the Netherlands over the period 1975 - 2010 are analyzed.

Chapter III. In this chapter, the effects of pesticides on freshwater macrofauna are investigated, taking into account environmental factors, the presence of other biota and temporal variation. To this purpose, field work was performed in the intensively used agricultural area (the flower bulb growing region of the Netherlands). Sampling and taxonomic identification of aquatic macrofauna, measurements of water chemistry parameters and pesticide concentrations in ditches bordering flower bulb fields was carried out during two consecutive years. A variance partitioning procedure, 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 (nitrate, nitrite, phosphate, dissolved organic carbon, dissolved oxygen, temperature, and macrophyte coverage), the presence of other biota, time (seasonal and annual variation), shared variance between different factors and unexplained variance.

Chapter IV. In this chapter, the potential of species trait approach in quantifying the effects of pesticides on aquatic macrofauna in the field is investigated. To this aim, macrofauna data previously collected in the field was analyzed (described in Chapter II). Each macrofauna taxon was classified according to the 54 trait modalities of nine species traits. After that, a variance partitioning procedure was applied to divide the variance in trait community composition into the variance explained by pesticides, environmental factors, time, shared variance between different factors, and unexplained variance. In addition, redundancy analysis (RDA) was performed to identify the relationships between species trait modality distributions, pesticides and environmental factors.

Chapter V. In this chapter, population responses of *Daphnia magna*, *Chydorus sphaericus* and *Asellus aquaticus* to pesticides in contaminated ditches around bulb fields were studied, taking into account environmental factors (nitrate, nitrite, phosphate, dissolved organic carbon, dissolved oxygen, and temperature). The responses of aquatic invertebrates to pesticides and environmental factors were ivestigated by means of in situ bioassays deployed in ditches adjacent to flower bulb fields.

Chapter VI. In this chapter, the responses of *Daphnia magna* to the insecticide imidacloprid in combination with food quality levels (expressed as the carbon: phosphorus ratio of algae cells) were investigated. To study the combined effects of imidacloprid and food limitation, *D. magna* juveniles exposed to different concentrations of imidacloprid were supplied with algae of varying nutritional quality.

Chapter VII. In this chapter, the results of the thesis are discussed within the scientific and social context. The implication of the study results to water management and environmental policy is highlighted.

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