Predicting effects of multiple stressors on aquatic biota.

Heugens, E.H.W.

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Chapter 1

General Introduction

The expanding human population and the associated rise in industrial and agricultural activities results in an ongoing pressure on aquatic ecosystems. Anthropogenic activities have changed the global climate and habitats, and increased the input of nutrients and large numbers of chemicals. Water pollution has greatly affected the distribution of aquatic species. Anthropogenic stressors are likely to alter species abundance and persistence, and to reduce biodiversity (Mooney and Godron 1983).

With respect to chemicals, risk assessment procedures have been developed to protect ecosystems against adverse effects. In laboratory toxicity tests, effects of single substances have been tested on few test species (algae, invertebrates, and fish) and the test results have been extrapolated to ecosystems (Van Leeuwen and Hermens 1995). Since levels of several contaminants have declined over the past decade (RIZA and RIKZ, 2001), results of standard toxicity tests suggest that these low levels of substances have no impact on ecosystems. However, the constant and optimal experimental conditions used in toxicity testing lack ecological realism, as environmental conditions in the field are generally variable and suboptimal. Also, in the field many species and substances occur simultaneously, while they are usually investigated separately in experiments. The differences between laboratory and field conditions are likely to result in changes in bioavailability and toxicity of substances, which complicates the extrapolation of results from standard toxicity tests to the field. Therefore, toxicity experiments have been performed under more ecological realistic conditions. For instance, the joint effects of combinations of chemicals were studied in mixture toxicity studies (Greco et al. 1995, Vighi et al. 2003), while interactions between biological species were studied in model ecosystems (Brock et al. 1992), experimental ditches (Van den Brink 1996), and enclosures (Caquet et al. 2000). A relatively new topic is the interaction between environmental factors and chemicals. Although much information is available about the responses of aquatic species to individual environmental factors, little is known about the interaction between, and joint effects of natural factors and chemicals on aquatic biota.

MULTIPLE STRESS

Many different definitions for stress have been formulated (as reviewed by Grime 1989, Hoffmann and Parsons 1994). Two viewpoints can be distinguished: stress is either defined as the *response* of a biological system (ranging from a cell to an ecosystem) to internal or external pressures acting on this system, or as the *agent* causing the response in a biological system, usually called the 'stressor' or 'stress factor' (Hoffmann and Parsons 1994). However, stressors cannot be identified without reference to the biological system on which they act (Koehn and Bayne 1989, Sibly and Calow 1989). The intensity of a stress response may vary from zero to complete inhibition and depends on the biological system, and on the nature, severity, and periodicity of the stressor (Grime 1989).

Environmental conditions in the field are seldom optimal and this would imply that organisms or populations living in natural systems are continually stressed. When deviations from optimal performance due to unfavorable conditions are not too large, compensation can occur. However, stressed organisms or populations are assumed to be more susceptible to additional unfavorable factors, for instance exposure to toxicants. On the other hand, exposure to a toxic substance may narrow the tolerance range for natural factors. In the present thesis, the term 'multiple stress' is used for the combination of natural stressors (abiotic and biotic) and chemicals. Following Grime (1989), the biological effect of multiple stressors is determined as the impairment of the normal functioning of individuals or populations, by reducing resource acquisition, survival, growth or fecundity relative to optimal conditions. Relatively little is known about the interactions between the two types of stressors. Therefore, knowledge of the interactions between multiple stressors, their joint effects, and underlying mechanisms is essential to assess the ecological relevance of multiple stressors, and to predict effects on aquatic species in the field.

OBJECTIVES

The present thesis addresses the joint effects of substances and environmental factors on aquatic biota and explores the mechanisms of their interaction by combining laboratory experiments and models. The objectives of this thesis are therefore:

- to review the present state of knowledge of joint effects of substances and natural factors on aquatic organisms,
- to assess the influence of multiple stressors at the individual and population level,

- to clarify whether patterns observed in multiple stressor studies are due to changes in exposure to chemicals or to a modified intrinsic sensitivity of the test organisms,
- and to quantify the interaction between compounds and environmental factors.

STRESSORS AND TEST SPECIES

Although many other natural stressors could have been chosen, this thesis focuses on temperature, food, and salinity. The choice for these three stress factors is extensively discussed in the literature review in Chapter 2. In short, all three factors are ecologically important and are highly variable in the field, which may cause stress responses in organisms. Further, they are likely to interact with substances, as they are major factors influencing the physiology of organisms.

Cadmium was chosen as a model toxicant. It still causes problems in Dutch inland waters, although levels are declining (CIW 2001). For this reason, cadmium is a priority and blacklisted substance in the Netherlands (VROM 2001). It is measurable in low concentrations and it is a nonessential element, which cannot be regulated by most organisms (Rainbow 1998). This makes cadmium an appropriate toxicant for use in accumulation experiments with small animals.

The water flea *Daphnia magna* was used as a model test species. Daphnids have been extensively used for toxicity testing. They are representatives of filter-feeding zooplankton and have a major contribution to food webs in many fresh waters (Lampert 1987). They are easy to culture in the laboratory and due to parthenogenetic reproduction under favorable conditions, populations of one genotype can be obtained. Further, an extensive amount of literature is available about the individual effects of the chosen stressors on *D. magna*.

OUTLINE

As stated above, this thesis aims to assess the joint effects of substances and environmental factors on aquatic biota, and to analyze the nature of their interaction by studying underlying mechanisms through experiments and models. The study departs from a literature study searching for studies concerned with the joint effects of multiple stressors in the aquatic environment. The results are given in Chapter 2, where the influences of temperature, nutritional state, and salinity on the responses of several aquatic species to various classes of chemicals are discussed. Quantitative relationships were derived from data presented in literature, which were used to discuss the suitability of uncertainty factors used in risk assessment procedures. From this literature review, several questions arose, which were investigated in subsequent experimental chapters. A stepwise approach was chosen to test successive situations of increasing complexity and ecological relevance. Chapter 3 addresses the question if temperature-dependent toxicity of chemicals is due to acceleration of physiological processes, and thus toxicant uptake rates, or increased sensitivity of the daphnids. With this purpose, data of toxicity and accumulation experiments were analyzed with the DEBtox model. Chapter 4 describes a chronic experiment with *D. magna* in which the influence of temperature and food level on the toxicity of cadmium was investigated at the population level. The DEBtox model was used to link effects on energy budgets of individuals to life history parameters determining population growth and to detect underlying mechanisms. Results of the model analyses are given in Chapter 5. In Chapter 6, the predictability of multiple stressors is discussed, and potential implications for risk assessment are mentioned.