

## *PhD Dissertation 02/2013*

### **The influence of competition on effect and recovery from pesticides in freshwater zooplankton communities**

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# The influence of competition on effect and recovery from pesticides in freshwater zooplankton communities

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1. Knillmann S, Stampfli NC, Beketov MA, Liess M (2012) Intraspecific competition increases toxicant effects in outdoor pond microcosms. *Ecotoxicology* 21 (7): 1857-1866. doi:10.1007/s10646-012-0919-y
2. Knillmann S, Stampfli NC, Noskov YA, Beketov MA, Liess M (2012) Interspecific competition delays recovery of *Daphnia* spp. populations from pesticide stress. *Ecotoxicology* 21:1039-1049. doi:10.1007/s10646-012-0857-8
3. Knillmann S, Stampfli NC, Noskov YA, Beketov MA, Liess M (2013) Elevated temperature prolongs long-term effects of a pesticide on *Daphnia* spp. due to altered competition in zooplankton communities. *Global Change Biology* 19: 1598–1609, doi: 10.1111/gcb.12151

**Abstract**

Intra- and interspecific competition play an important role in natural communities. In addition, toxicants are reported to alter biotic interactions. However, little is known about the influence of competition on toxicant effects within the aquatic community context. The present PhD thesis contributes to the understanding of how competition alters effect and recovery from pesticides of sensitive taxa under natural conditions.

Two microcosm experiments with zooplankton communities were conducted in 2008 and 2009 over a period of 4 and 5 months, respectively. In both experiments, communities were exposed to a pulse of the pyrethroid insecticide esfenvalerate (0.03, 0.3 and 3  $\mu\text{g/L}$ ). In the first experiment, the influence of competition on pesticide effects was investigated under an increased range of biotic interactions. For this purpose abiotic and biotic conditions were varied by shading and regular harvesting of the communities. In the second experiment, the combined effect of warming periods and exposure to esfenvalerate on the zooplankton communities was analysed.

Following the general introduction in Chapter 1, the results on the interaction of competition and pesticide exposure are described within the first experiment (Chapters 2 and 3) as well as the second experiment (Chapters 3 and 4), focused on the sensitive genus *Daphnia*. The results of Chapter 2 show that the pesticide-related survival of *Daphnia* spp. strongly depended on their densities before contamination, independent of the treatments of shading and harvesting. High densities before contamination were assumed to increase intraspecific competition for food and space and thus to affect the fitness of the organisms at the individual level. Consequently, high intraspecific competition enhanced the pesticide sensitivity of *Daphnia* spp. by a factor of up to 100 within the community context.

The recovery of *Daphnia* spp. from the effect of esfenvalerate is described in Chapter 3 (Experiment 1). The time needed for the recovery of populations was up to eight times longer than predicted by models using the population growth rates under optimal single species conditions. Interspecific

competition with closely related but less sensitive Daphniidae was detected as the underlying process. Following a pesticide-related reduction of sensitive *Daphnia* spp., less sensitive Daphniidae benefitted from the resources released and increased in abundance. This indirect increase in less sensitive taxa in turn delayed the recovery of *Daphnia* spp. from esfenvalerate, especially at concentrations leading to partial mortality of the populations (0.03 and 0.3  $\mu\text{g/L}$ ). The findings described in Chapters 2 and 3 were enabled by the exceptionally high number of replicates compared to similar experimental outdoor systems ( $n = 24$  per concentration level).

Chapter 4 is based on the second experiment, showing that the presence of short warming periods alone altered the community structure and thus the long-term biotic interactions. As a result, interspecific competition was prolonged under warming conditions, causing a delay in recovery of *Daphnia* spp. from 3  $\mu\text{g/L}$  esfenvalerate that exceeded the recovery under ambient conditions by a factor of two. This study highlights that changing environmental conditions may further increase the influence of competition on the recovery of populations from toxicant stress.

In conclusion, the thesis identifies and quantifies the role of intraspecific competition in increasing pesticide sensitivity, and interspecific competition in delaying the recovery of populations from pesticides in the community context. The outcomes can be used to reduce uncertainty and improve the predictive quality of the current risk assessment framework.

## Zusammenfassung

Intra- und interspezifische Konkurrenz spielen eine wichtige Rolle für Artgemeinschaften unter natürlichen Bedingungen. Zudem ist es bekannt, dass Schadstoffe biotische Interaktionen verändern können. Dennoch weiß man nur wenig über den Einfluss von Konkurrenz auf die Effekte von Schadstoffen im Kontext der Gemeinschaft. Die vorliegende Doktorarbeit trägt zum Verständnis bei, wie Konkurrenz den Effekt und die Wiedererholung von Schadstoffen bei sensitiven Taxa im Freiland verändert.

Zwei Mikrokosmenexperimente mit Zooplanktongemeinschaften wurden 2008 und 2009 über eine Periode von jeweils 4 und 5 Monaten durchgeführt. In beiden Experimenten wurden die Gemeinschaften mit einer Pulskontamination mit dem pyrethroiden Insektizid Esfenvalerat (0.03, 0.3 und 3  $\mu\text{g/L}$ ) behandelt. Im ersten Experiment wurde der Einfluss von Konkurrenz unter einem erhöhten Spektrum von biotischen Interaktionen untersucht. Hierfür wurden abiotische und biotische Bedingungen durch Beschatten und regelmäßiges Auslesen der Gemeinschaften variiert. Im zweiten Experiment wurde die Kombinationswirkung von Wärmeperioden und Pestizid auf die Mikrokosmengemeinschaften untersucht.

Nach der allgemeinen Einleitung in Kapitel 1, werden die Ergebnisse zu der Interaktion zwischen Konkurrenz und Pestizidexposition dargestellt. Der Schwerpunkt liegt auf der sensitiven Gattung *Daphnia* spp. im ersten Experiment (Kapitel 2 und 3), sowie auch im zweiten Experiment (Kapitel 4). Ergebnisse aus Kapitel 2 zeigen, dass die Überlebensraten von *Daphnia* spp. gegenüber Esfenvalerat stark von den Populationsdichten vor der Kontamination abhängen. Diese Abhängigkeit war unabhängig von der Behandlung mit Beschattung oder der Auslese von Organismen. Für hohe Dichten vor der Kontamination wurde angenommen, dass diese die intraspezifische Konkurrenz um Nahrung und Platz erhöhen und damit die Fitness der Individuen beeinträchtigen. Folglich nahm die Sensitivität von *Daphnia* spp. gegenüber dem Pestizid durch hohe intraspezifische Konkurrenz im Kontext der Gemeinschaft um einen Faktor von bis zu 100 zu.



Die Wiedererholung von *Daphnia* spp. von der Belastung mit Esfenvalerat ist im Kapitel 3 (Experiment 1) beschrieben. Die Zeit bis zur Wiedererholung der Populationsabundanz dauerte bis zu acht Mal länger als die Vorhersage von Modellen, die auf den Wachstumsraten von Populationen unter optimalen Bedingungen basieren. Als zugrunde liegender Prozess für die Verzögerung wurde die interspezifische Konkurrenz mit nahe verwandten, aber weniger sensitiven Daphniidae identifiziert. Nach der Abnahme von *Daphnia* spp. durch das Pestizid, konnten weniger sensitive Daphniidae von den freiwerdenden Ressourcen profitieren und anzahlmäßig zunehmen. Die indirekte Zunahme von insensitiven Taxa bewirkte wiederum eine verzögerte Wiedererholung von *Daphnia* spp. von der Pestizidbelastung. Dieser Zusammenhang war besonders deutlich bei Konzentrationen, die zu einer partiellen Mortalität der Populationen führten (0.03 und 0.3 µg/L). Die Resultate aus Kapitel 2 und 3 wurden zudem durch die außergewöhnlich hohe Anzahl an Replikaten im Vergleich zu ähnlichen Freilandssystemen ermöglicht (n = 24 pro Konzentration und Kontrolle).

Das Kapitel 4 basiert auf dem zweiten Experiment und zeigt, dass die Präsenz von kurzen Wärmeperioden die Gemeinschaftsstruktur und somit langfristig die biotischen Interaktionen veränderte. Daraus resultierte ein verlängerter Einfluss von interspezifischer Interaktion unter warmen Bedingungen, was die Wiedererholung von *Daphnia* spp. bei 3 µg/L im Vergleich zu den Kontrollbedingungen um den Faktor zwei verzögerte. Diese Studie hebt hervor, dass die Veränderung von Umweltbedingungen die Bedeutung von Konkurrenz für die Wiedererholung von Schadstoffbelastungen zusätzlich verstärken kann.

Abschliessend kann zusammengefasst werden, dass die vorliegende Arbeit qualitativ und quantitativ beschreibt, wie intraspezifische Konkurrenz die Pestizidsensitivität erhöht und auch wie interspezifische Konkurrenz die Wiedererholung von Populationen von Schadstoffen im Kontext der Gemeinschaft verlängert. Diese Ergebnisse können dazu verwendet werden um Unsicherheiten zu reduzieren und die Vorhersage von Schadstoffeffekten in der aktuellen Risikoabschätzung zu verbessern.

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**List of abbreviations**

ANCOVA	analysis of covariance
ANOVA	analysis of variance
CLASSIC	Community Level Aquatic System Studies - Interpretation Criteria
DCA	detrended correspondence analysis
d.f.	degrees of freedom
DMSO	dimethyl sulfoxide
DO	dissolved oxygen
EC	electrical conductivity
EPT	Ephemeroptera, Plecoptera, Trichoptera
ER	ecological risk
IPCC	Intergovernmental Panel on Climate Change
K <sub>oc</sub>	soil organic carbon partition coefficient
K <sub>ow</sub>	octanol water partition coefficient
LC50 [x h]	median lethal concentration after x hours
EC50 [x h]	median effective concentration after x hours
OECD	Organisation for Economic Cooperation and Development
PCA	principal component analysis
PGR	population growth rate
PRC	principal response curve
PVC	polyvinyl chloride
RDA	redundancy analysis
RFU	relative fluorescence units
SPEAR	species at risk
spp. (e.g. <i>Daphnia</i> spp.)	species pluralis
UV-radiation	ultraviolet radiation

## **General outline of the chapters**

The present thesis is divided into five chapters. Chapter 1 gives a general introduction into the topic of environmental factors as additional stressors for the assessment of toxicant effects. In addition, the relevance of investigating pesticides and the use of aquatic microcosms in the risk assessment of toxicants is introduced. The last section of Chapter 1 explains the concrete research questions and the concept of the experimental and analytical approach. The following Chapters 2, 3 and 4 present the scientific publications on the influence of competition on effect and recovery of sensitive taxa from pesticides. Finally, in Chapter 5 all main results are summarised and discussed. Furthermore, the representativity of the outcomes from the microcosm experiments for toxicant effects in the field is analysed. Subsequently conclusions are drawn and an outlook for future research and applications is given.

## **Chapter 1 - Introduction**

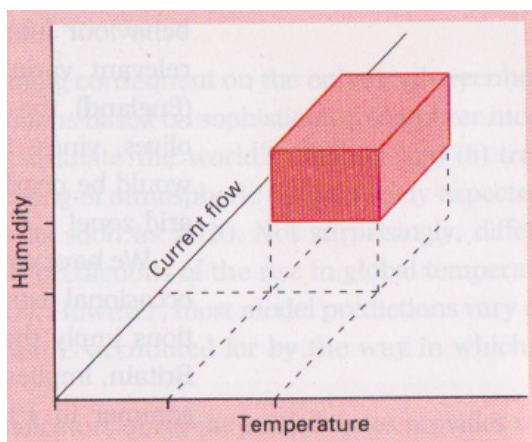
### **1.1 Environmental factors in Ecotoxicology**

Hutchinson (1957) defined the ecological niche of a species as a n-dimensional hypervolume with n environmental conditions. According to Hutchinson (1957) environmental conditions and resources can be optimal, but also sub-optimal or non-suitable leading to decreased survival and reproduction rates up to the point of exclusion of the population from the ecosystem. Stressful environmental conditions can be caused by both, abiotic and biotic factors outside the ecological optimum of species. Examples for abiotic stressors include sub-optimal pH (Thomsen and Friberg 2002), salinity (Baillieul et al. 1996, Hassell et al. 2006) and oxygen conditions (Nebeker et al. 1992, Heisey and Porter 1977). Abiotic factors influence the biotic interactions in a population or community. Biotic interactions as competition (Hülsmann 2001) and predation (Black and Dodson 1990) are very important parameters to affect the fitness of individuals. Competition can be again differentiated into intraspecific or interspecific competition. Following the definition of Birch (1957) both types of competition include exploitative competition for food and interference competition for space.

All the above mentioned environmental stress conditions occur in the wild and it was shown many times that they act as additional stressors when toxicants are released into the aquatic environment. An increase of the effects of a toxicant due to additional environmental stressors was reviewed by Heugens et al. (2001). In contrast to the relevance of environmental conditions in the wild, the current risk assessment is based on the sensitivity of species determined under optimal conditions. Results from such tests reflect the “toxicological sensitivity“ of species, which can be very different from the “context sensitivity“ under natural conditions (Liess and Beketov 2011). To determine the sensitivity of an organism to pesticides, heavy metals or other toxicants, we need to understand the range of effects that occur shortly after contamination and develop over time as delayed effects. In addition, the ability

of individuals, populations and communities to recover from toxicants under different environmental conditions must be assessed.

The aspect of stressful environmental conditions becomes further important when considering the impact of the current changes in the global climate and on existing ecological niches. According to the International Panel on Climate Change (IPCC, 2007), the average global temperature increased by 0.74°C from 1906 until 2005. Further observations indicate that not only the average temperature is rising, but also the length, frequency and intensity of warm periods (IPCC 2012). As mentioned above, also temperature has a strong influence on many physiological and ecological processes of aquatic organisms (Cairns et al. 1975). The niches described by Hutchinson (1957) will change due to an alteration of thermal conditions in relation to other environmental conditions as graphically shown in Fig. 1.1. This can affect biotic interactions, and species composition will shift towards a dominance of warm-adapted species as observed for marine copepod communities in the North Atlantic (Beaugrand et al. 2002). Similar shifts in community composition have been also reviewed by Walther (2010) and Wassmann et al. (2011). Therefore, it can be presumed that direct and indirect effects of changing temperatures can act as pronounced additional stressors to the response of organisms in communities and populations to toxicants.



**Fig. 1.1:** Ecological niches after the definition of Hutchinson (1957) in three dimensions of environmental conditions, modified after Begon et al. (1990).

*Environmental parameters and toxicant effects*

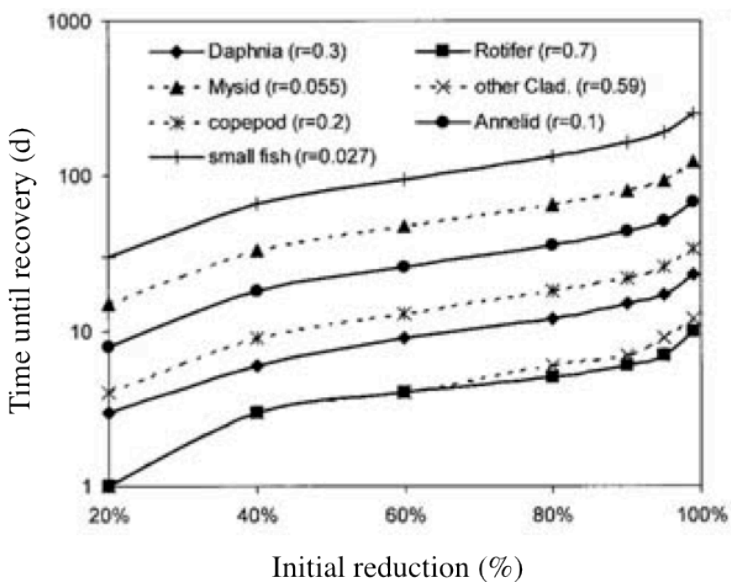
Several abiotic factors have been proven to increase toxicant effects at different levels of biological organisation (individual, population, community): high salinity (Schäfer et al. 2011), high temperature (Osterauer and Köhler 2008, Heugens et al. 2006), UV-B radiation (Liess et al. 2001, Duquesne and Liess 2003), and low oxygen (Van der Geest et al. 2002). Within biotic factors, predation and competition are also well known to exacerbate the effects of toxicants. At the population level, predator cues were detected to increase effects of the insecticide carbaryl on tadpoles (Relyea 2003, Relyea 2004). Enhanced combined effects of food limitation and toxicant exposure were shown at the individual level for daphnids exposed to chlorpyrifos (Rose et al. 2002). At the population level, food limitation increased the effects on mayfly larvae exposed to esfenvalerate (Beketov and Liess 2005), as well as on cladocerans exposed to cadmium (Chandini 1988). In comparison, augmenting population densities is expected to increase resource competition of the individuals for food and space. Similarly to food limitation as the only stressor, higher densities are observed to enhance short-term toxicant effects. This was for example confirmed at the population level for caddisfly larvae exposed to fenvalerate (Liess 2002) and within a multi-species system for daphnids and mosquito larvae likewise exposed to fenvalerate (Foit et al. 2011). However, within communities under conditions that resemble those in the wild, there is little knowledge about a quantitative relationship between intra- or interspecific competition and the response of sensitive populations to toxicants. The ability to quantify the effect of competition and other stressors under most realistic conditions is yet essential to include the extent of additional stressors into the existing risk assessment of toxicants.

*Relevance of environmental factors for the recovery of organisms from toxicants*

The long-term effect of a toxicant on a population can be assessed by the short-term impairment (lethal and sublethal) and in addition by the time to recover from this impairment. Hence, it is not only important to address the short-term effects of toxicants in biological systems, but also the recovery process of



species, populations and communities. Generation time is a very important parameter to estimate recovery of organisms from disturbances. A relative relationship between generation time and recovery was found for invertebrate communities in mesocosms (Sherratt et al. 1999, Beketov and Liess 2008) and in the field (Liess and von der Ohe 2005, Niemi et al. 1990). Taking this knowledge into account, the modelled prediction of recovery is often based on species-specific population growth rates (PGR's). The data base for PGR's originates mainly from single-species tests conducted under optimal laboratory conditions (Barnthouse 2004). According to the approach by Barnthouse (2004), sensitive taxa should recover at concentrations around LC50 within one generation time, defined as the time from birth of generation F0 until birth of generation F1 (Fig. 1.2).



**Fig. 1.2:** Time until population recovery depending on the initial reduction, modified after Barnthouse (2004).

In contrast to this consideration, several studies conducted at the community level in outdoor test systems detected times until recovery longer than 8 weeks for cladocerans, which were exposed to insecticides pulses

(Brock et al. 2000, López-Mancisidor et al. 2008). These durations until recovery are considerably longer than the generation times of most cladoceran species (Gillooly 2000). Consequently, there is a mismatch between optimal growth rates determined in laboratory investigations and the growth present in the field. Niemi et al. (1990) concluded from their review that there are more species-dependent and species-independent factors which are important for recovery than only the generation time as shown in Tab. 1.1.

**Tab. 1.1:** Factors relevant for recovery of organisms in aquatic systems according to Niemi et al. (1990).

Species dependent factors	Generation time and reproduction Presence of resistant stages Dispersal rate Biotic interaction (predation, competition)
Species independent factors	Changes in the habitat and productivity of the system Persistence of the toxicants Time of disturbance/contamination Presence of refugia

Liess and von der Ohe (2005) later applied many of the factors listed in Tab. 1.1 and added the species related toxicant sensitivity to create a trait based approach for the assessment of pesticides in running waters (SPEAR).

One important factor that may cause unfavourable environmental conditions and thus affect recovery is biotic interaction. Selective effects of toxicants indirectly disturb interactions like intra-/interspecific competition, predator-prey relationships and herbivore-producer interactions (Relyea and Hoverman 2006, Fleeger et al. 2003). Within the literature, two contrasting effects of competition on the strength and duration of toxicant effects in communities have been identified: (i) compensation and (ii) enhancement of long-term effects. Regarding compensation, many investigations have shown an

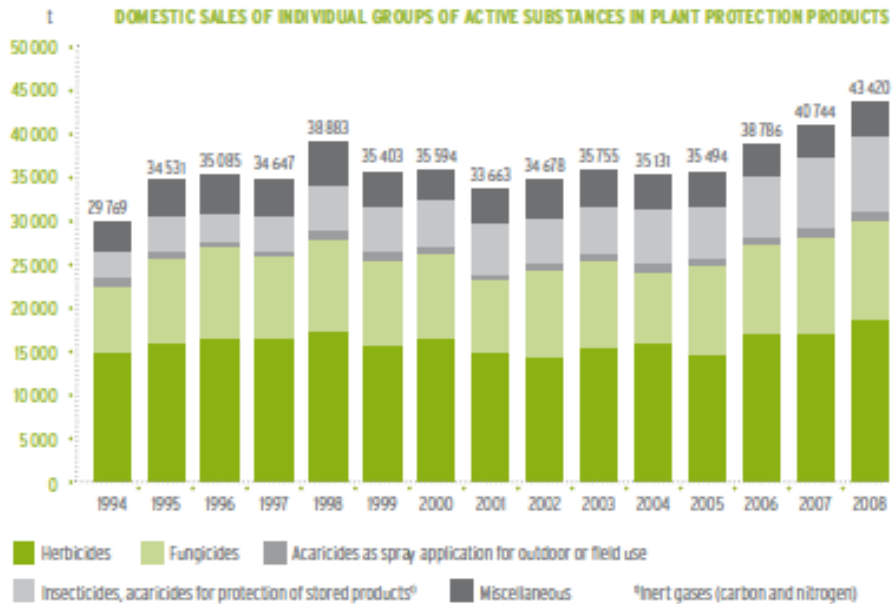
increase in less sensitive taxa following a reduction of sensitive taxa as reviewed by Fleeger et al. (2003) and Preston (2002). However, only little is known about how compensation with less sensitive species affects the recovery of sensitive species. Enhancement of long-term toxicant effects due to competition was observed in wild populations and quantified in experiments under laboratory conditions. In the field, for example, a delayed recovery of ecological effects from the oil spill Exxon Valdez in 1989 in Alaska was observed. Rockweed (*Fucus gardneri*) declined in abundance following the oil spill and thus facilitated an increase in algae and opportunistic barnacles. Peterson et al. (2001, 2003) assumed in their review on long-term effects of this event that the return of the rockweed and associated invertebrates was prolonged due to the increase in insensitive taxa. However, no direct links between prolonged recovery and indirect effects on competition have been identified and quantified under conditions that resemble those in the wild. To our knowledge, the only direct link between interspecific competition and prolonged recovery of sensitive taxa has been recently identified for a multi-species system under laboratory conditions. Recovery of *Daphnia magna* from fenvalerate was prolonged caused by competition with less sensitive mosquito larvae (Foit et al. 2011). Similar effects were detected at the population level. Although, *D. magna* recovered within a few days in abundance, the recovery of the age and size structure of the populations took much longer (Liess and Foit 2010). According to the authors, the delayed recovery of the structure can be explained by intraspecific competition between the different age and size classes.

## **1.2 The assessment of pesticide effects in relation to environmental factors**

### *Pesticides in the environment*

Pesticides are chemical compounds mainly applied in conventional agriculture to control, for example, unwanted weed (herbicides), insects (insecticides) and microorganisms (fungicides). On a global scale,  $2.36 \times 10^6$  kg of pesticides are used per year based on data from 2006 and 2007 (Grube et al. 2011). Public

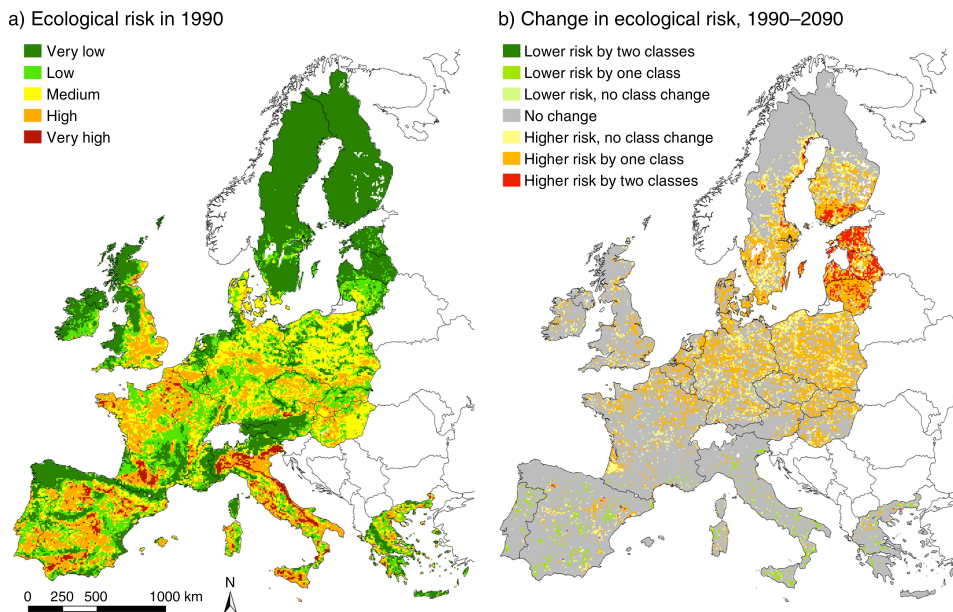
sales statistics in Germany even show an increase in the sold amount of pesticides from 2005 - 2008 (Fig. 1.3, UBA 2011).



Source: Federal Ministry for Food, Agriculture and Consumer Protection (BMELV): Statistisches Jahrbuch über Ernährung, Landwirtschaft und Forsten der Bundesrepublik Deutschland 2009, München-Hiltrup, verschiedene Jahrgänge

**Fig. 1.3:** Development of domestic sales of pesticides in Germany from 1994 until 2008 (UBA 2011).

Especially insecticides have strong toxic effects on non-target arthropods, including those in aquatic systems, due to their purpose and their wide distribution in the environment. Therefore, the upward trend of insecticides, but also other pesticides is alarming (Fig. 1.3). In acknowledgement of pesticide effects in the environment, the directive 2009/128/EG and the regulation no. 1107/2009 of the European Union on plant protection products demand a sustainable use of pesticides. Both also include the conservation of biodiversity and the prevention of unacceptable effects on non-target organisms. However, to reach that aim, a profound understanding of pesticide effects under realistic conditions and effect propagation from individual to ecosystem level is needed.



**Fig. 1.4:** Predicted development of the ecological risk (ER) in relation to the ecological risk in 1990 (Kattwinkel et al. 2011).

Another relevant aspect of pesticides in the aquatic environment is their interaction with current and future environmental alterations due to the global climate change. Global warming will increase volatilisation and degradation of pesticides and thus may reduce their concentrations in soil and water as reviewed by Noyes et al. (2009). However, Noyes et al. (2009) further reviews, that in contrast to the chemical fate, higher intensities of rain and storm events are likely to enhance the runoff and consequently the exposure to pesticides in aquatic systems. In addition to an increased runoff, changes in climate will shift agricultural practices to pristine areas. From agricultural practices in Europe during the last years it has been observed that rising temperatures correlate with an increase in the application of insecticides (Kattwinkel et al. 2011). Based on this relationship Kattwinkel et al. (2011) predicted, especially in Northern Europe, an increase in runoff and ecological risk (ER) according to future climate scenarios (Fig. 1.4).

*Assessment of pesticide effects in outdoor microcosms - artificial aquatic systems*

Ecotoxicological experiments under laboratory conditions are useful to identify the physiological sensitivity of organisms and to investigate isolated processes (e.g. combined effects of food limitation and toxicant stress) in one or multi-species systems (Campbell et al. 1999, Heger et al. 2001). However, the interactions of abiotic and biotic factors are much more complex in the field, altering the response of communities and populations to toxicants. For example, the community context was shown to be crucial for the effects of pesticides on amphibians (Relyea et al. 2005, Relyea and Hoverman 2008, Rohr and Crumrine 2005). To get a more realistic idea of processes in the field, observations from outdoor aquatic systems present a powerful tool to assess the complex interactions of environmental factors and pesticide exposure in lentic and lotic systems (Caquet et al. 1996, Heger et al. 2001, Campbell et al. 1999). Self-maintained outdoor microcosms, mesocosms or enclosures can represent specific field ecosystems. They also provide the possibility to identify direct and indirect pesticide effects, as well as to compare effects at different levels of biological organisation over several generations. Within such test systems, population and community data should be investigated for the ecological interpretation of the data as recommended by the existing guidance documents (Heger et al. 2001, Campbell et al. 1999).

For the understanding and the prediction of effects in the field, it is not only important to assess effects at the community and population level, but also to know which kind of endpoints are analysed. The measured endpoints at the different levels of biological organisation can be divided into “integrating“ and “differentiating“ endpoints as described by Liess and Foit (2010). The authors show, that integrating endpoints (e.g. at community level: total abundance, biomass) may underestimate time until recovery. Such an underestimation was explained by the fact, that a decline in sensitive size classes within a population (sensitive taxa) will be masked by compensation effects of less sensitive size classes (insensitive taxa) using the same resource. In comparison, differentiating endpoints (e.g. community structure via principal response curves) will take taxa

specific responses into account that are reflected at the population level (Van den Brink and Ter Braak 1999). Hence, for the evaluation of indirect toxicant effects on species interactions and consequently the recovery of sensitive species in the community context it is thus important to investigate differentiating endpoints.

### **1.3 Research questions and aim of the thesis**

Following the above described problems of pesticide effects in the environmental context, the aim of the thesis is to evaluate the relevance of competition for the risk assessment of pesticides in aquatic zooplankton communities. Competition was chosen as it is present in all ecosystems and important for the survival and fitness of species. In addition, despite the well studied relevance of competition as an additional stressor in ecotoxicology, there is only little knowledge about the extent of competition on the toxicant effect under conditions similar to those found in nature. Three research questions have been developed addressing the described gap of knowledge:

- 1. How and to what extent does intra- and interspecific competition affect the short-term effects of pesticides on sensitive taxa within the community context?*
- 2. How and to what extent does interspecific competition with less sensitive taxa affect the recovery of sensitive taxa from pesticides?*
- 3. What is the influence of abiotic factors, such as increased temperature, on competition within the community and the related pesticide effects?*

To answer the three research questions, two microcosm experiments were performed in 2008 and 2009 on the compound of the UFZ, Leipzig, Germany. The microcosms were designed to mimic shallow and small ponds in nature that are dominated by zooplankton species (Fig. 1.5). To increase the realistic conditions of the model systems, zooplankton communities were collected from several natural ponds and established together with natural pond sediment in the

microcosms. The effects of the widely used pyrethroid insecticide esfenvalerate on the communities were investigated as a model substance. In agreement with the guideline of the CLASSIC report on risk assessment with aquatic model systems (Heger et al. 2001), three concentrations of esfenvalerate were applied once in each experiment as a pulse exposure. Zooplankton communities matured over a period of 5 weeks before pesticide contamination. Community structure and population abundance were evaluated as endpoints.



**Fig. 1.5:** Microcosms in 2008

For the first experiment in 2008 with a duration of 4 months, the range of abiotic and biotic conditions was increased by shading and harvesting the communities. Shading and harvesting resulted in four treatments (“Shading/Harvesting“, “No Shading/Harvesting“, “Shading/No Harvesting“, “No Shading/No Harvesting“). Stampfli et al. (2011) investigated the effects of esfenvalerate on the community structure of the three treatments that represented a gradient of food availability and competition in the communities: “No Shading/Harvesting“, “No Shading/No Harvesting“, “Shading/No Harvesting“ (decreasing food availability/increasing competition from the left to the right). The authors detected a change in the community sensitivity to esfenvalerate by a factor of 100 between the three treatments. The change in sensitivity was related to



general unfavourable biotic conditions. Based on this outcome, the hypothesis that biotic interaction can explain the variation in short-term and long-term pesticide effects across all treatments is investigated. The underlying processes were analysed by means of the population dynamics of the sensitive taxa *Daphnia* spp. and their interaction with other taxa in the communities.

In the second experiment in 2009, the influence of warming periods and pesticide exposure on the community structure and the sensitive taxa *Daphnia* spp. was investigated over a time period of 5 months. The effect of increased temperature on pesticide effects and biotic interactions was assessed as an example of abiotic stressors. Furthermore, temperature is one of the most obvious parameters changing in relation to the global climate change (IPCC 2007). To create a shift of temperature conditions, three short warming periods of 1 week duration were applied with outdoor heaters. The warming periods increased the cumulative temperature without exceeding the ambient range under control conditions over the observation period.

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## **Chapter 2: Intraspecific competition increases toxicant effects in outdoor pond microcosms**

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## 2.1 Abstract

Competition is a ubiquitous factor in natural populations and has been reported to alter the ecological impact of xenobiotics. We investigated conditions that mirror the natural variation of environmental factors. For this, different treatments were applied to 96 outdoor pond microcosms by shading the ponds and harvesting the communities. Then, the effect of esfenvalerate (0.03, 0.3, and 3  $\mu\text{g/L}$ ) on populations of *Daphnia* spp. was investigated. The pesticide effect and the sensitivity of *Daphnia* spp. in the context of a zooplankton community was increased by intraspecific competition 11 days after contamination. This relationship was most pronounced at 0.03 and 0.3  $\mu\text{g/L}$  esfenvalerate, which were the concentrations that led to partial mortality. In contrast, interspecific interaction did not significantly alter the effect of the toxicant on *Daphnia* spp. Modelled concentration–response curves showed that the negative effects of the pesticide differed by a factor of up to 100 depending on the strength of intraspecific competition. In addition, a wider range of concentrations led to negative effects at high levels of intraspecific competition than at low levels. We argue that increased intraspecific competition reduces the availability of resources at the individual level and thereby increases the effect of contaminants. This knowledge about the interaction between competition and the response to toxicants is important in assessing the effects of these factors under field conditions.

**Keywords:** Competition, Esfenvalerate, Multiple stressors, *Daphnia*, Short-term effects, Community context

## 2.2 Introduction

It is well known that competition (Hülsmann 2001), predation (Black and Dodson 1990, Hanazato 1991), and unfavourable environmental conditions such as salinity stress (Baillieul et al. 1996) lead to reduced fitness and growth of individuals. These factors are all common in the wild. However, the sensitivities of different species to toxicants have mostly been determined within test systems

that are characterised by favourable conditions. The resulting estimations of sensitivity, which are also called toxicological sensitivities, are often used as the basis for risk assessments. Sensitivity to toxicants can be altered by several abiotic and biotic factors, as reviewed by Heugens et al. (2001). These effects can result in a “context sensitivity” that is substantially different from the toxicological sensitivity (Liess and Beketov 2011).

Competition, as defined by Birch (1957), includes exploitative competition for food and interference competition for space. Limited food availability, alone (Chandini 1988, Barry et al. 1995, Antunes et al. 2004) and in combination with predation (Beklioglu et al. 2010), is well known to have a negative influence on the response of aquatic organisms to toxicants. In addition, at high densities of individuals, which lead to both stronger interference and stronger exploitative competition, lower survival has been observed for populations of *Limnephilus lunatus* after exposure to pesticide (Liess 2002). At the community level, the presence or absence of interspecific competitors and predators in communities of amphibians has been shown to shape the response of species to endosulfan (Relyea et al. 2005, Rohr and Crumrine 2005). However, there is no knowledge about the quantitative relationship between competition and the strength of the short-term effects of pesticides within a community under conditions that are close to those found in the wild.

The aim of the present study was to quantify the extent to which intra- and interspecific competition affects the short-term sensitivity of populations of *Daphnia* spp. to pesticides under semi-field conditions. Through this approach, we attempt to understand the relevance of competition with respect to the risk assessment of toxicants in the field.

### **2.3 Materials and methods**

#### *General*

We aimed to establish pond communities with conditions that mirrored the natural variation of biotic and abiotic conditions. This was accomplished by conducting four different treatments in which harvesting and shading of the

communities were combined in different arrangements: “Shading/Harvesting”, “No Shading/Harvesting”, “Shading/No Harvesting”, and “No Shading/No Harvesting”. The treatments were designed to have subtle effects and to increase the range of the observed density (abundances) of species within the community, but not to result in significant changes in the investigated populations in the absence of the toxicant. The four different treatments were combined with exposure to four concentrations of esfenvalerate (0, 0.03, 0.3, and 3 µg/L). Six replicates were conducted for each of the 16 combinations.

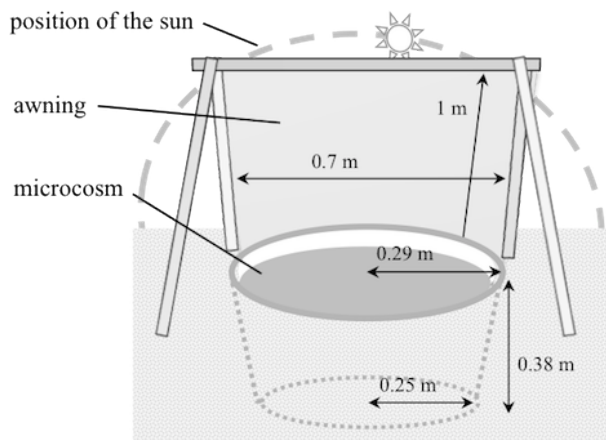
In the present study, we focused on the short-term effects of the pyrethroid esfenvalerate on *Daphnia* spp. 11 days after contamination. *Daphnia* is an abundant (Tab. 2.1) and toxicologically sensitive genus to esfenvalerate (Noskov 2011) within the zooplankton communities that were investigated in this study. The recovery processes of populations of *Daphnia* spp. until 59 days after contamination are presented in a separate paper (Knillmann et al. 2012). Changes in the structure and sensitivity of the entire communities are described in the publication by Stampfli et al. (2011), in which only the treatments “No Shading/Harvesting”, “No Shading/No Harvesting”, and “Shading/No Harvesting” were considered, because they represent a gradient of food availability and strength of competition.

#### *Microcosms: artificial pond systems*

Ninety-six outdoor microcosms were installed at the Helmholtz Centre for Environmental Research in Leipzig, Germany (51°21′13 N, 12°25′55 E). Each microcosm had a volume of 80 L and was filled with 60 L of water (tap water seeded with 1 L of natural pond water). The microcosms were maintained at a minimum water level of 60 L using tap water over the course of the experiment. Freshwater invertebrate communities and sediment were collected from five different natural ponds (forest and grassland habitats) within a radius of 15 km from the institute and used to establish the microcosm communities in late May and early June 2008. The natural pond sediment was mixed at a ratio of 1:1 with sand and distributed at the bottom of each tank to a thickness of approximately 1 cm. Furthermore, approximately 10 g of shredded leaves (*Populus* spp.) were

added to the microcosms. The collected invertebrate communities were distributed equally among all the microcosms using 250 mL dippers.

The treatments of shading and harvesting of the microcosms were established before contamination with esfenvalerate. For shading, awnings were positioned close to each pond at an angle of 45 so that the microcosms were shaded approximately from 12 a.m. to 4 p.m. each day (Fig. 2.1). All microcosms were shaded for 4 weeks until 4 days before contamination to enable comparable communities to develop in all the ponds. At this point, shading was removed from half of the microcosms (“No Shading/Harvesting”, “No Shading/No Harvesting”). For harvesting, biotic interaction was reduced in half of the microcosms (“Shading/Harvesting”, “No Shading/Harvesting”) by weekly harvesting 30% of the organisms in the communities using a net (10 x 12 cm, 250  $\mu\text{m}$  mesh size). The pond communities were stirred gently before harvesting to obtain a homogeneous distribution of the organisms. Harvesting was conducted from 2 weeks before contamination until the end of the experiment in mid-September 2008. Harvesting was started 10 days earlier than the shading was removed from half the microcosms on the assumption that more time would be required for the growth rate of invertebrates to adapt after harvesting was commenced than for algal growth to adapt after the awning was removed.



**Fig. 2.1:** Microcosm with awning for shading

### *Exposure to pesticide*

Esfenvalerate, (S)- $\alpha$ -cyano-3-phenoxybenzyl (S)-2-(4-chlorophenyl)-3-methylbutyrate, is a synthetic pyrethroid that is widely used in agriculture and is highly toxic to aquatic insects and crustaceans. We used the commercial formula Sumicidin Alpha EC (BASF, Limburgerhof, Germany), which is an emulsifiable concentrate that contains 50 g/L of the active ingredient esfenvalerate. On 4th July, 2008, the microcosms were contaminated with three different concentrations (0.03, 0.3, and 3  $\mu\text{g/L}$ ) of the pesticide, which was dissolved in dimethyl sulfoxide (DMSO). In all cases, the final concentration of DMSO in the ponds (10.6 mg/L, LC<sub>50</sub> [48 h] for *Daphnia magna* = 25,000 mg/L, Goto and Hiromi 2003) was kept below the solvent limit suggested by the OECD guidelines (2000). No solvent was added to the controls to ensure conditions that were fully undisturbed.

The exposure concentrations of esfenvalerate ranged around the laboratory-based acute LC<sub>50</sub> value for *D. magna* (LC<sub>50</sub> [48 h] = 0.37  $\mu\text{g/L}$ ), which was determined in preliminary laboratory studies and was similar to a previously published value of 0.27  $\mu\text{g/L}$  for the same species (Fairchild et al. 1992). The concentrations that were chosen for the present study were also in the range of those detected frequently in the field, namely, from trace concentrations to 0.166  $\mu\text{g/L}$  (Kelley and Starner 2004, Bacey et al. 2005) or even 0.76  $\mu\text{g/L}$  (Cooper et al. 2003). The actual concentrations of esfenvalerate in the open water were measured until 7 days after contamination (Tab. 2.1) by solid-phase extraction (Chromabond C18 Hydra columns, Macherey-Nagel, Dueren, Germany), followed by gas chromatography/mass spectrometry with single-ion monitoring (Varian CP-3800 gas chromatograph/Varian 2100T mass spectrometer, Rtx-5 columns, Restek, Bellefonte, PA, USA). The detection limit of the applied method is 0.01  $\mu\text{g/L}$ . Owing to its high values of  $\log K_{ow} > 6$  and  $K_{oc} = 215.000$  (Kelley 2004), most of the esfenvalerate that is added to a system is adsorbed rapidly into the sediment and particles, including algae. For this reason, the concentration of esfenvalerate decreased rapidly during the first few hours in all setups (Tab. 2.1). To ensure the highest possible degree of similarity for exposure and toxicity between shaded and non-shaded

microcosms, which will have different rates of photo-degradation and temperature-related toxicity (Kumaguru and Beamish 1981), contamination was initiated at around 6 p.m. At this time of day, there was no direct radiation on the ponds.

**Tab. 2.1:** Degradation of esfenvalerate.

Nominal concentration (µg/L)	Mean measured values of esfenvalerate (µg/L) and standard deviations at different time points after contamination (n = 12)					
	2 h	9 h	16 h	24 h	48 h	168 h
0.03	0.005 ± 0.01	n.d.*	n.d.	n.d.	-	-
0.3	0.07 ± 0.07	0.11 ± 0.07	0.04 ± 0.04	0.01 ± 0.02	0.004 ± 0.01	n.d.
3	1.80 ± 0.81	1.63 ± 0.72	0.25 ± 0.12	0.17 ± 0.05	0.15 ± 0.06	n.d.

\*n.d. = notectable (values below detection limit of 0.01 µg/L)

#### *Biological sampling and environmental parameters*

To determine species densities and identify the short-term effects of the pesticide, pelagic biological samples were collected at the following time points: 13 and 5 days before contamination, and 4 and 11 days after contamination. We included the time point of 11 days after contamination because it was observed previously that lethal effects of esfenvalerate or fenvalerate on aquatic macroinvertebrates still occurred 8 days and more after contamination (Beketov and Liess 2005). The samples were collected with a sampling tube (PVC, length = 31.7 cm, radius = 3.6 cm). First, the lid of the sampling tube was placed in the centre of each pond on top of the sediment. Before the tube was fitted onto the lid, the water was stirred gently to obtain a homogeneous distribution of organisms in the pond. Afterwards, the water from the tube (which contained 1.7% by volume of the water from the pond), including any organisms, was passed through a sieve (180 µm mesh size). The retained organisms were preserved in 70% ethanol, and then identified to the level of genus (within the order of Cladocera and the families of Chaoboridae, Culicidae, and Baetidae), suborder (within the order of Odonata and Coleoptera) or class (Ostracoda), and

counted under a microscope. For the genus *Daphnia*, we carried out further identification to species level in a subset of random samples (n = 17, replicates without pesticide exposure) that were selected from among the samples used for the other analyses at various time points. In this subset of samples, the presence of *Daphnia longispina* and *Daphnia pulex* was detected. The most abundant species within the genus was *D. pulex* (> 70%, with nearly 100% dominance before contamination with esfenvalerate). Other taxonomic groups that were found frequently in the pond communities are listed in Tab. 2.2.

**Tab. 2.2:** Densities of main invertebrate taxa in the communities that were not exposed to pesticide. The untransformed densities are displayed as the mean and standard deviation from 13 days before until 11 days after contamination.

Taxon	Abundances (Ind./L)
<i>Daphnia</i> spp.	54.7 ± 67.2
Other genera of Daphniidae	172.9 ± 125.2
Chydoridae	76.7 ± 180.5
Copepoda	34.2 ± 44.9
Ostracoda	6.2 ± 6.7
Baetidae	0.4 ± 1
Culicidae	1.9 ± 2.7
Chaoboridae	3.1 ± 3.3
Odonata	0.03 ± 0.2

Water temperature was recorded continuously with Handylog DK501-PL data loggers (Driesen & Kern, Bad Bramstedt, Germany). Differences in UV A + UV B radiation among the treatments were measured over the course of a sunny day and a cloudy day in July with a UV-meter (UV-Vis radiometer RM-21, Dr. Gröbel UV-Elektronik GmbH, Ettlingen, Germany). To monitor water quality and other differences among the treatments, additional parameters were measured on a weekly basis for a subsample of 32 microcosms over the

entire observation period. These parameters included the concentration of dissolved oxygen (DO) (WTW Multi 340i meter, WTW Instruments, Weilheim, Germany), pH (HI-98127, Hanna Instruments, Woonsocket, RI, USA), electrical conductivity (EC) (HI-98312, Hanna Instruments, Woonsocket, RI, USA), and concentration of chlorophyll a as a measure of algal density (relative fluorescence units-RFU, GEMINI XPS Fluorescence Microplate Reader, Molecular Devices, Sunnyvale, CA, USA).

### *Statistical analysis*

The data obtained by the counting of species were subjected to fourth-root transformation, as suggested previously for abundance data that are skewed (Quinn and Keough 2002). Differences in mean density ( $n = 24$  per concentration and control) among the different concentrations of toxicant and the control at the various time points were investigated by analysis of variance (ANOVA). The ANOVA was followed by pairwise t-tests for multiple comparisons and adjusted if the variances of the groups were not homogeneous. To correct the p values in multiple testing, we used the Bonferroni factor. In the case of non-normally distributed samples, the Kruskal–Wallis test for non-parametric data was applied, followed by a non-parametric multiple-comparison test (R-package *pgirmess*, function *kruskalmc*, Siegel and Castellan 1988). Data from 13 days and 5 days before contamination showed similar data ranges and a minor change in mean density of 16% (data not shown). Therefore, data from these time points were pooled together to minimise sampling-related variation before contamination. To analyse differences in physicochemical parameters and pesticide exposure among the treatments, we applied ANOVA and pairwise t-tests on untransformed data at the single time points.

Survival was calculated as the ratio of the population density obtained for the first samplings after contamination (4 days and 11 days after contamination) to the mean density before contamination (-9 days) for each microcosm. Factors that were expected to be relevant for the survival of *Daphnia* spp. were evaluated by analysis of covariance (ANCOVA, type II of sum of squares). As explanatory variables, we included pesticide concentration,



densities of *Daphnia* spp. before contamination, and the four treatments of shading and harvesting. Pesticide concentration and densities of *Daphnia* spp. before contamination were used as numerical variables and treatment as a categorical variable. The model was simplified by the stepwise removal of non-significant terms until the minimal adequate model was achieved (Crawley 2007). To investigate the influence of other species in the community on the pesticide-related survival of *Daphnia* spp., we conducted equivalent ANCOVA in which we included, in addition to the above-mentioned explanatory variables, the main taxa (Tab. 2.1) as numerical variables.

Linear models for the analysis of regression were produced to determine the strength of the relationship between the toxicant and the initial densities and survival of *Daphnia* spp. Differences in the variance of densities and survival were tested for all concentrations with Levene's test, followed by an F-test to compare single concentrations. For all linear models, we checked the residuals of the model for normal distribution, homogeneity of variance, and influential data points (Crawley 2007). We compared survival at low and high densities before contamination for the control and 0.03 and 0.3  $\mu\text{g/L}$  esfenvalerate (Student's t-test). Replicates with low and high densities of *Daphnia* spp. before contamination were grouped on the basis of the first quartile (lowest 25%) and the fourth quartile (highest 25%) of the population densities, respectively. The quartiles were chosen to represent at least four data points of the tested interval.

To derive concentration–response curves, we chose densities that represented the 10th, 50th, and 90th percentiles of the observed population densities before contamination. The survival of *Daphnia* spp. was predicted for three concentration–response curves, one for each selected level of density. Predictions of the survival at each concentration were based on the regression lines that were fitted for relationships between densities before contamination and survival of *Daphnia* spp. (11 days after contamination).

All statistical analyses and graphs were generated with R, version 2.14.1 (R Foundation for Statistical Computing, 2011).

## 2.4 Results

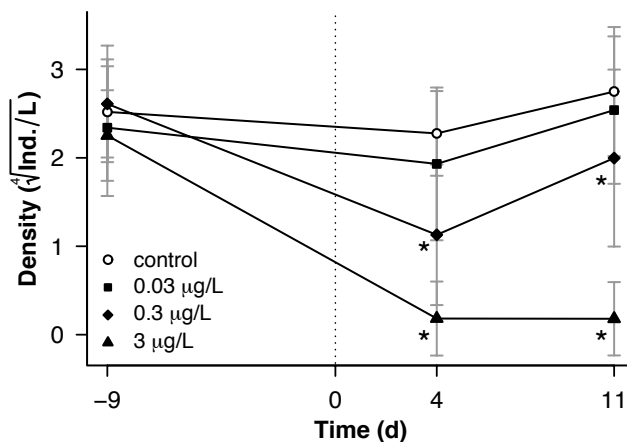
### *Influence of the treatments of shading and harvesting in the absence of pesticide*

The presence of the awning reduced the radiation at the surface of the microcosms (daily average difference between shaded and unshaded microcosms was 76% on both sunny and cloudy days). There was also a difference in water temperature between the shaded and unshaded microcosms from the time at which the awning was removed until the last sampling (minimum daily difference = 0.6 K, maximum daily difference = 3.3 K). Regarding chlorophyll a concentration, no differences could be observed between shaded and unshaded ponds. However, within the unshaded ponds, DO (mean +25.8%,  $p < 0.05$ ) was significantly increased until 11 days after contamination. EC decreased slightly and pH increased slightly, neither significantly, in unshaded ponds. On the basis of these physicochemical measurements, we assumed that shading had an indirect effect on algal growth.

There were no significant differences over the course of the experiment in the densities of *Daphnia* spp. among the four treatments of shading and harvesting in the absence of the pesticide. This finding provides evidence that we had accomplished our aim of establishing subtle biotic and abiotic changes in the microcosms.

### *Development of populations of Daphnia spp.*

The average development of populations densities of *Daphnia* spp. was analysed for the three tested concentrations of esfenvalerate from 9 days before contamination until 11 days after contamination across all treatments of shading and harvesting (Fig. 2.2). A clear concentration–response relationship was detected. At 0.03  $\mu\text{g/L}$ , an apparent but not significant decline (-15%) in mean density could be observed. At 0.3  $\mu\text{g/L}$ , mean densities decreased significantly by 50% relative to the control. At 3  $\mu\text{g/L}$ , mortality was very high and the size of the population of *Daphnia* spp. was reduced by more than 90% relative to the control.



**Fig. 2.2:** Mean densities with standard deviation of *Daphnia* spp. for the different concentrations of esfenvalerate across all treatments of shading and harvesting over time. Asterisks represent significant differences from the control (pairwise t-test or Kruskal–Wallis multiple comparison test,  $p < 0.05$ ). The time point before contamination (-9 days) represents the mean from 13 and 5 days before contamination.

#### *Influence of densities before contamination on pesticide-related survival of Daphnia spp.*

We assessed the effects of population densities before contamination and treatment with regard to shading and harvesting on the survival of populations of *Daphnia* spp. using ANCOVA. Data for the 3 µg/L concentration of esfenvalerate were removed from the analysis because of the high mortality rate at this concentration (see Section “Development of populations of *Daphnia* spp.”). We observed a significant influence of density before contamination on the effect of the pesticide on the survival of *Daphnia* spp., 4 and 11 days after contamination (Tab. 2.3). The treatment applied showed no significant influence at either time point. Pesticide-related mortality was still observed 11 days after contamination (data not shown), indicating that the effects of the pesticide, which included delayed effects, were still developing. Consequently, we looked at the survival 11 days after contamination to capture the full extent of the effect of the pesticide. However, minor recovery of some populations due to reproduction during that period could not be excluded. To determine the joint effect of esfenvalerate and population densities, we plotted

the densities before contamination against the survival of *Daphnia* spp. 11 days after contamination for all treatments of shading and harvesting. Survival was found to depend negatively on the population densities before contamination in communities that were exposed to concentrations of 0.03  $\mu\text{g/L}$  ( $r^2 = 0.67$ ) and 0.3  $\mu\text{g/L}$  ( $r^2 = 0.49$ ) esfenvalerate (Fig. 2.3). For the control, we also found a significant, but less pronounced, relationship ( $r^2 = 0.21$ ). At 3  $\mu\text{g/L}$  esfenvalerate, pesticide-induced mortality was too high to detect a significant relationship. The treatments of shading and harvesting affected the mean population densities before contamination and thus the survival of *Daphnia* spp. However, the differences in densities between the uncontaminated treatments were not significant. In addition, the observed relationships between the initial densities and survival were independent of the treatment (Tab. 2.3, Fig. 2.3).

**Tab. 2.3:** Analysis of covariance (ANCOVA) to analyse influences of pesticide concentration, densities of *Daphnia* spp. populations before contamination (-9 days), and treatment on survival of *Daphnia* spp. at 4 and 11 days after contamination. Data for 3  $\mu\text{g/L}$  were removed from the analysis.

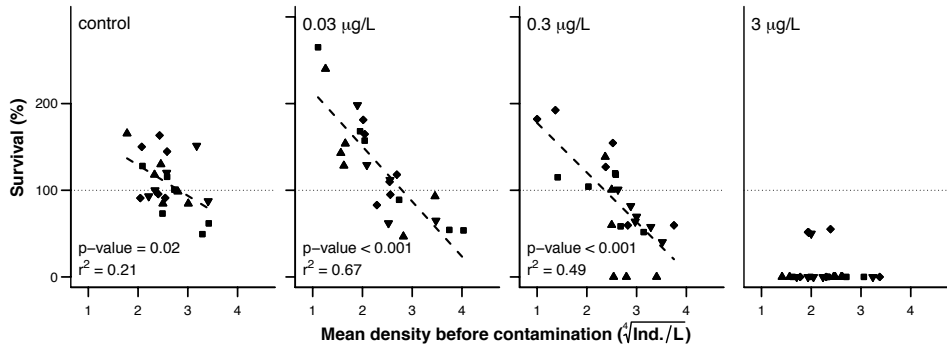
<b>Survival of <i>Daphnia</i> spp. 4 days after contamination (<math>r^2</math> adjusted = 0.41, <math>p &lt; 0.001</math>, <math>n = 70</math>)</b>			
Variable	d.f.	F	P
Concentration of esfenvalerate	1	19.973	< 0.001
Density of <i>Daphnia</i> spp. before contamination	1	28.271	< 0.001
Treatment	3	1.655	0.186
Residuals	64		
<b>Survival of <i>Daphnia</i> spp. 11 days after contamination (<math>r^2</math> adjusted = 0.59, <math>p &lt; 0.001</math>, <math>n = 71</math>)</b>			
Variable	d.f.	F	P
Concentration of esfenvalerate	1	5.541	0.022
Density of <i>Daphnia</i> spp. before contamination	1	88.113	< 0.001
Treatment	3	0.857	0.468
Residuals	65		

With regard to the variance, we did not detect differences in variance among the concentrations in relation to densities before contamination. In

contrast, the variance in the survival of *Daphnia* spp. after contamination was significantly higher with concentrations of 0.03 µg/L (F test,  $p = 0.005$ ) and 0.3 µg/L (F-test,  $p = 0.02$ ) esfenvalerate than in the control. As shown above, the variance at concentrations that showed partial mortality could be explained by the initial density (Fig. 2.3). Depending on the initial density of *Daphnia* spp., the survival of the populations varied from 265 to 47% at 0.03 µg/L esfenvalerate and from 192 to 0% at 0.3 µg/L. Owing to the strong lethal effect of esfenvalerate at 3 µg/L, we detected no change in the variance in survival compared with that of the control.

To understand further the relevance of the initial densities on toxicant-related survival, we analyzed the differences in survival at low and high densities of *Daphnia* spp. before contamination. This analysis was conducted for the control and at the concentrations that induced partial mortality (0.03 and 0.3 µg/L). Within the control, we found no significant differences between the tested groups, namely, low and high densities before contamination. In comparison, for 0.03 and 0.3 µg/L esfenvalerate, the toxicant-related survival was significantly lower for the group with high initial densities than for the group with low initial densities (t-test,  $p < 0.001$  and  $p = 0.02$ , respectively). Hence, high population density before contamination increased the toxicant sensitivity at concentrations that showed partial mortality.

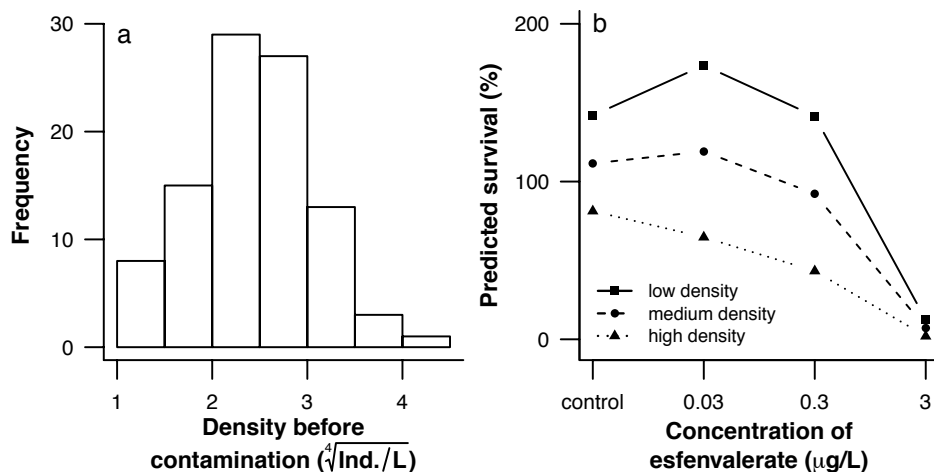
To assess the influence of other species on the pesticide survival of *Daphnia* spp., we applied ANCOVA as described above but added the densities of the main taxa (Tab. 2.1) before contamination as explanatory variables. Apart from *Daphnia* spp., none of the detected taxa showed a significant and a negative influence on the survival of *Daphnia* spp. at 11 days after contamination (ANCOVA, adjusted  $r^2 = 0.60$ ,  $df = 57$ ,  $p < 0.001$ ,  $n = 70$ ). The ANCOVA for the survival of *Daphnia* spp. at 4 days after contamination (ANCOVA, adjusted  $r^2 = 0.60$ ,  $df = 55$ ,  $p < 0.001$ ,  $n = 68$ ) demonstrated an additional significant influence of Copepoda ( $p = 0.03$ ). However, when we plotted the survival of *Daphnia* spp. as a function of Copepoda density for the different concentrations of pesticide and the control, no significant negative relationship was found.



**Fig. 2.3:** Dependence of the survival of *Daphnia* spp. 11 days after contamination on densities before contamination (-9 days) for all concentrations of esfenvalerate and treatments (black square “Shading/Harvesting”, black diamond “No Shading/Harvesting”, black up-pointing triangle “Shading/No Harvesting”, black down pointing triangle “No Shading/No Harvesting”). Significant linear regressions are represented by regression lines,  $r^2$ , and p values.

#### *Prediction of concentration–response curves at different densities before contamination*

To determine the consequences of initial population densities of *Daphnia* spp. on the effect of esfenvalerate, we predicted concentration–response curves at three levels of density before contamination. Survival was predicted on the basis of the regression lines shown in Fig. 2.3. The three levels of density represent the 10th (“low density”), 50th (“medium density”), and 90th (“high density”) percentiles of the densities observed before contamination (Fig. 2.4a). The predicted concentration–response curves revealed that populations with a low density before contamination only decreased at an esfenvalerate concentration of 3 µg/L. In contrast, populations with a high density were affected negatively at a concentration of 0.03 µg/L, which is two orders of magnitude below the concentration that had a negative effect at low densities (Fig. 2.4b). The concentration–response curve for high population densities showed a broader range of concentrations with partial effects than the range at low densities.



**Fig. 2.4:** Distribution of densities of *Daphnia* spp. before contamination (a) ( $n = 96$ , -9 days) and concentration–response curves for three different densities of *Daphnia* spp. before contamination (“low density”: 10th percentile = 1.7, “medium density”: 50th percentile = 2.5, “high density”: 90th percentile = 3.3) (b). Survival data for the three density levels are based on linear models, obtained from regressions of the pesticide survival and densities before contamination (Fig. 2.3). For 3  $\mu\text{g}/\text{L}$  esfenvalerate, no significant relationship was found. A fitted trend line is used here for the theoretical models.

## 2.5 Discussion

Previous analysis of the same data set as used in this study revealed differences in the sensitivity of the community structure and populations to esfenvalerate among the different treatments of shading and harvesting (Stampfli et al. 2011). Building on this previous work, we found that the population density of *Daphnia* spp. before contamination with the pesticide could explain the majority of the variability in survival after contamination across all treatments. An increased density of *Daphnia* spp. before contamination increased the effect and the sensitivity to the pesticide. This strong negative relationship between density before contamination and pesticide-related survival can be attributed to the fact that a high density of organisms intensifies competition for food and space. In addition to the variation in densities, we assume different levels of food

availability for *Daphnia* spp. as indicated by the measured treatment related alterations in oxygen, pH, and EC (see also Stampfli et al. 2011). Given that the relationships between initial density and survival were independent of the treatments of shading and harvesting, we argue that, in the present study, treatment-specific food levels did not play a major role in the population dynamics of *Daphnia* spp. Consequently, we related an increase in the density of *Daphnia* spp. to an increase in intraspecific competition. Furthermore, in microcosms that were not exposed to pesticide, we found a negative influence of intraspecific competition on the development of populations of *Daphnia* spp., which demonstrated the general relevance of competition in our systems.

A link between intraspecific competition and toxicant effects has been made previously. Food limitation is a well-studied factor that increases the response of aquatic invertebrates to insecticides (Rose et al. 2002, Beketov and Liess 2005) or heavy metals (Koivisto et al. 1992, Chandini 1989). In comparison to the manipulation of food levels, some studies have investigated the effect of high densities of organisms on the short-term effects of toxicants. At the population level stronger short-term effects of fenvalerate were detected in the presence of high densities of trichopteran larvae (Liess 2002), as well as of mosquito larvae and daphnids (Foit et al. 2011), than in the presence of low population densities.

Intraspecific competition was the factor that predominantly determined the short-term effects of the pesticide in the present microcosm communities, which resemble natural communities. This dominance of intraspecific competition in our test system, as compared with other biotic interactions, is noteworthy because competitors and predators of *Daphnia* spp. were detected in the system (Tab. 2.1). However, interspecific competition and predation were not found to cause significant alterations in the short-term effects of the toxicant in the present study. A possible explanation for the stronger intraspecific, than interspecific, competition is the high competitive strength of *Daphnia* spp. in our test system. *Daphnia* spp. were relatively abundant within the invertebrate community (Tab. 2.1) and did not decline until 2 months after contamination in the absence of the contaminant (Knillmann et al. 2012). The dominance of



intraspecific over interspecific competition for species with a competitive advantage has also been shown for mosquitoes (Armistead et al. 2008) and intertidal limpets (Creese and Underwood 1982). Foit et al. (2012) further showed in their multispecies test system that intraspecific competition, rather than interspecific competition, influences the acute effects of pesticides on a competitive species.

In the present study, the interaction between intraspecific competition and the effects of esfenvalerate could be quantified because of the wide range of initial densities of *Daphnia* spp. applied in the study and the large number of replicates for each concentration of pesticide. On the basis of this information, we determined that the toxicant sensitivity of populations could be altered by intraspecific competition by a factor of up to 100. Populations with a high density before contamination showed slight negative effects at a concentration, which is 100 times less than the concentration that had negative effects on populations at low and medium densities. This alteration in sensitivity is very relevant to the assessment of effect thresholds. The relevance is further highlighted when values from previous studies that considered the influence of environmental factors on toxicant effects are taken into account. Such factors include a wide range of stressors that increase the rate of acute and chronic effects due to toxicants (factor of increase in brackets): increased temperature (10) (Song et al. 1997, Osterauer and Köhler 2008), food limitation (2) (Chandini 1988, Pieters et al. 2005), increased salinity (10) (Wildgust and Jones 1998), low oxygen (2) (Van der Geest et al. 2002), UV radiation (30) (Liess et al. 2001), and competition (10) (Liess 2002).

In addition to the increased sensitivity to pesticide at high levels of intraspecific competition, we also identified a broader range of concentrations that showed partial mortality at high levels of competition than at low levels of competition. In the present investigation with the chosen concentration intervals, this effect range was identified to span two orders of magnitude for populations under high competition. In contrast, the ranges for populations at medium and low densities were much narrower, because these populations were only affected by a concentration of 3 µg/L esfenvalerate.

## 2.6 Conclusions

In this paper, we have described the importance of intraspecific competition for the actual effects of toxicant exposure. Under natural conditions, intraspecific competition is a ubiquitous factor that affects the availability of resources for organisms. Hence, factors such as competition must be considered when extrapolating toxicity data from the individual level to population effects. In addition, it is also relevant for basic understanding in ecotoxicology to determine the ecological consequences of a broader range of partial toxicant effects under high intraspecific competition in communities. This analysis should include the assessment of long-term effects and recovery in relation to short-term effects. A general framework in which competition and other biotic interactions can be considered within the risk assessment process is essential for a realistic prediction of the effects of a toxicant.

## 2.7 Acknowledgments

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**Chapter 3: Interspecific competition delays recovery of *Daphnia* spp. populations from pesticide stress**

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### 3.1 Abstract

Xenobiotics alter the balance of competition between species and induce shifts in community composition. However, little is known about how these alterations affect the recovery of sensitive taxa. We exposed zooplankton communities to esfenvalerate (0.03, 0.3, and 3  $\mu\text{g/L}$ ) in outdoor microcosms and investigated the longterm effects on populations of *Daphnia* spp. To cover a broad and realistic range of environmental conditions, we established 96 microcosms with different treatments of shading and periodic harvesting. Populations of *Daphnia* spp. decreased in abundance for more than 8 weeks after contamination at 0.3 and 3  $\mu\text{g/L}$  esfenvalerate. The period required for recovery at 0.3 and 3  $\mu\text{g/L}$  was more than eight and three times longer, respectively, than the recovery period that was predicted on the basis of the life cycle of *Daphnia* spp. without considering the environmental context. We found that the recovery of sensitive *Daphnia* spp. populations depended on the initial pesticide survival and the related increase of less sensitive, competing taxa. We assert that this increase in the abundance of competing species, as well as sub-lethal effects of esfenvalerate, caused the unexpectedly prolonged effects of esfenvalerate on populations of *Daphnia* spp. We conclude that assessing biotic interactions is essential to understand and hence predict the effects and recovery from toxicant stress in communities.

**Keywords:** Recovery, Competition, Toxicant, *Daphnia*, Community context, Indirect effects

### 3.2 Introduction

To evaluate the ecological effect of toxicants, the magnitude of their short-term effects and the duration of recovery for affected populations must be assessed. Models that predict the time required for recovery are often based on population growth rates (PGRs) that are obtained from analyses of single species conducted in a laboratory under optimal conditions (Barnthouse 2004). According to the approach used by Barnthouse, organisms are assumed to recover within one

generation time. At the community level, the recovery of the species in abundance was found to be related to the generation time within aquatic ecosystems after general disturbance (Niemi et al. 1990) and pesticide exposure (Liess and von der Ohe 2005).

However, in several cases, the actual recovery time in such test systems, or in the field, was found to be considerably longer than one generation time. For example, the generation time of short-living cladocerans rarely exceeds 4 weeks at a water temperature of 15°C, according to a review by Gillooly (2000). Nonetheless, populations of *Daphnia galeata* were still affected by the insecticide chlorpyrifos more than 11 weeks after contamination in an outdoor test system under Mediterranean climate conditions (López-Mancisidor et al. 2008). In addition, Brock et al. (2000) reviewed studies on semi-field systems where the recovery of cladocerans that were subjected to a single exposure to organophosphorous insecticides took longer than 8 weeks after contamination. It is worth noting here that the half-life for the dissipation of chlorpyrifos and other investigated organophosphates in the water only ranges from 1–2 days (López-Mancisidor et al. 2008, Van Wijngaarden et al. 2005, Tanner and Knuth 1995). The recovery of sensitive long-living freshwater organisms is expected to take even longer and it has been found in the field that sensitive species with a generation time of 4 months or longer have not fully recovered even 1 year after exposure to toxicants (Liess and von der Ohe 2005).

In the field, more parameters affect the recovery of sensitive organisms than the generation time and growth rates identified under optimal laboratory conditions. Here, biotic and abiotic conditions as well as the ability to recolonize within the ecosystem further determine time for recovery (Liess and von der Ohe 2005, Caquet et al. 2007, Schäfer et al. 2007). Organisms in the field are often exposed to unfavourable natural conditions that can lead to reductions in the fitness and growth of individuals, such as for example competition (Hülsmann 2001), predation (Black and Dodson 1990, Hanazato 1991), salinity stress (Baillieul et al. 1996) or unfavourable pH values (Thomsen and Friberg 2002). These environmental stressors increase the effect of toxicants as shown in the review by Heugens et al. (2001).

Toxicants are also known to indirectly alter predator–prey and herbivore–producer interactions and interspecific competition (Relyea and Hoverman 2006, Fleeger et al. 2003). Considering especially changes in interspecific competition, only a few studies have linked indirect effects with a prolonged recovery. One example where such a link has been suggested was for the ecological effects of the oil spill from the Exxon Valdez in Alaska in 1989. An initial direct decline in rockweed (*Fucus gardneri*) at the shoreline caused an increase in ephemeral algae and opportunistic barnacles. In turn, these increases might have contributed to prolong the recovery period of rockweed and thereby also the recovery of associated invertebrates, as reviewed by Peterson (2001) and Peterson et al. (2003). Another example is a study on lake acidification where sensitive zooplankton species did not recover until 1–6 years after the pH of the lake had been restored to control conditions. It was assumed that the recovery of species sensitive to acidification was delayed by competition from acid-resistant species (Frost et al. 2006).

However, to our knowledge, no direct connection has been established between increases in the abundance of less sensitive species and the delayed recovery of sensitive populations in a community context under conditions that closely resemble those in the field. The aim of the study described herein was to investigate the effects of a pyrethroid pesticide on daphnids in outdoor microcosms. By doing so we also investigated the relevance of indirect effects for the recovery of organisms from toxicants under different environmental conditions.

### **3.3 Materials and methods**

#### *General*

We established pond communities with variations in biotic and abiotic conditions that mirrored those found in the field. This was accomplished by the use of four different treatments that combined harvesting and the shading of communities: “Shading/Harvesting”, “No Shading/Harvesting”, “No Shading/No Harvesting” and “Shading/No Harvesting”. The treatments were designed to

produce subtle effects on the biotic and abiotic conditions in the pond communities.

In the present study, we focused on genera from the family Daphniidae with different sensitivities to esfenvalerate (sensitive and insensitive D.). Long-term effects of three concentrations of esfenvalerate on populations of sensitive and insensitive D. were investigated for a period of 59 days after contamination. Changes in the structure and sensitivity of the whole communities are presented in the publication by Stampfli et al. (2011), in which only the treatments “No Shading/Harvesting”, “No Shading/No Harvesting” and “Shading/No Harvesting” were considered, as they represent a gradient of food availability and competition strength.

*Microcosms: artificial pond systems*

Ninety-six outdoor microcosms were installed at the Helmholtz Centre for Environmental Research in Leipzig, Germany (51°21'13 N, 12°25'55 E). For every concentration and treatment of shading and harvesting, six replicate microcosms were established (n = 24 per level of concentration). Each microcosm had a volume of 80 L and was filled with 60 L of water (tap water seeded with 1 L of natural pond water). The microcosms were maintained at this volume over the course of the experiment. Communities of freshwater zooplankton and sediment were collected from five different natural ponds within a radius of 15 km from the institute and established in the microcosms at the end of May and beginning of June 2008. The natural pond sediment was mixed at a ratio of 1:1 with sand and distributed on the bottom of each tank to a thickness of approximately 1 cm. Furthermore, approximately 10 g of shredded leaves (*Populus* spp.) were added to the microcosms. The collected organisms were distributed equally among all microcosms.

Awnings were positioned close to each pond at an angle of 45° so that the microcosms were shaded at around noon each day (12–4 p.m.). All microcosms were shaded for 4 weeks until 4 days before contamination to enable comparable communities to develop in all ponds. In microcosms subjected to harvesting, biotic interaction was reduced by removing 30% of the

entire pond community each week using a net (10 x 12 cm, 250- $\mu$ m mesh size). Organisms were harvested from 2 weeks before contamination and continued until the end of the experiment in September 2008. The harvesting was started 10 days before the removal of the awning for the “No Shading” treatments because we assumed that more time would be required for the invertebrates to adapt to the reduction in biotic interaction than for algal growth to adapt to the increase in light.

#### *Pesticide exposure*

Esfenvalerate, (aS)-a-cyano-3-phenoxybenzyl (2S)-2-(4-chlorophenyl)-3-methylbutyrate, is a synthetic pyrethroid that is widely used in agriculture and is highly toxic to aquatic insects and crustaceans. We used the commercial formula Sumicidin Alpha EC (BASF, Limburgerhof, Germany), which is an emulsifiable concentrate that contains 50 g/L of the active ingredient, esfenvalerate. On 4th July 2008, the microcosms were contaminated with three different concentrations (0.03, 0.3, and 3  $\mu$ g/L) of the pesticide. The concentration of esfenvalerate decreased rapidly during the first hours in all setups. In addition, no significant differences in exposure among the different conditions of shading or harvesting were detected (for details, see Stampfli et al. 2011).

#### *Biological sampling and environmental parameters*

To determine species distributions and abundances, pelagic biological samples were collected and identified over the experimental period at the following time points: 13 and 5 days before contamination (mean: 9 days), and 4, 11, 16, 44, and 59 days after contamination. The samples were collected with a sampling tube (PVC, length = 31.7 cm, radius = 3.6 cm). The lid of the sampling tube was placed first in the centre of each pond on top of the sediment. Before the tube was fitted onto the lid, the water was stirred gently in order to obtain a homogeneous distribution of organisms in the pond. Afterwards, the water from the tube (which contained 1.7% by volume of the water from the pond), including any organisms, was passed through a sieve (180  $\mu$ m mesh size). The organisms obtained in this manner were preserved in 70% ethanol, identified to

the level of genus (Cladocera, Chaoboridae, Culicidae, Baetidae), order (Odonata, Copepoda) or class (Ostracoda, Arachnida) and counted under a microscope. The taxonomic groups that were relatively common in the pond communities are listed in Tab. 3.1.

**Tab. 3.1:** Abundances of main invertebrate taxa in the communities without pesticide exposure. The untransformed abundances are displayed with the mean and standard deviation from 9 days before until 59 days after contamination.

<b>Taxon</b>	<b>Abundances (Ind./L)</b>
<i>Daphnia</i> spp.	56 ± 60.3
other genera of Daphniidae	131 ± 128.6
Chydoridae	54.7 ± 140
Copepoda	29 ± 37.7
Ostracoda	12.8 ± 18.7
Baetidae	1 ± 2.1
Culicidae	1.3 ± 2.3
Chaoboridae	2.8 ± 2.9
Odonata	0.05 ± 0.3

Water temperature was recorded continuously with Handylog DK501-PL data loggers (Driessen & Kern, Bad Bramstedt, Germany). Differences in UV A + UV B radiation among the treatments were measured over the course of a sunny and a cloudy day in July with a UV meter (UV-VIS radiometer RM-21, Dr. Gröbel UV-Elektronik GmbH, Ettlingen, Germany). The presence of the awning reduced the radiation at the surface of the microcosms (average daily reduction due to the awning: 76% on both a sunny and a cloudy day). Water temperature also differed between the shaded and unshaded microcosms from the time at which the awning was removed until the last sampling point (minimum daily difference = -0.6 K, maximum daily difference = -3.3 K).

To monitor water quality in the different treatments, additional parameters were measured on a weekly basis for a subsample of 32 microcosms over the entire observation period. The additional parameters included the concentration of oxygen (WTW Multi 340i meter, WTW Instruments, Weilheim, Germany), pH (HI-98127, Hanna Instruments, Woonsocket, RI, USA), electrical conductivity (HI-98312, Hanna Instruments, Woonsocket, RI, USA), and the concentration of chlorophyll a as a measure of algal density (relative fluorescence units-RFU, GEMINI XPS Fluorescence Microplate Reader, Molecular Devices, Sunnyvale, CA, USA). No differences in chlorophyll a concentrations were observed between shaded and unshaded ponds. However, in unshaded ponds oxygen concentration and pH were significantly higher (mean +23.8% and +3.5%, respectively) and electrical conductivity decreased (mean -6.8%). On the basis of these measurements of physicochemical parameters, we assume that shading has an indirect effect on algal growth (Anderson et al. 1994, Falkowski and Raven 2007).

#### *Acute toxicity testing of esfenvalerate*

Acute toxicity tests were performed to generate most comparable information on toxicological sensitivity of the Daphniidae present in the microcosms. The following species were tested: *Daphnia longispina*, *Daphnia pulex*, *Ceriodaphnia reticulata* and *Simocephalus vetulus*. The detected LC50 (96 h) values for the investigated species were similar to those previously published (Beketov 2004, Lozano et al. 1992, Werner et al. 2002). Not enough individuals of *Scapholeberis* sp. could be found for a toxicity test. For this reason we used the only existing literature value of LC50 (96 h) = 0.84 µg/L for esfenvalerate (Noskov 2011) to classify the genera.

Individuals of *D. longispina*, *D. pulex*, *C. reticulata*, and *S. vetulus* were collected in permanent and temporary ponds from the floodplains of the River Elbe, near Rosslau, Germany (51°53'06 N, 12°15'55 E), in June 2009. The organisms from the field were adapted to laboratory conditions in natural pond water under a constant air temperature of 20°C for 24 h before contamination with esfenvalerate. The pond water was passed through filter paper (mesh

size: 1–2.5 nm) before the organisms were added for the toxicity tests. The electrical conductivity (EC) and pH of the used pond water were measured (HI-98312 and HI-98127, Hanna Instruments, Woonsocket, RI, USA) and are provided in Tab. 3.2.

For the acute toxicity tests with esfenvalerate, we applied the following concentrations: 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, and 3 µg/L. Ten replicates per control and per concentration of esfenvalerate were used. Individuals were each kept in a volume of 50 mL of medium (pond water, described above) and monitored every 24 h until 96 h after contamination. After 24 h of exposure, the medium for all test samples and controls was changed to fresh uncontaminated medium. The LC<sub>50</sub> after 96 h was calculated using the Trimmed Spearman–Karber method (Trimmed Spearman–Karber program, version 1.5, Hamilton et al. 1977).

#### *Statistical analysis*

The group of insensitive *D.* was generated by adding up the count data for all single genera in the family Daphniidae that were classified as insensitive taxa. Counted individuals and group data were fourth-root transformed, as suggested for skewed abundance data (Quinn and Keough 2002). Abundances of sensitive and insensitive *D.* were pooled for all treatments. Differences in mean abundance ( $n = 24$  per concentration and control) at the various time points among the different concentrations of toxicant and the control were investigated with analysis of variance (ANOVA). The ANOVA was followed by pairwise *t*-tests for multiple comparisons and adjusted if the variances of the groups were not homogeneous. In the case of non-normally distributed samples, the Kruskal–Wallis test for nonparametric data was applied, followed by a nonparametric multiple-comparison test (R-package *pgirmess*, function *kruskalmc*, Siegel and Castellan 1988).

The influence of pesticide-related survival, 2 weeks after contamination and treatment of shading and harvesting, on the abundances of sensitive *D.* at the end of the experiment (6 and 8 weeks after contamination) was investigated with an analysis of covariance (ANCOVA). The pesticide-related survival was



calculated as the ratio of the mean abundance from the samplings after contamination (11 and 16 days after contamination) to the mean abundance before contamination (-9 days) for each microcosm. Treatment was used as a categorical variable and pesticide survival of sensitive D. as a continuous variable. The models were simplified and validated in accordance with the work of Crawley (2007), by stepwise removal of nonsignificant terms until the minimal adequate model was reached.

Relations between abundances of sensitive and insensitive D. were tested for significance based on Pearson's product-moment correlation for normally distributed data (correlation coefficient indicated with  $r$ ) or Spearman's rank correlation (correlation coefficient indicated with  $\rho$ ). Outliers were identified by checking correlations for noteworthy data points in fitted linear regression lines and applied model validation according to Crawley (2007). We conducted a Principal Component Analysis (PCA) to assess correlations between sensitive D., insensitive D. and other taxonomic groups at pesticide concentrations with partial mortalities (0.03 and 0.3  $\mu\text{g/L}$ ). The selection of the linear multivariate method was based on the outcome of a preliminary Detrended Correspondence Analysis (DCA) following Leps and Smilauer (2003). The PCA was conducted and interpreted using correlation biplot scaling with centred and transformed species data (Zuur et al. 2007, Leps and Smilauer 2003). Species data were subjected to square-root transformation for reasons of most possible conformity with the previous univariate analyses. The concentration of the pesticide was  $\log(x + 1)$ -transformed and added by passive ordination.

For the predicted long-term concentration–response curves we chose three abundances of insensitive D. 6 weeks after contamination, representing different percentiles of the observed abundances (“low” = 10th percentile, “medium” = 50th percentile, “high” = 90th percentile). The abundances of sensitive D. for three concentration–response curves were predicted, one for each scenario of abundance of insensitive D. The predictions on the abundance of sensitive D. at control and every concentration (displayed in % to control) were based on the regression lines that were fitted for relations between

abundances of insensitive and sensitive D., 6 weeks after contamination.

Multivariate analyses were conducted using the program CANOCO 4.5 for Windows (Wageningen, Netherlands) in accordance with previous work and guides (ter Braak and Smilauer 2002, Leps and Smilauer 2003). The remaining statistical analyses and graphs were generated with R, version 2.14.1 (R Foundation for Statistical Computing, 2011).

### 3.4 Results

#### *Taxon classification according to toxicological sensitivity*

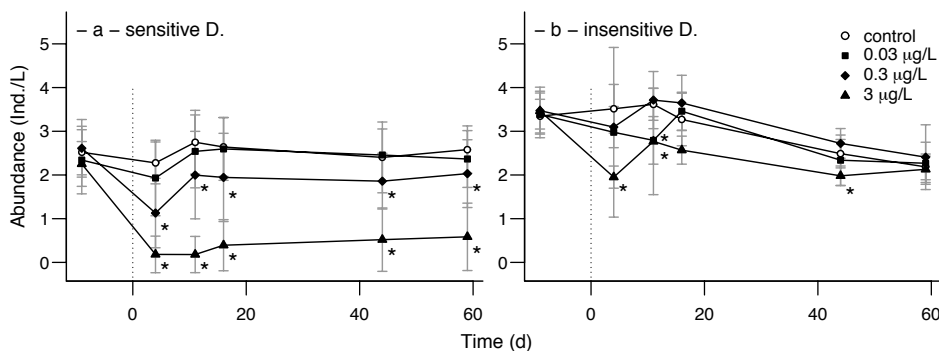
To classify taxa on the basis of their toxicological sensitivity, we determined the acute sensitivity to esfenvalerate of different genera from the family Daphniidae (Tab. 3.2). The LC50 values after 96 h of exposure for the genus *Daphnia* were found to be below the medium applied concentration of 0.3 µg/L esfenvalerate. For the other genera investigated, namely *Ceriodaphnia* and *Simocephalus*, LC50 values higher than 0.3 µg/L were found. Based on this information on toxicological sensitivity and the literature value for *Scapholeberis mucronata* (see “Acute toxicity testing of esfenvalerate” section), we divided the family Daphniidae into two groups: sensitive D. (*Daphnia* spp.) and insensitive D. (*Ceriodaphnia* spp., *Simocephalus* spp. and *Scapholeberis* spp.).

**Tab. 3.2:** LC50 values after 96 h with confidence intervals (CI) for the tested species and physicochemical parameters of the medium used.

Species	LC50 (µg/L) with CI	Physicochemical parameters	
		pH	EC (µS/cm)
<i>Daphnia pulex</i>	0.02 (0.01–0.04)	8.12	597
<i>Daphnia longispina</i>	0.15 (0.10–0.23)	7.9	604
<i>Ceriodaphnia reticulata</i>	0.44 (0.27–0.71)	7.91	610
<i>Simocephalus vetulus</i>	2.5 (1.86–3.07)	8.15	580

*Average population dynamics and influence of the pesticide*

The population dynamic of sensitive and insensitive D. was observed from 9 days before contamination until 59 days after contamination for control and all concentrations of esfenvalerate (Fig. 3.1). The data from the treatments of shading and harvesting was pooled to analyse the general influence of the pesticide under different environmental conditions. The treatments were supposed to induce subtle changes in the environmental conditions and to increase the variability of observed abundances, which is indicated by the standard deviation in Fig. 3.1. The aim of only introducing subtle changes was successful, as we found no clear trends and almost no significant differences between the treatments for sensitive and insensitive D. in abundances. Only the “Shading/Harvesting” treatment showed slight differences from the other treatments for sensitive D., 6 and 8 weeks after contamination ( $p < 0.05$ , data not shown).



**Fig. 3.1:** Average abundances and standard deviation of sensitive D. (a) and insensitive D. (b) for the control and the three concentrations of esfenvalerate from 9 days before until 59 days after contamination. Abundances were fourth-root transformed and averaged over all conditions of shading and harvesting. Asterisks indicate significant differences from the control ( $p < 0.05$ ).

Sensitive D. presented a clear concentration–response relationship (Fig. 3.1a). The population size of this group was reduced significantly upon exposure to 0.3 µg/L esfenvalerate (4 days after contamination: -50.4%) and 3 µg/L (4 days after contamination: -92%) and remained reduced until the end of

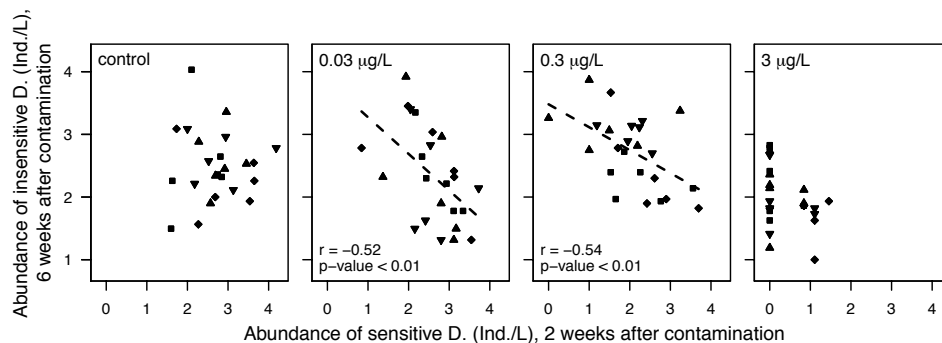
the experiment, more than 8 weeks after contamination. For the group of closely related but insensitive D. (Fig. 3.1b), no such clear concentration–response relationship was detected. Significant decreases in the size of insensitive D. populations were found only at some time points at the highest concentration of 3 µg/L (4 days after contamination: - 44.5%).

To assess the prolonged recovery period of sensitive D. after pesticide exposure we conducted an ANCOVA at pesticide concentrations with partial mortality (0.03 and 0.3 µg/L, Fig. 3.1). We found a significant influence ( $p < 0.001$ ) of the initial pesticide survival of sensitive D. 2 weeks after contamination on the abundance of sensitive D. 6 weeks after contamination. In contrast, for the different treatments of shading and harvesting, no significant effect was detected 6 weeks after contamination (ANCOVA, adjusted  $r^2 = 0.32$ ,  $df = 43$ ,  $p < 0.001$ ,  $n = 48$ ). Eight weeks after contamination, the influence of the initial pesticide survival was still significant ( $p < 0.01$ ). Again, the treatments showed no significant influence (ANCOVA, adjusted  $r^2 = 0.16$ ,  $df = 41$ ,  $p < 0.05$ ,  $n = 46$ ). The ANCOVA indicated that the recovery of *Daphnia* spp. depended only on the pesticide survival at 2 weeks after contamination, when sensitive populations were lastingly affected by esfenvalerate.

#### *Interspecific competition between sensitive and insensitive D.*

To understand the observed long-term influence of initial survival to esfenvalerate on the abundance of sensitive D., we examined the interactions between sensitive and insensitive D., a competing group of closely related but less sensitive taxa. We detected indirect effects of insensitive D. when their abundance at 6 weeks after contamination was plotted as a function of the abundance of sensitive D. 2 weeks after contamination (Fig. 3.2). Significant negative correlations between the abundances of sensitive and insensitive D. were detected at esfenvalerate concentrations of 0.03 µg/L ( $r = -0.52$ ) and 0.3 µg/L ( $r = -0.54$ ). At 3 µg/L, no clear pattern was detectable owing to the limited number or absence of survivors in the sensitive D. group. In addition, no correlation between the abundances of sensitive and insensitive D. was found in the control, which indicated that interactions between the two groups only

appeared when esfenvalerate was present.

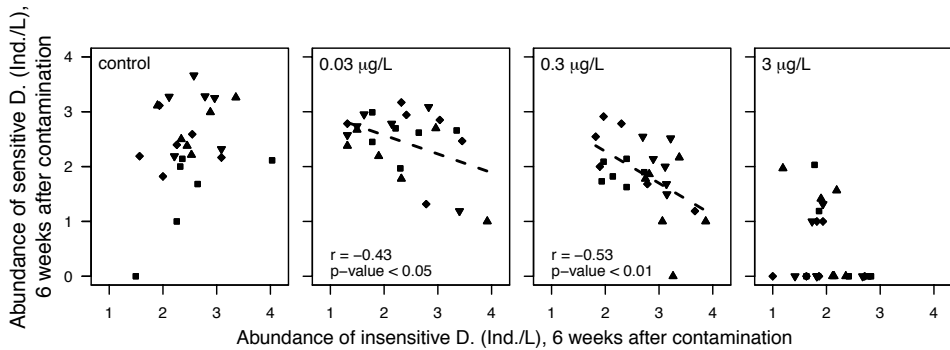


**Fig. 3.2:** Relation between abundance of insensitive D. (6 weeks after contamination) and the abundance of sensitive D. (2 weeks after contamination) for all concentrations of esfenvalerate and treatments (filled square = “Shading/Harvesting”, filled diamond = “No Shading/Harvesting”, filled triangle = “Shading/No Harvesting”, filled inverted triangle = “No Shading/No Harvesting”). Abundances were fourth-root transformed. Significant correlations are represented by  $r$ ,  $p$  values and fitted regression lines.

After indirect positive effects of pesticide exposure on the abundance of insensitive D. had been identified, we assessed the effect of this group on the recovery of sensitive D. To do so, we plotted the abundance of sensitive D. as a function of the abundance of insensitive D. at the same time point, 6 weeks after contamination (Fig. 3.3). We detected a negative correlation between the abundances of sensitive and insensitive D. at 0.03  $\mu\text{g/L}$  esfenvalerate ( $r = -0.43$ ), and the correlation was even more pronounced at 0.3  $\mu\text{g/L}$  esfenvalerate ( $r = -0.53$ ). Again, no correlation between the abundances of sensitive and insensitive D. was detected at 3  $\mu\text{g/L}$  esfenvalerate or in the control. The treatments had an influence on the abundances, but the observed relations were independent of the treatment (Figs. 2, 3).

The mean abundance of sensitive D. populations decreased slightly, but not significantly, at 0.03  $\mu\text{g/L}$ , and significantly at 0.3  $\mu\text{g/L}$  esfenvalerate, more than 8 weeks after contamination (Fig 1a). Eight weeks after contamination, the negative correlation between the abundances of sensitive and insensitive D. was weaker than that 6 weeks after contamination, but still significant when data for both 0.03 and 0.3  $\mu\text{g/L}$  esfenvalerate were combined (data not shown,

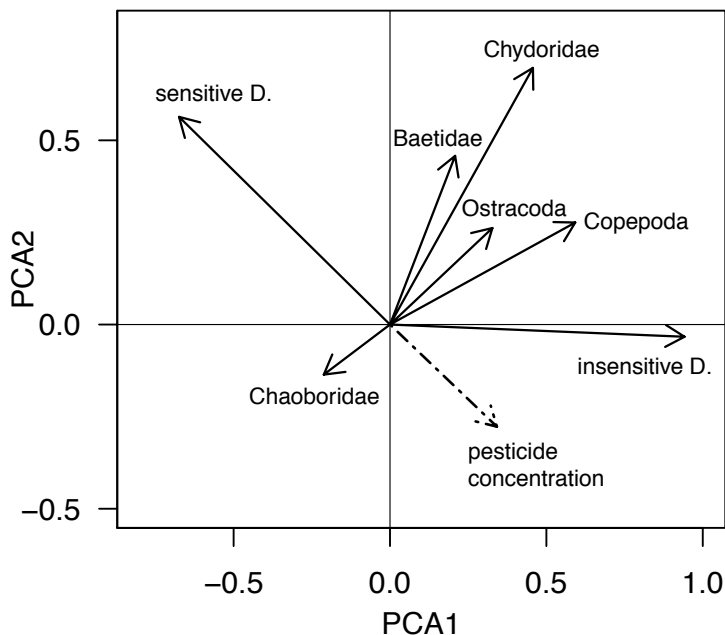
Spearman's  $\rho = -0.38$ ,  $p < 0.05$ ,  $n = 33$ ).



**Fig. 3.3:** Relation between abundance of insensitive D. and the abundance of sensitive D. 6 weeks after contamination for all concentrations of esfenvalerate and treatments (filled square = “Shading/Harvesting”, filled diamond = “No Shading/Harvesting”, filled triangle = “Shading/No Harvesting”, filled inverted triangle = “No Shading/No Harvesting”). Abundances were fourth-root transformed. Significant correlations are represented by  $r$ ,  $p$  values and fitted regression lines.

#### *Influence of other associated invertebrate taxa on sensitive D.*

We also analysed possible interactions of sensitive D. with other invertebrate taxa (8 taxon groups in total) 6 weeks after contamination using PCA. Data for pesticide concentrations with partial mortality of sensitive D. (0.03 and 0.3  $\mu\text{g/L}$ ,  $n = 48$ , Fig. 3.1a) was included. PCA1 explained 50.8%, and PCA2 accounted for a further 15.8% of the variation in the species data. The first four PCA axes together explained 91.4% of the observed variation. Following the interpretation of the correlation biplot diagram for relations between species (Fig. 3.4), insensitive D. are positively correlated with PCA1 and negatively related with sensitive D. Besides the insensitive D., none of the other taxon groups seemed to show a negative relation with sensitive D. Considering pesticide concentration, sensitive D. decreased in abundance with increasing pesticide concentration, whereas insensitive species abundances were positively correlated with pesticide concentration.

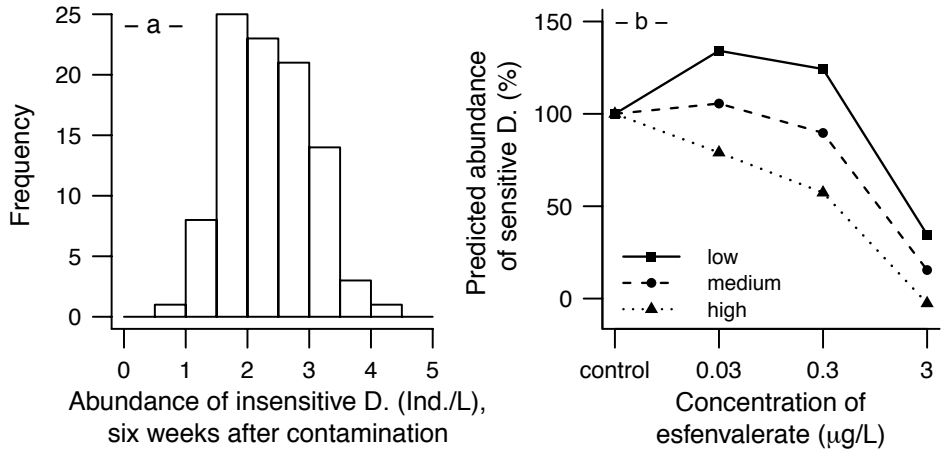


**Fig. 3.4:** PCA correlation biplot for the relations between species data of the microcosms and pesticide concentration, 6 weeks after contamination. Only data at concentrations with partial mortality (0.03 and 0.3  $\mu\text{g/L}$ ) were included.

*Concentration–response curves for sensitive D. according to interspecific competition*

On the basis of the result that the abundance of insensitive *D.* determined the recovery of sensitive *D.*, we predicted concentration–response curves for sensitive *D.* at three abundances of insensitive *D.* Abundances that were assigned as “low” (1.6 Ind./L), “medium” (2.3 Ind./L), and “high” (3.2 Ind./L) were chosen to represent the 10th, 50th, and 90th percentiles of the abundances of insensitive *D.* (Fig. 3.5a). The predicted concentration–response curves revealed that, with a low level of competitors, populations of sensitive *D.* only showed reduced abundances at 3  $\mu\text{g/L}$  esfenvalerate. In contrast, in high competitor presence, the abundance of sensitive *D.* was already affected slightly at 0.03  $\mu\text{g/L}$  esfenvalerate, which is two orders of magnitude below the effective concentration at low levels of interspecific competition (Fig. 3.5b). Furthermore, the shape of the concentration–response curve at high levels of interspecific

competition was flatter than that for low interspecific competition.



**Fig. 3.5:** Distribution of abundances of insensitive *D.* (fourth-root transformed) with observed frequencies 6 weeks after contamination (a) ( $n = 96$ ) and concentration–response curves for abundance of sensitive *D.* at three different densities of insensitive *D.* (“low”: 10th percentile = 1.6 Ind./L, “medium”: 50th percentile = 2.3 Ind./L, “high”: 90th percentile = 3.2 Ind./L) (b). Predicted abundance data for sensitive *D.* in % (relative to control) is based on linear models for the relations between sensitive and insensitive *D.*, 6 weeks after contamination (Fig. 3.3). For the control and 3  $\mu\text{g/L}$  esfenvalerate, no significant correlations were found. Here a fitted trendline was used for prediction of the concentration–response curves.

### 3.5 Discussion

*Generation time can only be used as a relative measure of time to recovery*

Taxa of the Daphniidae family responded to esfenvalerate in accordance with their toxicological classification into sensitive and insensitive *D.* The abundances of sensitive *D.* were significantly reduced at concentrations of 0.3 and 3  $\mu\text{g/L}$  until the end of the experiment, whereas insensitive *D.* were only affected at 3  $\mu\text{g/L}$  esfenvalerate at some time points during the experiment.

The generation times of organisms have proved to be important for predicting the relative recovery time of aquatic communities in mesocosms



(Sherratt et al. 1999, Beketov et al. 2008) and in the field (Liess and von der Ohe 2005, Niemi et al. 1990). However, in the current study, the actual recovery times differed from the recovery time that was predicted in the model by Barnthouse (2004) on the basis of generation times. According to this model, populations of sensitive D. should have recovered in abundance within 7 days after an initial reduction of 50% (0.3 µg/L) or within 16 days after an initial reduction of more than 90% (3 µg/L) upon exposure to the toxicant. Thus, in our study, the recovery times of the populations of sensitive D. were at least eight times longer than expected at 0.3 µg/L esfenvalerate and three times longer than expected at 3 µg/L. Similar prolonged recovery times were also observed in previous studies on the effects of pesticide in the field (Liess and von der Ohe 2005) and in test systems with complex communities (Brock et al. 2000, López-Mancisidor et al. 2008).

When the time required for the recovery of sensitive populations was compared with the time derived for the recovery of the community by principal response curves (PRC) and redundancy analysis (RDA) using the dataset presented here, differences from controls were only detected up to 16 days after contamination at 0.3 µg/L (Stampfli et al. 2011). The reason for this apparent difference in effects is that multivariate analyses such as PRC or RDA are based on the structure of the entire community. Due to the dominating presence of species in the study, that were not affected by the pesticide on the long-term, these analyses probably detected other results than observed for sensitive D. alone.

#### *Interspecific competition delays the recovery of sensitive species*

Experiments at the population level have shown that the exposure to toxicants can reduce competition and increase the abundance and survival rate of surviving conspecifics (Moe et al. 2002, Postma et al. 1994, Beketov and Liess 2005, Liess 2002). However, we assert that within communities surviving individuals of sensitive species do not benefit from increased resources after a disturbance if less sensitive and fast developing taxa are present. An increase in the abundance of less sensitive species following a reduction in the abundance of

sensitive taxa has been observed in many studies (Friberg-Jensen et al. 2003, Roessink et al. 2005, Gustafsson et al. 2010, López-Mancisidor et al. 2008) and reviewed by Relyea and Hoverman (2006) and Fleeger et al. (2003). In addition, sub-lethal effects of the toxicants can also lower the profit from resources of affected individuals, as already suggested in a review by Forbes et al. (2001). Esfenvalerate/fenvalerate are known to reduce filtration rates (Day and Kaushik 1987) and the fecundity of daphnids (Reynaldi et al. 2006) or mayflies (Beketov and Liess 2005). In the present study, no negative interactions between sensitive and insensitive *D.* were detected in the control. In contrast, upon exposure to concentrations of pesticide that caused partial mortality, negative interactions between sensitive and insensitive *D.* were found at densities of individuals that were comparable to those in the control conditions. These results indicate that survivors of sensitive *D.* might have been weakened by esfenvalerate, which probably increased the indirect effects on interspecific interaction.

We did not only observe an increase in the abundance of insensitive taxa after exposure to the toxicant, but also determined that the amount of less sensitive organisms was correlated with long-term effects on sensitive *D.* under all treatments of shading and harvesting. By quantifying the influence of insensitive *D.* on the recovery of sensitive *D.*, we determined that the abundance of sensitive populations can change by a factor of up to 100 depending on the abundance of competitors. Multivariate statistical analyses showed that other taxonomic groups did not interact with sensitive *D.* as strongly as competitors that were closely related to the species, namely insensitive *D.* This finding is related to the concept that interspecific competition is higher for closely related taxa that use similar niches and resources.

To date, only a few studies have linked indirect effects of toxicants on field communities with the delayed recovery of sensitive species, for example, as shown for the recovery of rockweed after an oil spill (Peterson 2001). At the population level, a similar delay in the recovery of population structure due to the lack of resources has been revealed. Liess et al. (2006) investigated populations of *D. magna* and found that, after a short-term pesticide disturbance,

while recovery in terms of abundance took a few days, the size structure of the populations only approached that of the control after 2 months. It was argued that the rapid development of small individuals after exposure to pesticide consumed all available resources and interrupted the long-term growth of large individuals. This hypothesis was confirmed later (Liess and Foit 2010) and a further very recent multispecies study has shown that the recovery in abundance of *D. magna* from fenvalerate is delayed by a high level of interspecific competition with mosquito larvae, which are less sensitive (Foit et al. 2012). To the best of our knowledge, this multispecies system under laboratory conditions is unique in proving a direct connection between indirect effects of pesticides and the delayed recovery of sensitive species.

*A high number of replicates facilitates the identification of recovery processes*

As already mentioned, an explicit link between interspecific competition and recovery of complex communities was previously not established. This might be because the number of replicates within community test systems (e.g., microcosms, mesocosms) is restricted by the fact that these systems are very cost and labour-intensive. As an example, we selected all the studies from the review by Fleeger et al. (2003) that showed decreases and increases in the abundance of different taxa after exposure to toxicants in aquatic test systems. These reviewed experimental studies employed an average of three replicates per concentration. In contrast, we were able to use 24 microcosms for each concentration of toxicant, which enabled us to identify factors that could explain the variance in the recovery of sensitive *D.*

### **3.6 Conclusion**

The results of the study reveal that the persistence of disturbance in terms of population density by a pesticide depends strongly on the strength of interspecific competition when resource limitation is present. Given that competition is prevalent in natural communities, these biotic interactions need to be considered when predicting the recovery of affected populations. For species

with a long life cycle in particular, the time needed to recover from a disturbance might reach several years or even decades if recovery is prolonged by a factor of three to eight. These findings are of crucial relevance for the risk assessment of toxicants as within the respective frameworks the duration of recovery is a relevant parameter for acceptability of effect (i.e., the EU regulation on plant protection products, EU 1107/2009).

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**Chapter 4: Periods of elevated temperature prolong long-term effects of toxicants by altered competition**

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#### 4.1 Abstract

Considerable research efforts have been made to predict the influences of climate change on species composition in biological communities. However, little is known about how changing environmental conditions and anthropogenic pollution will affect aquatic communities in combination. We investigated the influence of three short-term periods of warming alone and in combination with pulse exposure to the insecticide esfenvalerate (0.03, 0.3, and 3  $\mu\text{g/L}$ ) on zooplankton communities in 55 outdoor pond microcosms. Temperatures in the warming periods were measured to be within the natural range of water temperatures during the experimental period, but the abundance of some zooplankton taxa increased selectively under warming compared to ambient conditions. This resulted in a shift in the community composition that had not recovered by the end of the experiment, 8 weeks after the last warming period. Short-term effects of the pesticide on the community structure and the sensitive taxa *Daphnia* spp. did not differ between the two temperature regimes. In contrast, the recovery time of sensitive taxa under warming conditions was twice as long as that at ambient temperature. In detail, at the concentration that caused a high level of short-term mortality (3  $\mu\text{g/L}$ ), the warming conditions led to long-term effects on *Daphnia* spp. until at least 4 months after the pesticide pulse. In all setups, we identified interspecific competition as an underlying mechanism that determined the time until recovery. However, interspecific competition under warming conditions was prolonged and thus delayed recovery of *Daphnia* spp. from esfenvalerate. These results show that, for realistic prediction of the combined effects of temperature and toxicants on sensitive species, the impact of the two stressors on the competitive balance within the community needs to be considered.

**Keywords:** *Daphnia* spp., community context, esfenvalerate, microcosm, recovery, interspecific competition

## 4.2 Introduction

Changing environmental conditions are omnipresent. Among these environmental conditions, temperature is particularly well studied and its global average is predicted to continue to increase in the future (IPCC 2007, 2012). In addition to the average temperature, the frequency of periods of extreme high temperature is also predicted to increase. As a biotic response to altered temperatures and other associated parameters, shifts in the temporal and biogeographical range of species have been observed in the field over recent years (Root et al. 2003, Parmesan 2006, Parmesan and Yohe 2003, Wassmann et al. 2011). In turn, these shifts lead to a lasting increase in the proportion of species better adapted to a warmer environment within many communities, as shown for example in copepod communities in the North Atlantic (Beaugrand et al. 2002) and the distribution of fish in the North Sea (Perry et al. 2005).

Changing environmental conditions are also expected to affect the presence of toxicants and their effects on organisms in aquatic and terrestrial ecosystems. With the predicted increases in temperature, agriculture will show a geographical shift in Europe to higher latitudes, this will expose previously unpolluted freshwater systems to pesticides (Noyes et al. 2009, Kattwinkel et al. 2011). In previous studies, modelling by Kattwinkel et al. (2011) and the review by Noyes et al. (2009) indicated that it is likely that more extreme rainfall and storm events will cause higher levels of pesticide run-off from agricultural land, which will result in increased concentrations of pesticide in running and standing waters.

In addition to alterations in pesticide exposure, sensitivity to toxicants increases with unfavourable environmental conditions. For example, this has been observed for stressful conditions related to salinity (Schäfer et al. 2011, Wildgust and Jones 1998), oxygen concentration (Van der Geest et al. 2002), food limitation (Beketov and Liess 2005, Rose et al. 2002, Koivisto et al. 1992), competition combined with altered solar radiation (Stampfli et al. 2011), and predation (Beketov and Liess 2006). With a focus on environmental conditions

that will be altered by the predicted climate change (IPCC 2007, 2012), Stampfli et al. (2013) found that changing the water levels in microcosms increased the community sensitivity of zooplankton to esfenvalerate by a factor of ten. In this previous paper, the authors discuss how the sensitivity to pesticide was altered by differences in competition and water quality under fluctuating water conditions. Other factors such as UV-B radiation were shown to enhance the sensitivity of amphipods to a toxicant, copper, under both laboratory (Liess et al. 2001) and Antarctic field conditions (Duquesne and Liess 2003).

In relation to increased temperatures, many studies at the individual level have shown that toxicants reduce the tolerance of organisms to high temperatures and thereby induce metabolic stress. This was shown for oysters exposed to cadmium (Lannig et al. 2006) and fish exposed to organochlorines (Patra et al. 2007). The combined effects of increased temperatures and toxicants have also been shown to have sublethal effects on the fecundity of amphipods (Jacobson et al. 2008) and the rate of population growth of daphnids (Heugens et al. 2006). In comparison, within the community context, Van Wijngaarden et al. (2005) and López-Mancisidor et al. (2008) detected no differences in the short-term sensitivity of the zooplankton community to chlorpyrifos between Mediterranean and temperate climate conditions. However, the recovery of the community from the pesticide was delayed under warmer conditions due to algal blooms, as discussed by the authors (Van Wijngaarden et al. 2005, López-Mancisidor et al. 2008). Broomhall (2002) also showed that exposure to endosulfan under varying temperature increased the subsequent sensitivity of tadpoles to predation. These findings indicate that changes in biotic interactions can occur under the combined effects of warm conditions and the presence of toxicants.

Altered biotic interactions, including competition, can prolong the period required by sensitive taxa to recover. Competition with less sensitive taxa reduces the availability of resources for surviving individuals of sensitive taxa. Indeed, a direct association between prolonged recovery of sensitive daphnids from pesticide exposure and interspecific competition with insensitive species has been identified (Foit et al. 2011, Knillmann et al. 2012). However, there is

no available information about direct links between affected biotic interactions in communities and toxicant effects under conditions of increased temperature. Given that temperatures are expected to change under global climate change, the influence of temperature on the sensitivity of organisms to toxicants is very important for future risk assessment in the field of ecotoxicology.

Consequently, we designed an outdoor microcosm experiment to expose zooplankton communities to periodic periods of warming and a pulsed contamination with esfenvalerate. Using this approach, we aimed to assess the effect of the two stressors at the community and population levels. Furthermore, we wanted to determine the influence of changes in biotic interactions due to increased temperatures on the response of sensitive species to pesticides.

### **4.3 Material and methods**

#### *General*

The aim of the periodic warming was to increase the cumulative daily temperature without exceeding the maximum water temperature observed under ambient conditions over the study period. Three periods of warming that each lasted 1 week were applied at intervals of 3 weeks. The influence of the warming periods was analysed by comparison with communities kept under ambient temperatures. Both the communities subjected to warming and those kept at ambient conditions were exposed to the pesticide esfenvalerate at three concentrations (0.03, 0.3 and 3  $\mu\text{g/L}$ ). The different regimes of temperature and pesticide exposure resulted in two experimental setups: “ambient conditions” and “warming conditions”. The setup of ambient conditions was replicated twelve and six times for the control and each concentration, respectively, whereas the setup with warming periods was replicated ten and five times for the control and each concentration, respectively. In the present study, we focused on changes in the community structure of zooplankton. At the population level, we investigated the dynamics of *Daphnia* spp. and *Simocephalus* spp. in more detail.

### *Microcosms*

Fifty-five outdoor microcosms were installed at the UFZ - Helmholtz Centre for Environmental Research in Leipzig, Germany (51°21'13 N, 12°25'55 E). Each microcosm had a volume of 80 L and was filled with 60 L of water (tap water seeded with 0.5 L of natural pond water). The microcosms were maintained at a minimum water level of 60 L over the course of the experiment using tap water. Natural pond sediment was mixed at a ratio of 2:1 with sand and distributed at the bottom of each tank to a thickness of approximately 1 cm. Furthermore, approximately 10 g of shredded leaves (*Populus* spp.) were added to each microcosm. Invertebrate communities (mainly zooplankton) were collected from three natural ponds within a radius of 15 km of the research centre and distributed equally among all microcosms.

The warming periods were simulated using electric heaters for outdoor use (100 W, HT100, Tetra GmbH, Melle, Germany). The heaters (28.5 × 3 cm) were inserted into the microcosms and attached to the microcosm walls below the water surface.

### *Exposure to esfenvalerate*

The zooplankton communities in the microcosms were exposed to the widely used pyrethroid pesticide esfenvalerate. We used the commercial formula Sumicidin Alpha EC (BASF, Limburgerhof, Germany), which contains 50 g/L of the active ingredient, esfenvalerate. Microcosms were contaminated on 18th June, 2009, with one of three concentrations of esfenvalerate: 0.03, 0.3, and 3 µg/L. These concentrations correspond to the range of esfenvalerate concentrations that are detected frequently in the field (Kelley and Starner 2004, Bacey et al. 2005, Cooper et al. 2003). Esfenvalerate was applied 3 days after the start of the first warming period. Regarding the toxicity of pyrethroids, it is known that they possess a negative temperature coefficient (Kumaguru and Beamish 1981, Hinkle et al. 1989, Harwood et al. 2009), which means that their toxicity decreases with increasing temperature. Consequently, to minimise changes in toxicity caused by marked differences in temperature, the heaters were switched off from 12 hours before until 12 hours after contamination.

**Tab. 4.1:** Mean exposure concentrations and standard deviation of esfenvalerate in the microcosms. The re-measurement was performed one year later (2010) than the original experiment with the same setup of treatments and microcosms. Detection limit of esfenvalerate was 0.01 µg/L.

Nominal concentration (µg/L)	Time after contamination (h)	Actual concentration (µg/L), n= 3	
		Warming	Ambient
0.03	2	0.03 ± 0.004	0.03 ± 0.001
0.3	2	0.25 ± 0.031	0.28 ± 0.092
3	2	2.70 ± 0.173	2.10 ± 0.100
Re-measurement			
3	2	3.54 ± 0.344	3.47 ± 0.532
3	14	2.47 ± 0.488	2.72 ± 0.653
3	48	0.80 ± 0.083	0.71 ± 0.078

As a result of a technical failure, exposure concentrations could not be measured in the original experiment. Thus, we determined peak exposure concentrations (2 hours after contamination) for the two temperature regimes using an analogously designed microcosm experiment that was conducted in August 2009 (Tab. 4.1). A further experiment was performed in August 2010 to follow the dissipation of esfenvalerate until 48 hours after contamination. For this microcosm experiment, which was performed in the absence of zooplankton communities, we repeated the pesticide treatments under ambient and warming conditions as described above. In both experiments, the measured exposure concentrations showed no consistent differences between ambient and warming conditions (Tab. 4.1), even though increased temperatures are expected to enhance the degradation of pesticides (Noyes et al. 2009). Esfenvalerate is characterised by high values of  $\log K_{ow}$  ( $> 6$ ) and  $K_{oc}$  (215.000) (Kelley 2004), which cause applied esfenvalerate to be adsorbed rapidly into sediment and particles, including algae. Thus, the concentrations of esfenvalerate decreased rapidly during the first few hours in all setups (Tab. 4.1, for details on pesticide



application and dissipation in previous microcosm experiments until seven days after contamination, see also Stampfli et al. 2011, Knillmann et al. 2012). The concentrations of esfenvalerate were measured by Eurofins Umwelt Ost GmbH (Jena, Germany). Samples were extracted using solid-phase extraction, which was followed by gas chromatography–mass spectrometry (GC-MS, Agilent Technologies Inc., Palo Alto, USA). The detection limit of the applied method was 0.01 µg/L.

*Biological sampling and environmental parameters*

Biological samples of the zooplankton community were taken at the following time points in relation to the pesticide contamination (time point = 0): -15, -1, 8, 29, 36, 55, 64, 84, and 119 days. The samples were collected with a sampling tube (PVC, length = 31.7 cm, radius = 3.6 cm). The lid of the sampling tube was placed first in the centre of each pond on top of the sediment. Before the tube was fitted onto the lid, the water was stirred gently to obtain a homogeneous distribution of organisms in the pond. Afterwards, the water from the tube (which contained 1.7% by volume of the water from the pond), including any organisms, was passed through a sieve (180-µm mesh size). The organisms that were obtained in this manner were preserved in 70% ethanol. Subsequently, samples were counted and identified under a microscope to the level of genus (Cladocera, Chaoboridae, Culicidae, Baetidae), order (Odonata, Copepoda) or class (Ostracoda, Arachnida). The main detected taxa and their abundances in the absence of the pesticide are shown in Tab. 4.2.

Water temperature was recorded continuously every hour with Handylog DK501-PL data loggers (Driessen & Kern, Bad Bramstedt, Germany) in a subset of five microcosms assigned to warming and five assigned to ambient conditions. Electrical conductivity (EC, HI-98312, Hanna Instruments, Woonsocket, USA), pH (HI-98127, Hanna Instruments, Woonsocket, USA), turbidity (Turbiquant 1100 IR, Merck Chemicals, Darmstadt, Germany) and dissolved oxygen concentration (DO, WTW Multi 340i Meter, WTW Instruments, Weilheim, Germany) were recorded weekly between 7 and 9 a.m. for a subsample of three replicates for each treatment.

**Tab. 4.2:** Densities of main invertebrate taxa in the communities that were not exposed to pesticide. The untransformed densities are shown as the mean and standard deviation from 15 days before until 119 days after contamination.

<b>Taxa</b>	<b>Abundances (Ind./L) under ambient conditions</b>	<b>Abundances (Ind./L) under warming conditions</b>
<i>Daphnia</i> spp.	48.4 ± 53.3	33.1 ± 28.8
<i>Simocephalus</i> spp.	4.2 ± 8.3	9 ± 11.9
<i>Scapholeberis</i> spp.	6.2 ± 8.2	7.9 ± 11.9
<i>Chydorus</i> spp.	34.3 ± 73.7	34.6 ± 60.7
<i>Pleuroxus</i> spp.	0.7 ± 2.5	6.9 ± 18.3
<i>Alona</i> spp.	0.3 ± 1.3	0.6 ± 2.4
Ostracoda	28.3 ± 23	39 ± 32.3
Copepoda	14.3 ± 21.8	28.1 ± 40.3
Culicidae	0.4 ± 3.2	0.1 ± 0.4
Baetidae	0.5 ± 1.6	1 ± 2.4
Chaoboridae	1.6 ± 2.1	3.2 ± 3.3
Odonata	0.1 ± 0.4	0.2 ± 0.7

### *Statistics*

To identify the environmental variables that had a significant influence on the communities over time, we performed redundancy analysis (RDA) using the technique of forward model selection (Leps and Smilauer 2003). Species data were included as dependent variables and physico-chemical measurements (see section 2.4) as independent variables. In addition, the temperature regime was included as a categorical independent variable and time as a covariable for the RDA. Species were  $\ln(4x+1)$ -transformed before performing multivariate analyses as suggested previously by Van den Brink et al. (2000) to avoid false discrepancies between zero-abundance values and low abundance values in the data. Before the RDA, we tested for differences in the physico-chemical data between the control and the three pesticide concentrations under each temperature regime. These differences were evaluated using analysis of variance

(ANOVA), followed by pairwise t-tests for multiple comparisons, which were adjusted if the variances of the groups were not homogeneous. In the case of non-normally distributed samples, the Kruskal–Wallis test for nonparametric data was applied, followed by a nonparametric multiple-comparison test (R package *pgirmess*, function *kruskalmc*, Siegel and Castellan 1988). To correct the p-values in multiple testing, Holm’s correction was applied.

The influence of the warming periods and toxicant on community structure was assessed with the help of principal response curves (PRC). Monte Carlo permutation tests using an F-type statistic based on the eigenvalue of the components were applied to test the significance of the PRC (first principal components) (Leps and Smilauer 2003, Van den Brink and Ter Braak 1999). To interpret the species response, species weights between -0.5 and 0.5 were considered to be weakly or not related to the changes displayed in the PRC (Van den Brink and Ter Braak 1999). The differences in community structure between the treatments of ambient and warming conditions were tested for every treatment by RDA followed by a Monte Carlo permutation test. In a similar manner, we tested the influence of different pesticide concentrations on the community structure for each of the four setups. The temperature treatment or the  $\ln(x+1)$ -transformed pesticide concentration was used as an explanatory variable for each RDA conducted.

To focus on the relationships between taxa during the period after contamination, we performed an additional principal component analysis (PCA). The PCA is an unconstrained ordination method, which also includes variability in the species composition that is not related to explanatory variables (for rationale, see Beketov and Liess 2008). To determine interpretable PCA axes, we used the broken-stick method, as recommended for simple and consistent evaluation of dimensionality in PCA (Jackson 1993). The PCA was interpreted using correlation biplot scaling with centred and transformed species data as described by Zuur et al. (2007) and Leps and Smilauer (2003). The  $\ln(x+1)$ -transformed pesticide concentration, time variable and temperature regime was added by passive ordination. The selection of linear multivariate methods (RDA, PCA, and PRC) was based on the outcome of preliminary detrended

correspondence analysis (DCA) in accordance with the work of Leps and Smilauer (2003).

For the analyses of zooplankton at the population level, counted abundance data were subjected to fourth-root transformation as suggested for skewed abundance data (Quinn and Keough 2002). Differences in sample means among the different concentrations of toxicant and the control at the various time points were investigated by ANOVA, followed by multiple comparisons as described above for the physico-chemical data. The initial pesticide effect was calculated as the relative change in the mean abundance from the sampling 7 days after contamination to the abundance 1 day before contamination for each microcosm. The influence of the initial pesticide effect on the recovery of *Daphnia* spp., as well as the relationships between *Daphnia* spp. and *Simocephalus* spp., were investigated by linear analysis of regression. For the relationships between the two taxa, we investigated the abundance of *Daphnia* spp. at time point “i” depending on the preceding growth of *Simocephalus* spp. and vice versa. The preceding growth was defined as the absolute difference from the preceding time point “i - 3 weeks” until time point “i”. For all linear models, we checked the residuals of the model for a normal distribution, homogeneity of variance, and influential data points (Crawley 2007).

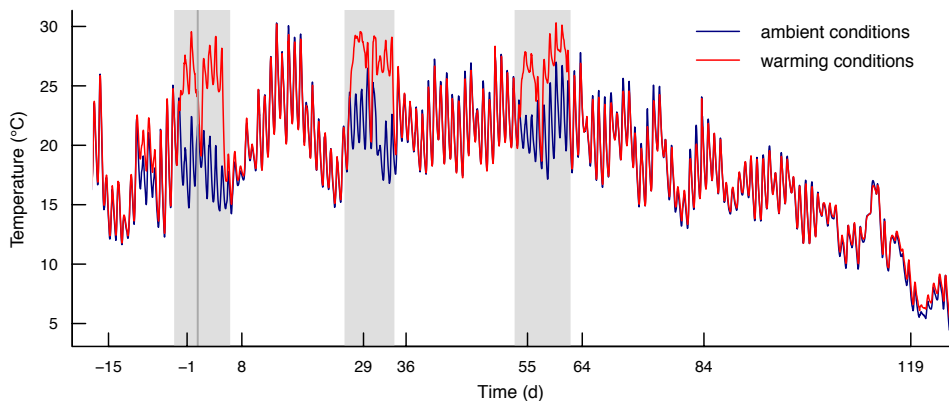
Multivariate analyses were conducted using the program CANOCO 4.5 for Windows (Wageningen, Netherlands) in accordance with previous work and guides (ter Braak and Smilauer 2002, Leps and Smilauer 2003). The remaining statistical analyses and graphs were generated with R, version 2.15.1 (R Foundation for Statistical Computing, 2011).

#### **4.4 Results**

##### *Changes in environmental conditions as a result of warming*

Temperature, pH, DO, and turbidity were identified as having a significant influence on the microcosm communities over the experimental period (RDA,  $p = 0.002$ ). The subset of environmental variables that included temperature

regime, pesticide concentration, and time as a covariable explained 15.9% of the total variance. Among the identified physicochemical parameters, we only found significant differences in the measured temperatures between the two temperature regimes during the warming periods (ANOVA,  $p < 0.05$ ). During the warming periods, water temperature was increased by a daily average of 6.2 K relative to ambient conditions (Fig. 4.1). The maximum and minimum differences in temperature per hour were 10.4 K and 0.2 K, respectively. Water temperatures during the warming periods never exceeded 30.3°C per hour, which proved to be very similar to the maximum as for water temperatures under ambient conditions over the whole experimental period (30.3°C).

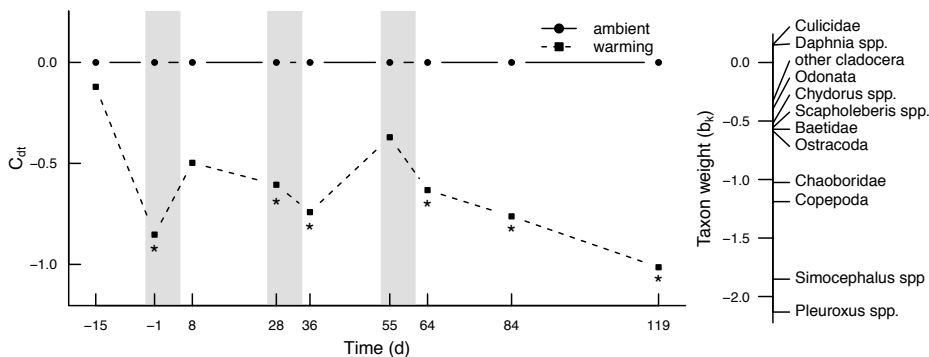


**Fig. 4.1:** Profile of the water temperature under ambient and warming conditions over the experimental period.

#### *Influence of warming on community structure*

We observed a significant difference in community composition between ambient and warming conditions (Fig. 4.2, PRC, Monte Carlo permutation test,  $p = 0.002$ ). According to the RDA, followed by Monte Carlo permutations at single time points, the structure of the community was altered significantly up to the end of the experiment, 8 weeks after the last warming period. No negative effects on species were detected. Some taxa, especially *Pleuroxus* spp. and *Simocephalus* spp., showed highly positive taxon weights, which indicated an increase in abundance (Fig. 4.2, taxon weights). In comparison, other taxa,

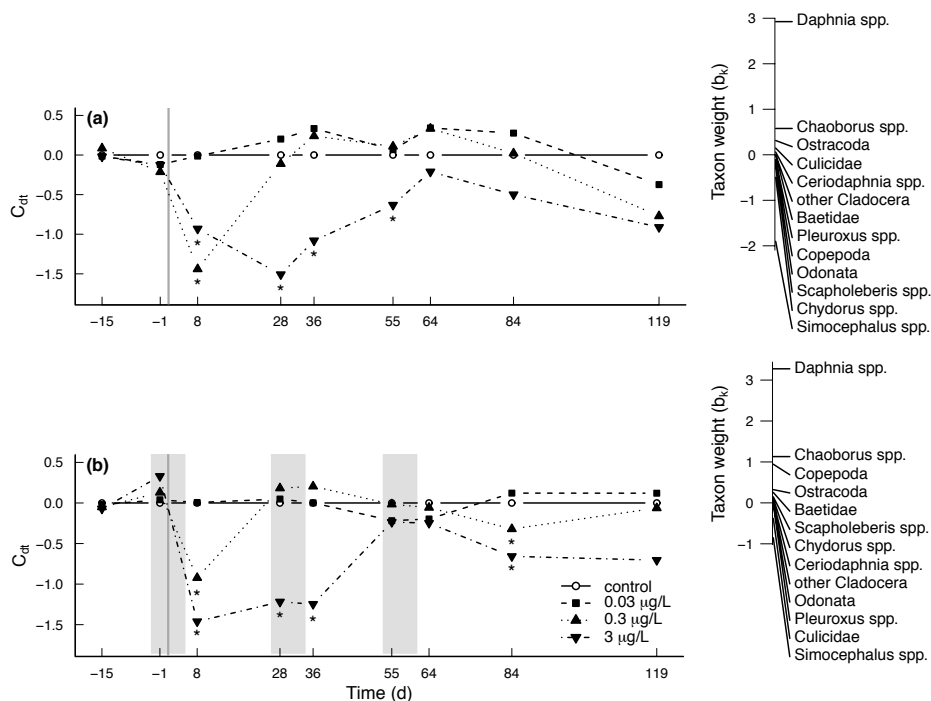
including *Daphnia* spp. and Culicidae, did not react negatively or positively to the warming in terms of their overall abundance.



**Fig. 4.2:** Principal response curve (PRC) showing the differences in community structure between ambient and warming conditions. Asterisks indicate significant differences in the community at specific time points (Monte Carlo permutation test following RDA,  $p < 0.05$ ). The grey bars indicate the warming periods.

#### *Effects of warming combined with pesticide exposure on the community*

In general, we observed significant effects of esfenvalerate on community structure under both ambient and warming conditions (Fig. 4.3, PRC,  $p = 0.002$ ). At esfenvalerate concentrations of 0.3 and 3  $\mu\text{g/L}$ , we observed significant short-term effects on the community, 8 days after contamination, under both temperature regimes (Monte Carlo permutation test following RDA,  $p < 0.05$ ). For both treatments at 0.3  $\mu\text{g/L}$ , recovery occurred by 4 weeks after contamination. In contrast, at 3  $\mu\text{g/L}$ , the time until recovery differed according to the temperature regime. Under ambient conditions, significant pesticide-related effects were observed relative to the control until more than 7 weeks (55 days) after contamination. Under warming conditions, communities showed 3  $\mu\text{g/L}$  significant differences from the control communities only until 5 weeks (36 days), but effects reappeared 12 weeks after contamination at the highest and intermediate concentrations tested. With regard to the investigated community, the taxon of *Daphnia* spp. was affected the most negatively by esfenvalerate, in terms of its abundance, whereas *Simocephalus* spp. increased the most following esfenvalerate exposure under both temperature regimes (Fig. 4.3, taxon weights).

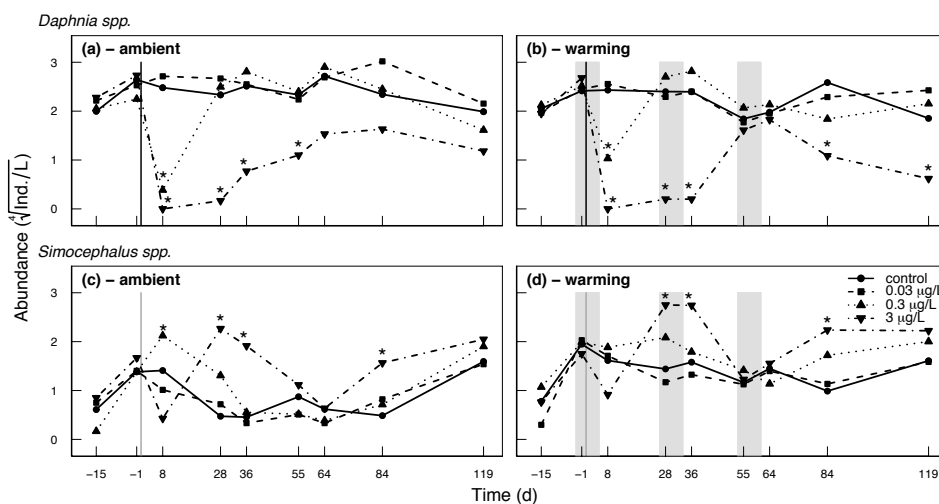


**Fig. 4.3:** Principal response curves (PRC) representing the effect of esfenvalerate on community structure under ambient (a) and warming conditions (b). Asterisks indicate significant differences in the community at specific time points (Monte Carlo permutation test following RDA,  $p < 0.05$ ). The grey bars (b) indicate the warming periods.

When population dynamics were evaluated, *Daphnia* spp. showed strong decreases in abundance at 0.3 µg/L under both ambient (-84%) and warming conditions (-57%) (Fig. 4.4a-b). However, populations recovered until 4 weeks (28 days) after contamination under both treatments. At 3 µg/L, initial declines in abundance were close to -100% under both conditions. Under ambient conditions, we detected pesticide-related effects on *Daphnia* spp. until 8 weeks (55 days) after contamination. In contrast, populations under warming conditions showed effects at 3 µg/L esfenvalerate until the end of the experiment at 16 weeks. Hence, negative short-term effects on *Daphnia* spp. at 0.3 µg/L esfenvalerate did not differ significantly between the two treatments, but

recovery in terms of abundance took at least twice as long under warming conditions as under ambient conditions.

For populations of *Simocephalus* spp., we observed no significant negative effects at any concentration under either temperature regime (Fig. 4c-d). In fact, populations of this less sensitive taxon were even increased significantly at concentrations of 0.3 and 3  $\mu\text{g/L}$  esfenvalerate at some time points during the experiment. In general, abundances of *Simocephalus* spp. were higher under warming conditions than under ambient conditions (+206%, absolute abundance averaged over the control and the three concentrations of esfenvalerate, data not shown).



**Fig. 4.4:** Mean densities for *Daphnia* spp. (a, b) and *Simocephalus* spp. (c, d) for the different concentrations of esfenvalerate under ambient and warming conditions. Asterisks represent significant differences from the control (pairwise t-test or Kruskal–Wallis multiple comparison test,  $p < 0.05$ ). The grey bars (b, d) indicate the warming periods.

#### *Interspecific competition and its influence on recovery*

We wanted to investigate the long-term effects of esfenvalerate on *Daphnia* spp. under warming conditions and evaluate more closely the influence of the initial pesticide effect at 4 and 12 weeks after contamination (for definition of the initial pesticide effect, see Materials and methods). The time point of 12 weeks

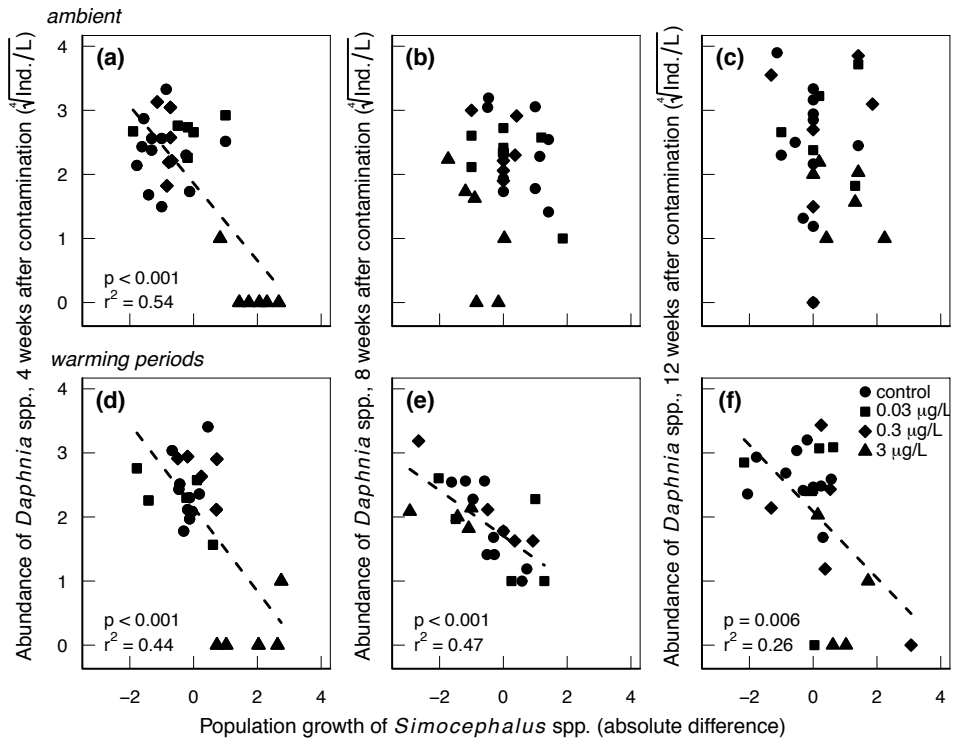


after contamination was included, because populations of *Daphnia* spp. started to decrease again at this point after a previous increase before the 12-week time point under warming conditions.

Under ambient conditions, the abundance of *Daphnia* spp. at 4 weeks after contamination depended on the strength of the short-term pesticide effect (linear regression, d.f. = 28, adjusted  $r^2 = 0.36$ ,  $p < 0.001$ ). At 12 weeks after contamination, the initial pesticide effect showed no significant influence on the abundances. In contrast, under warming conditions, we detected a significant influence of the initial pesticide effect on the abundances of *Daphnia* spp. at 4 weeks (d.f. = 23, adjusted  $r^2 = 0.35$ ,  $p = 0.001$ ) and 12 weeks (d.f. = 22, adjusted  $r^2 = 0.44$ ,  $p < 0.001$ ) after contamination. Hence, under the regime with warming periods, the influence of the short-term effect of pesticide on long-term reductions of *Daphnia* spp. was more pronounced than under ambient conditions.

In further comparison of the population dynamics of *Daphnia* spp. and *Simocephalus* spp., the abundances of the two taxa seemed to develop in an antagonistic manner (Fig. 4.4). This relationship was especially pronounced at 0.3 and 3  $\mu\text{g/L}$  esfenvalerate, and was exhibited under both ambient and warming conditions. To understand the relationship, we plotted the abundance of *Daphnia* spp. at 4, 6, and 8 weeks after contamination (which equated to 3 weeks after each warming period) as a function of the preceding population growth of *Simocephalus* spp. (for definition, see Materials and Methods) for all concentrations of esfenvalerate (Fig. 4.5). Under ambient conditions, we identified a significant negative relationship between abundances of the two species at 4 weeks after contamination ( $p < 0.001$ , adjusted  $r^2 = 0.54$ ), but not at later time points. In comparison, under periodic warming conditions, we detected a significant negative relationship at 4 weeks ( $p < 0.001$ , adjusted  $r^2 = 0.44$ ), 6 weeks ( $p < 0.001$ , adjusted  $r^2 = 0.47$ ), and 8 weeks after contamination ( $p = 0.006$ ,  $r = 0.26$ ). To avoid missing any significant relationships under ambient conditions owing to the specific selection of time points, we assessed whether there was any relationship at other time points, including 36, 64 and

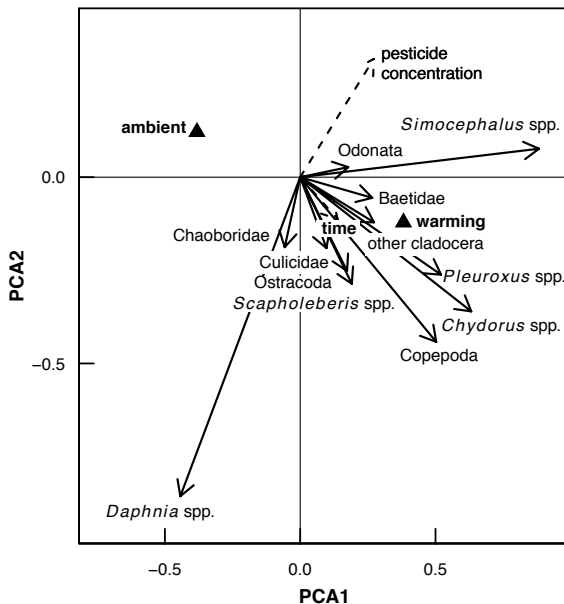
119 days after contamination. However, no significant relationships were detected.



**Fig. 4.5:** Abundance of *Daphnia* spp. as a function of the population growth of *Simocephalus* spp. (for definition, see Material and Methods) for the time points of 4 weeks, 8 weeks, and 12 weeks after contamination under ambient and warming conditions. Significant linear regressions are represented by regression lines,  $r^2$ , and  $p$ -values.

Before the analyses, we checked whether the abundance of *Simocephalus* spp. changes in a manner dependent on the population growth of *Daphnia* spp. to assess the direction of the relationship between the taxa. Significant relationships between *Simocephalus* spp. and the preceding growth of *Daphnia* spp. were only detected under warming conditions, at 8 weeks ( $p = 0.02$ , adjusted  $r^2 = 0.18$ ) and 12 weeks ( $p = 0.009$ , adjusted  $r^2 = 0.23$ ) after

contamination. Hence, under warming conditions, interspecific competition between *Simocephalus* spp. and *Daphnia* spp. was prolonged in both directions.



**Fig. 4.6:** Correlation biplot for the species data of the microcosms in relation to pesticide concentration, temperature regime, and time derived from the PCA with normalised variables. Data from 8 days until 119 days after contamination were included. Continuous independent (bold font) and dependent variables (normal font) are shown as arrows for better understanding. Triangles represent the independent categorical variable of temperature regime.

We also assessed interactions of *Daphnia* spp. with other taxa in the microcosm communities. The performed PCA included species data as response variables, and time, temperature regime, and pesticide concentrations as environmental variables. According to the broken-stick method, the first three axes were determined to be interpretable. It was found that 25.9% of the total variability in the community was explained by the first PCA axis, 17% by the second PCA axis, and a further 13.3% by the third PCA axis. Relationships between the taxa and the environmental data were displayed and interpreted with a correlation biplot (Fig. 4.6). According to the correlation biplot, *Daphnia* spp. were

associated negatively with pesticide concentration and *Simocephalus* spp., as well as to a lesser extent with Odonata. However, when *Daphnia* spp. were plotted as a function of the abundance of growth of Odonata, no significant relationship was found.

#### 4.5 Discussion

In this study on the effects of increased temperature and pesticide effects, warming conditions alone altered community structure up to the end of the experiment, 8 weeks after the last warming period. Considering the combined effects of warming and pesticide exposure, short-term effects of esfenvalerate on community structure and sensitive taxa were the same regardless of the temperature regime. This detection of similar short-term effects can be explained by the fact that the peak exposure to esfenvalerate occurred under comparable temperature conditions in both setups (see Material and methods). In contrast, long-term effects of esfenvalerate on populations of *Daphnia* spp. were detectable much longer under periodic warming than under ambient conditions. Recovery of *Daphnia* spp. under conditions of periodic warming was not observed until the end of the experiment, 4 months after contamination, this effect was also reflected at the community level.

##### *Long-term change of community structure by warming alone*

The change in community structure over the long term under warming conditions was in accordance with the outcome of previous studies using model test systems, which also revealed alterations of community composition due to an increase of temperature. Examples include zooplankton and phytoplankton communities in alpine ponds (Strecker et al. 2004) and experimental microcosms (McKee et al. 2002).

In the present study, some taxa increased in abundance under warming, such as *Simocephalus* spp. and *Pleuroxus* spp., whereas others, such as *Daphnia* spp., did not change. This is supported by the findings of a laboratory investigation of Laberge and Hann (1990), who observed that *Simocephalus*

*vetulus* was more tolerant to high temperatures than *Daphnia pulex*. Such selective changes in the abundance of species within our pond community caused a new competitive balance of species. A new competitive balance of species can lead to altered biotic interactions, as described in previous studies in which the competitive dominance of species shifted towards warm-water species following an increase in water temperature. Examples include cyanobacteria (Watermann et al. 1999, Jöhnk et al. 2008), fish (Daufresne et al. 2004), copepods (Beaugrand et al. 2002), and daphnids (Vijverberg and Vos 2006).

*Altered biotic interactions delay recovery from pesticide stress*

Despite similar short-term effects under the two temperature regimes and a presumably faster rate of degradation of pesticides under warmer conditions, as reviewed by Noyes et al. (2009), long-term effects on the community and on sensitive taxa were more pronounced under warming than under ambient conditions. Prolonged recovery of zooplankton communities from exposure to chlorpyrifos under a Mediterranean climate, as compared with a temperate climate, was also observed previously in experimental ditches and microcosms (Van Wijngaarden et al. 2005, López-Mancisidor et al. 2008).

To explain the underlying processes behind the long-term effects in the present study, we hypothesise that the combined effects of warming and toxicant enhance the sensitivity of *Daphnia* spp. to competition and thus are responsible for long-term effects of the toxicant. Evidence that supports this hypothesis is found in the literature on sublethal effects of warming and toxicants at the individual and population level. These findings are essential to draw conclusions on the propagation of such effects up to the community level. At the individual level, it was reported that combined exposure to diazinon and high temperature reduced the hatching rates of zebrafish (Osterauer and Köhler 2008). In addition, Jacobson et al. (2008) found that thermal stress and the fungicide fenarimol synergistically decreased the fecundity of amphipods. Such negative effects at the individual level can cause a decline in population abundance, as shown for the population growth of *Daphnia magna* exposed to elevated temperatures and cadmium (Heugens et al. 2006). In terms of the propagation of effects up to the

community level, Broomhall (2002) observed that exposure to endosulfan under varying temperatures increased the sensitivity of tadpoles to predation, 3 weeks after contamination. These findings are in line with our hypothesis that the period of recovery of *Daphnia* spp. from exposure to esfenvalerate is prolonged under warming conditions owing to an increased sensitivity to interspecific competition across all concentrations of esfenvalerate and in the control. Interspecific competition in relation to *Daphnia* spp. was observed under ambient conditions, but only until 4 weeks after contamination. We also detected that, under warming conditions, the initial pesticide effects were important for the presence of long-term effects, independently of the pesticide treatment. Initial pesticide effects were strongest at 3 µg/L and thus led to a significant decrease in average abundances up to the end of the experiment.

Under both temperature regimes, interspecific competition of *Daphnia* spp. was most pronounced with *Simocephalus* spp., with the latter taxon being very insensitive to esfenvalerate within the investigated community. As determined in an earlier experiment, the two taxa differ markedly in terms of sensitivity. *Daphnia* spp. are toxicologically very sensitive to esfenvalerate (LC50 [96 h] = 0.02–0.15 µg/L, Knillmann et al. 2012), whereas *Simocephalus* spp. were observed to be relatively insensitive (LC50 [96 h] = 2.5 µg/L, Knillmann et al. 2012). Differences in sensitivity to a stressor such as a toxicant cause indirect effects that are a well-studied phenomenon in eco(toxico)logy. Insensitive species benefit from the resources that are made available by the toxicant-induced impairment of sensitive species and thus increase in abundance. Such indirect effects of toxicants have been described in several reviews that are related mainly to model ecosystems (Relyea and Hoverman 2006, Fleeger et al. 2003, Preston 2002), but also from studies in the field (Peterson 2001, Liess and von der Ohe 2005). Indirect effects on insensitive taxa can be enhanced by the fact that sublethal effects of toxicants reduce the use of resources by affected surviving individuals of sensitive species (Forbes et al. 2001). Esfenvalerate/fenvalerate are known to have sublethal effects on filtration rates (Day and Kaushik 1987) and the fecundity of daphnids (Reynaldi et al. 2006, Day 1987).

Indirect increases in insensitive species have in turn been associated with a delayed recovery of sensitive species. From observations in a field study, Frost et al. (2006) concluded that the recovery of sensitive zooplankton species from acidification was delayed by interspecific competition with acid-resistant species. Furthermore, a direct quantitative relationship between interspecific competition and long-term effects on abundance was identified recently for the recovery of daphnids from exposure to pyrethroids under laboratory (Foit et al. 2011) and semi-field conditions (Knillmann et al. 2012). Analogously, at the population level, abundances of *D. magna* recovered within a week in abundance, but intraspecific competition between individuals of different size classes delayed the recovery of population structure (Liess and Foit 2010). However, the present study is the first to reveal a direct connection between enhanced interspecific competition and recovery from exposure to a toxicant under increased temperatures.

Furthermore, the competition between sensitive and less sensitive taxa explains the apparent recovery and delayed effects (that is, effects that increase in magnitude or appear long after contamination) on *Daphnia* spp. at 3 µg/L esfenvalerate under warming conditions. Populations of *Daphnia* spp. recovered to control levels during the period in which *Simocephalus* spp. were declining, that is, at a time when interspecific competition was reduced. After the subsequent increase again of *Simocephalus* spp., delayed effects on *Daphnia* spp. became apparent. Thus, periods of warming within the natural temperature range, constituted with the alteration of competitive balance a latent stressor when toxicant stress was also present. Previously, delayed effects were observed for freshwater arthropods upon pesticide exposure (Liess 2002, Beketov and Liess 2005, Daam et al. 2009, Van der Hoeven and Gerritsen 1997), as well as an apparent recovery of copepods exposed to lufenuron in mesocosms (López-Mancisidor et al. 2008).

## **4.6 Conclusions**

For realistic prediction of the combined effects of temperature and toxicants on sensitive species, the impact of the two stressors on the competitive balance within the community needs to be considered. Temperature and pesticides are only one example, the competitive balance can also be disrupted by other combinations of changing environmental conditions and toxicants. Those species that are affected negatively by the environmental factors under consideration will experience reduced competitive strength and thus will be affected lastingly by the toxicants. Such stressed species can include those that are already endangered or those particularly vulnerable to suffer from future environmental changes.

## **4.7 Acknowledgements**

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## Chapter 5 - Discussion and outlook

### 5.1 Influence of intraspecific competition on the short-term sensitivity of *Daphnia* spp. to pesticides

In Experiment 1, the treatment of shading and harvesting caused differences in community sensitivity to the pesticide by a factor of 100 due to general biotic stress (Stampfli et al. 2011). Further analyses revealed that for the sensitive taxa *Daphnia* spp., the pesticide-related survival strongly depended on the densities of the densities before contamination. This dependency explained the variation in the survival of *Daphnia* spp. across all treatments of shading and harvesting (Experiment 1 - Chapter 2). Such a strong influence of the densities before contamination can be explained by the fact that high densities increase the competition for food and space and thus reduce resources at the individual level. This finding is supported by previous studies, where an increase in the pesticide effect due to high densities of trichopteran larvae (Liess 2002) as well as mosquito larvae and daphnids (Foit et al. 2011) before contamination was observed. Food limitation, as the only factor to increase exploitative competition, is also well known to enhance the toxicant effects on aquatic organisms (Rose et al. 2002, Beketov and Liess 2005, Koivisto et al. 1992, Antunes et al. 2004).

Intraspecific competition was the major factor determining the strength of the pesticide effect on *Daphnia* spp. within the microcosm communities. The relatively low relevance of interspecific competition for the alteration of the pesticide effect (Experiment 1 - Chapter 2) was also observed by Foit et al. (2011). In their multi-species systems the authors detected a stronger influence of intraspecific competition than interspecific competition on the pesticide effects on mosquito larvae and daphnids. Especially for species with competitive advantages and relatively high abundances, intraspecific competition can have a stronger influence on population dynamics than the interspecific competition shown for mosquitoes (Armistead et al. 2008) and intertidal limpets (Creese and Underwood 1982).



The present work shows that (i) intraspecific competition has a strong influence on the pesticide-related survival of *Daphnia* spp. in the community context and determined (ii) a quantitative link between individual density and pesticide effect. High intraspecific competition increased the short-term pesticide sensitivity of *Daphnia* spp. by a factor of up to 100 (Experiment 1 - Chapter 2). Other environmental factors, such as high temperature (Osterauer and Köhler 2008, Song et al. 1997) and intraspecific competition at the population level (Liess 2002) increased pesticide effects by a factor of ten. UV B radiation in combination with food limitation increased effects of copper by a factor of 30 (Liess et al. 2001). This comparison with other factors highlights that intraspecific competition is a very relevant parameter to consider when predicting toxicant effects on complex communities.

## **5.2 Recovery from pesticides of sensitive taxa in the community context**

The time until recovery of *Daphnia* spp. was up to eight times longer in the mesocosm experiments (Experiment 1 - Chapter 3, Experiment 2 - Chapter 4) than the predictions by models based on population growth rates under optimal laboratory conditions (Barnthouse 2004) (Tab. 5.1). It is well established that generation times of species are related to recovery times in mesocosms (Beketov et al. 2008, Sherratt et al. 1999), but also under field conditions (Liess and von der Ohe 2005, Niemi et al. 1990). However, the differences between the results of this work and the predictions by Barnthouse (2004) indicate that generation times of species can be used to estimate a ranking of relative recovery between taxa, but not the absolute time until recovery. Comparable absolute times until recovery from pesticides of sensitive taxa within the community context were also detected in other aquatic model systems (López-Mancisidor et al. 2008, Brock et al. 2000) and in the field (Liess and von der Ohe 2005).

**Tab. 5.1:** Predicted recovery times of *Daphnia* spp. by Barnthouse (2004) and observed recovery times in the two microcosm experiments.

Experiment	Concentration of esfenvalerate ( $\mu\text{g/L}$ )	Initial reduction by esfenvalerate (%)	Predicted recovery following Barnthouse (d)	Observed recovery (d)	Factor of increase from predicted to observed recovery
Exp. 1	0.3	50	7	> 59	8.4
Exp. 2 (ambient conditions)	0.3	84	13	28	2.2
Exp. 2 (warming conditions)	0.3	57	8	28	3.5
Exp. 1	3	> 90	16	> 59	3.7
Exp. 2 (ambient conditions)	3	> 90	16	64	4.0
Exp. 2 (warming conditions)	3	> 90	16	> 119	7.4

Interspecific competition with less sensitive daphnids was seen to delay the recovery of *Daphnia* spp. in abundance. This relation between competition and recovery was observed in both microcosm experiments and was probably enhanced by sub-lethal effects of esfenvalerate on *Daphnia* spp., since this insecticide was previously observed to sub-lethally affect feeding rates and the reproduction of daphnids (Day and Kaushik 1987, Reynaldi et al. 2006). The strength of interspecific competition depended on the short-term effects at all concentration levels (Experiment 2 - Chapter 4) and for concentrations leading to partial mortality of *Daphnia* spp. populations (0.03 and 0.3  $\mu\text{g/L}$ , Experiment 1 - Chapter 3). In addition, the influence and the duration of species interactions increased with the presence of changing environmental factors such as increasing temperature (Experiment 2 - Chapter 4). Interactions of *Daphnia* spp. with other taxa that could have affected their recovery were not detected (Experiment 1 - Chapter 3, Experiment 2 - Chapter 4).

*Interspecific competition prolongs recovery*

Indirect effects of toxicants on less sensitive species in communities are well investigated. A toxicant-related reduction of sensitive taxa will provide more resources for competing but less sensitive taxa and cause an indirect increase of these, as reviewed by Fleeger et al. (2003) and Relyea and Hoverman (2006). Despite the fact that indirect effects have often been described, very few studies have linked the increase in less sensitive species to a subsequent prolonged recovery of the more sensitive species to the toxicant. For the recovery of lake communities from acid conditions, Frost et al. (2006) related the observed long-term effects on acid-sensitive species to interspecific competition with acid-resistant species. In addition, at the population level Liess and Foit (2010) detected a recovery of *Daphnia magna* in abundance within a few days, but the structure of the population only recovered after 2 months due to intraspecific competition between the different size and age classes. To my knowledge, there is only one recent study that directly linked and quantified interspecific competition with a prolonged recovery of sensitive taxa. This study by Foit et al. (2011) showed, in a multi-species system, that the recovery of *D. magna* was delayed by competition with less sensitive mosquito larvae.

*Influence of warming on recovery*

Warming periods without pesticide exposure caused long-term changes in the structure of the microcosm communities (Experiment 2 - Chapter 4). The presence of warming periods as a single factor caused no negative effects on any of the identified zooplankton taxa, but selectively increased the abundances of some taxa, such as *Simocephalus* spp. or *Pleuroxus* spp. The selective changes in the community are supported by previous findings on *Simocephalus* spp. and *Daphnia* spp. under laboratory conditions (LaBerge and Hann 1990). The long-term alteration of the community structure under warming periods presumably altered the competitive balance of species. Similar shifts in the competition strength were observed in the field, as shown for example for cyanobacteria (Watermann et al. 1999, Jöhnk et al. 2008) and copepods (Beaugrand et al. 2002).

The combination of warming periods and esfenvalerate did not alter short-term effects on the community structure and populations of *Daphnia* spp. In contrast, the time for recovery of *Daphnia* spp. from esfenvalerate (3 µg/L) was doubled under combined warming and toxicant exposure when compared to toxicant exposure under ambient conditions. In detail, after pesticide exposure, interspecific competition of *Daphnia* spp. with the less sensitive *Simocephalus* spp. was detected under both temperature regimes (Experiment 2 - Chapter 4). However, the susceptibility of *Daphnia* spp. to interspecific competition was prolonged under warming conditions. Findings from previous studies have shown that pesticide effects at the population level increase under high temperature (Jacobson et al. 2008, Heugens et al. 2006). In comparison, Broomhall (2002) also detected at the community level that tadpoles became more sensitive to predation after exposure to endosulfan under varying temperatures. Nevertheless, the microcosm study with warming periods is the only one that related a more pronounced interspecific competition – based on changes in environmental parameters – to a delayed recovery of sensitive species.

### **5.3 Representativity of the outcomes for pesticide effects in the field**

#### *Micro- and mesocosms as the “worst case scenarios” for a risk assessment of pesticides*

Micro- and mesocosm studies are often considered to be the worst case scenarios, a conservative approach for the risk assessment of pesticide effects in the field (Heger et al. 2001). This is assumed because toxicant exposure is present in the whole system and the model systems are often isolated from upstream recovery areas. The microcosms of the present study were designed to mimic small ponds. Such ponds also represent hydrologically isolated systems in nature. In addition, natural ponds or streams can generally be isolated to a higher degree from re-colonisation via air, since there is not always a nearby recovery resource from “control treatments” as in the experimental setups (Griffith et al. 1998, Petersen et al. 1999, Trekels et al. 2011).

In the investigated exposure scenario, the pesticide was only applied once in both experiments. The three concentrations of esfenvalerate (0.03, 0.3 and 3  $\mu\text{g/L}$ ) were chosen around the acute LC50 value of *D. magna* (LC50 [48h] = 0.37  $\mu\text{g/L}$ , Chapter 2) and appeared to be in the range of those detected frequently in the field (Kelley and Starner 2004, Cooper et al. 2003). In addition, esfenvalerate was measured to disappear within a few days from the open water (Experiment 1 - Chapter 2, Stampfli et al. 2011). The effects of pesticides increase with increasing exposure time, as shown for paraoxon-methyl (Duquesne et al. 2006) and fenvalerate/esfenvalerate (Reynaldi and Liess 2005, Cold and Forbes 2004) under laboratory conditions. Therefore, the outcomes from microcosm studies on communities exposed to pesticides with short half-lives may underestimate the effects of more persistent toxicants in the field. A very recent study on the risk assessment of pesticides in streams also detected much higher pesticide effects in the field than estimated in model stream systems (Schäfer et al. 2012). As possible reasons for such differences, the authors discuss for example the exposure to multiple stressors, toxicant mixtures in the field, as well as statistical approaches to define environmentally safe concentrations. As a conclusion, meso- and microcosm do not represent the worst case scenario. They represent a realistic scenario, which may rather underestimate the pesticide effects in lentic and lotic systems. In contrast, the effects on species with a high dispersal rate are likely to be overestimated in the field.

#### *Relevance of other biotic interactions and the community composition*

It has been shown in several previous studies that other biotic interactions, such as predation, also enhance pesticide effects at the population level (Beketov and Liess 2006, Relyea 2003), as well as at the community level under changing temperature conditions (Broomhall 2002). Furthermore, toxicant effects have been observed to indirectly alter predator–prey and herbivore–producer relationships (Fleeger et al. 2003, Relyea and Hoverman 2006). However, in the present work, competition was the major biotic factor to influence short-term effects and recovery in present experiments. Predatory taxa, such as

*Chaoborus* spp. and Odonata, were frequently detected in the investigated microcosms (Experiments 1 and 2). However, *Chaoborus* spp. was strongly affected by esfenvalerate (LC50 [96h] = 0.016 µg/L, unpublished data) and Odonata occurred in very low abundances, which made it difficult to interpret relationships between predators and sensitive prey taxa. Composition and shifts in the algae composition were not assessed. Therefore, it cannot be excluded that such changes can indirectly influence effect/recovery of sensitive taxa and the zooplankton community from the pesticide.

With regards to the investigated communities in general, communities in artificial aquatic systems can differ from those found in the field. In a mesocosm study by Liess and Beketov (2011) the proportion of sensitive long-living species in the communities was low compared to those in the field, despite a period of 2 years for establishing communities originating from uncontaminated sites. This further supports the conclusion from the previous section (*Micro- and mesocosms as the “worst case scenario” for risk assessment*). Relative effects and underlying processes can be extrapolated from mesocosms and microcosms to the field, but recovery of communities and species with low dispersal rates in the field is probably underestimated.

#### *Experimental set-up to identify effects of combined stressors*

During the first microcosm experiment in 2008, 24 replicates were used for the control treatment and each concentration level of esfenvalerate (Experiment 1 - Chapters 2 and 3). Compared to other community test systems, such an amount of replicates is high. Selected studies from the review by Fleeger et al. (2003) for example only employed an average of three replicates, and a minimum of only two replicates is suggested by the guidelines (Campbell et al. 1999, Heger et al. 2001). The high number of replicates enabled a meaningful detection and quantification of competition as underlying process in the community experiments. Hence, to analyse the interaction of toxicant effects with different biotic, but also abiotic, factors a sufficient number of replicates is needed.

#### **5.4 Implications for the ecological risk assessment and outlook**

Intra- and interspecific competition are ubiquitous factors in natural systems. Consequently, competition is important for the assessment of toxicant effects, as the present work shows that competition increases the effect and also delays population recovery from toxicants. A prolonged recovery, up to eight times longer than expected under optimal conditions, is of particular relevance for the protection of long-living species. In addition, the effect strength of competition – and hence the time for recovery – can be altered by changing environmental conditions. In this context, it has to be considered that the sustainable use of pesticides, including recovery of non-target organisms and the protection of biodiversity, is further required by EU regulation 1107/2009 and the directive 2009/128/EG on plant protection products. Such goals can only be reached if ecological processes like those described in the present work are strongly considered and integrated into the existing risk assessment.

##### *How to assess and predict realistic toxicant effects in the field?*

The assessment of realistic effects in the field is a highly complex challenge due to the multitude of biotic and abiotic interactions. In general, there are two existing approaches to assess and predict realistic effects:

- top-down (traditional ecology): bio-indicator systems (e.g. saprobic index, EPT)
- bottom-up (traditional toxicology): toxicological experiments (e.g. LC50, EC50)

To combine both approaches, underlying ecological processes must be understood. This is shown for example with the trait-based indicator system SPEAR (species at risk, Liess and von der Ohe 2005), where the toxicological sensitivity of species is combined with ecological parameters (e.g. generation time, recovery potential). The identification and quantification of competition as an additional stressor in the present work improves the understanding of

“general stress” on organisms under natural conditions and should be included in an extended combined approach. Such an extended combined approach would not only consider the toxicological sensitivity and ecological species-specific characteristics, but also the competitive stress (e.g. presence of competing and less sensitive species). By doing so, direct and indirect effects of toxicants on populations and communities could be predicted. Especially the indirect effects are important, as they induce a chain of effects in the community. The more we know about biotic interactions between species/trophic levels and the relative toxicological sensitivity of species, the better will be the prediction of toxicant effects.

This also applies to the identification of ecosystems which are especially vulnerable to toxicants due to their specific conditions of competition and biotic interactions in general. Based on the findings on competition described here, it can be hypothesised that communities experiencing unexpected changes (anthropogenic and natural) will be particularly sensitive to toxicants. Changing environmental conditions can directly affect the toxicant sensitivity of species, but also indirectly alter biotic interactions and thus again affect sensitivity, including effect and recovery. In contrast, systems with constant environmental conditions (e.g. springs, tropical ecosystems) are likely to be characterised by high interspecific competition and less intraspecific competition. Hence, competition in such systems might mainly affect recovery from toxicants. These hypotheses present an interesting and important aspect for future investigations.

## 5.5 References Chapter 5

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