Dirty water and disease pesticide mediated interactions between Daphnia and their parasites

Author(s):
Buser, Claudia Carolina

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Dirty water and disease: Pesticide mediated interactions between *Daphnia* and their parasites

A dissertation submitted to
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for the degree of
Doctor of Sciences

presented by
CLAUDIA CAROLINA BUSER
Master of Science in Biology, University of Zürich
born 05.05.1982
citizen of Basel (BS)

accepted on the recommendation of
PD Dr. Piet Spaak
Prof. Dr. Paul Schmid-Hempel
Dr. Christoph Haag

2011
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Communities in the wild are subject to a multitude of abiotic and biotic stressors. In turn, environmental stressors acting on the individual level can further influence population and community structure. In my doctoral research, I studied the effect of parasites and pesticides on host individuals as well as on hosts in experimental communities. I used several *Daphnia* (water flea) taxa and their endoparasites, as well as the pesticide diazinon. **Chapter 1** introduces these animals and the pesticide, and additionally includes a short review about host-parasite-environment interactions.

Pesticides are one of the most ubiquitous pollutants in water bodies. Although the effects of chemical contamination can permeate whole ecosystems, many toxicological studies are conducted using a single clone of one species. **Chapter 2** shows variation in mortality rates towards the exposure of the common pesticide diazinon within and between three different *Daphnia* species (*D. magna*, *D. pulicaria* and *D. galeata*). Not taking that variation into account by using only one clone for assessing the hazardous potential of a chemical might lead to an underestimation of risk towards other, more sensitive clones in the community.

In **Chapter 3**, I investigated the combined influence of disease and chemical pollution on host fitness. I exposed *D. magna* clones to the parasite *Pasteuria ramosa* and the pesticide diazinon. The clones were hatched from different sediment layers from a pond in Belgium where they co-occur with different densities of this parasite. Although I did not observe visible signs of infection, *D. magna* clones had reduced survival when simultaneously exposed to both parasites and chemical pollution. I did not find that host clones were adapted to pesticides or parasites over time.

Effects of parasites and/or pesticides on single individuals may shape the structure and dynamic of the whole community. However, extrapolation of experimental results from tests on single individuals to a community level may not always be accurate given that, for example, competition between individuals is not considered. In **Chapter 4**, I followed changes in experimental *Daphnia* communities after exposure to the fungal parasite *Metschnikowia* sp. and/or the pesticide diazinon. On a community level, including competition within and among taxa, dynamics were altered by parasite and pesticide exposure. One taxon went extinct under parasite and combined stress treatments, and I also show clonal/taxon sensitivity towards the pesticide. Furthermore, the density of all adult
females was significantly reduced in the parasite treatment, but not in the pesticide treatment. In general, the parasite exposure inserted stronger selection pressure than the pesticides.

Not only hosts are affected by pollutants. Parasites can be affected as well. Parasite virulence can be strongly dependent on host condition and can be mediated by environmental variation. In **Chapter 5** the environmental and evolutionary effects of diazinon on parasite fitness and virulence were tested. Parasites exposed for several generations to the pesticide evolved towards higher fitness and lower virulence, a finding that could have a significant impact on disease dynamics.

Using epidemiological models, I examined the impact of environment-dependent virulence on parasite virulence and disease spread in **Chapter 6**. The results suggest that the underlying relationship between virulence and host environment can drastically affect whether disease spreads as well as the intensity of the epidemic.

In summary, the studies I conducted for my doctoral thesis show that two forms of environmental stress, parasites and pesticides, can mediate the fitness of single individuals, affect the structure and evolution of communities, and determine whether disease can spread. Parasites act together with ecologically-relevant levels of pesticide pollution to impact host individuals and communities, but the strength of the combine effects highly depends on host genotype and the organismal level (i.e., individual, community) considered. Moreover, pesticides not only reduce host fitness and alter community structure, but also shape the evolution of parasites towards higher fitness and lower virulence. These outcomes may affect the theoretical spread of disease in environments of varying quality. I discuss the implications of these findings, as well as future research directions in **Chapter 7**.
Zusammenfassung


Zusammenfassung


Chapter 1

Introduction and outline of the thesis

Claudia C. Buser
Parasites in ecosystems

Parasites are ubiquitous and play an important role in host ecology and evolution. For example, parasites are a driving factor for the maintenance of sexual reproduction (Jaenike 1978; Hamilton 1980; Bell 1982; Hamilton et al. 1990), and they can alter the genetic structure of their host populations (Sasaki 2000) and structure of host communities (Anderson & May 1979, 1982; Hudson & Greenman 1998; Boots & Sasaki 2003). Host-parasite interactions may result in continuous coevolutionary changes (Hamilton 1980), in which parasites evolve new adaptations to infect and hosts evolve new adaptations to resist parasites, also known as the Red Queen hypothesis (van Valen 1977; Jaenike 1978; Bell 1982). This type of coevolution can result in host and parasite genotypes oscillating in frequency over time (Jaenike 1978; Hamilton 1980). Such oscillatory dynamics were observed in for example snails or Daphnia (Dybdahl & Lively 1998; Decaestecker et al. 2007; Jokela et al. 2009; Koskella & Lively 2009). Although host and parasites genotypes are major determinants of infection success (Lambrechts et al. 2006), these host-parasite interactions can be influenced by environmental conditions (Lazzaro & Little 2009; Wolinska & King 2009), having impact on populations and even whole ecosystems (Fig. 1).

Environment and host-parasite interactions

As most parasites cannot live outside their host for extended periods, parasite success is strongly linked with host ecology and evolution. Consequently, if environmental factors affect host fitness, they likely also highly impact on parasite fitness. Host \times parasite \times environment interactions can mediate parasite prevalence, disease dynamics and, ultimately affect populations and whole ecosystems (Fig. 1).

Environmental stress can make hosts more susceptible to parasites. Abiotic and biotic factors can affect immune defence. In general, the immune system is energetically costly to maintain and therefore, environmental stress, such as poor feeding conditions (Schmid-Hempel 2005) or pollution (McDowell et al. 1999), can lead to a weaker immune system. This enables parasites to infect more easily. Increased infectivity when hosts experience environmental stress has been observed in several host-parasite systems: pesticide exposures increases infectivity in oysters (Chu & Hale 1994) and amphibians (Rohr et al. 2008) predation increases infectivity in crustaceans (Yin et al. 2011).
Thus, parasite fitness can increase in a poor host environment due to reduced host immune function (Sheldon & Verhulst 1996; Moret & Schmid-Hempel 2000). Conversely, not only hosts, but also parasites can be negatively affected when the host is exposed to environmental stress, for example, due to reduced resources available for the parasite growth (Pulkkinen & Ebert 2004; Tschirren et al. 2007; Seppälä et al. 2008; Hall et al. 2009b). Furthermore, parasites themselves can be killed by the environmental stressor itself due to higher sensitivity to the stressor (Pietrock & Marcogliese 2003). Also, parasite virulence has been repeatedly shown to be influenced by environmental variation in a variety of animal-parasite systems (empirical examples in Lazzaro & Little 2009; Wolinska & King 2009). The relationship between virulence and environmental quality can take many forms (see examples in Thomas & Blanford 2003). Often, mortality of infected hosts is increased in bad environments, for example by starvation (Jokela et al. 1999; Ferguson & Read 2002; Brown et al. 2003; Tseng 2004; Jokela et al. 2005) or exposure to pesticides (Coors et al. 2008; Coors & De Meester 2011), microorganism-enriched water (Cornet & Sorci 2010) and high temperatures (Mitchell
& Read 2005). Less frequently reported, parasites can induce higher host mortality when environmental conditions are good (e.g., Vale et al. 2011).

Parasite transmission can be affected by environmental stressors too. Environmental stress can increase (e.g., Murray et al. 1998) or reduce (e.g., Coors & De Meester 2011) the production of parasite transmission stages, or transmission stages could be sensitive towards direct exposure (Pietrock & Marcogliese 2003). Furthermore, transmission rate is linked with host density (e.g., Ebert 1995; Bittner et al. 2002), and therefore environmental stressors reducing host density could affect parasite transmission.

Transmission rate, host and parasite fitness, host immunocompetence and parasite virulence all can influence disease occurrence, the timing of epidemics and epidemic intensity (Fig. 1). Environmental parameters can also spark epidemics (Johnson et al. 2006; Johnson et al. 2009). Temperature and predators, for example, can jointly drive the timing of epidemics (Hall et al. 2006).

The emergence of disease in fresh water can be imputed to anthropogenic changes (Daszak et al. 2001). Epidemiological models are increasingly integrating the environment to determine how local conditions affect spread of diseases (Lafferty & Holt 2003; Hall et al. 2007; Hall et al. 2009a). As infection disease models suggest that the likelihood and impact of an epidemic increases with host density (Anderson & May 1978), stressors depressing population density should reduce the chance of an epidemic. Lafferty and Holt (2003) suggest that stress reduces density and/or host quality, and therefore transmission rate is lowered. On the other hand, the spread of disease can be enhanced by improved environmental conditions (Hall et al. 2007).

Over the last few years, studies including the effect of environmental factor on host × parasite interactions have become more frequent (e.g., Wolinska & King 2009). Nevertheless, there is still a general need for more empirical studies testing predictions of how environmental stress influence the different steps in Fig. 1. The study system (host-parasite-environmental stress) used to answer the aims (see below) of this thesis is presented below.

**Host-parasite system and pesticides as environmental stressors**

**The host Daphnia**

The genus Daphnia comprises planktonic crustaceans belonging to the Cladocera (crustacean) which inhabit freshwater habitats (water bodies from small temporary pools to huge lakes) throughout the world. Over 100 species are included in the genus. Some species can form hybrid complexes which co-exist with the parental species in Daphnia communities (e.g., Schwenk & Spaak 1995; Keller et al. 2008; Petrusek et al. 2008; Yin et al. 2010).
Many *Daphnia* species have a cyclic parthenogenetic reproduction mode (Fig. 2). Under good conditions, *Daphnia* produce clones inside their brood pouch which are released as fully-developed juveniles. When conditions are deteriorating, most *Daphnia* clones are able to switch to sexual reproduction. The sexual eggs must be fertilized by asexually-produced males. These sexual eggs are encapsulated and protected by an ephippium. They enter into diapause until environmental clues (e.g., light, warmer temperature) induce hatching. Otherwise, dormant eggs can accumulate in the sediment. A new field of resurrection ecology is using dormant eggs as a tool to address ecological, evolutionary and toxicological based questions.

![Fig. 2: Life cycle of a cyclic parthenogenic *Daphnia*. Drawing by Dita B. Vizoso, Fribourg University (Ebert 2005)](image)

*Daphnia* is a model organism in ecology, evolutionary biology, and toxicological and biomedical research. The animal has a cyclic parthenogenetic reproduction mode, short generation time, central role in ecosystems, worldwide distribution, small size, and quickly responds to environmental changes.
**Daphnia and their parasites used in this thesis**

*Daphnia* species are host of different parasites, carrying a large number of epi- and endobionts (Green 1974). Most parasites of *Daphnia* are microparasites, belonging to bacteria, fungi and microsporidians (Ebert 2005). In my thesis, I worked with two different *Daphnia* taxa-parasite systems: a) *Daphnia magna* and *Pasteuria ramosa*, b) *Daphnia* of the *D. longispina* hybrid complex (specifically, *D. galeata* and its hybrids *D. galeata × longispina*) and *Metschnikowia* sp..

a) *D. magna* and *P. ramosa*

*D. magna* is found in temporary and permanent ponds. *P. ramosa* is a bacteria parasitizing *Daphnia* (Ebert 2005) and co-occurring with *D. magna* in the field (e.g., Little & Ebert 2000; Jansen et al. 2010). This Gram-positive bacterium, belonging to the family of the Alicyclobacillaceae (e.g., Ebert et al. 1996), is an extracellular parasite infecting the hemolymph (Metchnikoff 1888). Infected hosts grow large, the body becomes darkish and non-transparent (Fig. 3), and infected hosts become infertile. *P. ramosa* is transmitted horizontally through spores released from dead infected hosts. *P. ramosa* spores are found in pond sediments and remain infective for decades (Decaestecker et al. 2004).

![Fig. 3: *D. magna* uninfected (left) and infected (right) with *P. ramosa* (photo and copyright by Ebert (2005)).](image)

b) *D. galeata*, the hybrid *D. galeata × longispina* and *Metschnikowia* sp.

*D. galeata* is one of the three parental species (*D. cucullata* G. O. Sars, 1862, *D. galeata* G. O. Sars, 1863 and *D. longispina* O. F. Müller, 1776) belonging to the *D. longispina* hybrid complex (Petrusek et al. 2008). *D. galeata × longispina* hybrids can co-occur with parental
species and dominate *Daphnia* communities of European lakes (e.g., Keller et al. 2008; Petrussek et al. 2008; Yin et al. 2010). The yeast parasite *Metschnikowia* sp. (family Hemiascomycetes, Wolinska et al. 2009) commonly infects *Daphnia* populations in Europe and the United States (Hall 2005; Caceres et al. 2006; Yin et al. 2010; Wolinska et al. 2011). Needle-like ascospores accumulate in the body cavity and are released and horizontally transmitted after host death (Green 1974)(Fig. 4). Infected *Daphnia* have fewer offspring and a reduced lifespan (Duffy & Hall 2008; Lohr et al. 2010).

**Fig. 4:** *D. galeata* uninfected (left) and infected (right) with *Metschnikowia* sp. (photo and copyright by Yin et al. (2011)).

**The pesticide diazinon and the interaction with *Daphnia***

Many chemicals find their way into the water (e.g., through surface run-offs from agricultural fields and urban areas after rain events). Among anthropogenic chemicals, pesticides are harmful because they were originally designed to kill a variety of target species. Pesticides are known to affect fitness of individual organisms, as well as to change community and ecosystem structure from non-target individuals (reviewed in Hanazato 2001; Rohr & Crumrine 2005). Organophosphosphate pesticides can be regularly detected in the aquatic environment (Giddings et al. 2000; Strom et al. 2003; Pedersen et al. 2006; Gaworecki et al. 2009; Wittmer et al. 2010). They were introduced as replacements for the persistent organochlorine pesticides (e.g., DDT), which caused adverse health effects in non-target species (Woodwell et al. 1967). Organophosphosphate pesticides can have toxic effects on the immune systems and immune functions of invertebrates, fish and higher vertebrate wildlife (reviewed in Galloway & Handy 2003). Because of its wide use in agriculture and
households, the organophosphate diazinon (O,O-diethyl O-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphothionate; Fig. 5; initially produced in 1952 by Ciba-Geigy, later Novarits and then Syngenta) is among the most used pesticides and is still frequently measured in aquatic environments. For example, diazinon is found in Switzerland around Lake Greifensee (e.g., Singer et al. 2010; http://www.umweltschutz.zh.ch; Wittmer et al. 2010) or Greece (Konstantinou et al. 2006). It can be detected in surface waters mainly in the ng/L range, but also acute concentrations (μg/L range) in short pulses have been recorded (Wittmer et al. 2010). Exposure to a sub-lethal diazinon concentration causes a delay in reproduction, a decrease in fecundity and offspring size and higher adult mortality in *Daphnia magna* (Sanchez et al. 1998).

![Chemical structure of Diazinon](structure.png)

Fig. 5: Chemical structure of Diazinon (Structure retrieved from toxipedia.org/display/toxipedia/Diazinon)

**Daphnia, their parasites and pesticide interactions**

Pesticides and biotic stressors, like parasites, can interact in the wild. Coors found increased parasite virulence and/or increased host susceptibility to infections, when exposing *D. magna* to the pesticide carbaryl (Coors & De Meester 2008; Coors et al. 2008; Coors et al. 2009; Coors & De Meester 2011). Moreover, Jansen found that natural *Daphnia* populations are able to rapidly evolve resistance towards the pesticide carbaryl, but at the cost of a greater susceptibility towards the parasite (Jansen et al. 2011a; Jansen et al. 2011b).

**Aim of the thesis**

This thesis aimed to examine the influence of pesticide pollution on host and parasite fitness, as well as parasite virulence. It additionally focuses on the effect of multiple stressors on host-parasite interactions and the consequences on population dynamics and disease spread.
Specifically, aims of the chapters are:

- **Chapter 2**: to highlight the importance of testing a wide variety of genotypes in toxicity tests, and to show that there is *Daphnia* genetic and species variation in sensitivity to the pesticide diazinon.
- **Chapter 3**: to test whether the combined exposure to parasites and pesticides affect the fitness of *Daphnia magna* and whether these fitness consequences change through time as a population evolves.
- **Chapter 4**: to follow the taxa and clonal dynamics in experimental *Daphnia* communities after parasite and pesticide exposure.
- **Chapter 5**: to investigate the environmental and evolutionary effects of pesticide exposure on parasite fitness and virulence.
- **Chapter 6**: to explore how eight relationships between parasite virulence and environmental quality alter the ability of parasites to spread across an environmental gradient using epidemiological models.
- **Chapter 7**: to summarize the above findings and discuss the importance of multiple stressors, as well as G×E×E interactions, in an ecological and evolutionary context, and to highlight future research questions.

**Outline of the thesis**

There are many studies showing the harmful effects of the pesticide diazinon on *Daphnia*. Although it is common to conduct toxicology studies experiments on single clones, **Chapter 2** highlights the importance of considering the genetic diversity of test organisms. I further show that acute toxicity of diazinon affects *Daphnia* species and conspecific clones differently. In ecosystems, organisms are exposed to natural stressors, in addition to anthropogenic inputs. Therefore, in **Chapter 3** I tested the combined influence of disease, a natural stressor, and chemical pollution on host fitness by exposing *Daphnia magna* clones hatched from different sediment layers to the parasite *Pasteuria ramosa* and the pesticide diazinon. Effects observed in life-history experiments on single individuals are useful to test the effect on changes in susceptibility and life history traits for the specific combination of abiotic and biotic stressors. However, extrapolating these results to predict the effect on communities is difficult given that interactions between and within taxon are not taken into account. In **Chapter 4** I followed changes in experimental *Daphnia* communities (consisting of two taxa and three clones per taxon), after exposure to the fungal parasite *Metschnikowia* sp. and/or the pesticide diazinon. I tracked changes in density, prevalence, genetic composition, fecundity and size of *Daphnia* over more than a 3 month period. In addition to the hosts, parasites exposed to pesticide could also be affected, yet the evolutionary consequences of long-lasting exposure to pesticides on parasites have not been studied in great detail. In **Chapter 5**, I tested whether there were environmental and
evolutionary effects of diazinon on parasite fitness and virulence. If an environmental stress factor has high impact on parasite virulence, this might significantly impact disease dynamics in aquatic systems. Although theoretical studies have found that the environment can affect host-parasite population dynamics in nature, little is known of the influence of the interaction between environment and virulence on the spread of disease through a host population. Assuming different virulence relationships with the environment, I used an epidemiological model in Chapter 6 to explore how condition-dependent virulence and environmental quality alter disease dynamics.

Final discussion, overall conclusions and future perspectives are presented in Chapter 7.

References


Chapter 2

Variation within and between species cannot be ignored in toxicity tests

Claudia C. Buser, Jennifer Fox, Andreas Kretschmann & Piet Spaak

(In preparation, target journal Aquatic Toxicology)

Abstract

Although chemical contamination can affect whole ecosystems, many toxicological studies of aquatic pollutants are still conducted on only a single clone of a single Daphnia species. Variation in sensitivity towards a toxicant within and between species is not taken into account, which could lead to inaccurate estimates of the harm of the toxicant. In this study we show that there is substantial variation within and between three different Daphnia species in their responses to the common pesticide. To estimate the effect on a population or even on ecosystems level we therefore suggest conducting toxicological experiments with more than one clone.

Keywords

Acute toxicity test, Daphnia clonal variation, diazinon, ecology, fitness
**Introduction**

Chemical contaminants are very widespread in the environment. Aquatic ecosystems in particular encounter many chemical stressors because runoff into rivers and lakes concentrates chemicals from across a watershed. Watersheds are important and for humans widely used as drinking water and food sources, though we are also affected (directly or indirectly) by pollutants in ponds and lakes.

Although scientists from different fields try to assess the toxic effect of chemical contaminants on individual organisms in order to predict potential impacts of chemicals in the environment, the fields of toxicology and ecology are still quite separate (Chapman 2002). While in ecology genetic variation is taken into account when testing for effects of stressors, in toxicology it is still common to test the toxic potential of a potential toxicant on only one clone of a single species. For example, many toxicology studies on aquatic organisms focus on a single *Daphnia magna* reference clone (Adema 1978). This provides toxicologists with a good estimate of the toxic potency of a chemical and leads, for example, to fast decisions about whether and in which concentrations a pesticide can be used in agriculture. But natural populations normally harbour a genetically diverse set of individuals, all potentially adapted to quite different environments, and thus the harm of a chemical compound can vary between and also within different taxa. This variation in response can be due to variation in traits, such as size, mode of respiration, feeding type and habit and life-cycle duration (Baird & Van den Brink 2007; Rubach et al. 2010), variation in biotransformation processes and in sensitivity of the target site (e.g., a certain enzyme) inside an organism (Keizer et al. 1995; Barata et al. 2001; Damasio et al. 2007), or because of adaptation of a species towards the chemical compound (Klerks & Weis 1987).

Acute toxicity tests are one common method for assessing the hazardous potential of chemicals (OECD/OCDE 2004). They focus on acute toxic effects like immobilization and mortality of organisms after short-term exposure. Therefore they are appropriate for the estimation of effects of chemicals present in the environment in rather high concentrations and short pulses e.g., due to an accidental spill or a fast degradation. These acute effects observed on an individual level (e.g., mortality after exposure to short pulses of acutely toxic concentrations) might have a long-term effect at the population level.

Organophosphorous insecticides are regularly detected in aquatic environments, e.g., in surface waters due to surface run-off from agricultural fields after rain events (Strom et al. 2003; Pedersen et al. 2006; Wittmer et al. 2010). The organophosphate diazinon (O,O-diethyl O-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphothionate) is an insecticide widely applied in
agricultural systems (e.g., Konstantinou et al. 2006; Wittmer et al. 2010) and has been shown to affect and to be highly toxic to aquatic animals (Ankley et al. 1991; Ceron et al. 1996; van der Geest et al. 2000; Marcial et al. 2005; Gaworecki et al. 2009). Acute toxic effects (immobilization, mortality) of diazinon are related to inhibition of acetylcholinesterase (AChE), an enzyme essential for proper functioning of the nervous system (Chambers 1992; Maxwell et al. 2006). Diazinon is often found in aquatic environments (Miles & Harris 1978; Konstantinou et al. 2006; Singer et al. 2010). It can be detected in surface waters mainly in the ng/L range, but also acute concentrations (μg/L range) in short pulses have been recorded (Wittmer et al. 2010).

Plankton crustaceans are very abundant and sensitive to environmental conditions, for example to organophosphorous insecticides (Vaal et al. 1997). They are near the bottom of aquatic food webs and are ecologically important as both grazers of algae and food sources for fish and other invertebrates. Daphnia, belonging to the Cladocera, are one genus of planktonic crustaceans often used as model organism in fields like ecology and toxicology. There are a dozens of Daphnia species differing in size and life history traits (Koivisto 1995), distributed all over the world. For example Daphnia pulicaria is found in permanent, usually clear-water, ponds and lakes. Daphnia galeata Sars., 1863 is found in small to very large permanent lakes, and Daphnia magna is typically found in temporary and permanent ponds (Hebert 1978). Since D. magna is easy to culture in the lab (Adema 1978; Baudo 1988), this species is commonly used for toxicity tests (Baudo 1988; ISO_10706 2000).

Many studies have shown the negative effects of diazinon on Daphnia (Fernandez-Casalderrey et al. 1994; Sanchez et al. 1998; Jemec et al. 2007). Most of these studies test acute toxicity using only one clone and do not consider the variation within and between taxa. In nature we rarely find Daphnia populations consisting of only a single clone, so it makes sense to investigate how results obtained from “one clone studies” relate to the variation that can be obtained by doing experiments with several clones. Ideally the “one clone” would be somewhere in the middle of the distribution of the many clones, and would not be an extreme genotype that shows a behaviour quite different from clones chosen from natural environments.

In this study, we highlight the importance of taking the genetic diversity of organisms into account when performing toxicity tests. We show that the toxic response to acutely toxic diazinon concentrations affect Daphnia species and even clones within a species differently.
Material and Methods

Acute toxicity test
Acute toxicity tests were performed according to the OECD Guideline 202 (OECD/OCDE 2004). Diazinon (CAS 333-41-5, 99.5 % purity) was obtained from Ultra Scientific Analytical Solutions (N. Kingstown RI, USA). For exposure experiments diazinon was dissolved in acetone and spiked into filtered lake water. We tested 6 different concentrations of diazinon (0.00, 0.13, 0.25, 0.50, 1.00, and 2.00 µg/L), with lake water containing the same amount of acetone as in the treatments as a control. Nominal exposure concentrations of diazinon were verified with LC-MS/MS as described in (Kretschmann et al. 2011b). As measured diazinon concentrations were within ± 20 % of the nominal concentrations, for data analysis nominal concentrations were used.

Six clones of Daphnia magna, four clones of Daphnia pulicaria, and four clones of Daphnia galeata were used. The clones were selected from different lakes from several countries as listed in Table 1, to increase genetic variation. All clones had been cultured in the laboratory for at least 1 year. Prior to the experiment, clones were raised in multiple source jars for two generations under standard laboratory conditions. From the second or later clutch of the third generation we put 5 juveniles (<24 h old) in 10 mL of medium in 50-mL jars, with 3 replicates for each clone in each of the 7 treatments (6 diazinon concentrations and acetone control). Animals were assessed for survival after 48 h. Daphnia were not fed during the experiment. Experiments were performed at a temperature of 20 ± 1 °C and a photoperiod of 16 h light and 8 h dark.

Data analysis
Acute toxicity data were evaluated with the program SigmaPlot version 10.0. LC₅₀ values (concentration at which 50 % of the tested organisms died) and the hill slope (steepness of the dose-response curve) were obtained by fitting a sigmoidal dose-response curve (four-parameter logistic equation) to the plot of the fraction of surviving animals (%) vs. log-transformed diazinon concentrations.

To test if mortality differed within and among taxa according to diazinon concentrations we used a generalized linear model in SPSS 15.0 with proportion of animals surviving after 48 h as the dependent variable, the concentrations and taxa as fixed factors, and the clones nested within taxa as a random factor.
Table 1: Overview and distribution of the clones used in the acute toxicity test

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Clone</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. galeata</em></td>
<td>ENDI</td>
<td>Lago di Endine, Italy</td>
</tr>
<tr>
<td></td>
<td>SEGR</td>
<td>Lago del Segrino, Italy</td>
</tr>
<tr>
<td></td>
<td>ZUG</td>
<td>Lake Zug, Switzerland</td>
</tr>
<tr>
<td></td>
<td>ZURI</td>
<td>Lake Zurich, Switzerland</td>
</tr>
<tr>
<td><em>D. magna</em></td>
<td>CN2-4</td>
<td>Czech Republic</td>
</tr>
<tr>
<td></td>
<td>EK1-1</td>
<td>United Kingdom</td>
</tr>
<tr>
<td></td>
<td>HO-1</td>
<td>Hungary</td>
</tr>
<tr>
<td></td>
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<td>Germany</td>
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<tr>
<td></td>
<td>M10</td>
<td>Belgium</td>
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<tr>
<td></td>
<td>M12</td>
<td>Germany</td>
</tr>
<tr>
<td><em>D. pulicaria</em></td>
<td>A16</td>
<td>Czech Republic</td>
</tr>
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<tr>
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<td>REZ</td>
<td>Czech Republic</td>
</tr>
</tbody>
</table>

Results

Acute toxicity test on three *Daphnia* species

A comparison of the LC$_{50}$ values reveals that *D. pulicaria* and *D. galeata* had a lower LC$_{50}$ than *D. magna* (Table 2, Fig. 1). The LC$_{50}$ of *D. pulicaria* was almost 50% lower than that of *D. magna* (0.878 µg/L versus 1.512 µg/L).

Table 2: Summary of LC$_{50}$ values and hill slope for *D. galeata*, *D. magna* and *D. pulicaria*.

<table>
<thead>
<tr>
<th>Species</th>
<th>R</th>
<th>Best fit hill slope value ± SE</th>
<th>Best fit LC$_{50}$ (µg/L) value ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. galeata</em></td>
<td>0.9960</td>
<td>-0.099 ± 0.025</td>
<td>1.112 ± 0.022</td>
</tr>
<tr>
<td><em>D. magna</em></td>
<td>0.9997</td>
<td>-0.122 ± 0.005</td>
<td>1.512 ± 0.006</td>
</tr>
<tr>
<td><em>D. pulicaria</em></td>
<td>0.9995</td>
<td>-0.070 ± 0.014</td>
<td>0.878 ± 0.013</td>
</tr>
</tbody>
</table>

The generalized linear model revealed highly significant main effects of diazinon concentration ($\chi^2 = 1955.187$, df$ = 6$, $p < 0.001$), species ($\chi^2 = 81.264$, df$ = 2$, $p < 0.001$, Fig. 1), and clone ($\chi^2 = 146.691$, df$ = 12$, $p < 0.001$, Fig. 2) on survival. The higher the diazinon concentration, the lower the proportion of animals that survived (Fig. 1 & Fig. 2). The three species differed in their response, with *D. magna* showing higher survival than the other two species.
Fig. 1: Fitted dose-response curves show the variation in survival (mean ± SE) after 48 h of diazinon exposure among the three *Daphnia* species. The LC50 values for each species are back transformed.

There was also a significant interaction for species × diazinon concentration ($\chi^2 = 121.448$, $df = 12$, $p < 0.001$). For example, in the absence of diazinon or at low diazinon concentrations fewer animals of *D. pulicaria* died compared to *D. galeata*, but at higher diazinon concentrations *D. pulicaria* was the most sensitive species (Fig. 1). We also observed similar interactions within the species on a clonal level (clone × diazinon concentration: $\chi^2 = 428.965$, $df = 66$, $p < 0.001$, Fig. 2). Within different clones of the species *D. magna*, the clones CN2-4 and M10 were found to be the most resistant ones (see Fig. 2B). Survival of the CN2-4 clone was not affected by any diazinon concentration (in all concentrations 100% survival). Survival of the M10 clone was affected only at the highest diazinon concentration applied (proportion of M10 clones surviving at 2 µg/L: 0.65 ± 0.236 (mean ± SE); proportion of CN2-4 clones surviving at 2 µg/L: 1.0 ± 0).
Fig. 2: Proportion (mean ± SE) of clones surviving after 48 hours at different concentrations of diazinon within the (A) *D. galeata*, (B) *D. magna*, (C) *D. pulicaria*.

**Discussion**

The species and even clones within a single species reacted significantly differently from each other when exposed to diazinon for 48 h. In our acute test *D. magna* was the species least sensitive to diazinon, whereas *D. galeata* and *D. pulicaria* have a higher mortality rate when exposed to the pesticide. The difference in sensitivity could be due to several (not mutually exclusive) reasons. First, larger animals often have a lower sensitivity towards toxicants. For example, a positive relationship between EC$_{50}$ (concentration at which 50 % of the tested organism show an effect) values and body size was found by Vesela and Vijverberg (2007) when four different *Daphnia* species were exposed to zinc. In other interspecies comparisons large-sized *Daphnia* tended to be more tolerant to toxic substances than smaller ones (Winner
& Farrell 1976; Koivisto et al. 1992). A correlation between sensitivity and size would explain why the larger *D. magna* is less sensitive than the two smaller species in our experiments. This hypothesis does not hold true for the finding that *D. pulicaria* is more sensitive than *D. galeata* in our study; however the size difference between these two species is not as great as with *D. magna*. Also this size-hypothesis does not explain the high variation within taxa, as the size differences within a taxa are small. Second, the difference in sensitivity could be due to differences in the habitats the species live in. *D. magna* lives more in shallow ponds, which are unpredictable habitats with large temporal and spatial variability in abiotic factors; whereas the other two species live in a larger water habitats that are more stable. Adaptation to natural abiotic stress may increase pollution tolerance (Fisher 1977; Leblanc 1985). For example, Koivisto et al. (1992) show that *Daphnia* species living in ponds were less sensitive to copper than lake-living cladocerans. Finally, the observed difference in sensitivity towards diazinon might also be due to different intrinsic properties, like different reactivities/sensitivities of biotransforming enzymes as well as of the target site AChE. Keizer et al. (1995) explained differences in the toxicity of diazinon towards different fish species by an observed variation in AChE sensitivity toward inhibition by diazoxon, the active metabolite of diazinon, in combination with the ability to biotransform diazinon. A correlation between the sensitivity towards diazinon and the sensitivity of AChE has also been shown for *D. magna* (Kretschmann et al. 2011a).

In our study we not only showed variation between species; we also demonstrated that clones within a species show very different levels of sensitivity to the pesticide. An ideal model clone should be in the middle of the distribution to provide a good mean estimate for the harm within the species. In our example, if only the CN2-4 or M10 clone of the species *D. magna* was used for assessing the hazardous potential of a chemical this might lead to an underestimation of risk towards other, more sensitive clones.

When assessing the effects of chemicals on aquatic organisms, it makes sense to choose an ecologically significant species, such as *Daphnia*, that may be near the base of the food web. Concentrations affecting this species could have an impact on species higher up in the food chain. Therefore we recommend the use of more than one clone in toxicology studies in order to take into account the variation within and between species and to minimize the risk of underestimating the effects of a chemical (Cairns 1984; Chapman 2000). If (for whatever reason) it is possible to perform an experiment with only a single clone, we recommend using a clone known to be in the middle of the sensitivity distribution.
Acknowledgment

We thank Nora Brede for her help setting up the experiment and Christoph Haag for comments on the manuscript. The study was financially supported by the Swiss National Science Foundation (SNF) and the ETH Board (CCES-GEDIHAP).

References


Chapter 3

Combined exposure to parasites and pesticides causes increased mortality in the water flea *Daphnia*

Claudia C. Buser, Mieke Jansen, Kevin Pauwels, Luc De Meester & Piet Spaak

(In preparation, target journal Freshwater Biology)

Abstract

Organisms are exposed to multiple biotic and abiotic environmental stressors, which can influence dynamics of individual populations and communities. Species may also genetically adapt to both natural (e.g., disease) and anthropogenic (e.g., chemical pollution) stress. We set out to study fitness consequences of exposure to both parasites and pesticides in the water flea *Daphnia* and to quantify whether these fitness consequences change through time as a population evolves. We exposed *Daphnia magna* clones hatched from dormant eggs isolated from different time layers of a natural dormant egg bank to the parasite *Pasteuria ramosa* and the insecticide diazinon. While our experimental treatments for unknown reasons failed to induce disease in the *Daphnia* we did observe a reduced survival of *D. magna* when exposed to both parasites and pesticides. No increased mortality upon exposure to individual stressors was observed. As also no induction of phenoloxidase activity was found, we cannot confirm if the difference in survival was related to immune suppression. We did not observe an evolutionary change in fitness response of the *Daphnia* clones hatched from different time horizons upon exposure to stressors.

Keywords

*Daphnia magna*, diazinon, fitness, multiple stressors, *Pasteuria ramosa*, parasites, resurrection ecology, dormant eggs
Introduction

In recent years, the importance of multiple stressors on the health of organisms has received increased attention (e.g., Marcogliese & Pietrock 2011). It is well known that responding to additional disturbances leads to higher costs, which can result in a reduction of fitness of exposed individuals (reviewed in Relyea 2003; Sih et al. 2004). Anthropogenic stress is expected to increase diseases in natural populations (e.g., Daszak 2000). Pesticides, for example, are used worldwide in agriculture, and have been shown to increase susceptibility to parasite infections (e.g., Chu & Hale 1994; Gendron et al. 2003; Coors & De Meester 2008; Rohr et al. 2008; King et al. 2010; Kreutz et al. 2010).

Studies considering abiotic and biotic stressors are often conducted with genotypes sampled over a spatial gradient (King et al. 2010; Schoebel et al. 2010; Bryner & Rigling 2011; Jansen et al. 2011). Although this approach does not directly track changes over time (De Meester et al. 2007), it provides valuable information patterns of genetic adaption to local environmental conditions (Kawecki & Ebert 2004). In organisms that produce dormant eggs, however, “resurrection ecology” can be a powerful tool for studying microevolutionary responses to environmental change (Hairston et al. 1999; Jeppesen et al. 2001; Kerfoot & Weider 2004). In lake and pond sediments, dormant stages of different organisms can remain viable for decades or longer (Hairston 1996). Thus it is possible to hatch dormant eggs from a dated sediment core in the laboratory and compare these “old” genotypes in experiments with current genotypes from the same pond or lake (Kerfoot et al. 1999). Dormant eggs from different time periods can therefore be used to examine evolutionary responses to environmental changes. Several resurrection ecology studies have provided evidence for microevolutionary responses over only a few generations (e.g., Hairston et al. 1999; Cousyn et al. 2001; Hairston et al. 2001). For example, Hairston et al. (1999) showed that *Daphnia* hatched from a period when toxic cyanobacteria were present in Lake Constance were more resistant to a diet with cyanobacteria. Cousyn et al. (2001) documented microevolutionary changes in phototactic behaviour in a natural *Daphnia* population in response to changes in fish predation pressure and Pauwels et al. (2010) showed that the same population also evolved with respect to phenoloxidase activity, a component of the invertebrate innate immunity. Decaestecker et al. (2007) using both *Daphnia* and its microparasites hatched from different sediment layers, showed very strong parasite-host co-adaptive responses.

To our knowledge, there are no studies observing the effect of multiple stressors over a timescale of several decades. In the present study we set out to test whether the combined exposure to a parasite and a pesticide affected the fitness of *Daphnia magna* (Straus, 1820) hatched from different sediment layers.
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Material and Methods

Origin and culture conditions of the host-parasite system and the pesticide

*Daphnia magna* host clones were used from a previous study (Decaestecker et al. 2007). The hatched *Daphnia* came from seven different sediment layers of the Belgian pond OM 2 situated in Heverlee (50°50’22’’N, 4°39’18’’E), covering a time period from about 17 - 28 years (Decaestecker et al. 2007). This pond is characterized by epidemics of *Pasteuria ramosa* (Metchnikoff, 1888), a parasite of *Daphnia magna* (Decaestecker et al. 2007), as well as intense agricultural activity in the catchment (Coors et al. 2009). In total, we used 13 genetically different clones, two from each sediment layer, except from the oldest, where only one clone could be used.

*P. ramosa* spores used for infection were obtained by exposing different individuals of a single *D. magna* clone (M10, see Cousyn et al. 2001) originating from the pond Oud Heverlee to the first few centimetres of the sediment core from the pond Knokke Nat (Belgium 51°21’25’’N, 3°19’50’’E), as described in Jansen et al. (2010). The parasites were collected from a different pond than the host clones used in the experiment to avoid possible effects of co-adaption of hosts and parasites (Decaestecker et al. 2007). *P. ramosa* from Knokke Nat have been shown to heavily infect *D. magna* clones from OM 2 (Jansen et al. 2010). To increase the amount of *P. ramosa* spores, infected M10 clones were grown for 21 days and used to infect Daphnia juveniles of the same clone for another generation, before using them in the experiment as described in Coors et al. (2008). Infected Daphnia become sterilized, are darkish-red and become larger in body size (Ebert 2005) and are therefore easily recognizable by eyes. Grounded up, non-infected *Daphnia* from the M10 clones were used as placebo in the treatments without parasites.

The organophosphate diazinon (O,O-diethyl O-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphothoniate, CAS 333-41-5, 99.5 % purity, Ultra Scientific Analytical Solutions, N. Kingstwon RI, USA) is a pesticide developed in 1952 that was heavily used between 1970 and the early 1980s in many parts of the world. Nowadays, it is still frequently used in agriculture and households, and can thus be detected in aquatic environments (Konstantinou et al. 2006; Singer et al. 2010). Acute toxic effects of diazinon (e.g., immobilization and death) are related to the inhibition of acetylcholinesterase, an enzyme essential for proper function of the nervous system (Chambers 1992; Maxwell et al. 2006). Exposure of *D. magna* to sub-lethal concentrations of diazinon leads to a delay in reproduction, a decrease in the number and size of offspring, and higher adult mortality (Sanchez et al. 1998).
Experimental set-up and procedures
Thirteen clones (from seven sediment layers) were exposed to *P. ramosa* and diazinon in a full factorial design, and replicated six times, resulting in 312 experimental units. To minimize maternal effects at the start of the experiment, the clones were kept under standard experimental laboratory conditions [1:5 diluted Aachener Daphnien Medium (ADaM, Klüttgen et al. 1994), 20 ± 2 °C, 16 h:8 h light/dark photoperiod, fed daily with $2 \times 10^5$ *Scenedesmus obliquus* cells/mL] for at least two generations. To start the experiment ten third-clutch juveniles (less than 24 h old) were put together in 250 mL glasses containing 20 mL medium. The pesticide treatment consisted of a concentration of diazinon (0.25 μg/L), which is sub-lethal for *Daphnia*, whereas the pesticide-free control treatment included the addition of the same amount of the solvent (acetone 0.008 %). Concentrations of diazinon were verified with spot test with LC-MS/MS as described in Kretschmann et al. (2011b) and were all within ± 20 % of the nominal concentrations. Individuals belonging to the parasite treatment were exposed on day 0 and day 2 to $375 \times 10^2$ mature spores of *P. ramosa* per mL (Jansen et al. 2011). All glasses belonging to the control treatment were treated with ground up, non-infected, *Daphnia* of the M10 clone. On day five all *Daphnia* were transferred into new glasses (500 mL) containing 400 mL diluted ADaM medium, which was renewed every third day thereafter. Animals were checked for infections, the number of offspring was counted and the survival rate was checked daily (and the dead individuals removed) till the end of the experiment. On day 21, 1 μL haemolymph was taken from two animals per glass and frozen at -21 °C in 100 μL phosphate buffered saline ( PBS) buffer. Phenoloxidase (one component of the invertebrate innate immune response, Söderhäll & Cerenius 1998) was activated with chymotrypsin (Sigma aldrichchemie GmbH, Buchs, Switzerland) and quantified on a spectrophotometer (absorbance 475 nm) following Mucklow et al. (2004). All traits were quantified as a mean or proportion per set of 10.

Statistical analyses
Statistical analyses were performed using SPSS 19.0. In total, seven experimental units were lost due to handling errors and excluded from the analysis. Differences in survival rate, reproductive output as well as phenoloxidase activity were analysed using linear mixed effects models, with exposure to parasites (yes/no), exposure to diazinon (yes/no) and sediment layer as fixed factors and clone nested in sediment layer as random factor. We did not include the interactions between the fixed factors and the random factor clone, as our aims were not related to clonal variation. The individual reproductive output was calculated (total amount of offspring born on day x divided by the total amount of living females on day x, for x going from day 4 to 21, Van Doorslaer et al. 2009). All residuals were distributed normally.
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Results

In this experiment no visible signs of infections could be observed at day 21. Nevertheless, significant differences in survival rate were observed when *Daphnia* were exposed to both stressors: The proportion of surviving individuals was significantly reduced when *Daphnia* were exposed to parasite spores and pesticides simultaneously, but not when they were exposed to a single stressors (Table 1; Fig. 1). There was neither a significant effect of these factors or their interactions on the individual reproductive output nor on phenoloxidase activity (see Table 1). Furthermore, there were no interaction effects with sediment layer, nor a main effect of sediment layer (all \( p > 0.1 \), see Table 1). All variables were highly variable among clones (Table S1).

Table 1: Results of linear mixed effects models describing the influence of exposure to parasite spores, exposure to diazinon, sediment layer and interactions among these factors on survival, reproduction and phenoloxidase activity (PO).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Source</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival rate</td>
<td>parasite (P)</td>
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<td>271.034</td>
<td>3.008</td>
<td>0.084</td>
</tr>
<tr>
<td></td>
<td>diazinon (DZ)</td>
<td>1</td>
<td>271.034</td>
<td>2.876</td>
<td>0.091</td>
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<tr>
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<td>6</td>
<td>6.036</td>
<td>1.936</td>
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<tr>
<td></td>
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<td>271.087</td>
<td>5.099</td>
<td><strong>0.025</strong></td>
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<td></td>
<td>P × S</td>
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<td>271.037</td>
<td>0.689</td>
<td>0.659</td>
</tr>
<tr>
<td></td>
<td>DZ × S</td>
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<td>271.037</td>
<td>1.601</td>
<td>0.147</td>
</tr>
<tr>
<td></td>
<td>P × DZ × S</td>
<td>6</td>
<td>271.093</td>
<td>0.982</td>
<td>0.438</td>
</tr>
<tr>
<td>Reproductive output</td>
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<td>267.060</td>
<td>0.873</td>
<td>0.351</td>
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<td>0.280</td>
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</tr>
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<td>0.629</td>
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<tr>
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<td>267.067</td>
<td>1.042</td>
<td>0.398</td>
</tr>
<tr>
<td></td>
<td>DZ × S</td>
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<td>267.154</td>
<td>1.642</td>
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</tr>
<tr>
<td></td>
<td>P × DZ × S</td>
<td>6</td>
<td>267.162</td>
<td>1.321</td>
<td>0.248</td>
</tr>
<tr>
<td>PO</td>
<td>parasite (P)</td>
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<td>227.054</td>
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<td>0.561</td>
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<td>0.526</td>
<td>0.469</td>
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<td>5.898</td>
<td>1.521</td>
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<tr>
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<td>P × DZ</td>
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<td>0.341</td>
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</tr>
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<td>P × S</td>
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<td>227.071</td>
<td>1.225</td>
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<td>227.597</td>
<td>1.603</td>
<td>0.147</td>
</tr>
<tr>
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<td>P × DZ × S</td>
<td>6</td>
<td>227.608</td>
<td>0.803</td>
<td>0.569</td>
</tr>
</tbody>
</table>
Fig. 1: Reaction norms of mean proportion of surviving individuals per glass (± SE) as a function of exposure to the pesticide diazinon (no/yes) and parasite spores (no= open circles/yes= closed circles).

Discussion
Our key result is an increased mortality upon exposure to both parasites and a pesticide, even though no parasite infections could be detected visually (see further). Many studies showed hosts in poor condition have higher parasite-induced mortality than hosts in good condition (e.g., Braune & Rolff 2001; Krist et al. 2004; Jokela et al. 2005). The negative effect of two stressors on survival is much stronger compared to individual effects. Immune suppression under direct exposure to pollutants is a well-known phenomenon (e.g., McDowell et al. 1999; Aggarwal et al. 2008) and also diazinon is known to have immunomodulatory effects (Galloway & Handy 2003; Oostingh et al. 2009; Holmstrup et al. 2010). In this study we did not observe a decrease in phenoloxidase activity in animals exposed to the pesticide diazinon. This could either be because phenoloxidase activity is not affected by diazinon, which stays in contrast to other pesticides (Campero et al. 2008), or due to the fact that phenoloxidase was measured 17 days after pesticide exposure of the Daphnia. The Daphnia which did not die might have recovered from pesticide stress over the day 21 period, resulting in identical phenoloxidase activity between pesticide treatments. Kretschmann et al. (2011a) observed a slow recovery from diazinon exposure after transferring D. magna from media containing diazinon into clean water. According to their model, a recovery from diazinon exposure would take place within approximately 17 days. Nevertheless, our results indicate that the defence against parasite spores, even if they do not cause a visible infection, is associated with costs for D. magna. Yin et al. (2011) showed that defence against predators induced stronger infectivity of parasites, while Coors et al. (2008) showed that exposure to pesticides increased disease progression in Daphnia. Here, we show the reverse, that the enhanced costs of
defending against parasites spores, even if they do not cause visible infections, increases mortality upon exposure to a pesticide.

There are several potential reasons why *D. magna* clones in our study did not get infected by *P. ramosa*. First of all, it is possible that either during parasite isolation from the sediment or during enrichment of parasite spores, M10 clones were infected by parasite spores that were not infective for OM2 clones, even though *P. ramosa* from Knokke Nat have been shown to heavily infect *D. magna* clones from OM 2 (Jansen et al. 2010). It is possible that our additional round of infection of clone M10 resulted in a bottleneck with respect to the number of parasite lineages, and that a strain was selected which by chance did not infect OM2 clones. Alternatively, it may be that, for unknown reasons, the number of viable *P. ramosa* spores decreased to below threshold levels in the time between estimating their density and using them in the experiment. Low spore concentrations result in failure to induce disease (Ben-Ami et al. 2008). The observed increased mortality in our combined parasites × pesticides exposure treatment may thus be associated with either exposure to a below-threshold concentration of parasite spores or exposure to spores that were not infective.

We did not find any effect of sediment layer on any measured trait, which is in contrast to Decaestecker et al. (2007), who found a correlation between parasite density and years as well as with the study of Pauwels et al. (2010), who found differences in levels of *Daphnia* phenoloxidase activity over time. The fact that our spore solution did not cause visible infections in this study as well as having few clones per sediment layer could be the reason why we do not find any sediment layer effect. In case of the diazinon treatment alone, we never expected to find a sediment layer effect. First of all, OM 2 is surrounded by farm land, but the historical use of diazinon is not well documented. Therefore we do not know if diazinon was ever present in this lake. Additionally, it might be diazinon degraded too fast not allowing enough time for the hosts to adapt. Nevertheless, we encourage scientist to study evolutionary responses to environmental changes using dormant eggs, as this approach is straightforward to document microevolutionary changes in natural populations. Also multiples stressors should be included, since this is a more realistic representation of natural situations.

**Acknowledgment**

We thank several members of the Luc De Meester’s group for their help during the experiment and Andreas Kretschmann for helping verifying the diazinon concentration. The manuscript improved by comments from Andreas Bruder, Christoph Tellenbach and Mat
Seymour. This research was funded by the ETH Board (CCES-GEDIHAP), the Mobility Support from Eawag and the IWT, Flanders.

References


Multiple stress on *Daphnia* hatchlings


## Web appendix

### Table S1: Descriptive statistic (mean, standard deviation (SD), number of vials (N)) for survival, reproductive output and phenoloxidase activity for each clone exposed to the four treatments.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Sediment layer</th>
<th>Treatment</th>
<th>Survival mean SD N</th>
<th>Reproductive output mean SD N</th>
<th>Phenoloxidase activity mean SD N</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2-2</td>
<td>1</td>
<td>Control</td>
<td>0.24 0.18 5</td>
<td>6.39 2.03 5</td>
<td>63.97 28.63 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diazinon (D)</td>
<td>0.35 0.27 6</td>
<td>4.32 2.87 6</td>
<td>86.52 65.65 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parasite (P)</td>
<td>0.30 0.30 6</td>
<td>2.95 1.96 6</td>
<td>132.10 23.21 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &amp; D</td>
<td>0.18 0.21 6</td>
<td>3.28 2.76 6</td>
<td>25.85 21.81 3</td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>0.47 0.32 6</td>
<td>3.52 0.57 6</td>
<td>19.83 27.18 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diazinon (D)</td>
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<td>2.60 1.64 6</td>
<td>4.52 7.02 4</td>
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<td>0.38 0.12 6</td>
<td>4.51 0.60 6</td>
<td>8.57 10.06 6</td>
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<td>3.12 1.09 6</td>
<td>59.31 41.63 4</td>
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<td>D2-3</td>
<td>1</td>
<td>Control</td>
<td>0.56 0.46 6</td>
<td>3.41 1.35 6</td>
<td>47.32 91.88 4</td>
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<td></td>
<td>Diazinon (D)</td>
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<td>85.39 148.79 4</td>
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<td></td>
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**Chapter 4**

**Disease and pollution alter Daphnia community structure**

Claudia C. Buser, Piet Spaak & Justyna Wolinska

(submitted to Freshwater Biology)

**Abstract**

Environmental stressors can influence population and community structure. But a majority of experimental work on multiple environmental stressors have been tested on an individual level only. In the present work we followed changes in experimental *Daphnia* (waterflea) communities (consisting of two taxa and three clones per taxon) after exposure to the fungal parasite *Metschnikowia* sp. and/or the pesticide diazinon. We found a significant shift in taxon and clonal composition under both stressors. Strikingly, one taxon went extinct in the parasite and parasite + pesticide treatments. While the pesticide had no effect on total parasite prevalence, one taxon was more infected when additionally exposed to the pesticide. Furthermore, the density of all adult females was significantly reduced in the parasite treatment, but not in the pesticide treatment. Our results demonstrate that dynamics in *Daphnia* communities are altered by parasites and pesticide exposure. Given *Daphnia*’s key-role in aquatic food webs, converting primary production to fish food, this may lead to a higher stress on aquatic ecosystems.

**Key words**

Competition, *Daphnia galeata*, frequency-dependent selection, hybrids, *Metschnikowia* sp., parasite, pesticide
Introduction

Communities are subject to a multitude of abiotic and biotic stressors. One approach to understand the effect of these multiple stressors on communities is to study the effect of stressors on single individuals and extrapolate these findings to the higher community level. The effect of multiple stressors on the individual level has been investigated for several taxa across the plant and animal kingdom. It has been shown that a combination of abiotic and biotic stressors is often more harmful than either stressor alone (e.g., Hanazato & Dodson 1995; Folt et al. 1999; Relyea 2003; Sih et al. 2004).

Abiotic stressors (e.g., temperature change, eutrophication, acidification) are very abundant and can affect the functioning and structure of ecosystems (e.g., Bronmark & Hansson 2002; Brede et al. 2009). Pesticide pollution is one frequently occurring stressor in aquatic habitats as pesticides are washed out from agricultural fields after rain events (Pedersen et al. 2006; Wittmer et al. 2010). For example, zooplankton exposed to a chronic concentration of pesticides often have fewer and smaller offspring and suffer increased mortality (reviewed in Hanazato 2001). Among biotic stressors, parasites seem to play an especially important role. They affect the fitness of host individuals and thus can alter the structure and the diversity of host populations as well as competition among species (e.g., Combes 1996; Hudson & Greenman 1998; Jokela et al. 2003; Duncan & Little 2007; Wolinska & Spaak 2009). Parasite-mediated selection can be increased or decreased by additional biotic or abiotic stressors in the host environment, like extreme temperature or food shortage (reviewed in Wolinska & King 2009).

Parasites and pollution may interact to enhance the damage to and selection on host populations. For example, exposure to pesticides can increase host susceptibility to infection, as well as enhance parasite virulence, as shown for amphibians (Gendron et al. 2003; Rohr et al. 2008; King et al. 2010), fish (Kreutz et al. 2010), oysters (Chu & Hale 1994) and planktonic crustaceans (Coors & De Meester 2008; Coors et al. 2008; Coors et al. 2009; Coors & De Meester 2011). Many of these studies have been conducted on the individual and population level. But, the effects of pesticide exposure on the relative genotypes frequencies is rarely investigated. Hanazato & Yasuno (1987) observed changes in zooplankton species composition after exposure to insecticides, and Relyea & Hoverman (2008) demonstrated that pesticide exposure lowered zooplankton diversity and abundance. However, the extent to which the combined effect of parasites and pesticides alter host composition on a community or population level remains largely unexplored.
In this study, we examined how *Daphnia* (waterflea) communities are affected by exposure to the parasite *Metschnikowia* sp. and pesticide diazinon. More specifically, we followed taxa and genotype dynamics in experimental communities when exposed to both stressors in a full factorial design. As *Daphnia* reproduce by cyclical parthenogenesis, we could easily follow the frequencies of the two taxa and three genotypes (clones) per taxon. We predicted that parasite and pesticide exposure would additively decrease host density and increase parasite prevalence because effects of stressors are worse in combination than alone (Hanazato & Dodson 1995; Folt et al. 1999). However, predictions for the effects of stressors on clonal and taxa composition are difficult to form given the conflicting results on whether *Metschnikowia* sp. infects different taxa and clones to the same extent (Stirnadel & Ebert 1997; Wolinska et al. 2009; Yin et al. 2011) or not (Duffy & Sivars-Becker 2007; Duffy et al. 2011). If the latter is true, the parasite would infect various taxa and clones to different extents, possible altering the competition among taxa and clones.

**Materials and Methods**

**Host-parasite system**

As a model host, we used *Daphnia* (waterflea) belonging to the *D. longispina* hybrid complex. This complex consists of three parental species (*D. cucullata* G. O. Sars, 1862, *D. galeata* G. O. Sars, 1863 and *D. longispina* O. F. Müller, 1776, as revised in Petrusek et al. 2008) and their interspecific hybrids, and dominates *Daphnia* communities of European lakes (e.g., Keller et al. 2008; Petrusek et al. 2008; Yin et al. 2010). The yeast parasite *Metschnikowia* sp. (family Hemiascomycetes, Wolinska et al. 2009) commonly infects *Daphnia* populations in Europe and the United States (Hall et al. 2005; Caceres et al. 2006; Wolinska et al. 2011). Needle-like ascospores accumulate in the body cavity and are released only after host death (Green 1974). Infected *Daphnia* have fewer offspring and a reduced lifespan (e.g., Duffy & Hall 2008; Lohr et al. 2010).

**Pesticide exposure**

The organophosphate diazinon (O,O-diethyl O-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphothionate) is one of the most used and applied pesticide in agriculture and households, and is thus detected in several aquatic environments (Miles & Harris 1978; Singer et al. 2010). Acute toxic effects of diazinon (like immobilization, mortality) are related to the inhibition of acetylcholinesterase, an enzyme essential for proper function of the nervous system. Exposure of *Daphnia magna* to a sub-lethal concentration leads to a delay in reproduction, a decrease in the number of offspring and offspring size and higher adult mortality (see Sanchez et al. 1998). Based on the results of sub-lethal concentrations of
diazinon (CAS: 333-41-5, 99.5 % purity, Ultra Scientific Analytical Solution, N. Kingstown RI, USA) on *D. galeata* (see supplementary material, S1) we set our pesticide concentration to 0.1 μg/L. Similar diazinon concentrations as used in this study have been measured in natural systems (e.g., Konstantinou et al. 2006; Wittmer et al. 2010).

**Origin and care of host and parasite**

Both host and parasite were isolated from Ammersee, a natural lake in Germany (surface area: 46.6 km²; coordinates: 48°00′34″N, 11°07′02″E), two years prior to the experiment (in autumn 2008). The *Daphnia* community of this lake is dominated by *D. galeata* and *D. galeata × D. longispina* hybrids (Yin et al. 2010). Three *D. galeata* and three *D. galeata × longispina* hybrid clones were selected from a larger collection of sampled clones (taxon and multilocus genotype assignment based on 15 microsatellite loci, for methods see Yin et al. 2010). *Metschnikowia* was maintained on a single *D. galeata × longispina* clone (AMM_12) by adding uninfected juveniles into the infected culture every second week (Lohr et al. 2010). Uninfected and infected *Daphnia* cultures were kept under standardized conditions (20 °C, 16:8 light-dark photoperiod, fed three times a week with 1.0 mg C/L green algae, *Scenedesmus obliquus*). Before the experiment, the uninfected *Daphnia* clones were raised in 10 L buckets filled with lake water (Greifensee, Switzerland; 0.45-μl-filtered and UV-light sterilized), to obtain enough individuals.

**Experimental set-up and procedures**

We tested whether the presence of parasite and/or pesticide alters the competition between *Daphnia* taxa (hereafter we use the term "taxon" for each of the two *Daphnia* forms tested: parental *D. galeata* and *D. galeata × longispina* hybrids) and within taxa (three clones per taxon tested). Experimental communities were exposed to four treatments: control (lake water only), parasite, pesticide and parasite + pesticide. Each treatment was replicated five times. The diazinon, dissolved in acetone, was added at a final concentration 0.1 μg/L; the respective amount of acetone was also added to non-pesticide treatments. To obtain parasite spores for experimental infection, 24 juveniles (four per clone; 3-6 days old) were placed together in a 200 mL jar (one-hundred jars were prepared). Then, 300 heavily infected/uninfected *Daphnia* of clone AMM_12 were crushed and equally distributed to the corresponding jars (i.e., fifty infected and fifty uninfected jars were prepared). The water was stirred daily to re-suspend the spores and maximise *Daphnia* spore contact. *Daphnia* were daily fed with *S. obliquus* at an amount of 1.0 mg C/L. Four days later (i.e., on experimental day 1), the contents of the jars were added into the respective aquarium (see below).
On day 1, twenty aquaria (4 treatments × 5 replicates) were prepared, containing 4 L of lake water. Each aquarium was set up with three *D. galeata* and three *D. galeata × longispina* clones (40 adult females of each clone). In addition, the contents of five spore/control jars was added into each aquarium, resulting in a total of 360 *Daphnia* in each aquarium. Every day 200 mL water was added (with or without diazinon), reaching a final volume of 8 L after 22 days. *Daphnia* were daily fed with 0.3 mg C L$^{-1}$ *S. obliquus*. Magnetic stirrers were turned one minute per day to provide a uniform distribution of parasite spores, pesticide and algae. Under same experimental conditions, the diazinon degrades at a rate of 2.8 % per day (supplemental information S2). To compensate for that loss, a corresponding amount of diazinon/acetone was added to the aquaria every 2nd week. The experiment lasted 99 days. On day 57, all animals of one control aquarium died because of a handling error.

**Data collection**

*Daphnia* were first sampled on day 29 and then again every second week (i.e., day 43, 57, 71, 85 and 99). From each aquarium three replicate samples were collected after stirring the water using a Plexiglas tube (diameter: 3 cm) and *Daphnia* were preserved in 100 % EtOH (infection estimates from the ethanol preserved samples are similar to those estimated from live samples; J.W. pers. observation, see also Wolinska et al. 2011). The water level of each aquarium was refilled to 8 L. The number of infected and uninfected adult females was counted under a stereomicroscope at a magnification of ×16.0 (Leica M 205 C) to assess *Daphnia* density and parasite prevalence. In addition, from the samples collected at day 29 and 99, from each aquarium one subsample of 24 randomly selected adult females were individually assessed for their body size (from top of the eye to the base of the spine), fecundity (presence/absence of eggs in the brood pouch), infection status (presence/absence of parasite spores in the body cavity) and taxon/clonal identity. Scoring presence/absence of eggs in the brood pouch of *Daphnia* preserved in EtOH can lead to underestimation of fecundity, but since all samples were treated the same, a possible underestimation is unlikely to be treatment-biased.

*Daphnia* were genotyped at three diagnostic microsatellite loci (SWID 12, SWID 14 and SWID 10, Brede et al. 2006). The DNA was extracted using HotSHOT DNA extraction method (Montero-Pau et al. 2008). PCR was carried out using the following cycle profile: an initial step of 15 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 1.5 min at 54 °C and 1 min at 72 °C; with a final extension step of 30 min at 60 °C. The total reaction volume was 12 μl, containing 6 μl Qiagen multiplex PCR mix, 3.4 μl water, 0.6 μl primer mix and 2 μl DNA template. PCR products were analysed on a sequencer (Applied Biosystems 3730xl
DNA Analyzer). The microsatellite peaks were scored using Applied Biosystems GeneMapper® software version 4.0.

**Statistical analyses**

Statistical analyses were performed using linear models in R 2.10.1 for Windows (R Development Core Team, 2008). Our model simplification proceeded as follows: from each initial model we removed higher order terms in order, starting with the least significant term. We tested whether the exclusion of a term resulted in a significantly poorer model using likelihood ratio tests as recommended by Crawley (2007). All relevant factors belonging to the minimum adequate model for each analysis are summarized in Table 1. For the linear mixed effects models, the highest posterior density (HPD) intervals and associated $p$-values for fixed effect parameters were derived using Markov Chain Monte Carlo (MCMC) sampling methods with the pvals.fnc command of the LANGUAGE R library (Baayen et al. 2008). All data satisfied the assumptions of parametric tests (inspected with q-q plots and plots of residual against fitted values). In the analyses of parasite prevalence and host susceptibility, only data from aquaria exposed to parasite treatment were included. To correct for repeated measures we included sampling date as random factor in all the performed analysis (Pinheiro & Bates 2000; Crawley 2007).

**Host density, parasite prevalence and host susceptibility.** To examine treatment effects on host density (measured as the number of adult females/L) or parasite prevalence (proportion of infected adult females), two separate linear mixed effects models (with the lmer function) were employed, using either host density or parasite prevalence as dependent variable, exposure to parasite (yes/no; only in the host density model) and to pesticide (yes/no) as fixed effects and sampling date as well as aquarium as random effects. To test for the influence of pesticide addition on the susceptibility of certain taxa, a generalized linear mixed effects model (glmer function) was used, with an individual being infected (yes/no) as binomial dependent variable, exposure to pesticide (yes/no) and taxon identity as fixed effects and sampling date as well as clone (nested within taxon) as random effects.

**Host taxon and clonal composition.** To test for treatment effects on taxon or clonal composition (Agresti 2002), two separate log-linear models were used (with the glmer function, family= poisson distribution). The counts of individuals were treated as dependent variable, exposure to parasite (yes/no) and to pesticide (yes/no) and taxon/clonal identity as fixed effects and sampling date and aquarium as random effects. For these and the following tests, only data collected at day 29 and 99 were analysed.
Host fecundity and body size. A generalized linear effects model (with the glmer function) was conducted with the presence of eggs (yes/no) as dependent variable to test for treatment effects on host fecundity. Differences in the body size of adult *Daphnia* were examined using a linear mixed effects model, with size of adult females as dependent variable. In both models, exposure to parasite (yes/no) and to pesticide (yes/no) and taxon identity were treated as fixed effects, whereas the sampling date and aquarium were random effects.

Results

Host density, parasite prevalence and host susceptibility. The density of adult females was significantly reduced by exposure to parasites, but not to pesticides (Table 1; Fig. 1). Although pesticide exposure had no effect on total parasite prevalence (Table 1; Fig. 2), susceptibility of the different taxa was affected (see significant pesticide-by-taxon interaction; Table 1). At day 29, *D. galeata × longispina* hybrids exposed to pesticides were more infected than non-exposed hybrids, but this effect was not visible in *D. galeata* (Fig. 2).

![Fig. 1: Changes in mean density (number of adult females per litre ± SE) across the different treatments: control (white circles), pesticide (white squares), parasite (black circles), and parasite + pesticide (black squares).](image)

![Fig. 2: Changes in mean parasite prevalence (proportion of infected adult females ± SE) across the different treatments: parasite (black circles), and parasite + pesticide (black squares). Parasite prevalence per taxa at day 29 is shown (empty symbols). Samples from day 99 were also genotyped, but the community consisted of *D. galeata* only (see Fig. 3).](image)

Host taxon and clonal composition. Taxa and clonal composition changed when parasites or pesticides were added to the aquaria (see the significant parasite-by-taxon/clone and pesticide-by-taxon/clone interactions; Table 1). At day 29, hybrids were more abundant than *D. galeata* across all treatments (Fig. 3). At day 99, however, the no hybrid taxon could be detected in all the parasite and parasite + pesticide treatments (Fig. 3). At this same time point, six clones...
were still present in the control treatment, five clones remained in the pesticide treatment, and the same two *D. galeata* clones (AMM_47 & 66) remained in the parasite and parasite + pesticide treatments (Fig. 4).

![Fig. 3](image1.png)

**Fig. 3:** Changes in mean taxon frequency (± SE) across the different treatments: (A) control, (B) pesticide, (C) parasite, and (D) parasite + pesticide (grey: *D. galeata*; white: *D. galeata × longispina* hybrids).

![Fig. 4](image2.png)

**Fig. 4:** Changes in mean clonal frequency (± SE) across the different treatments: (A) control, (B) pesticide, (C) parasite, and (D) parasite + pesticide. *D. galeata* clones are shown in grey (AMM_10, 47, 66), and *D. galeata × longispina* hybrid clones are shown in white (AMM_30, 39, 45).
**Host fecundity and body size.** In the parasite and parasite + pesticide treatments, females were more fecund than in those treatments not exposed to parasites (Table 1; Fig. 5). *D. galeata* were more fecund when additionally exposed to pesticide whereas hybrids were more fecund when not exposed to pesticides (see significant pesticide-by-taxon interaction; Table 1). *Daphnia* exposed to parasites were generally smaller than unexposed *Daphnia* (Fig. 6). In addition, hybrids were smaller than *D. galeata*, but only in those treatments not exposed to parasites (see significant parasite-by-taxon interaction; Table 1, Fig. 6).

![Fig. 5](image1.png)  
**Fig. 5:** Changes in mean proportion of fecund females (± SE) across the different treatments: control (white circles), pesticide (white squares), parasite (black circles) and parasite + pesticide (black squares).

![Fig. 6](image2.png)  
**Fig. 6:** Changes in mean body size (± SE) of adult females across the different treatments: control (white circles), pesticide (white squares), parasite (black circles) and parasite + pesticide (black squares).

**Discussion**

Parasite exposure had a high impact on the experimental *Daphnia* communities. As expected, parasites reduced *Daphnia* host density, a finding consistent with other *Daphnia*-microparasite experimental systems (Ebert et al. 2000; Decaestecker et al. 2005). It was previously shown that *Metschnikowia*-infected *Daphnia* had fewer offspring and a higher mortality rate than uninfected ones (e.g., Duffy & Hall 2008; Lohr et al. 2010). Conversely, in our experiment, treatments involving parasite exposure resulted in a higher proportion of fecund females. Given that fecundity of *Daphnia* is strongly dependent on resource availability (Lampert 1978), this result could be due to parasites lowering *Daphnia* density and consequently freeing up more resources per host individual.
### Table 1: Statistical models and test statistics for the effect of parasites and pesticides on *Daphnia* host individual traits and community composition. We used three kinds of linear models: linear mixed effect model (lmer), generalized linear mixed effect model (glmer) and log-linear model. Details concerning the model construction and simplification are provided in the text.

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<th>MCMCp/a/bc.</th>
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</tbody>
</table>

*a* for lmer  
*b* for glmer and log-linear model (except for the clonal frequency model, where $\chi^2$ are presented)  
*c* single effects not included in the table because they are not meaningful in log-linear models  
*d* the overall effect of parasite/pesticide on clonal composition was tested by removing the corresponding interaction term and comparing this new model with the model including all two-way interactions. ΔDf= 5

Parasites directly altered *Daphnia* species composition. Although two taxa coexisted in a parasite-free environment, *D. galeata × longispina* hybrids went extinct under parasite pressure. Differences in the susceptibility of the studied taxa may be the cause. At day 29, *D. galeata* were less infected than hybrids, despite previous findings that *Metschnikowia* infected different taxa and clones to the same extent (Stirnadel & Ebert 1997; Wolinska et al. 2009; Yin et al. 2011). Our result that hybrids had higher parasite prevalence may support the ‘hybrid susceptibility’ hypothesis (Fritz et al. 1994) or be explained by parasite-driven, negative frequency-dependent selection (Hamilton 1980). At day 29, the more frequent taxon (hybrids) were the most infected, and at the end of the experiment, hybrids had disappeared.
and *D. galeata* were common. At this point, *D. galeata* had higher prevalence of infection. Such a mechanism has been shown to be responsible for the promotion of genetic diversity in *Daphnia* and other invertebrate host populations (Duffy 2008; Wolinska & Spaak 2009; Berenos et al. 2011; King et al. 2011). As we did not look at the parasite genetic variation, we cannot confirm, that the observed pattern is due to coevolution. Alternatively, the parasite strain used in the experiment may have adapted to the hybrid taxon, as the parasite was maintained for two years on a hybrid clone. This is unlikely, however. Yin et al. (2011) did not find a different infection rate among *D. galeata* and hybrid clones, when exposed to a parasite strain raised on a hybrid *Daphnia* clone. Also, Duffy & Sivars-Becker (2007) found no evidence for *Metschnikowia* adaptation towards different host clones after five host generations.

Contrary to our expectations that pesticide exposure would decrease *Daphnia* density due to delay in reproduction, decrease in number of offspring or reduced survival (Sanchez et al. 1998), density did not differ from the control treatment. This could be the result of pesticide-tolerant clones replacing sensitive clones, a mechanism described as pollution-induced community-tolerance hypothesis (Blanck & Wangberg 1988). This mechanism should not affect density, but only taxon and clonal composition. Indeed, in our experiment both taxon and clonal composition were influenced by pesticide exposure. The observed increase in *D. galeata* frequency in the aquaria containing pesticides, is plausible with respect to the finding that *D. galeata* had higher fecundity in the pesticide treatments whereas the hybrid fecundity was marginally lower when exposed to the pesticide. In contrast to our expectations, the pesticide did not affect the number of the total infected adult females. However, we observed that the hybrids (but not *D. galeata*) were more infected when exposed to the pesticide compared to unexposed hybrids. Although there are examples that hybrids can be more susceptible to parasites (i.e., ‘hybrid susceptibility’ hypothesis of Fritz et al., 1994), the sensitivity of hybrids and parental taxa towards pesticides has not previously been compared. Our observations of reduced hybrid fecundity under pesticide exposure, as well as the higher susceptibility of hybrids, indicate that hybrids are more affected by both environmental stressors, parasites and pesticides. This combination of negative responses to both stressors could lead to the observed higher infection rate under pesticide exposure.

Studies tracking genetic changes of a community exposed to a pesticide as well as studies examining multiple stressors, especially those taking competition within and among taxa into account are very rare. The here employed experimental design is a potentially promising tool for a verification if effects on individual levels are also observed in communities as well as help to interpret more complex observations of field studies (Marcogliese & Pietrock 2011).
Although previous studies did observe synergistic effect of the pesticide and parasite stressors on individual level (e.g., Coors & De Meester 2008), this is a first study using a mixture of taxa and several clones to investigate the relationship between the two stressors. Under the tested experimental conditions (using pesticide concentration found in natural habitats) parasite exposure was a stronger selection factor than pesticide exposure. However, since there are often concentration effects of stressors (e.g., Little & Killick 2007; Ben-Ami et al. 2008; Oda et al. 2011), a larger experiment using several pesticide concentrations and spore loads would better verify whether interaction effects were present. Nevertheless, our study revealed that parasites, as well as pesticide exposure, alter the structure of Daphnia host communities.

The observed changes in a Daphnia community are most likely due to varying sensitivity of the taxa/clones to the biotic and abiotic stressors. Communities exposed to stress lose less-tolerant taxa and clones, resulting in a decrease of diversity (Altizer et al. 2003; Relyea & Hoverman 2008). Such a loss in diversity may make the community more vulnerable to additional stress. Given that parasites are ubiquitous and anthropogenic stressors are becoming increasingly pervasive, our study shows that the genetic diversity of Daphnia populations might be at higher risk. Given Daphnia’s key-role in aquatic food webs, converting primary production to fish food, this may lead to a higher stress on aquatic ecosystems.

Acknowledgment
We thank Esther Keller and Markus Möst for helping sampling the aquaria and Christine Dambone for providing the Daphnia food. The Genetic Diversity Centre at ETH Zurich allowed us to use their genotyping facility. Further we thank Christoph Tellenbach and Blake Matthews for intense statistical discussions. The manuscript was substantially improved by comments from Kayla C. King. This research was funded by the ETH Board (CCES-GEDIHAP).

References
Chapter 4  Competing taxa exposed to multiple stressors


Little, T., and S. C. Killick. 2007. Evidence for a cost of immunity when the crustacean Daphnia magna is exposed to the bacterial pathogen Pasteuria ramosa. Ecology 76:1202-1207.


Web appendix

S1: Sub-lethal effects of diazinon on *Daphnia galeata*

**Material & Methods**

We explored sub-lethal effects of diazinon on two *Daphnia galeata* clones following the OECD guidelines for reproductive test (OECD/OCDE 1998). We tested five different concentrations of diazinon (0.00256, 0.0064, 0.016, 0.04 and 0.1 µg/L). As controls, we used 1) lake water without diazinon and 2) lake water without diazinon but containing the same amount of the solvent acetone as in the treatments. Nominal exposure concentrations of diazinon were verified with LC-MS/MS (liquid chromatography-mass spectrometry/mass spectrometry) as described in Kretschmann et al. (2011). As measured diazinon concentrations were within ± 20% of the nominal concentrations, for data analysis nominal concentrations were used. We distributed the offspring (<24 h old) of the third clutch singly in 50 mL jars with five different diazinon concentrations and two controls. Each treatment was replicated 10 times, resulting in 140 experimental units (7 concentrations × 2 clones × 10 replicates). Under experimental conditions (20 °C, 16:8 light-dark photoperiod) the medium was changed every third day and the *Daphnia* were fed with 1 mg C/L of the algae *Scenedesmus*. The following parameters were recorded: survival after 21 days (experiment was terminated then), time of first offspring released and number and body size of the offspring of the first clutch.

To test for differences in the life history traits we conducted a univariate ANOVA using SPSS 19.0 with the life history trait (number of survived individuals, time of first offspring released and number and body size of the released offspring as dependent factor, the different diazinon concentrations as a fixed factor and clones as a random factor. All data were tested for the assumptions of parametric tests (inspected with q-q plots and plots of residual against fitted values) and, if necessary, transformed. The two sided Dunnett t-test treats one group as a control and compares all other groups against it. From that we were able to figure out which concentrations are different from the control and, consequently, determine the threshold concentration.

**Results**

Different diazinon concentrations significantly affected survival rate (F= 13.35, df= 6, p= 0.003), the number of offspring (F= 6.68, df= 6, p= 0.018), the size of offspring (F =4.66, df =6, p =0.042) and the time of first offspring released (F= 5.79, df= 6, p= 0.025). We found
a clone effect on the average offspring size \( (F = 45.19, df = 1, p < 0.001) \) and the number of offspring of the first clutch \( (F = 7.386, df = 1, p = 0.030) \).

Survival was only affected at the concentrations 0.016µg/L and 0.1µg/L. The time to release the first juveniles was delayed and the number of offspring in the first clutch decreased when the concentration was equal to or higher than 0.04µg/L (first juveniles released: 0.04 µg/L \( (p = 0.024) \), 0.1 µg/L \( (p < 0.001) \); number of offspring in first clutch: 0.04 µg/L \( (p = 0.003) \), 0.1 µg/L \( (p = 0.015) \)).

**Fig. S1:** The effect of different diazinon concentrations on survival rate (A), the number (B) and the time of offspring of the first clutch released (C) and the size of offspring of the first clutch (D).

**Conclusions**

For further experiments the diazinon concentration of 0.1 µg/L was chosen. This concentrations was above the threshold and showed significant differences compared to the control for all measured traits.
S2: Degradation of diazinon under experimental conditions

Material & Methods

Measurements of diazinon degradation was carried out with 14C labelled diazinon ([Pyrimidinyl-6-14C]-Diazinon, 99.21 % radiochemical purity, supplied by American Radiolabeled Chemicals, St. Louis, USA) under the same experimental conditions (20 °C, 16:8 light-dark photoperiod) and in the same room as the main experiment itself. Eight litre of lake water (filtered through a membrane of 0.45µl) were filled into a 20 litre aquaria and 5 µg/L 14C labelled diazinon was added (concentration high enough to be measured with the method described and below the limit of the permitted maximum dose 20 kBq). During 14 days, water samples (1 mL) were taken every 1 to 2 days and 10 mL of Ecosicint A scintillation cocktail (National Diagnostics, Hessle, UK) immediately added to a water subsample. Following the protocol of Ashauer et al. (2006), radioactivity was quantified with liquid scintillation counting (Beckman LS6000 TA Liquid Scintillation Counter, Beckman Instruments Inc., Fullerton, CA, USA) and blank controls were used to correct for background activity.

Results

Measured diazinon concentrations over 14 days as well as the best fit function is shown in Fig. S2. Diazinon is degrading 2.8 % per day.

![Graph showing degradation rate of 14C labelled diazinon during 14 days. Best fitted regression line and it’s equation are included in the graph.]

**Fig. S2:** Degradation rate of 14C labelled diazinon during 14 days. Best fitted regression line and it’s equation are included in the graph.
Conclusions
To compensate for that loss, a corresponding amount of diazinon/acetone was added to the aquaria every 2\textsuperscript{nd} week.

Acknowledgments
We thank Roman Ashauer and Andreas Kretschmann for helpful advices and measuring the diazinon concentrations.

Reference supplementary material
Chapter 5

Evolutionary and environmental effects of pesticide exposure on parasite fitness and virulence

Claudia C. Buser, Justyna Wolinska & Piet Spaak

(Journal of Evolutionary Biology, in review)

Abstract
Parasite fitness and virulence can be altered by the environment, especially if conditions persist for several parasite generations. In this study, we compared the evolutionary and environmental effects of pesticide exposure to parasite fitness and virulence using the planktonic crustacean Daphnia galeata and the yeast Metschnikowia sp. Environmental effects of pesticide exposure resulted in host genotype specific interactions concerning parasite spore load. Additionally, host fecundity was reduced. Under evolutionary pressure of pesticide exposure, parasites evolved towards higher spore loads and lower virulence. Since the concentration of pesticides reflected natural concentrations, our results indicate that pesticide pollution might have an important impact on disease dynamics in aquatic systems.

Key words

Daphnia, experimental evolution, Metschnikowia sp., parasite, pesticide
Introduction

The effect of the environment on host-parasite interactions depends on the sensitivity of hosts to particular environmental conditions. Consequently, parasite fitness (measured, for example, as infection rate and/or spore load: Ebert 1998; Jensen et al. 2006; Vale & Little 2009) and virulence (measured as mortality of infected hosts and/or host sterilisation: Garnick 1992; Poulin & Combes 1999; Alizon et al. 2009) depend on the environment in which the host is living.

Parasite fitness can increase in stressful host environments due to reduced host immune function (Sheldon & Verhulst 1996; Moret & Schmid-Hempel 2000). Empirical studies have shown that stressed hosts are more frequently infected (Chu & Hale 1994; Oppliger et al. 1998; Rohr et al. 2008a) and parasites have higher spore production (Bedhomme et al. 2004; Yin et al. 2011). Conversely, other studies have shown that parasites experience a fitness loss when hosts are stressed, due to reduced resources available for parasite growth (Pulkkinen & Ebert 2004; Tschirren et al. 2007; Hall et al. 2009). Parasite virulence can also be further influenced by all variety of environmental conditions (reviewed in Thomas & Blanford 2003; Lazzaro & Little 2009; Wolinska & King 2009). Generally, in environments which are stressful for hosts, parasite virulence increases, as observed under higher host mortality rates (Jokela et al. 1999; Coors & De Meester 2011).

The impact of environmental changes on parasite evolution could be especially important if the altered conditions persist for several parasite generations. Aquatic habitats in particular are extensively impacted by human activity, in ways that may affect the evolution of parasites. For example, pesticide runoff from agriculture into water bodies is likely to select for fast-growing, early-transmitted parasites (Mennerat et al. 2010) to overcome a short host life span (Nidelet et al. 2009). Indeed, for obligate killers, parasite virulence can evolve to increase when extrinsic host mortality is high (Ebert & Weisser 1997). Pesticides have been shown to affect parasite fitness and virulence across many host-parasite systems; for example, in parasites of amphibians (Gendron et al. 2003; Rohr et al. 2008b; King et al. 2010), fish (Kreutz et al. 2010), oysters (Chu & Hale 1994) or planktonic crustaceans (Coors & De Meester 2008; Coors et al. 2008; Coors et al. 2009; Coors & De Meester 2011). However, the evolutionary consequences of long-lasting host exposure to pesticides on parasite fitness and virulence have not been studied in great detail.

In the present study, we investigated environmental and evolutionary effects of host exposure to pesticides on parasite fitness and virulence. We used the planktonic crustacean Daphnia galeata (G. O. Sars, 1863) and the yeast Metschnikowia sp. (family:
Hemiascomycetes; Wolinska et al. 2009) as a host-parasite model system. Needle-like ascospores accumulate in the body cavity and are released only after host death (Green 1974). As a stressor, we applied diazinon, which is a commonly used pesticide (Konstantinou et al. 2006; Singer et al. 2010). Standard toxicological tests on *D. magna* showed that environmental effects of diazinon exposure cause a delay in reproduction and earlier death, as well as a decrease in offspring number and body size (Sanchez et al. 1998). *D. magna* is a pond species whereas *D. galeata* inhabits large, permanent lakes (e.g., Keller et al. 2008; Petrušek et al. 2008; Yin et al. 2010). Such habitats are frequently affected by pesticide pollution (e.g., Konstantinou et al. 2006; Relyea & Hoverman 2006). Moreover, lake *Daphnia* population are commonly infected by a variety of parasite species; with the parasites used in the present study, *Metschnikowia* sp., being particularly common (Caceres et al. 2006; Duffy et al. 2010; Wolinska et al. 2011). *Metschnikowia* sp. reduces *D. galeata* reproduction and life span (Lohr et al. 2010). In addition, parasite fitness (i.e. infectivity and spore production) is higher in *D. galeata* exposed to a natural stressor (a predator signal, Yin et al. 2011). However, the effect of diazinon or any other pollutant on *Metschnikowia* sp. is not known.

Pesticide exposure has been already shown to increase parasite infectivity (Coors & De Meester 2008, 2011), but to conversely decrease parasite spore load (Coors & De Meester 2011) in the *D. magna-Parasitica ramosa* bacteria system. However, the above mentioned studies investigated only the environmental effect of pesticide exposure over a maximum of 21 days. Here, we studied both the environmental and the evolutionary effects of pesticide exposure on parasite fitness and virulence. We expected that parasites raised on hosts exposed to the pesticide for several generations would evolve adaptations to that stressor, showing higher infectivity and spore production than the parasites which has been raised in a control treatment, when environmental effect of the pesticide was tested.

**Material and Methods**

**Origin and care of the host-parasite system and the pesticide**

Two different *Daphnia galeata* clones were selected from a larger collection of clones isolated in 2008 from the lake Ammersee in Germany (clone_10 and clone_66; taxon and multilocus genotype assignment were based on 15 microsatellite loci, for methods see Yin et al. 2010) and used as the experimental hosts. The clones were kept under standard laboratory conditions (20 °C, 16:8 light-dark photoperiod, fed with 1.0 mg CL^{-1} of the green algae *Scenedesmus obliquus*) for two years. The horizontally-transmitted yeast *Metschnikowia* sp. was isolated from the same lake, also in 2008 (Yin et al. 2011) and used as model parasite. Before the experiment, *Metschnikowia* sp. isolate was raised for 99 days in independent ten
Experimental *Daphnia* communities (starting with a mixture of three *D. galeata* and three *D. galeata × longispina* clones isolated from Ammersee, including clone_10 and clone_66). The *Daphnia* communities were set up in ten 20L aquaria containing lake water filtered through a membrane of 0.45µL. In five of those aquaria, *Daphnia* were exposed to the sub-lethal dose of diazinon (CAS 333 41 5, 99.5 % purity, Ultra Scientific Analytical Solution N., Kingstown RI, USA), 0.1µg/L (similar diazinon concentrations have been measured in natural systems; e.g. Konstantinou et al. 2006; Wittmer et al. 2010). In the other five aquaria, which served as a control, *Daphnia* were exposed to the corresponding amount of the solvent acetone. After day 99, only two *D. galeata* clones (clone_47 and clone_66) remained whereas clone_10 disappeared in both treatments (Claudia C. Buser, Piet Spaak & Justyna Wolinska, unpublished data). For the two experimental spore solutions, a mixture of heavily-infected *Daphnia* collected from each aquaria of the corresponding treatment (i.e., with or without diazinon) were ground up.

**Experimental set-up and procedures**

We conducted a life-history experiment with a fully crossed design, in which parasites (*Metschnikowia* sp.) were raised for 99 days either on a host community exposed to diazinon (hereafter referred to as PD: parasite-diazinon), or on a host community exposed to control conditions (PC: parasite-control). In addition to using these two different parasite sources (PD or PC), experimental *Daphnia* were either exposed to diazinon or to the solvent acetone (control). This resulted in twelve experimental treatments: 2 (host clone) × 3 (parasite history: PD, PC and no parasites: noP) × 2 (diazinon and no diazinon). The four treatments not exposed to parasite spores were replicated 10 times, whilst the other eight treatments were replicated 20 times, for a total of 200 experimental units.

Experimental *Daphnia* (younger than 24h, third brood of second generation) were kept individually in 5 mL lake water with or without diazinon. On day 5, 80 individuals (40 from each clone) were exposed to the parasites raised with diazinon (PD), and 80 to parasites raised without diazinon (PC), while the remaining 40 animals were inoculated with the appropriate amount of uninfected crushed *Daphnia* (noP), to avoid possible bacteria or alarm cue effects. The concentration of spores in the parasite treatments was 450 spores/mL (similar as in Duffy & Sivars-Becker 2007). *Daphnia* were fed daily with 1.0 mg CL\textsuperscript{-1} *S. obliquus* except on day 7 and 9, when no food was added to promote spore uptake (Hall et al. 2007). On day 8, 5 mL of fresh diazinon or control medium was added, and on day 10, the *Daphnia* were transferred to 30 mL of the corresponding medium. From then on, the media was changed every third day throughout the experiment. Juveniles were counted and removed daily. The body length (from top of the eye to beginning of the spine) of three juveniles was measured from the first clutch
of each female. Dead *Daphnia* were ground up and the number of spores was determined under a stereo microscope as described in Lohr et al. (2010), using a Bürker counting chamber. The experiment lasted 44 days until all animals had died.

**Statistical analyses**
All statistical analyses were performed using SPSS 17.0. Twenty-eight individuals died before day 10 (i.e., when infections first became visible) and were excluded from the analyses. Differences in parasite infectivity were analysed using a generalized linear model, and parasite spore load per lifetime was analysed using ANOVA; with diazinon (no/yes), parasite history (PD/PC) and clonal identity as fixed factors. In the ANOVA analyses of host life-history traits (survival, total number of juveniles, age at reproduction and 1st clutch juvenile size), the same fixed factors were used with the exception that parasite treatment included the third level (i.e. noP: no parasites, in addition to PD/PC), and only infected animals were included in the parasite-exposure treatments. A Tukey HSD test was used to determine if PD and PC treatments differed. All data were tested for the assumptions of normality and, if necessary, transformed using the Rankit function (Conover & Iman 1982).

**Results**

*Parasite fitness traits.* Neither parasite history (PD, PC; i.e. evolutionary effects), nor diazinon presence in the *Daphnia* medium (i.e. environmental effects), had a significant influence on parasite infectivity (Fig. 1a). However, there was a main effect of clone, with clone_66 being more frequently infected than clone_10 (Table 1; Fig. 1). Parasite spore load was affected by evolutionary effects of diazinon exposure: spore load was higher when *Daphnia* was exposed to parasites raised with diazinon (PD) than in *Daphnia* exposed to parasites raised in control media (PC; Fig. 1b). Although there was no direct environmental effect of diazinon exposure, parasite spore load was higher under diazinon exposure for clone_66, whereas for clone_10 parasite spore load was higher without diazinon exposure (see significant diazinon × clone interaction; Table 1).

*Host traits to determine parasite virulence.* Infection generally reduced *Daphnia* survival (Fig. 2a, Table 1). However, *Daphnia* exposed to parasites evolved under diazinon exposure (PD) died later than *Daphnia* exposed to parasites evolved under control conditions (PC); Tukey test: $p = 0.001$. Environmental diazinon exposure did not affect *Daphnia* survival, but the uninfected clone_66 died earlier in the diazinon treatment (parasite exposure × diazinon × clone interaction; Table 1).
Similarly, the production of juveniles was also reduced in the parasite-exposed *Daphnia* (Fig. 2b, Table 1). The evolutionary effect was important here as well: *Daphnia* exposed to parasites evolved under diazinon exposure (PD) produced more offspring than those exposed to parasites evolved under control conditions (PC); Tukey test: \( p = 0.02 \). Environmental diazinon exposure resulted in lower production of offspring by the uninfected clone_66, whereas uninfected clone_10 produced more offspring then (parasite exposure \( \times \) diazinon \( \times \) clone interaction; Table 1). This result reflects the clonal differences concerning survival (see above).

**Other host traits.** Neither the reproductive age of *Daphnia*, nor the size of juveniles was dependent on evolutionary or environmental effects of diazinon exposure (Table 1). However,
there were clonal differences for both traits: clone_10 reproduced earlier (Fig. 2c) and produced smaller juveniles (Fig. 2d), but the difference in size disappeared in juveniles from infected mothers (parasite exposure x clone interaction; Table 1).

**Table 1:** Results of a generalized linear model and several ANOVA’s describing the influence of parasite, medium and clone identity on the different dependent variables.

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<td>parasite history (P_H)</td>
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<td>P_E x C</td>
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<td>0.461</td>
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*PC and PD differ (Tukey test: for exact p-values see the main text)
Discussion

The parasite strains that were confronted with long-lasting diazinon exposure (PD) produced significantly more spores than those raised on a host community exposed to control conditions (PC). In contrast, such an effect was not observed under environmental (short-lasting) exposure to pesticides. Apparently, the parasites had evolved during the long-lasting, pre-culturing conditions. Although in our study the parasites were evolving on host communities originally consisting of six different clones, by the end of the 99 days only two clones (clone 66 and 47) were left. However, as the clonal composition of experimental host communities did not differ between the communities exposed and unexposed to pesticides (Claudia C. Buser, Piet Spaak & Justyna Wolinska, unpublished data), we are confident that the observed evolutionary effects are due to the pesticide treatment and not due to clonal differences. Therefore, diazinon might have selected for parasite genotypes with high spore production in the pre-culturing conditions. Indeed, the parasite selection experiment for either pesticide or control conditions was likely to be started with a mixture of parasite strains. This is because a parasite was originally isolated from a field-infected Daphnia. Co-infections with multiple strains and species are a rule and not an exception in nature (e.g., Read & Taylor 2001), and Daphnia are often co-infected by several strains of a given parasite taxon (Ben-Ami 2008). Similarly to our results, an increase in parasite reproductive success was commonly observed in the field of parasite control. For example, it was suggested that drug therapy should eventually lead to more fertile nematodes (i.e., higher parasite fitness), and therefore can act as a selection pressure on parasite evolution (Leignel & Cabaret 2001). In contrast, malaria protozoan parasites adopted reproductive restraint when stressed by drugs (Reece et al. 2010).

Although there was no main effect of environmental (i.e. short-lasting) diazinon exposure on parasite spore production, there was a difference between the two tested clones: one clone produced more spores without pesticide stress, whereas the other clone produced more spores under diazinon exposure. A similar effect of host genotype-by-environment interaction on parasite fitness has been detected across other host-parasite systems (reviewed in Wolinska & King 2009). The other studied parasite fitness trait, infectivity, also differed among the clones, but was neither affected by evolutionary nor by environmental pesticide exposure. This suggests that genetics are more important than host physiology, as potentially influenced by environment (Vale & Little 2009).

Overall, host fitness was reduced due to parasite exposure; infected Daphnia had lower reproductive output and shorter lifespan (as in Lohr et al. 2010; Yin et al. 2011). However, parasite virulence differed according to the parasite evolutionary history: parasites that
evolved with diazinon (PD) were less virulent than parasites raised on a host community not exposed to diazinon (PC). Hosts infected with PD produced more offspring and had higher survival. Such long-lasting pesticide exposure leading to evolution of lower virulence contrasts with most studies testing for environmental effects of pesticide exposure, which have observed an increased parasite virulence, particularly in *Daphnia*-microparasite systems (e.g., Coors et al. 2008; Coors & De Meester 2011). Moreover, an increase in parasite virulence under conditions stressful for hosts is commonly observed in other systems, also as a consequence of evolutionary treatment. For example, Mennerat et al. (2010) suggested in their review that intensive fish farming is likely to select for more virulent parasites. However, repeatedly used imperfect vaccines do not affect parasite virulence at all (Gandon et al. 2001; Alizon & van Baalen 2005). Thus, most studies testing for environmental or evolutionary effects on parasite virulence are contrary to our findings. It could be that the environmental stressor applied here, diazinon, suppresses the transmission of parasites which consequently leads to lower virulence (e.g., Frank 1996). Indeed, transmission-blocking vaccines can select for lower virulence if there is competition among the parasite strains (Gandon et al. 2001).

Parasite fitness and virulence seem to be interconnected; the later the host dies the longer the duration of spore production. Also in our experiment, parasites showing an elevated spore load (the PD parasites) caused less host mortality. Diazinon somehow causes a decoupling of parasite growth and virulence. The conventional wisdom (avirulence hypothesis) suggests that lower parasite virulence, and therefore increasing parasite fitness, should be favoured by natural selection as there is more time to exploit hosts (discussed for example in Anderson & May 1979; Alizon et al. 2009). Alternatively, the “virulence-transmission trade-off” hypothesis, in which an increased transmission rate increases virulence is now commonly accepted (Anderson & May 1982; Ewald 1983). Although our pattern of virulence evolution is more in line with the avirulence hypothesis, it is still possible that virulence decreases and transmission rate increases assuming a trade-off between those two traits. Alizon & van Baalen (2005) modelled that an additional activation of the hosts immune response leads to a decrease in parasite virulence and increase of transmission rate. It is possible that diazinon could have activated hosts immune response. Organophosphosphate pesticides have been shown to affect the immune function of invertebrates, fish, and higher vertebrate wildlife (reviewed in Galloway & Handy 2003).

Previous work has focused on acute effects of pollution on host and parasite fitness, as well as on parasite virulence (e.g., Coors & De Meester 2008; Coors et al. 2008; Kreutz et al. 2010). Here, we additionally considered the evolutionary implications of parasite exposure to
diazinon (host community exposed for 99 days) by using a mixture of parasite spores from parasites evolved in five independent experimental lines. We therefore tested for a mean evolutionary response in the presence/absence of the pesticide. The evolutionary effects of pesticide exposure (resembling natural concentrations) increased parasite fitness, but decreased parasite virulence, and might therefore have an important impact on disease dynamics in aquatic systems. More empirical studies testing the effect of a persistent environmental stress on parasites would improve our understanding of the evolution of virulence in nature.

**Acknowledgment**
We thank Esther Keller and Christine Dambone for assistance in the lab and providing food for the *Daphnia* as well as Christoph Tellenbach and Otto Seppälä for statistical advice. The manuscript was substantially improved by comments from Hanna Hartikainen, Kayla C. King, Tom Little and Samuel Alizon. This research was funded by the ETH Board (CCES-GEDIHAP).

**References**


Chapter 6

Environment-dependent virulence affects disease spread in host populations

Claudia C. Buser & Kayla C. King
(In preparation)

Abstract
Parasite virulence can be mediated by variation in the host environment. Empirical work has documented the environmental conditions under which virulence is expected to increase or decrease. However, less attention is given to how condition-dependent virulence affects the epidemic spread of disease in a host population. Here, we examine epidemiological models in which parasite virulence (i.e., infected host mortality rate) is environment-dependent. We explicitly explore how eight qualitatively different relationships between parasite virulence and environmental quality alter the ability of a parasite to invade in “poor” and “good” host environments. Our results suggest that these relationships can drastically affect whether highly virulent parasites can spread in the population. The study highlights the consequences of environmental quality for epidemics. We further discuss how variation in the shape of the virulence-environment relationships may also affect the strength of selection on natural host populations across time and space.

Keywords
Condition-dependent virulence, environment, host-parasite interactions
Introduction

The spread of disease epidemics is highly dependent on certain host and parasite traits, such as reproduction, transmission and virulence (e.g., Anderson & May 1981; Anderson & May 1986). Parasite virulence, which can refer to parasite-induced mortality rate or reduction in host fitness, may play an especially important role (Frank 1996). Disease dynamics can be affected by changes in virulence caused by relatedness among infecting strains (Frank 1992, 1996), host population structure (Boots et al. 2004) and parasite transmission mode (Day 2001), for example.

Parasite virulence has also been repeatedly shown to be influenced by environmental variation in a variety of host-parasite systems (Lazzaro & Little 2009; Wolinska & King 2009). However, the effects on virulence differ depending on the environmental factor of interest. Most empirical data to date show that infected hosts die more in environments where hosts are stressed in poor conditions: mortality of infected hosts increase because of exposure to near-starvation conditions (Jokela et al. 1999; Ferguson & Read 2002; Brown et al. 2003; Tseng 2004; Jokela et al. 2005), pesticides (Coors et al. 2008; Coors & De Meester 2011) and high temperatures (Mitchell & Read 2005). Virulent effects may also be reduced if hosts are better able to tolerate infection in improved environmental conditions (Vale et al. 2011). Conversely, when conditions are good, parasites might also induce a higher host mortality (see "high food" example in Vale et al. 2011). Overall, the relationship between virulence and environmental quality can take many directions (see additional examples in Thomas & Blanford 2003) and likely varies among host-parasite interactions. The exact shape of the relationship, however, over a range of conditions remains largely speculative and untested for most systems.

Epidemiological models are increasingly integrating the environment to determine how local conditions affect epidemiology (e.g., Lafferty & Holt 2003; Hall et al. 2007; Hall et al. 2009), as well as host-parasite coevolution (Mostowy & Engelstaedter 2011). Indeed, theoretical studies have found that abiotic conditions can accurately describe some host-parasite population dynamics in nature (e.g., Hall et al. 2006; Hall et al. 2009). However, little is known of the influence of condition-dependent virulence on the spread of disease in a host population. Here, we explored how eight different relationships between virulence and environmental quality alter the ability of a parasite to invade across a gradient of host conditions.
**Model and Results**

We use a ‘classic’ differential SI model (Anderson & May 1981), with disease-induced mortality and mass-action transmission, to follow how different functional relationships between virulence and environmental quality affect the spread of disease across an environmental gradient (ranging from bad to good). We are interested in virulence from an ecological perspective, as the immediate effect of parasite infection on host mortality, not as a parasite trait.

The change in susceptible host (S) and infected host densities (I) was tracked as follows:

\[
\frac{dS}{dt} = b(S + I)(1 - c(S + I)) - \beta SI - dS \tag{1A}
\]

\[
\frac{dI}{dt} = \beta SI - dI - \alpha I \tag{1B}
\]

The rate of change in the densities of susceptible hosts (equation 1A) is dependent on the number of susceptible and infected hosts, the strength of density dependence on birth rate (c), the birth rate of uninfected and infected hosts (our parasite does not affect birth rate of the infected animals) (b), the transmission rate (β) and the natural background mortality (d). The rate of change in densities of infected hosts (equation 1B) depends on transmission rate (β) as well as host death, as a result of natural background mortality (d) or mortality due to infection, otherwise known as virulence (α).

We calculate all possible equilibria: dS/dt=dI/dt=0, the equilibrial density of susceptible hosts with no infected hosts (disease-free or boundary equilibrium, \(S^*_\text{bnd}\)) and persisting with parasites (the endemic or interior equilibrium, \(S^*_\text{int}\)):

\[
S^*_\text{bnd} = \frac{(b - d)}{bc} \quad S^*_\text{int} = \frac{(\alpha + d)}{\beta} \tag{2}
\]

To examine the conditions under which disease can spread in a host population, we calculate \(R_0\), the parasite reproductive ratio. This is the expected number of secondary infections generated from a single infected host individual. Calculating \(R_0\) is a standard approach to analysing similar epidemiological models (Anderson & May 1986).

\[
R_0 = \frac{S^*_\text{bnd}}{S^*_\text{int}} \tag{3}
\]
Parasites can spread in a population when $R_0 > 1$. As $R_0$ increases, so does disease spread. By substituting $S^\text{bad}_t$ and $S^\text{int}_t$ from equation 2 into equation 3, we see that $R_0$ increases when $\beta$ and $b$ are high and $c$, $\alpha$ and $d$ are low. It is never the case that $d > b$, which excludes the possibility of $R_0 < 0$.

$$R_0 = \frac{\beta(b-d)}{bc(d+\alpha)}$$  \hfill (4)

We assume our environment ($E$) varies between 0 ("bad") and 1 ("good") and affects birth rate (equation 5) in a positive linear fashion. As the quality of environment improves (as $E$ approaches 1), more resources are available for reproduction. Therefore, $b_r$ is the innate birth rate in an ideal, good environment.

$$b = b_r E$$  \hfill (5)

We set environmental conditions to alter virulence/the mortality rates of infected hosts ($\alpha$) in eight mutually exclusive functional relationships (see also Fig. 1):

$$\alpha_1 = \alpha_r E$$  \hfill (6)

$$\alpha_2 = \alpha_r \sqrt[3]{E}$$

$$\alpha_3 = \frac{\alpha_r}{1 + e^{-(16E+8)}}$$

$$\alpha_4 = \alpha_r (1 - E)$$

$$\alpha_5 = \alpha_r - \alpha_r \sqrt[3]{E}$$

$$\alpha_6 = \alpha_r - \left(\frac{\alpha_r}{1 + e^{-(16E+8)}}\right)$$

$$\alpha_7 = 4\alpha_r E(1 - E)$$

$$\alpha_8 = 4\alpha_r E(E-1) + \alpha_r$$

We set the following parameters: $\beta=0.001$, $c=0.01$ and $d=0.01$. The reference birth rate of hosts ($b_r$) is 0.9. Five levels of peak reference parasite virulence are examined to explore parameter space between 0.01 and 0.2 (1-20% of infected animals die at peak virulence). Values of $\alpha$ vary from 0 to $\alpha_r$. As a reminder, the influence of the environment on $R_0$ depends
on how the environment affects the ability of the parasite to harm the host or on the ability of the host to resist the parasite. Virulence may increase as the availability of more host resources can boost parasite exploitation rates ($\alpha_1$: positive linear, $\alpha_2$: positive cube root, $\alpha_3$: positive sigmoidal). In contrast, virulence may decrease in a good environment from higher host resistance/tolerance and less conflict over shared resources ($\alpha_4$: negative linear, $\alpha_5$: negative cube root, $\alpha_6$: negative sigmoidal). Some parasites may have a fitness optimum in an intermediate environment ($\alpha_7$: negative quadratic). However, others may be successful in a bad environment because of weakened host resistance (Coors et al. 2008; Rohr et al. 2008) and also in a good environment as hosts may have higher resource levels to exploit (Seppälä et al. 2008; $\alpha_8$: positive quadratic).

**Fig. 1:** Relationships between virulence and host environmental quality, from “bad” to “good”. Environmental conditions can have a (a) linear, (b) cube root, (c) sigmoidal, or (d) quadratic relationship with virulence.
We delineate changes in the reproductive ratio \( (R_0) \) with environment \( (E) \) by calculating the partial derivative. Changes in \( R_0 \) with environmental quality depend on two components:

\[
\frac{\partial R_0}{\partial E} = R_0 \left[ \frac{1}{b-d} \frac{\partial b}{\partial d} + \frac{1}{\alpha+d} \frac{\partial \alpha}{\partial d} \right]
\]

(7)

Component A encompassed the dependence of birth rate on environmental quality and component B, the dependence of virulence. With increasing birth rate and virulence, component A and B become smaller, respectively. As component B increases relative to A disease spread decreases. To examine the specific changes in \( R_0 \) with improved environmental quality, we consider the different functional relationships between virulence and environmental quality: positive relationship \( (\alpha_1, \alpha_2, \alpha_3) \), negative relationship \( (\alpha_4, \alpha_5, \alpha_6) \), intermediate optimum \( (\alpha_7) \) and intermediate minimum \( (\alpha_8) \). For \( \alpha_1, \alpha_2 \) and \( \alpha_3 \), parasite spread reduces as a consequence of improved environmental conditions (Fig. 2a, b, c). The reduction in \( R_0 \) is less pronounced in the positive cube root relationship \( (\alpha_2) \) (Fig. 2b), but more so in the positive sigmoidal relationship \( (\alpha_3) \); (Fig. 2c). For the negative virulence-environment functional relationships \( (\alpha_4, \alpha_5 \) and \( \alpha_6) \), \( R_0 \) increases with environmental quality (Fig. 2d, e, f). Virulence with an intermediate optimum \( (\alpha_7) \), allows for those parasites to spread in either poor or pristine environments only (Fig. 2g). Conversely, parasites with minimal virulence in intermediate environments \( (\alpha_8) \) are inhibited from spreading in environments of either bad or good quality (Fig. 2h).

Less virulent parasites were found to spread in all conditions (Fig. 2). However, the ability of highly virulent parasites (i.e., \( \alpha_r = 0.2 \)) to spread strongly depended on the environmental conditions and the virulence-environment relationship. Under the positive cube root \( (\alpha_2) \) relationship, virulent parasites are barely able to reproduce and transmit in poor environments and not at all as conditions improved. In poor environments, virulent parasites with a positive linear \( (\alpha_1) \), a positive sigmoidal \( (\alpha_3) \), and a negative quadratic relationship \( (\alpha_7) \) could spread, and the positive sigmoidal relationship \( (\alpha_3) \) resulted in the best conditions for virulent epidemics. In good environments, however, virulent parasites were successful given negative relationships \( (\alpha_4, \alpha_5, \alpha_6) \) and the negative quadratic relationship \( (\alpha_7) \). Virulent parasites were able to spread across the largest range of environments under the positive quadratic relationship \( \alpha_8 \), but not under extremely poor or good conditions.
Fig 2.: Spread of parasites ($R_0$) of low to high virulence (five lines from lightest grey to black represent $\alpha_1 = 0.01, 0.05, 0.10, 0.15, 0.20$) in “bad” (0) to “good” (1) environmental conditions. Eight relationships between virulence and environmental quality are shown. Virulence increases with quality – (a) linear, (b) cube root, (c) sigmoidal – or decreases with quality (d) linear, (e) cube root, (f) sigmoidal. Parasite virulence may also be highest in intermediate environments (g) negative quadratic, or lowest in intermediate environments (h) positive quadratic.

Discussion

There is mounting empirical evidence that environmental conditions can play an important role in mediating host-parasite interactions (Lazzaro & Little 2009; Wolinska & King 2009). Our results suggest that while less virulent parasites easily spread over a range of environments, as virulence increases, parasites depend more on environmental quality and the conditions under which they can optimally exploit hosts (i.e., virulence-environment functional relationship). In addition, it is when their virulence is reduced by environmental conditions that otherwise highly virulent parasites are able to spread. For example, a larger intensity of disease spread requires low virulence caused by condition-dependence: among functional relationships $\alpha_1$-$\alpha_3$ (positive relationship), $R_0$ is highest in $\alpha_3$ (positive sigmoidal, Fig. 2c) because poor environments maintain the lowest levels of virulence. Virulence limits
disease spread in the present model, a finding in agreement with other epidemiological models without a trade-off between virulence and transmission (see Keeling & Rohani 2007), albeit in a constant environment. However, we show how virulence interacts with the environment and plays out across a range of environmental conditions.

The best conditions for disease spread may not always be optimal for parasite fitness over the long term. If parasite virulence has a positive functional relationship with environmental quality (Fig. 2a-c), epidemics are more likely to occur in a poor environment. The combination of poor conditions and parasite spread could be devastating for a host population and suboptimal for parasite fitness, given that bad environmental conditions may additionally impair other aspects of host health (e.g., growth, maintenance; Strong & James 1993; Boersma & Vijverberg 1994). Therefore, negative relationships with environment quality (Fig. 2d-f) may be the most optimal for parasite epidemics in the long term. Indeed, the majority of studies examining condition-dependent virulence show parasite virulence at its peak under stressed host conditions (e.g., Jokela et al. 1999; Ferguson & Read 2002; Tseng 2004). As a result, compared to conditions in a laboratory setting which are ideal, parasites may do more damage to hosts, and thus have greater impact on host populations, in the wild where hosts experience stressful, natural environmental conditions (Jaenike 1992; Jaenike et al. 1995).

By mediating the impact of infection on hosts, environmental factors can alter the strength of parasite-mediated selection (see examples in Lazzaro & Little 2009; Wolinska & King 2009). Our results suggest a mechanism by which the environment may do this: favour the spread of virulent parasites in some environments and not others. Environments conferring an advantage to epidemics of highly virulent parasites may impose stronger selection than those in which they cannot spread. Under relationship $\alpha_3$ (increasing sigmoidal), for example, epidemics of virulent parasites can occur in a poor host environment, but are inhibited in a good host environment (Fig. 2c). In addition, while some relationships yield little change in $R_0$ across the range of environmental conditions (Fig. 2b, 2g), parasite reproduction can greatly vary in others (Fig. 2c, 2f, 2h). Rapid changes in the strength of selection may result from environmental fluctuations or geographic clines depending upon the environment-virulence relationship. Thus, variation in environmental conditions across populations or within populations over time may contribute to host-parasite coevolutionary dynamics (Mostowy & Engelstaedter 2011) or the geographic mosaic of coevolution (Thompson 2005).

Our model makes important predictions of how the interaction between parasite virulence and environmental condition affects disease spread. It emphasizes the need for studies that
examine the relationship between environmental factors and virulence in natural systems vulnerable to environmental variation over space or time. Ideal candidates would be organisms and parasites which interact across seasons or a range of habitats, perhaps even those that are anthropogenically-degraded.

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Reference
Chapter 7

Concluding remarks and recommendations for future research

Claudia C. Buser
With this thesis, I aimed to detect pesticide-mediated effects on host-parasite interactions. Specifically, I examined the influence of pesticide pollution on host and parasite fitness, as well as virulence, and tested the effect of multiple stressors on host population dynamics and disease spread.

Firstly, I would like to discuss the effect of pesticide on host fitness and host community structure without taking parasites into account. I showed that both acute and chronic exposure to the pesticide diazinon reduced host fitness. By exposing *Daphnia* to acute effect diazinon concentrations (Chapter 2), test species and clones varied in survival, and therefore in sensitivity, towards the toxicant. Also, the exposure to chronic and sub-lethal diazinon concentrations reduced the number of offspring (Chapter 5), but for only one of the two clones, pesticides caused higher mortality and lower fecundity. In Chapter 4, I found that the fecundity of the competing taxa depended on the pesticide: *D. galeata* was more fecund when additionally exposed to pesticide whereas the hybrids were more fecund when not exposed. Pesticides have been previously shown to affect host life-history traits (see for example Hanazato 2001), but pesticide concentrations used in most experimental tests are often higher than concentrations found in aquatic systems (Relyea & Hoverman 2006). Thus, I aimed to use ecologically-relevant diazinon concentrations (e.g., Konstantinou et al. 2006; Wittmer et al. 2010), and I found that such sub-lethal concentrations drastically reduce host fitness. In general, variation in response to pesticide exposure can be due to variation in traits, such as size, mode of respiration, feeding type, habit, and life-cycle duration (Baird & Van den Brink 2007; Rubach et al. 2010), as well as variation in biotransformation processes and in sensitivity of the target site (e.g., a certain enzyme) inside an organism (Barata et al. 2001). Additionally, it can be because of adaptation towards the chemical compound (Klerks & Weis 1987). Consistently observing this genetic variation in sensitivity towards the pesticide diazinon suggests that more than one genotype should be used in toxicology studies. This is necessary to take into account the variation within and between species and to minimize the risk of underestimating the effects of a chemical in the wild (Cairns 1984; Chapman 2000).

Differences in the sensitivity of single genotypes to anthropogenic effects can be manifested at the community level. Pesticides did not reduce host community density, but changed the genetic structure (Chapter 4), indicating that pesticide-tolerant clones replace sensitive clones. In the Chapter 4 experiment, pesticide concentrations measured in natural habitats were able to change the genetic structure of a mesocosm experimental community. Similarly, Hanazato & Yasuno (1987) observed changes in zooplankton species composition after
ecologically-relevant exposure to insecticides, and Relyea & Hovemann (2008) demonstrated that pesticide exposure lowered zooplankton diversity and abundance in mesocosms. A loss in diversity, through exposure to a single stressor can highly impact communities making them more prone to future stressors (Spielman et al. 2004; Reusch et al. 2005). For example, reductions in genetic diversity may influence host or parasite evolutionary rates (Altizer et al. 2003). A reduction in host population diversity can also increase the risk of getting infected (Elton 1958; Sherman et al. 1988; Schmid-Hempel 1998; Alternatt & Ebert 2008) or lead to increased severity in the effects of epidemics on host populations (Duffy & Sivars-Becker 2007; Duffy & Hall 2008; Lively 2010). Furthermore, in a population exposed to pesticides, resistance towards this chemical compound may evolve. Evolving resistance can be associated with evolutionary costs and can lead to further synergistic interactions between a pesticide, and for example, parasite exposure (Jansen et al. 2011).

In this thesis, I further examined whether pesticide exposure affected parasite fitness and virulence upon single (environmental) or several (evolutionary) host generation exposures to the pesticide diazinon. In Chapter 3, exposure to the pesticide for one generation increased virulence. Pesticides has been shown to increase parasite fitness and virulence in several host-parasite systems; parasites of amphibians (Gendron et al. 2003; Rohr et al. 2008; King et al. 2010), fish (Kreutz et al. 2010), and oysters (Chu & Hale 1994). Also virulence of a bacterial parasite, Pasteuria ramosa, was demonstrated to increase when it’s host, Daphnia magna, was exposed to the pesticide carbaryl (Coors & De Meester 2008; Coors et al. 2008; Coors et al. 2009; Coors & De Meester 2011). Interestingly, in Chapter 5, whether diazinon exposure caused lower or higher parasite virulence (i.e., production of host offspring) and/or parasite fitness (i.e., spore load) was highly dependent on the Daphnia clone infected by the parasite. In Chapter 5, the evolutionary consequences of long-lasting pesticide exposure on parasite fitness and virulence were more obvious than the environmental effects due to parasite evolution. Persistent pesticide exposure over several host generations resulted in higher parasite fitness (i.e., spore load) and lower virulence (i.e., host mortality rate and host reproduction). It would be very interesting to test, if the observed decreases in virulence is stable over time.

In general, parasite virulence is increased when hosts are exposed to pesticides in the short-term. From our virulence-environment relationships built into the epidemiological model in Chapter 6, this would correspond with a negative (decreasing) relationship (see Fig. 1d, e or f in Chapter 6). Following the assumptions of the model, I would expect that disease would be
less likely to spread in a host population inhabiting an environment of bad quality than in a population in good condition. Higher background mortality, as well as reduced transmission, select for parasites to adapt and reduce their virulence (Garnick 1992; Bull 1994; Ebert & Herre 1996; Frank 1996; Ebert & Weisser 1997; Day 2001). This was observed in Chapter 5, in which parasites under pesticide stress evolved towards lower virulence, and consequently higher fitness. Changes in parasite fitness and virulence can have consequences on disease dynamics, as well as host population dynamics. In Chapter 4, we were not able to detect such effects likely because the chosen timescale of 3 months was too short. Nevertheless, the results of Chapter 5, in addition to the simulations of Chapter 6, improve our understanding of external factors that may affect the evolution of virulence and the spread of disease.

A major goal of thesis was to determine the effect of multiple stressors on host fitness and community structure and the consequences of pesticide-meditated effects on host-parasite interactions, as well as the spread of disease. In our studies, a first clear effect of simultaneous exposure to parasite spores and pesticide was found in Chapter 3: Whereas each stressor alone did not affected host survival and parasite spores itself were not infectious, I found a reduced survival of Daphnia by exposure to parasite spores which did not cause infection in combination with pesticides. It has been shown that a combination of abiotic and biotic stressors is often more harmful than either stressor alone (e.g., Hanazato & Dodson 1995; Folt et al. 1999; Relyea 2003; Sih et al. 2004; Marcogliese & Pietrock 2011). The effect of multiple stressors can be synergistic, antagonistic or additive, varying by response level (Crain et al. 2008). Mostly synergistic or no effect of simultaneous exposure has been observed (Holmstrup et al. 2010). In the Daphnia-endoparasite system clear interactions between pesticide and parasites were found, namely increased host mortality and host castration (Coors & De Meester 2008; Coors et al. 2008; Coors & De Meester 2011).

Pesticides, including diazinon have been shown to have immunomodulatory effects (Oostingh et al. 2009; Holmstrup et al. 2010). The immune system seems to be condition- and genotype-dependent (Carius et al. 2001; Mucklow & Ebert 2003; Rolff & Siva-Jothy 2003) which can lead to genetic variation in resistance (e.g., Dybdahl & Lively 1998; Baer & Schmid-Hempel 1999) and infection (e.g., Lively 1989; Ebert 1994). I repeatedly found that if the host is susceptible, and if pesticides enhance parasite virulence depends on the host genotype. For example, in Chapter 5, I observed interactions between host genotype, parasite and pesticide: in one clone, parasite spore load was higher when the clone was exposed to diazinon, whereas for the other clone, parasite spore load was higher when the clone was not exposed to
diazinon. G×E×E interactions were also observed when looking at taxon susceptibility in Chapter 4. I observed that the *Daphnia* hybrids (but not *D. galeata*) were more infected when exposed to the pesticide compared to unexposed hybrids. In this experiment, reduced hybrid fecundity under pesticide exposure, as well as the higher susceptibility of hybrids, indicates that hybrid *Daphnia* species are more adversely affected by the environmental stressors, parasites and pesticides. Interactions between host-parasites and environment are increasingly being considered, particularly at the genotype level, to affect host-parasite interactions and coevolution (Lazzaro et al. 2008; Wolinska & King 2009).

In the studies conducted in this thesis I found effects of multiple stressors on an individual level, but host density and community composition was not affected by simultaneous exposure (Chapter 4). At that level, parasite stress was the stronger selection factor. This could be due to the low pesticide concentrations used in that experiment. In general, a dose effect is very important. For example, depending on the amount of parasite dose (e.g., Ebert et al. 1998) or chemical concentrations (see Chapter 2) used in the experiment, the harm of a single stressor on host fitness can vary, and this could also affect the interplay between those stressors.

In summary, the effects of anthropogenic activities can be manifested at the genotype, population, and community levels of host-parasite interactions. Both parasite and pesticide stress can mediate host fitness and community structure. Pesticides can affect key parasite traits: fitness and virulence, and parasites can act together with ecologically-relevant levels of pesticide pollution to enhance harm to host health. However, the strength of the combined exposure to pesticides and parasites are highly variable, and must not be generalized. Effects depend on host genotype, as well as the pesticide type, parasite dose, and the target host level (individual, community). Nevertheless, given that parasites are ubiquitous, and anthropogenic stress is a major cause of disease emergence in wildlife populations (Daszak et al. 2001), predicting joint effects of stressors are important. In fact, combined effects of multiple stressors must be considered for risk assessment of environmental pollution or conservation biology, to make predictions more ecologically realistic and useful. Moreover, the genetic diversity of exposed populations might be at higher risk, resulting in changes in the genetic structure and opportunity for selection in whole ecosystems.
Discussion & Conclusion

Chapter 7

Recommendations for future research direction

Resurrection ecology
Using hatchlings from dormant eggs is a strong tool to study environmental effects on a decade-long evolutionary scale. In Chapter 3 I failed to show evolutionary responses to multiple stressors over time, as I did not have a visible sign of infection and therefore I could not quantify infection success (spore density) over time. Nevertheless, from this study I learned that for investigating multiple stressors over several decades it would be an advantage to use several stressors which are all historically well documented or measurable in the sediment. For example, using an approach as in Chapter 3, the anthropogenic stressor could be a heavy metal (e.g., lead) which is accumulating in the sediment. In general, resurrection ecology creates great opportunities to answer questions related to disturbance ecology and can be applied to questions concerning disturbance effects on natural populations on a multi-decade scale. Having knowledge of the distribution of environmental stressors in the past provides a mechanism to follow changes in community structure, for example, by linking genetic variation through time to environmental changes along extended time axes.

Combining field studies with mesocosm and laboratory studies
Another approach is to do field studies to examine the influence of abiotic stressors on host-parasite interactions (e.g., Minguez et al. 2009; King et al. 2010; Marcogliese et al. 2010; Wenger et al. 2010). Such field studies reflect realistic conditions, but are sometimes difficult to interpret; it is difficult to control for confounding factors. The combination of field studies and controlled mesocosms or laboratory studies helps to better interpret effects of multiple stressors. Furthermore, potential confounding factors (e.g., trait-mediated effects or density-mediated effects) can be disentangled and accounted for. Moreover, genes involved in host-parasite interactions can be identified from laboratory experiments and reconciled with studies in natural populations in stressed versus non-stressed habitats. For example, one can examine the difference in expression of the candidate genes in more or less polluted habitats.

Interaction of disease and anthropogenic stress factor
When testing the effect of multiple stressors on host fitness in Chapter 3 we observed that combined exposure of parasite spores and pesticide-reduced host survival significantly compared to when exposed to a single stressor. This suggests that under certain conditions the immune system can cope with one stress, but is compromised with multiple stressors. Moreover, I demonstrated that host clones vary in susceptibility to parasites based on their
ecological habitat. The next step is to test on a range of clones varying in susceptibility to one parasite strain (genetic diversity of susceptibility to parasites) if an anthropogenic stress factor can influence the development of the disease. Are less susceptible clones fitter when exposed to combined stress factors than highly susceptible clones? The expectation would be that less susceptible individuals may have a stronger immune system, one that is less affected by a second stressor, like diazinon, than those with high parasite susceptibility. Such a study could be further expanded by using several parasite strains instead of one strain and exposing them in a matrix (e.g., Carius et al. 2001), but under varying concentrations of pesticide. Revealing G×G×E effects, the results would show if anthropogenic stress affects host-parasite specificity.

**Epidemiological models including environment**

Epidemiological models are increasingly integrating the environment to determine how local conditions affect disease spread (e.g., Lafferty & Holt 2003; Hall et al. 2007; Hall et al. 2009), as well as host-parasite coevolution (Mostowy & Engelstaedter 2011). Our model from **Chapter 6** could be expanded to ask: (1) What is the optimal level of virulence in a fluctuating environment (when virulence is environment-dependent), and (2) How does the optimal level change with different functional relationships between virulence and environmental quality? In my opinion, models have to be tested with empirical data. Therefore, the relationships between environmental factors and virulence should be quantified in natural systems vulnerable to environmental variation over space and time. Ideal candidates would be organisms and parasites which interact across seasons or a range of habitats, perhaps those that are anthropogenically-degraded.

**References**


CLAUDIA CAROLINA BUSER

EDUCATION

2008 – present  Ph.D. position, Department of Aquatic Ecology, Eawag, Dübendorf, and Institute of Integrative Biology, ETH Zürich, Switzerland

2007 – 2008  Master of Science in Biology, Systematics and Evolution, Zoological Museum, University of Zürich, Switzerland

2003 – 2007  Bachelor of Science in Biology, University of Zürich, Switzerland

LANGUAGES

German  First language

Spanish  Fluent in spoken and written

English  Fluent in spoken and written

French  Fluent in spoken and written

PUBLICATIONS


MANUSCRIPTS IN REVISION

**Buser, C.C.**, King, K.C. Environment-dependent virulence and transmission affect disease spread in host populations.


SUBMITTED MANUSCRIPTS


**Buser, C.C.**, Wolinska, J, Spaak, P. Evolutionary and environmental effects of pesticide exposure on parasite fitness and virulence. *Journal of Evolutionary Biology, in review.*

Kashian, D., **Buser C.C.** The role of diapausing eggs in disturbance ecology. Book chapter in Resurrection ecology, C. W. Kerfoot, ed.

MANUSCRIPTS IN PREPARATION

**Buser, C.C.**, Fox, J.A., Kretschmann, A., Brede, N., Spaak, P. Variation within and between taxa cannot be ignored in toxicity test.

**Buser, C.C.**, Jansen, M. Pauwels, K., De Meester, L. Spaak, P. Combined exposure to parasites and pesticides causes increased mortality in the water flea *Daphnia.*


INTERNERSHIP

02/2009 - 06/2009 Laboratory of Aquatic Ecology and Evolutionary Biology, Katholieke Universiteit Leuven, 3000 Leuven, Belgium
## WORK EXPERIENCES

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<tr>
<td>10/2006 – 04/2007</td>
<td>Research assistant</td>
<td>Zoological Museum, University of Zürich, Switzerland</td>
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<tr>
<td>07/2006 – 10/2006</td>
<td>Research assistant</td>
<td>Marine Biological Association of the United Kingdom, Plymouth, United Kingdom</td>
</tr>
<tr>
<td>07/2003 – 09/2003</td>
<td>Voluntary service</td>
<td>Foundation for information and research on marine mammals (firmm), Pedro Cortés, 11380 Tarifa, Spain</td>
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## TEACHING EXPERIENCES

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<tr>
<td>2008</td>
<td>Supervising undergraduate student</td>
<td>Department of Aquatic Ecology, Eawag, Switzerland</td>
</tr>
<tr>
<td>2007</td>
<td>Tutor in the reproduction course</td>
<td>Zoological Museum, University of Zürich, Switzerland</td>
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<tr>
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<td>Tutor in the systematic zoology course</td>
<td>ETH Zürich, Switzerland</td>
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<td>2007 and 2005</td>
<td>Tutor in systematic zoology course</td>
<td>University of Zürich, Switzerland</td>
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<td>2004</td>
<td>Tutor in chemistry course</td>
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## INVITED PRESENTATION

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<td>10/2010</td>
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<td>Buser C.C., Wolinska J., Spaak P.</td>
<td>“The influence of a pesticide on host-parasite interactions”</td>
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## CONFERENCE PRESENTATIONS

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<tr>
<td>08/2011</td>
<td>ESEB meeting, Tübingen, Germany</td>
<td>Buser C.C., Ward P.I., Bussière L.I.</td>
<td>“Maternal perception of the larval environment affects offspring performance but not paternity” (poster)</td>
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<td>03/2011</td>
<td>GEDIHAP- annual meeting, Zürich, Switzerland</td>
<td>Buser C.C., Wolinska J., Spaak P.</td>
<td>“Disease and pollution alter Daphnia community structure” (talk)</td>
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02/2011  Eco-Evolutionary Dynamics, Leuven, Belgium: Spaak P, **Buser C.C.** Schöbel C, Wolinska J “The coexistence of hybrid and parental *Daphnia* – the role of parasites” (talk)

11/2010  SETAC, Portland, USA: **Buser C.C.**, Wolinska J., Spaak P. “The influence of an insecticide on *Daphnia* individuals and populations exposed to parasites” (poster)

06/2010  ASLO Summer Meeting, Santa Fe, USA: **Buser C.C.**, Wolinska J., Jansen M., Spaak P. “The influence of a pesticide (Diazinon) on *Daphnia*-parasite interactions” (talk)


10/2008  Symposium on Sexual Selection, Sperm Competition & Cryptic Female Choice in Honour of Paul Ward, Zürich, Switzerland: **Buser C.C.**, Bussière L.I. “Do female yellow dung flies adjust oviposition and paternity according to perceived levels of larval competition?” (talk)

04/2008  BEES, Zürich, Switzerland: **Buser, C.C.** “Do female dung flies adjust paternity according to the level of larval competition?” (talk)

09/2007  Fortgeschrittenenseminar in Evolutionsbiologie, Kilchberg, Switzerland: **Buser C.C.** “Oviposition behaviour of the yellow dung fly“ (talk)

### WORKSHOP

09/2009  Host-Parasite Coevolution Workshop, Frauenchiemsee, Germany
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