Ecological and evolutionary dynamics in *Daphnia* metapopulations

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Science is always wrong.
It never solves a problem without creating ten more.[1]

George Bernard Shaw

To know that no one before you has seen an organ you are examining, to trace relationships that have occurred to no one before, to immerse yourself in the wondrous crystalline world of the microscope, where silence reigns, circumscribed by its own horizon, a blindingly white arena — all this is so enticing that I cannot describe it.[2]

Vladimir Nabokov

[1] I chose this quotation from G. B. Shaw, as it describes in my view fundamental principles of science. In science – contrary to religion – there should be no dogmata which are unquestionable (even this quotation itself…). Our work is not the absolute truth, and therefore it may be wrong. However, by discovering the unknown, we come to a better understanding. We scientists should not hesitate to disapprove existing knowledge and to say that something is wrong or different. We should question very much the work of ourselves, as well as the work of others. We should be courageous enough to state new hypotheses, which will increase our understanding of nature. In my eyes this quotation also contains the fundamental principle of hypotheses. As a first step, a null hypothesis is formulated, which states that there is no difference at all. By rejecting the hypothesis, and thus stating its wrongness, science makes progress. Most of the time when we get a step further, new questions arises, and it may be worth to pursue some of them. Also in my work I questioned some existing statements, collected data and rejected hypotheses. This added some new aspects to the understanding of the studied *Daphnia* metapopulations. Last but not least, new questions arose as well.

[2] This quotation summarizes all hours I was sitting at the microscope, was out on the islands in the archipelago around Tvärminne or wrote some R code late at night: beyond all difficulties I found beauty.

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Summary

Many animal and plant species occur in metapopulations that are assemblages of spatially delimited local populations coupled by some degree of migration. The occupation of habitat patches may change over time due to local extinction and colonisation. Migrants colonise empty habitat patches, which often leads to founder effects. They may also invade existing populations, and thereby increase local genetic diversity. Besides gene flow in the focal species, migrants may evade parasites or parasites may co-migrate with their hosts. Often, migrants are not a random subset of their population of origin, and populations may differ in their contribution of migrants. Due to the evolutionary and ecological significance of migrants, it is important to know their number and populations of origin. Parasites may drive the evolution within host populations. But they also influence the success of migrants and thereby gene flow between populations.

I studied ecological and evolutionary dynamics in *Daphnia* metapopulations. Three interacting species of *Daphnia* – namely *D. magna*, *D. longispina* and *D. pulex* – occur sympatrically along the coast of southwest Finland. They live in ephemeral freshwater rock pools of various size and reproduce asexually during most of the summer. The sexually produced migration stage, the so-called ephippium, is essential to survive harsh environmental conditions such as desiccation during summer or the freezing during winter. There exist two different hypotheses on the origin of migrants in this metapopulation. One hypothesis assumes a Levins' type metapopulation, with no differences between the patches, while the other suggests a mainland-island model, where long-lived populations in large patches are the source of migrants. In a first step, I quantified the ephippium production of populations in various sized natural rock pools and in containers under outdoor conditions. Populations in larger habitats produced more ephippia but the increase was much smaller than the increase in habitat size, and the numerical dominant populations in small rock pools produced a substantial number of ephippia.

In the next chapter, I show that desiccation, which is a common phenomenon in the natural rock pools, is not detrimental for the populations. This year's ephippia are sufficient to survive a desiccation event and an ephippium bank from previous years is not required. I developed a mathematical model to predict desiccation for more than 500 individual pools over 25 years. During warm and dry periods, evaporation is high and especially shallow pools with a small surface area and vegetation tend to dry up. Nevertheless, also these pools with a high risk of desiccation are often inhabited by *Daphnia* populations. Populations in these ephemeral pools are usually short-lived, but ephippia are especially exposed to passive dispersal by wind or birds in the sediments of desiccated pools. I showed that populations in small pools (less than about 300 l volume) produced about 50 % of all ephippia. However, 90 % of all exposed ephippia originated from these populations. Exposition of ephippia on dry sediments is practically non-existing in pools larger than 1000 l. This analysis suggests that populations in small ephemeral pools are most relevant for the metapopulation dynamics.

Consistent with the predictions and the functional understanding of the production of migrants, I found increased colonisation rates after warm and dry summers. The weather in southwest Finland changed in accordance to global climate change predictions, and this led to increased dynamics in the metapopulations of the three *Daphnia* species. It is the first time that an influence of climate change on metapopulation dynamics has been shown. Furthermore, I also found changes in the whole metacommunity composition, as the three species reacted differently to climate change.

In the final chapters, I did not focus on migrants themselves, but investigated proximate effects of migration. All ephippia can migrate, but the successful invasion and establishment depends on fitness components of the hatchlings. For example, migrants infected with the microsporidium *Octosporea bayeri* are less successful than uninfected migrants. I was interested in further correlations between the fitness of a host and its natural infection status and compared the cost of resistance hypothesis with the inbreeding-infection hypothesis. I experimentally confirmed that the naturally observed infection status has a genetic basis. However, I did not find a difference in competitive abilities between naturally uninfected and cured but former infected genotypes. This suggests that resistance genes segregate independently of other fitness associated genes in this system.

A consequence of migration and dispersal is either the establishment of low-diversity and potentially parasite-free populations in newly colonized habitat patches or the introduction of new host genotypes into already existing populations, which increases local genetic diversity. Parasites may either co-migrate with their hosts or arrive independently in *D. magna* populations. I thus compared the epidemiology of *O. bayeri* in host populations of low and high genetic diversity. Following parasite prevalence over two years, I showed that the parasite spread less successful in host populations of higher genetic diversity. In the long-term, this may influence coevolution and hamper local adaptation of the parasite.

Zusammenfassung

Vorbemerkung: Diese etwas ausführlichere Zusammenfassung richtet sich an allgemein interessierte Leserinnen und Leser ohne spezielle Vorkenntnisse in der Evolutionsbiologie oder Ökologie. Die genauen Ausführungen meiner Arbeit finden sich im englischsprachigen Teil.

In meiner Arbeit habe ich Wasserflöhe der Gattung *Daphnia* untersucht. Wasserflöhe sind planktonisch lebende Krebse, welche zwischen 2 und 6 mm gross werden. Sie leben in Süsswasserteichen und ernähren sich von Algen und Bakterien, die sie aus dem Wasser filtrieren. Sie haben eine zyklisch parthenogenetische Fortpflanzung: Ein Weibchen kann sich sowohl asexuell wie auch sexuell fortpflanzen. Im ersten Fall sind alle Nachkommen eine genetisch identische Kopie der Mutter, während im zweiten Fall der Nachwuchs – so wie es bei den meisten Tieren oder dem Menschen der Fall ist – die Hälfte seines Genoms von der Mutter und die andere Hälfte vom Vater erhält.

Bei den Wasserflöhen entwickeln sich sexuell produzierten Eier nicht direkt zu jungen Wasserflöhen, sondern werden jeweils paarweise in eine Chitinhülle verpackt, welche sie vor unwirtlichen Umweltbedingungen schützt. In diesem Dauerstadium, Ephippium genannt, überleben sie sowohl das Austrocknen der Teiche wie auch das mehrmonatige Gefrieren im Winter. Zudem ist das Ephippium das Migrationsstadium, welches vom Winde verweht, oder im Gefieder von Vögeln hängen bleiben kann.

Ich habe meine Studien in Südfinnland gemacht. Dort leben die Wasserflöhe in sogenannten Schärentümpeln (englisch “rock pool”) auf den Inseln des dem Festland vorgelagerten Archipels. Vor allem im Winter herrschen sehr raue klimatische Bedingungen: starke Winde, Abrieb durch das Packeis des Baltischen Meeres und Wellen, welche über die Inseln spülen. Dadurch sind viele Inseln ohne Vegetation. Nur in der Mitte der grösseren Inseln hat es Bäume und kleine Moore. Die Schärentümpel selbst sind typischerweise vegetationslose, mit Regenwasser gefüllte Vertiefungen im Granitgestein. Ihre typische Grösse liegt zwischen 100 und 1000 Liter Wasservolumen.

Entlang der Küste Südfinnlands hat es zehntausende von Schärentümpeln, und viele davon sind von Wasserflöhen besiedelt. Die von mir studierten Wasserflöhe bilden eine sogenannte Metapopulation. Eine Metapopulation wird sowohl durch die räumliche Unterteilung der Gesamtpopulation in einzelne Teilpopulationen, wie auch durch die Prozesse von lokalem Aussterben, Migration und Neukolonisation definiert. Die Metapopulation umfasst alle von Wasserflöhen bewohnten Schärentümpel. Ephippien können in einen unbewohnten Tümpel gelangen und dort eine neue Population gründen. Jedes Jahr sterben rund 20 % aller Populationen aus, und im Schnitt werden gleich viele neue Tümpel kolonisiert. Während wir also über die ganze Metapopulation eine stabile Zahl von Populationen haben, wechseln die tatsächlich bewohnten Tümpel von Jahr zu Jahr. Das Studium von Metapopulationen ist interessant, weil viele Pflanzen- und Tierarten in solch räumlich strukturierten Populationen vorkommen, und durch menschliche Aktivitäten der Lebensraum von vielen Arten in kleine Teile zerschnitten wird.

Für das Funktionieren der Metapopulation ist entscheidend, dass Migration und Kolonisation stattfindet. In der von mir untersuchten Metapopulation weiss man aber nicht, in welchen Populationen wie viele Migranten gebildet werden. Es gibt zwei Hypothesen bezüglich der Bedeutung einzelner Populationen. Die eine Hypothese gewichtet alle Populationen gleich, während die zweite Hypothese besagt, dass für das Fortbestehen und Funktionieren der Metapopulation nur einige langlebige Populationen in sehr grossen Tümpeln entscheidend sind. Ich habe in meiner Arbeit untersucht, in welchen Populationen wie viele Ephippien gebildet werden. Ich erfasste die Ephippienproduktion in unterschiedlich grossen Habitaten in einem Experiment in Plastikgefässen und gleichzeitig in 34 natürlichen Tümpeln. Ich kann zeigen, dass die Zunahme der Habitatgrösse einen positiven Einfluss auf die Ephippienproduktion hat. Die Zunahme ist aber nicht proportional zur Grösse, und Habitate mit einem 1000-fach grösseren Volumen produzieren nur etwa sieben Mal mehr Ephippien. Ein Grossteil der Ephippien wird also in den vielen Populationen in kleinen Tümpeln produziert.

Die kleinen Tümpel trocken oft aus. Dies wurde zumeist als negativ für die Wasserflöhe angesehen, und deshalb wurden die Populationen in diesen Tümpeln auch als weniger wichtig erachtet. Beim Austrocknen stirbt zwar die planktonische Population, andererseits sind die Ephippien in den trockenen Sedimenten exponiert und können leicht vom Winde verweht werden. Ich entwickelte ein mathematisches Modell, mit dem ich das Austrocknen von Tümpeln vorhersagen kann. Damit habe ich die wichtige Variable “Austrocknen” erstmalig quantifiziert. Weiter konnte ich zeigen, dass Wasserflöhe bei erneutem Auffüllen der Teiche mit Wasser nach einer Trockenheit rasch schlüpfen und lebensfähige Populationen bilden. Diese Erkenntnis übertrug ich auf einen Langzeitdatensatz, in dem die Besiedlung von über 500 Tümpeln während der vergangenen 25 Jahren studiert wurde. In diesem Datensatz wird deutlich, dass auch austrocknungsgefährdete Tümpel regelmässig von Wasserflöhen bewohnt sind.

Wenn man die Verteilung der Populationen in den natürlichen Tümpeln, die darin produzierten Ephippien und die jeweilige Austrocknungswahrscheinlichkeit zusammennimmt, wird deutlich, dass die meisten Migranten aus Populationen in kleinen und austrocknungsanfälligen Tümpeln kommen. Es ist sogar möglich, dass die Migranten aus kurzlebigen Populationen in kleinen Tümpel die ganze Metapopulationsdynamik aufrechterhalten. Dies ist das zweite Beispiel von einer Metapopulation, in der die Populationen in kleinen und unstabilen Habitaten wichtig für die Gesamtdynamiken sind.

In ausgetrockneten Tümpeln sind die Ephippien exponiert und können so leicht migrieren. Auch in Südfinnland wurden die Sommer wegen der weltweiten Klimaveränderung wärmer und trockener. Im gleichen Zeitraum konnte ich eine Zunahme der Kolonisierungsereignisse beobachten. Ich fand die Zunahme bei drei verschiedenen Wasserfloharten, wobei das Ausmass artspezifisch war. Damit zeige ich das erste Mal einen Einfluss der Klimaveränderung auf Metapopulationsprozesse und schlussendlich auf die Zusammensetzung der Lebensgemeinschaften in den Schärentümpeln.

In den beiden letzten Teilen meiner Arbeit untersuchte ich den Einfluss von Parasiten auf Wasserflohpopulationen. Wie praktisch alle Lebewesen sind auch Wasserflöhe mit Parasiten konfrontiert. Die Wasserflohpopulationen in den Teichen werden von bis zu zehn verschiedenen Parasiten und Epibionten (auf den Wasserflöhen wachsenden Algen und Einzeller) befallen. Nicht jeder Wasserfloh und nicht jede Population ist aber gleich krank. Ich testete, ob es einen Zusammenhang zwischen dem Krankheitsstatus eines Wasserflohs und seiner Fitness gibt. Dabei verwendete ich das parasitische Mikrosporidium *Octosporea bayeri*. Mikrosporidien sind einzellige, mit den Pilzen verwandte Parasiten. In den befallenen Wasserflöhen wächst der Parasit in den Fettzellen und im Ovar des Weibchens. Kranke Tiere haben weniger Nachwuchs und sterben früher.

Ich sammelte gesunde und kranke Wasserflöhe, heilte sie und verglich danach ihre Fitness mit der Fitness von anderen Wasserflöhen. Dabei starteten in meinem Experiment immer die Nachkommen von zwei Wasserflöhen in gleicher Anzahl. Die beiden Wasserflohtypen konnte ich mit Allozym-Elektrophorese unterscheiden. Sie vermehrten sich asexuell, und am Ende des Experiments bestimmte ich ihre relative Häufigkeit. Dies ist ein Mass für ihre Fitness, d. h., wie gut sie sich gegenüber anderen Wasserflöhen behaupten können. Ich fand keinen Fitnessunterschied zwischen gesunden und geheilten, ehemals kranken Wasserflöhen. Daraus schloss ich, dass es keine Kosten für Resistenzgene gibt und dass Gene für Konkurrenzfähigkeit und Gene für Resistenz unabhängig voneinander an die Nachkommen weitergegeben werden.

Schlussendlich untersuchte ich, wie sich der Parasit *O. bayeri* in Wasserflohpopulationen von geringer und von hoher genetischer Diversität ausbreitet. Das Experiment fand unter Freilandbedingungen statt, und ich beobachtete den Prävalenzverlauf des Parasiten während zwei Jahren (Prävalenz = Prozent infizierter Wirte). Ich fand, dass sich der Parasit in Wasserflohpopulationen von hoher genetischer Diversität schlechter ausbreitet, und dass er nie so hohe Prävalenzwerte erreicht wie in den parallel beobachteten Wasserflohpopulationen von geringer genetischer Diversität.

Meine Arbeit trägt dazu bei, evolutionäre und ökologische Dynamiken in Metapopulationen besser zu verstehen. Die an den Wasserflöhen gewonnenen Erkenntnisse können teilweise auf andere Organismen übertragen werden.

Introduction

Metapopulations and metacommunities

Many animal and plant species occur in metapopulations that are assemblages of spatially delimited local populations coupled by some degree of migration (Hanski, 1999, Hanski & Gaggiotti, 2004, Levins, 1968). Migrants can colonise suitable habitat patches that are currently unoccupied. Thereby, the spatial arrangement of populations changes over time. Individual populations have a finite lifespan and eventually go extinct, while the overall stability is guaranteed by colonisations balancing local extinctions (Levins, 1970). Thus, metapopulations are defined by both the local dynamic processes and by their spatial structure (Hanski & Gaggiotti, 2004).

The study of metapopulations has received great attention since the mid 1980s, especially in the context of anthropogenic habitat fragmentation (Hanski & Gaggiotti, 2004), and the concept of metapopulations has become a key issue in modern population ecology. Metapopulations have been studied in almost every taxa, such as bacteria (Keymer et al., 2006), mites (Gonzalez et al., 1998, Ellner et al., 2001), butterflies (Ehrlich & Hanski, 2004, Saccheri et al., 1998), mammals (Lambien et al., 2004, Crone et al., 2001), or plants (Burdon & Thrall, 1999, Thrall & Burdon, 2003, Antonovics, 2004). Meanwhile, also an immense number of theoretic studies are dealing with metapopulations (e. g. reviewed in Ehrlich & Hanski, 2004, Hanski, 1999, Hanski & Gaggiotti, 2004, Holyoak et al., 2005).

Open questions and a lack of empirical data in metapopulation biology are especially concerning migration and gene flow (Clobert et al., 2001, Hanski & Gaggiotti, 2004). Migration is a key process in metapopulations: migrants balance local extinctions of populations by colonising new, previously empty habitat patches (Clobert et al., 2001, Townsend et al., 2000). They may not only colonise empty patches, but also invade into already established populations, leading to gene flow. By that, they can rescue resident populations from extinction due to demographic, stochastic or genetic processes (called “rescue effect”, (Brown & Kodrick-Brown, 1977), see also effects due to hybrid vigour in Ebert et al. (2002)). While mutations create genetic diversity *de novo*, migrants will spread and introduce new alleles from other populations at much higher rates than they would occur by mutation. Thus, local genetic diversity may increase due to immigrants. However, newly established populations may have a low genetic diversity due to founder effects (Ebert et al., 2002, Haag et al., 2005, Haag et al., 2006). Finally, migration can be a way to escape parasites (Torchin et al., 2003, Altermatt et al., 2007), but at the same time parasites may also co-migrate with its hosts. Ultimately, the long-term survival of a metapopulation may depend on the balance between local extinction and colonization and thus on the ability to produce enough migrants (Levins, 1970, Ovaskainen & Hanski, 2002).

In the original metapopulation model (Levins, 1970) and in subsequent extensions, migration and the production of migrants was seen as a fixed trait of any individual in the metapopulation. Later models (reviewed by Hanski, 1999) considered migration as a function of the population density in the patch of departure, the patch size and the distance between patches. All these models assumed a positive relationship between population size and contribution of migrants. This led to one of the most commonly cited generalities of metapopulation theory: smaller and less frequently occupied habitat patches are less important for the overall metapopulation dynamics (Hanski, 1994, Hanski, 1999). An extension thereof is the mainland-island metapopulation (Harrison, 1991, Harrison et al., 1988), with populations in one or more very large patches – the mainland – with negligible risk of extinction. The remaining populations in small habitat patches run a high risk of extinction. Although the mainland-island model is strongly based on the extinction risk of individual populations, it also implicitly makes a statement about the origin of migrants: migrants are predominantly or exclusively originating from the permanent "mainland" populations, while "islands" are only receiving immigrants (Hanski, 1999, Kawecki, 2004, Harrison, 1991).

From new empirical and theoretical studies it emerges that previous assumptions and conclusion about dispersal and migration in metapopulations were far too simple (reviewed in Clobert et al., 2004). Indeed, understanding how and which individuals are migrating has become of prime importance, as we need to identify key populations in the context of habitat fragmentation and global climate change (Clobert et al., 2004, Thomas & Hanski, 2004). Theoretical studies on condition-dependent migration now include ecological, genetic and social factors (Brown & Kodrick-Brown, 1977, Clobert et al., 2001, Clobert et al., 2004). Still, empirical data on the various aspects of dispersal are scarce (Bullock et al., 2002) and have been addressed only recently. They showed, for example, a genetic predisposition of becoming a migrant (Haag et al., 2005), increased dispersal from heavily disturbed patches (Bates et al., 2006) and a higher success of immigrants due to local parasites (Altermatt et al., 2007) or hybrid vigour (Ebert et al., 2002). These studies illustrate that it is important to know which factors influence migration and migrant production to better understand the dynamics of metapopulations.

The increased isolation of populations due to habitat fragmentation reduces successful migrations, which might accelerate local extinction (Hanski & Gaggiotti, 2004, Watts et al., 2005). Furthermore, in conservation biology, the limited conservation efforts must often be allocated either to a single large or to several small populations (SLOSS, for a list of relevant literature see Ovaskainen, 2002), and the former is mostly favoured. Runge et al. (2006), however, showed that estimates of emigration rather than patch size are important for delineating sources and sinks and for evaluating how local populations interact in metapopulation dynamics. Often, models implicitly treat emigration as mortality and small populations that produce many emigrants will thereby not classified as key populations (Runge et al., 2006). A notable example is given by Crone et al. (2001, coincidently studied in the same archipelago whereas I did my studies). They showed in a vole metapopulation that the ephemeral populations rather than the more permanent populations in large habitat patches are the important sources of migrants. They concluded that the stable long-term equilibrium in that metapopulation is only allowed by the ephemeral high-emigration subpopulations (Crone et al., 2001).

I studied *Daphnia* metapopulations in southwest Finland that inhabit rock pools on skerry islands. The three interacting species *Daphnia magna*, *D. longispina* and *D. pulex* occur in sympatry. Therefore they are also a good example of a metacommunity. A metacommunity is defined as a set of local communities that are linked by dispersal (Leibold et al., 2004, Holyoak et al., 2005, Wilson, 1992). Each local community consists of some or all species that interact within a single patch. A metacommunity can also be seen as the sum of all metapopulations of different, interacting species that use the same set of habitat patches. Again, the processes and the interactions among them at different spatial scales are central rather than the spatial structure alone (Leibold & Miller, 2004, Holyoak et al., 2005). Dispersal connects different patches, while populations within patches are influenced by interspecific competition (Leibold et al., 2004, Bengtsson, 1989).

In my work, I measured the numerical production of migration stages in *D. magna* and related it to characteristics of their population of origin. I developed a model to predict desiccation of pools, as migration stages may get exposed to passive dispersal in dry pools (Vanschoenwinkel et al., 2007). Long-term data show changes in colonisation dynamics in all three *Daphnia* species due to climate change, supporting my conclusions. Finally, I investigated epidemiological and genetic components of *D. magna* genotypes and populations that can influence or be influenced by migration.

The study system

Waterfleas of the genus Daphnia

I did all my studies in a metacommunity of the three planktonic crustaceans *Daphnia magna* Straus, *D. longispina* O. F. Müller, and *D. pulex* De Geer (Crustacea: Cladocera) in southwest Finland near Tvärminne Zoological Station. On the surrounding skerry island (Fig. 1), these three species are widely distributed and inhabit ephemeral freshwater rock pools (Bengtsson & Ebert, 1998, Ebert et al., 2001, Pajunen & Pajunen, 2003, Ranta, 1979, Pajunen, 1986, Lagerspetz, 1955). They are filter-feeding on algae and bacteria. The chapters one, four, five and six of my thesis deal only with *D. magna*, while chapters two and three incorporate data on all three species.

Here, I describe intrinsic characteristics of rock pool *Daphnia* populations, as they differ in several aspect from conspecifics in lakes. All *Daphnia* populations in the rock pools represent metapopulation systems, with frequent extinction and colonisation (Pajunen & Pajunen, 2003, Hanski & Ranta, 1983). Within a given year, the average percentage of pools containing *D. magna* or *D. longispina* is about 17 % and about 6 % for *D. pulex*. Depending on the species, yearly mean extinction probabilities vary between 16 % and 23 % (Pajunen & Pajunen, 2003).

The three species either occur singly or coexist in the same rock pool, though they have slightly different ecological preferences (Pajunen & Pajunen, 2003, Ranta, 1979, Pajunen & Pajunen, 2007). On average, *D. magna* occurs in smaller pools, *D. pulex* in intermediate-sized pools and *D. longispina* in larger pools (Bengtsson, 1988, Lagerspetz, 1955, Ranta, 1979, Pajunen & Pajunen, 2007). Furthermore, *D. magna* is most tolerant to high salinity and prefers pools with higher calcium content (Pajunen & Pajunen, 2007). High salinity values are found in pools close to the sea or in desiccating pools.

All three species reproduce by cyclical parthenogenesis (Zaffagnini, 1987), except for some populations of *D. pulex*, that are obligate parthenogenetic (Haag & Ebert, 2004). Cyclical parthenogenesis means that phases of asexual reproduction are intermitted by sexual reproduction. As a result of sexual reproduction, resting eggs (so-called ephippia) are produced. These ephippia guarantee survival in an unstable environment and can outlast unfavourable conditions such as freezing during winter or desiccation of pools during summer (Ebert, 2005, Ranta, 1979). Due to several hatching stimuli per year, long-lasting resting egg banks are absent in the rock pools (Pajunen & Pajunen, 2003). The ephippia also serve as dispersal stages that migrate passively either by wind or birds (Brendonck & Riddoch, 1999, Ranta, 1979, Maguire, 1963, Vanschoenwinkel et al., 2007). The sedimented

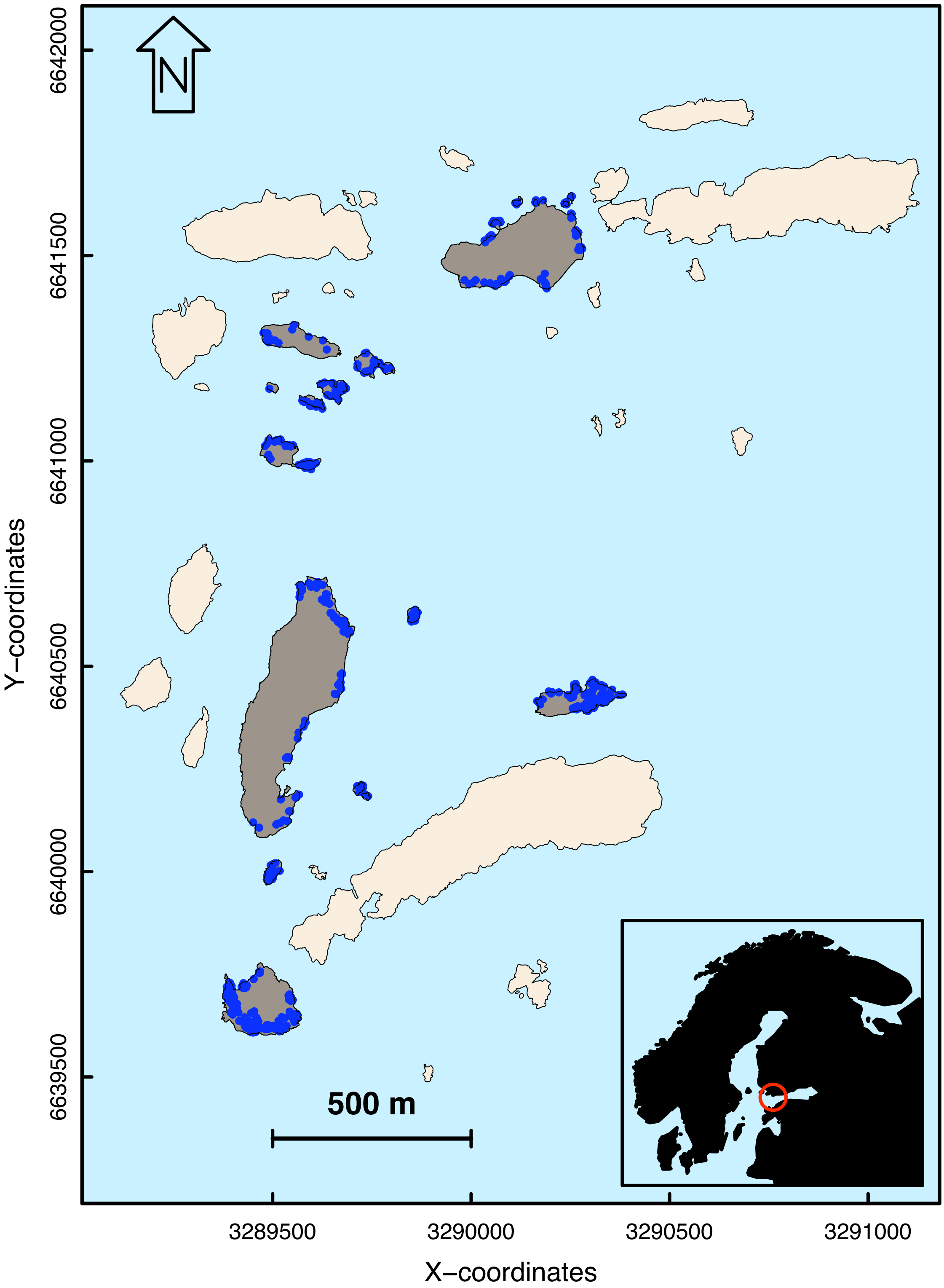

Fig. 1. Study area in southwest Finland. The map shows all islands that were included in the long-term monitoring (brown) as well as island that were not monitored (light brown). Rock pools are indicated by blue dots. The grid follows the Finnish coordinate system. The location of the study area within Scandinavia is indicated by a red circle in the inserted map.

ephippia are particularly exposed to migration in desiccated rock pools (see figure 2.19 in Ebert (2005)). Ephippia can migrate into rock pools that are already inhabited by a conspecific *Daphnia* population (= invasion) or into rock pools that have no conspecific *Daphnia* populations (= colonisation). Due to asexual reproduction, large planktonic populations can develop within a few weeks. This may be followed by an influx of further immigrants over time. Genetic data of populations in newly colonized rock pools suggest that most rock pools get colonized by a single or very few individuals, which leads to strong bottlenecks (so-called founder-effects, Haag et al., 2005). These bottlenecks lead to inbreeding depression and hybrid vigour after an influx of further immigrants (Ebert et al., 2002, Haag et al., 2006). Genetic diversity increases with the age of the populations (Haag et al., 2005), and so does parasite load (Ebert et al., 2001).

The survival period of a population in a rock pool ranges from less than a year to more than 20 years (Pajunen & Pajunen, 2003). Populations go extinct for various reasons. The most common causes are rock pools being washed out by waves from the surrounding Baltic Sea (Pajunen & Pajunen, 2003), parasite epidemics (Bengtsson & Ebert, 1998, Ebert, 2005), competition with other *Daphnia* species (Bengtsson, 1989) and changes in habitat qualities. Desiccation of a pool is detrimental for the planktonic animals, while ephippia can survive and the population does not necessarily go extinct (Pajunen & Pajunen, 2003, Ranta, 1979).

The *Daphnia* metacommunity in southwest Finland provides excellent possibilities to study evolutionary and ecological questions. Also, a large data set on metapopulation dynamics exists, as on 13 islands (Fig. 1) more than 500 potential habitat patches have been monitored for the presence or absence of any of three *Daphnia* species over 25 years (Pajunen, 1986, Pajunen & Pajunen, 2003, Pajunen & Pajunen, 2007). There are two hypotheses that describe these *Daphnia* metapopulations differently with respect to persistence of individual populations and the origin of migrants. Hanski & Ranta (1983) suggested a Levins'-type metapopulation in an extinction-colonization equilibrium where all populations contribute migrants equally. Pajunen & Pajunen (1986, 2003) favoured a mainland-island model with the populations in large and long-lasting rock pools being the dominant sources of migrants.

Parasites

Parasites are ubiquitous in nature (Ebert, 2005, Hudson & Greenman, 1998, Minchella & Scott, 1991, Poulin, 1998). They harm the infected hosts to various degrees and thereby impose selection. Usually only a subset of individuals within a host population is infected, either due to genetic or due to epidemiological reasons. For example, hosts of one sex, of a certain age, in certain phenotypic conditions, with a higher inbreeding level or of specific genotypes may be more often found to be infected by parasites (Bandi et al., 2001, Brown et al., 2000, Keller & Waller, 2002, Little et al., 2002).

The herein studied *Daphnia* are parasitized to various extents and especially *D. magna* is host to many parasite and epibiont species (Ebert et al., 2001). The most common parasite in our study area is the microsporidium *Octosporea bayeri* Jírovec, 1936 (Ebert et al., 2001). *Octosporea bayeri* is specific to *D. magna*. It is vertically as well as horizontally transmitted and reduces host fecundity and survival (Vizoso & Ebert, 2004, Vizoso et al., 2005). *Octosporea bayeri* can be found in about 45 % of all *D. magna* populations. In natural populations, prevalence cycles characteristically. Prevalence is typically low to intermediate in frequency in spring and reaches levels towards 100 % at the end of the summer (Lass & Ebert, 2006). Vertical transmission is 100 % during the asexual reproduction of the host. Transmission through the sexually produced ephippia is the less successful the longer the diapause and the more outbred the host (Ebert et al., 2007, Lass & Ebert, 2006). Local adaptation of the parasite occurs on a population level and promotes immigration of allopatric hosts (Altermatt et al., 2007).

Aims of the thesis project

During my thesis, I studied ecological and evolutionary aspects related with migration in *Daphnia* metapopulations. I was especially interested in the production and origin of migration stages and the consequences of migration. In a previous study we showed that uninfected migrants have a higher immigration success compared to infected migrants and that immigration success is positively correlated with parasite prevalence in the resident population (Altermatt et al., 2007). Furthermore, immigrant genes have an advantage due to hybrid vigour (Ebert et al., 2002). However, nothing was known on the production of migration stages in different populations within the metapopulation.

To fill this gap, I measured the seasonal production of ephippia in natural rock pools and in artificial mesocosms varying in size over five orders of magnitude. Special emphasis was given on the ephippium production of populations in small and potentially unstable habitats. I then developed a model to predict desiccation of single rock pools. Desiccation is Janus-faced, as it is detrimental for the planktonic population, but may at the same time expose ephippia to passive migration (Ranta, 1979). Thus, deteriorating environmental conditions may lead to both high mortality and emigration (see also Crone et al., 2001, Pokki, 1981, Vanschoenwinkel et al., 2007). Furthermore, I wanted to combine the production of migration stages in individual pools and their subsequent exposition during droughts, to make a global budget of potential migrants within the whole metapopulation and to identify key populations.

Many studies document unidirectional correlations between climate change and biological phenomena such as altered phenologies (usually a shift towards earlier times in the year), shifts in species ranges (usually polewards and/or to higher altitudes), extinctions (due to changes in the habitat or increased stochasticity), changes in habitat qualities, altered life histories, community compositions, ecosystem functions, or genetic changes (Balanya et al., 2006, Parmesan, 1996, Parmesan et al., 1999, Parmesan & Yohe, 2003, Roy & Sparks, 2000, Thomas et al., 2004, Walther et al., 2002, McLaughlin et al., 2002). As desiccation of the pools is related to the weather in a mechanistically simple way, I hypothesised an influence of the ongoing climate change on metapopulation processes. Also in Tvärminne, the weather became warmer and drier within the last 25 years. I thus analysed the long-term metapopulation data with respect to changes in colonisation dynamics and metacommunity composition over time.

We know that uninfected hosts have a higher migration success than infected hosts (Altermatt et al., 2007). I was interested in further correlations between the fitness of a host and its natural infection status. I compared the cost of resistance hypothesis (Kraaijeveld & Godfray, 1997, Carton et al., 2005) with the inbreeding-infection hypothesis (Coltman et al., 1999, Keller & Waller, 2002, Reid et al., 2003). The former predicts that resistant genotypes pay a cost of being resistant and are less fit in the absence of parasites, while the latter predicts that the susceptible individuals are also less fit due to inbreeding depression. In the context of migration, this may even increase the difference in migration success between uninfected and infected individuals: in the case of the inbreeding-infection scenario, the immigration success of infected individuals will be additionally decreased due to inbreeding depression, while in a cost of resistance scenario, the uninfected individuals may profit from their resistance when immigrating into infected resident populations, but not when immigrating into uninfected resident populations.

A consequence of migration and dispersal is either the establishment of newly colonized habitat patches with low-diversity and potentially parasite-free populations or the introduction of new host genotypes and potentially co-migrating parasites into already existing populations (Ebert et al., 2001, Haag et al., 2005). Due to asexual reproduction of *Daphnia*, populations in newly colonised habitat patches can be monoclonal (Haag et al., 2006). On the other hand, repeated invasion into existing populations may drastically increase their genetic diversity (Haag et al., 2005). As migration influences the genetic diversity of both host and parasite populations, I was interested in the epidemiological dynamics in different host-parasite populations. Whereas parasite dynamics have been followed in plant populations or plant communities of different diversities (Garrett & Mundt, 1999, Mitchell et al., 2002, Zhu et al., 2000), nothing was known about epidemiological dynamics in animal host populations that differ in their genetic diversity.

Outline

Chapter 1 comprises data on the production of ephippia by *D. magna* populations that inhabit various sized habitat patches. As the ephippia are the migration stages, their number and origin is important to understand metapopulation dynamics. Ephippium production was followed both in experimental mesocosms as well as in 34 natural rock pools over two years. I show that the increase in habitat size results in an under-proportional increase in ephippium production. Furthermore, I show experimentally that desiccation of the habitat is not detrimental for the *Daphnia*, as viable populations could build up within the same season again. Combined with data on the phenology of ephippium production, this indicates that populations in small habitat patches may substantially contribute to the total number of ephippia in the whole metapopulation.

Chapter 2 describes an individual-pool-based model to predict desiccation events and drought lengths in 530 rock pools over a period of 25 years. I introduce desiccation risk as a new variable to explain patterns of occupancy of *Daphnia* populations and to predict ephippium exposure in desiccated pools. My model is based on both meteorological variables as well as pool characteristics. Water influx by precipitation, water loss by evaporation, and eventually desiccation can be predicted retrospectively. I calibrated the model with evaporation measurements from 50 pools during one summer and correlate predicted desiccation with data from a survey of all 530 pools. In a further step, I correlate the presence of the three species *D. magna, D. longispina* and *D. pulex* and occupation lengths to the desiccation risk of the different habitat patches. I suggest that desiccation risk is a better variable explaining long-term occupancy of a habitat patch than pool volume alone. Long-lasting populations occur mostly in pools with a low desiccation risk. However, populations are also often found in desiccation-prone pools. As ephippia are exposed to passive migration in desiccated pools, these short-lived populations may contribute significantly to the total number of migrants and play an important role in metapopulation dynamics.

Chapter 3 presents for the first time data that relate changes in metapopulation processes and consecutively in metacommunity structure to global warming. Consistent with climate change models and global long-term climatic measurements, the weather became significantly warmer and drier in the herein studied metacommunity of the three species

D. magna, *D. longispina* and *D. pulex* in southwest Finland. In parallel, the colonisation rates of all species increased. I show that colonisation rates are significantly higher after warm and dry summers, and that the magnitude of the change differs between the three species. The increased migration rates are mechanistically explained by the exposition of resting stages in desiccated pools. Eventually, the whole metacommunity composition changes. This ongoing process literally incorporates the aspects described in chapters 1 and 2. It integrates the production of migration stages in different populations and their exposition in habitat patches that differ in their desiccation risk.

Chapter 4 contains estimates on the number of migration stages that were produced in the whole *Daphnia magna* metapopulation over 25 years. The model combines the production of migration stages of individual pools (chapter 1), size distributions of occupied pools and their predicted risk of being desiccated (chapter 2) to get overall estimates on migration stage production. Estimates are given for each year separately, depending on the yearly number of occupied rock pools and the time they contained water. Overall, the yearly number of migration stages that are produced ranges between several hundred thousand to about 1.1 millions. The model suggests that about 50 % of all migration stages are produced by populations in small to medium sized pools (10–300 l) and that 90 % of all migration stages are exposed in such populations. Finally, I show that colonisation rates do not depend on the number of ephippia that are produced, but rather on the amount of ephippia that are exposed during desiccation events.

Chapter 5 contrasts predictions of two evolutionary hypotheses that predict a correlation between the fitness of a genotype in the absence of infection and the likelihood to become infected. The cost of resistance hypothesis predicts that resistant genotypes pay a cost of being resistant and are less fit in the absence of parasites, while the inbreeding-infection hypothesis predicts that the susceptible individuals are less fit due to inbreeding depression. I use the *D. magna–O. bayeri* system to test these hypotheses, which are not only relevant within populations, but can also influence the success of migrants in the whole metapopulation. First, I experimentally confirm that the naturally observed infection status has a genetic basis. I then estimate the relative fitness from uninfected and cured but former infected genotypes from each of 22 populations. As I find no difference in competitive abilities between these genotypes, my data are neither in support of the cost of resistance nor the inbreeding-infection hypothesis. This suggests that resistance genes segregate largely independently of other fitness associated genes in this system.

Chapter 6 presents an experiment, where I follow the prevalence of the parasite *O. bayeri* in replicated *D. magna* host populations under outdoor conditions over two years. Due to colonisation of empty habitat patches or invasion into existing populations, host populations differ greatly in their genetic diversity. Therefore, I compare host populations of both low and high genetic diversity that get infected by the parasite. I use two different parasite treatments, namely an isolate of high genetic diversity and an isolate of low diversity. Parasites establish and spread less successful in host populations of high genetic diversity compared to host populations of low diversity, while the parasite isolate has no effect on prevalence. In the long-term, the observed difference in parasite spread may influence host-parasite coevolution and local adaptation.

Chapter 7 concludes this thesis. It briefly summarizes the results from the previous chapters and shows open questions and possible directions for future research.

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Chapter 1

The influence of pool volume and summer desiccation on the production of the resting and dispersal stage in a *Daphnia* metapopulation

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Conditional accepted in Oecologia

Abstract: Dispersal is a key process in metapopulations, as migrants genetically connect populations and enable the colonization of empty habitat patches. Sub-populations may differ in their numerical contribution of migrants within a metapopulation. This has strong implications on evolutionary and ecological dynamics and has led to two different hypotheses about the herein studied *Daphnia* metapopulation: while sub-populations are assessed equally in the production of migrants by some authors, others postulated long-lived core populations in large "mainland" habitat patches as the dominant source of migrants. We studied the production of ephippia, which are the resting and dispersal stage, in the natural *Daphnia* metapopulation and in mesocosm experiments and tested for effects of habitat size and summer desiccation. We found that a 1000-fold increase in rock pool volume resulted on average only in a 2.8-fold increase in ephippium production. Mesocosm experiments confirmed these results: a 1000-fold increase of the mesocosms' volume resulted in a 7.2-fold increase in ephippium production. Additionally, we showed that ephippium production did not depend on the initial population size. Thus, populations in small pools may contribute only marginal fewer potential migrants in the whole metapopulation than populations in large pools. In a second mesocosm experiment we found that summer desiccation, which is typically occurring in small pools, is not detrimental for the populations. *Daphnia* hatched out of ephippia that were produced earlier within the same season, and built up viable populations again. The substantial production of ephippia by populations in small pools suggests that these populations might be important for metapopulation dynamics and for the metapopulation's global stability.

Introduction

Many animal and plant species occur in metapopulations that are assemblages of spatially delimited local populations coupled by some degree of dispersal (Levins, 1970, Hanski & Gaggiotti, 2004). Dispersal is the process that genetically connects separated populations and enables the colonization of empty habitats (Clobert et al., 2001, Townsend et al., 2000), counteracting local extinction. Ultimately, the long-term survival of a metapopulation depends on the balance between local extinction and colonization and thus on the ability to produce migrants (Ovaskainen & Hanski, 2004). An understanding of dispersal and the ability to identify key populations for species survival is also important in the light of habitat fragmentation. Due to the increased isolation of populations, there will be fewer successful migrants. In order to set priorities for conservation, one would like to know the number of migrants and their populations of origin (Hanski & Gaggiotti, 2004, Watts et al., 2005). To obtain realistic estimates and to incorporate potential feedbacks between different factors that influence the production of migrants, migrant production must be studied in the field.

Theoretical studies indicate the significance of dispersal within metapopulations (Brown & Kodrick-Brown, 1977, Clobert et al., 2001), but empirical data on the various aspects of dispersal are still scarce (Bullock et al., 2002). In the original metapopulation model (Levins, 1970) and in subsequent extensions of it, dispersal and the production of migrants was seen as a fixed trait of any individual in the metapopulation. Later models (Hanski, 1999) considered dispersal as a function of the population density in the patch of departure, the patch size and the distance between patches. A more specific model is the mainland-island metapopulation (Harrison, 1991), with populations in one or more very large patches – the mainland – with negligible risk of extinction. The remaining populations in small habitat patches run a high risk of extinction. Although the mainland-island model is strongly based on the extinction risk of individual populations, it also implicitly makes a statement about the origin of migrants: migrants are predominantly or exclusively originating from the permanent "mainland" populations in large patches, while populations in small “island” patches are only receiving immigrants (Hanski, 1999, Kawecki, 2004). Population size is assumed to be proportional to patch size. Thus, these models do not only make statements about the extinction risk of populations, but also assume a positive relationship between population size and contribution of migrants. Yet, a different type of models focuses on condition-dependent dispersal that include ecological, genetic and social factors (Clobert et al., 2001). Empirical studies are starting to incorporate such factors as well, for example, a genetic predisposition of becoming a migrant (Haag et al., 2005), increased dispersal from heavily disturbed patches (Bates et al., 2006), changes in dispersal dynamics due to climate change (Altermatt et al., 2008) and a higher success of immigrants due to local parasites (Altermatt et al., 2007) or hybrid vigor (Ebert et al., 2002). These studies illustrate that it is important to know which factors influence dispersal and migrant production to better understand the dynamics of metapopulations. Data on the origin of migrants are especially needed to make predictions about gene flow, local adaptation and parasite dispersal (Clobert et al., 2001).

We studied the influence of habitat size and summer desiccation on the production of the resting and dispersal stage (ephippium) in a natural metapopulation of the crustacean *Daphnia magna*. *Daphnia magna* commonly occurs in freshwater rock pools along the coast of the Baltic Sea and is an ideal model system to study metapopulation processes (Bengtsson & Ebert, 1998, Ebert et al., 2001, Green, 1957, Hanski & Ranta, 1983, Pajunen, 1986, Pajunen & Pajunen, 2003). Metapopulations in southwest Finland and east Sweden consist of many thousands of populations that occur in rock pool habitats (Pajunen & Pajunen, 2003, Ebert et al., 2001, Bengtsson & Ebert, 1998). *Daphnia* reproduce both asexually as well as sexually. An ephippium is produced by the female *Daphnia magna* as a result of sexual reproduction. It consists of a part of the maternal carapace that forms a protective shell around up to two eggs, comparable with a plant seed capsule containing two seeds. The ephippium is released during the next moult. The enclosed eggs may survive desiccation, freezing and can be passively dispersed.

While ephippium production of some *Daphnia* species has been quantified in single lakes (Cáceres & Tessier, 2004, Kerfoot et al., 2004, Cáceres, 1998), nothing is known about the ephippium production of *Daphnia* inhabiting small rock pools (<10 m^3 water volume) and how it relates to pool size. Ephippia are essential for survival in such metapopulations for two reasons (Hanski, 1999, Hanski & Ranta, 1983). First, they are the dispersal stage (Maguire, 1963) and dispersal is mainly depending on the production of ephippia. Ephippia are passively dispersed by wind or birds (Maguire, 1963) and allow for long-distance dispersal. Functionally, ephippia are very similar to plant seeds and the same dispersal mechanisms may operate (Bullock et al., 2006, Figuerola & Green, 2002). In the herein studied *Daphnia* metapopulation, extinction and colonization in local patches occurs at a high annual rate (Pajunen, 1986, Pajunen & Pajunen, 2003), stressing the importance of dispersal. Second, ephippia are essential to endure freezing in winter and droughts in summer. Due to the obligate freezing of the rock pools, only populations that produce ephippia can survive during winter (Pajunen, 1986, Pajunen & Pajunen, 2003). Thus, ephippium production can be used both as a surrogate of a population's migrant production and a population's long-term survival ability. *Daphnia* populations in lakes tend to produce ephippia towards the end of the season (Cáceres, 1998, Cáceres & Tessier, 2004). In unpredictable habitats such as rock pools, however, the seasonal timing of ephippium production may be different, as ephippia must be produced before possible droughts during summer. We thus monitored the phenology of ephippium production in natural populations.

There are two hypotheses that describe these *Daphnia* metapopulations differently with respect to persistence of individual populations and the origin of migrants. Hanski and Ranta (1983) suggested a Levins-type metapopulation in an extinction-colonization equilibrium where all populations contribute migrants equally. Pajunen and Pajunen (1986, 2007) favored a mainland-island model with the long-lived populations in large rock pools being the dominant sources of migrants. Populations in small pools have a higher risk of extinction for example due to frequent desiccation and are generally more short-lived (Bengtsson, 1989, Altermatt et al., in Prep.). Pajunen and Pajunen (2003) classified short-lived populations in small pools as less important for metapopulation dynamics (mainland-island model). The two hypotheses are based on different assumptions about the production of migrants in individual populations. Hanski and Ranta (1983) did not assume that dispersal is related to any pool variable other than the presence of other *Daphnia* species. Pajunen and Pajunen (1986, 2003) assumed that the origin of migrants is associated with population persistency. Long-lived populations in large pools should produce more migrants, while populations in small pools are usually short-lived and should not contribute migrants due to the unpredictability of their habitat with respect to desiccation. None of the earlier studies quantified ephippium production and it is not understood which populations produce how many ephippia (Pajunen & Pajunen, 2003). However, the origin and number of ephippia is a relevant aspect for distinguishing between these two hypotheses.

To fill this gap, we measured the seasonal production of ephippia in natural populations as well as in experimental populations in mesocosms. The rock pools and containers respectively varied in size over five orders of magnitude. We further included initial population size as a factor. Especially populations in unstable habitats may go more often through population bottlenecks and be recruited out of a few individuals. Special emphasis was given on the ephippium production of populations in pools that may dry-up frequently. We tested, whether populations can produce ephippia before a drought and then build up viable populations from hatchlings afterwards within the same season, which has not yet been shown. A population is classified as "viable" when the planktonic animals are able to produce ephippia, and thereby guarantee long-term survival. Intentionally, we did not relate densities of adult *Daphnia* throughout the season with pool size, as densities vary strongly on short timescales (personal observation). By using the number of ephippia, we have a measurement that integrates over the whole time-span and all densities. It gives number of potential migrants, which is relevant in an evolutionary and ecological context. Also, we did not intend to make mechanistic statements on ephippium production of adult *Daphnia*. Our findings will improve our understanding about the origin and number of migrants in a metapopulation and focus on the significance of the numerous populations in habitat patches that are small or where the environment is less predictable.

Material and Methods

The natural system

The freshwater crustacean *Daphnia magna* Straus, 1820 (Crustacea: Cladocera) is widely distributed along the coast of the Baltic Sea. It inhabits rock pools ranging from 10 to 30000 litre in volume on the skerry islands of southwest Finland (Pajunen & Pajunen, 2003). *Daphnia* hatch from ephippia at the beginning of May and the populations are usually in their planktonic phase until autumn (September/October). During this time, *D. magna* reproduces predominantly asexually, intermitted by periods of sexual reproduction when ephippia are produced. Various stress-linked factors such as high population density (Banta & Brown, 1929, Carvalho & Hughes, 1983), food limitation (Kleiven et al., 1992), increased salinity and short-day photoperiod have been suggested as triggers for sexual reproduction and production of ephippia. Only ephippia can survive unfavourable conditions such as the obligate freezing during winter or the occasional desiccation of pools during summer (Ebert, 2005). They also serve as wind-drifted dispersal stages (Ranta, 1979, Maguire, 1963). The ephippia rest on the bottom of the pools until a hatching stimulus occurs. *Daphnia* females can produce ephippia that do not contain eggs and only consist of a shell. In all our studies and experiments, only ephippia containing eggs were counted. Ephippia without eggs were excluded. Empty ephippia, however, were rare and their proportion was negligible.

Most rock pools contain little to no sediments and are washed out frequently during autumn storms. Thus, contrary to lakes, rock pools do not have yearly strata of sediments. A *Daphnia* population consist both of the planktonic animals and the ephippia. Local extinction of a population only occurs, when all individuals in both of these two life-stages are dying. Several diapause termination cues occur per year (Pajunen & Pajunen, 2003). In accordance to other publications, we define local extinction when no *Daphnia* have been observed within 18 months (Pajunen & Pajunen 2003; Altermatt et al. 2008). An average population persists for about three to five years (Pajunen & Pajunen 2003). Populations that persist for less than about three years are called short-lived, while populations that persist for 10 to up to 25 years are called long-lived (Pajunen & Pajunen 2003).

Ephippium production of natural populations

We quantified the production of ephippia by populations in natural rock pools of various volumes throughout the season. We chose populations in 34 rock pools on 14 different islands near Tvärminne Zoological Station, Finland (59° 50' N, 23° 15' E). All these rock pools had contained a *D. magna* population in the previous year. We measured the longest axis of each pool, the greatest width perpendicular to this and the maximal water depth to estimate the volume of the rock pool as an inverted pyramid (width * length * depth / 3; following the method of Ebert et al. (2001)). The measurements were done in 2005 and 2006 when pools were filled with water up to their maximal level. The volume estimates of the chosen rock pools (between 24 and 24200 L) spanned almost the entire spectrum of pools in that metapopulation. We also estimated the surface (width * length).

We measured ephippium production representative rock pools in 2005 (18 rock pools) and 2006 (20 rock pools, 4 of them were used also in 2005). We did not include the very small proportion of pools with a thick layer of soft sediments in our study. In May 2005 and 2006, we placed large glass petri dishes (diameter either 182 or 193 mm) in each of these rock pools at depths that were representative for the rock pool. At that time, *Daphnia* populations start growing. The petri dishes passively collect all particles that sink from the water column above them, including the ephippia produced by the *Daphnia*. Ephippia of *D. magna* do not float but only sink to the bottom of the pool. Before the study, we confirmed that the petri-dishes did not collect resuspended ephippia from the bottom of the pool. In 2005, we focused on temporal changes of the ephippium production throughout the season. All rock pools were equipped with one trap and the content of each trap was collected at four different time points (after about 21, 50, 97 and 127 days). In 2006, we focused on differences in the total ephippium production throughout the season between populations in pools of different sizes. Thus, ephippia were sampled only twice in 2006 (after about 54 and 105 days). In 2006 two petri dishes were used per rock pool whenever the pool was sufficiently large (17 out of 20 rock pools) to obtain a better estimate for each population's ephippium production. The arithmetic mean of the ephippium number in the two petri dishes was then used in the analysis. By using two traps per pool we could also affirm that our method gave representative catches (thus little variation between the petri dishes within a rock pool). The depth of the traps in the rock pools was recorded at each sampling to calculate the water volume above the traps. The last sampling was done on 3 September 2005 and 6 September 2006 respectively; the monitored time thus spanned the entire season when *Daphnia* produced significant numbers of ephippia. There may be still some *Daphnia* in September and October, but then water temperatures are so low that ephippium production becomes insignificant. All ephippia in the collected sediments were counted with a stereomicroscope at 10-fold magnification. Ephippium production per day per trap (adjusted for the two slightly different sizes of the petri dishes) as well as per day and water volume was calculated.

Habitat size experiment

We measured the influence of habitat size (water volume) and of the initial number of *D. magna* on the populations' seasonal ephippium production in an experiment. We used four different, parasite-free *D. magna* genotypes that were collected in rock pools on four different islands near Tvärminne Zoological Station in spring 2003 and 2004 and were kept in their asexual phase since then. At the end of April 2005, mass cultures of each genotype starting from one single female were established. The *D. magna* were kept in their exponential growth phase in artificial medium (Klüttgen et al., 1994) at room temperature and fed *ad libitum* with the green alga *Scenedesmus obliquus*. On 22 May 2005 the experiment was started with females of the same age class (about 10 days old animals, i.e. most of them had their first asexual egg clutch in their brood chamber). The animals were released into plastic containers (= mesocosms) filled with water from a natural rock pool filtered with a 20-µm filter. Per 10 litre of filtered pool water, 30 ml of a horse manure suspension (10 kg horse manure suspended in 60 L of seawater) and 0.5 litre of seawater were added to increase the nutrient content and the salinity of the water. Containers of seven different sizes were used (1, 2, 5, 8, 30, 75 and 320 L). Per container size class and treatment we had four (sizes 1 to 30 L), two (size 75 L) and one replicate (size 320 L) respectively, giving in total 46 container replicates. The containers were arranged within 32 m^2 in a latin square outdoors on an island next to natural rock pools. Each container received all four *D. magna* genotypes. We had two different treatments where we introduced the genotypes either at the same density (one animal per clone and litre = "same density" treatment) or at the same number per container (one animal per clone = "same number" treatment). During the whole season the water volume was kept constant, and losses due to evaporation were replaced with deionised water. After 100 days, which is close to the length of the natural season and comparable to 10 to 12 asexual generations, the sediments in all replicates were sampled and frozen at –20 °C. The numbers of ephippia were counted with a stereomicroscope at 10-fold magnification.

Sunlight is the energy source for the primary production of algae, which are the food of the *Daphnia*. Sunlight irradiation is proportional to the surface and not the volume of the water body. We therefore analyzed the production of ephippia both using water volume as well as water surface area as explanatory variable.

Desiccation-Experiment

Desiccation of rock pools is common during summer droughts (Pajunen & Pajunen, 2003). Therefore, we tested the influence of desiccation intermitting the ongoing asexual reproduction of a *Daphnia magna* population. We performed an experiment in plastic containers where we could standardize both desiccation and the genetic composition of the *Daphnia* populations. In natural pools we could not have excluded desiccation/refilling at different rates (due to different catchment areas, evaporation rates etc., personal observations). We were interested if a forthcoming drought can stimulate ephippium production. It is known that *Daphnia* hatch after droughts in rock pools with persisting populations (Lass & Ebert, 2006), but it is unclear if these animals hatch from ephippia of the previous year or from ephippia that did not pass a winter diapause. We thus tested if – after refilling with water – *D. magna* can hatch out of ephippia that were produced immediately before the drought without any additional winter diapause. This has never been tested before. However, it has important implications for dynamics in pools that dry up within the first year of a population's presence.

For the experiment, *D. magna* populations were kept under outdoor conditions in containers containing 5 litre of water from a rock pool filtered through a 20-µm filter and charged with horse manure suspension and seawater (analogous to the previous experiment). We measured absolute ephippium production before (A, letters represent in the following the symbols used in Fig. 5), during (B_1) and after desiccation (B_2, desiccation treatment) as well as ephippium production in control populations without desiccation (C_1, C_2). At the end of April 2005, a mass-culture of one of the *D. magna* genotypes used in the habitat size experiment was established (analogous to the previous experiment). The experiment was started on 31 May 2005 and 20 adult females were released into each of 75 plastic containers. The containers were placed outdoors next to natural rock pools on an island. After 33 days, 11 randomly chosen replicates were destructively sampled and all sediments including the ephippia collected and stored at –20 °C (A, ephippium production before evaporation). To study the effect of desiccation we added wicks to increase evaporation in 32 randomly chosen containers of the remaining 64 replicates. The wicks were made out of three layers of Whatman filter paper (size 20 x 40 cm) clamped between two plastic grids with a mesh size of 1 cm. In each container we fixed one wick with cable ties in an upright position. The lower half of the wick reached the bottom of the container, while the upper half exceeded the container and was exposed to wind. In the 32 control replicates, the same wicks were installed, but the filter paper was interrupted at the water surface level. This prevented an increased evaporation rate. During a natural period of dry weather in July 2005, the water in all replicates of the desiccation treatment evaporated completely within 16 days. In the controls, the water volume decreased only slightly and never fell below 4 litre. There was in total 2.4 mm of rainfall during these 16 days (weather data from the Tvärminne Zoological Station's weather station). On 19 July, all wicks were removed, and the desiccated containers were covered with a lid to keep out rainwater. Containers remained outdoors over the whole time. On 31 July, these desiccated replicates were refilled with 4 litre of deionised water and the dried sediments were resuspended. From then on we daily visually checked all replicates and recorded the occurrence of the first hatchlings. We also recorded when these animals became adult and produced their first clutch of asexual eggs. At the same time of refilling the containers, all sediments including ephippia were destructively sampled in half of the remaining control treatment replicates and the density of the planktonic *D. magna* population was reduced to 5 % (about 50 animals) to mimic the bottleneck of the populations after hatching in the desiccation treatment (D). The other 16 controls were not manipulated. In 16 randomly chosen replicates of the refilled desiccation treatment, the number of ephippia and the number of hatchlings was counted on 9 August. By then the *Daphnia* had already hatched again, but not yet started to produce ephippia. The shells of empty resting eggs are long-lasting, and can be studied even after hatching of the *Daphnia*. Therefore, the number of ephippia produced before the desiccation event could be determined retrospectively (B_1, all ephippia counted. We then subtracted the number of ephippia that were open due to hatching, which gave the decrease indicated by the dotted line). The ephippia in the remaining replicates (16 of the desiccation treatment B_2, 16 of the continuous control treatment C_2 and 16 of the density reduction treatment D) were collected and stored at –20 °C on 10 September. All ephippia were counted with a stereomicroscope at 10-fold magnification to determine the ephippium production during the different phases and in the different treatments.

Analysis

All variables were log-transformed prior to the analysis to fulfil the requirements of the ANOVAs and ANCOVAs. If transformed data still did not fulfil the requirements, nonparametric tests were used. Statistical analyses were performed with R (R Development Core Team, 2007). Statistical comparison of estimated and theoretical slopes was performed according to Scherrer (1984). Number of ephippia per day per trap were used when comparing ephippium production relative to pool volume. By doing so, differences in the volume of the water column above the different ephippium traps were not taken into account. The range of the water column volume above the trap was more than three orders of magnitude smaller than the range of the numbers of ephippia collected per trap. Also, the potential bias is conservative with respect to our interpretation of the data that small pools have an over-proportional ephippium production. Pool surface and not pool volume was used when comparing the depths of the ephippium traps and pool size to avoid any potential autocorrelations between depths and size. To avoid pseudoreplication, only the data from 2006 were used from the four rock pools sampled in both years. In that year, we had two traps in each of these rock pools instead of one as in 2005, thus our estimates of ephippium production are more precise. For comparing the results of the habitat size experiment with the natural pool data, a population's total ephippium production per rock pool was calculated by extrapolating the ephippium production per litre of water above the trap to the total volume of the rock pool (ephippia * L^{-1} * volume of the rock pool).

Results

Ephippium production of natural populations

Ephippia were produced throughout the summer, but the production per day per trap varied significantly between the four measurement periods in 2005 (Kruskal-Wallis test, $C^2 = 12.91$, df = 3, $p = 0.005$, Fig. 1a). As expected, the production of ephippia was low early in the season (May), increased to a maximum in July and decreased afterwards. The same pattern was also seen when ephippium production per day and litre was used as response variable (Kruskal-Wallis test, $C^2 = 12.27$, df = 3, $p = 0.007$, Fig. 1b). There was also a significant difference in the total number of ephippia produced between the different populations (Kruskal-Wallis test, $C^2 = 35.51$, df = 17, $p = 0.005$), ranging from 1 to 2266 ephippia (mean = 220; median = 41). There was little variation between the two traps within one pool, indicating that the method gave representative estimates.

The number of ephippia produced per day per trap by populations in 34 natural rock pools (data from 2005 and 2006 combined) correlated negatively with the volume of the rock pools (ANCOVA, $F_{1,31} = 14.6$, $p = 0.0006$, Fig. 2), meaning that the productivity (ephippia produced per trap or volume) was higher by populations in small pools. There was no significant difference between the two years (ANCOVA, $F_{1,31} = 1.8$, $p = 0.18$, Fig. 2). The depths of the ephippium traps were slightly deeper in larger pools and thereof the water column above the trap was larger, though the correlation was not significant (linear model between pool surface ($\log(m^2)$) and depth of ephippium trap ($\log(cm)$), $F_{1,32} = 1.75$, $R^2 = 0.088$, $p = 0.09$). We extrapolated the ephippium counts per trap to the whole pool to estimate the total number of ephippia produced by populations in pools of various size (see below and Fig. 4).

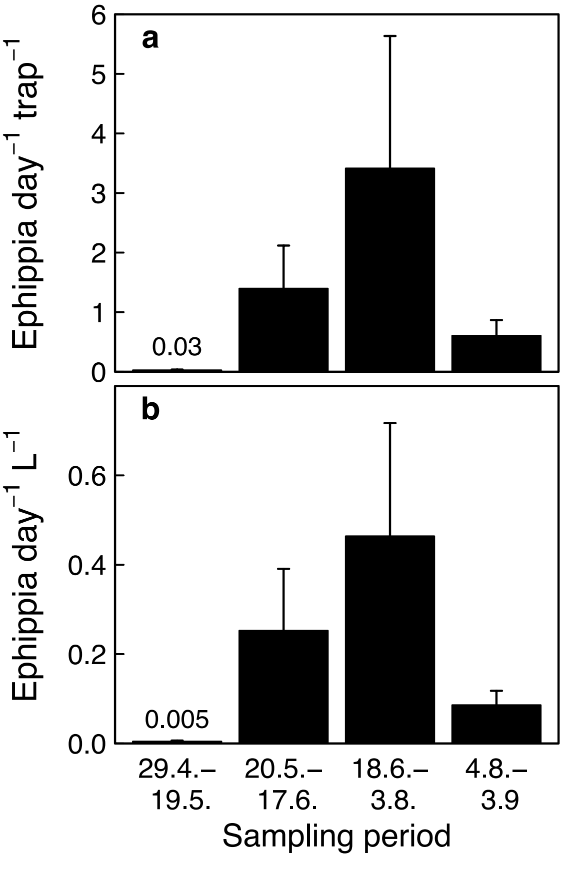

Fig. 1. Mean (± SE) ephippium production of *Daphnia magna* populations in 18 natural rock pools during four time periods in summer 2005. The number of ephippia produced differed significantly between the four periods and the different rock pools. a) Mean number of ephippia produced per day per trap. b) Mean number of ephippia produced per day and water volume above the trap.

Habitat size experiment (mesocosms)

There was a significant positive correlation between the total number of ephippia produced during one season and the water volume of the mesocosm containers inhabited by the *Daphnia* populations (Fig. 3). However, the increase of ephippium counts with volume was significantly smaller than an increase with a slope of one (on a log-log scale; same density treatment $t_{21} = 11.06$, $p < 0.0001$; same number treatment $t_{21} = 12.38$, $p < 0.0001$). An increase with a slope of one (1:1 line) would indicate a proportional increase and thus a constant productivity per volume. As the increase was lower than one, the populations' productivity significantly decreased with increasing container size. A 1000-fold increase in habitat volume resulted only in a 5 to 11-fold increase in the populations' ephippium production ("same density treatment": $\log_{10}(\text{ephippia day}^{-1}) = 2.64 + 0.35 * \log_{10}(\text{volume})$; "same number treatment": $\log_{10}(\text{ephippia day}^{-1}) = 2.61 + 0.22 * \log_{10}(\text{volume})$). The initial number of *D. magna* females in spring did not influence the overall production of ephippia per season (Fig. 3a, 3b and Tab. 1a).

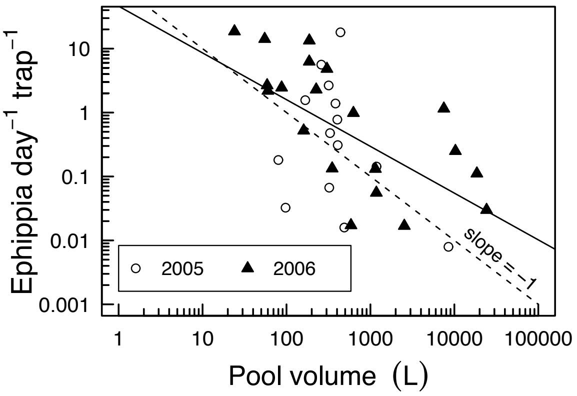

Fig. 2. Correlation between pool volume and the ephippium production of *Daphnia magna* populations in 34 natural rock pools in 2005 and 2006. The rock pool water volume ranged from 24 L to 24200 L. The solid line is the overall linear regression $\log_{10}(y) = 1.66 - 0.731 * \log_{10}(x)$; the dashed line is the 1:1 line with slope – 1 for visualization. Both axes on logarithmic scale.

The results were very similar when using water surface area instead of water volume as the explanatory variable (Fig. 3c, 3d and Tab. 1b): the total number of ephippia produced by a population increased with the water surface area of the container in both treatments ("same density treatment": $\log_{10}(\text{ephippia day}^{-1}) = -0.55 + 0.54 * \log_{10}(\text{surface})$; "same number treatment": $\log_{10}(\text{ephippia day}^{-1}) = -0.14 + 0.37 * \log_{10}(\text{surface})$). Again, this increase was significantly smaller than an increase with a slope of one (on a log-log scale; same density treatment $t_{21} = 4.57$, $p = 0.0002$; same number treatment $t_{21} = 5.99, p < 0.0001$).

We then combined the data from the habitat size experiment with the extrapolated data from the natural pools (Fig. 4). The numbers of ephippia produced relative to inhabited water volume were in agreement between these two independent studies. In both studies, the increase in habitat volume resulted in a much smaller increase in the populations' total ephippium production: a 1000-fold increase in habitat volume resulted only in a 2.8 to 7.2-fold increase in a population's absolute ephippium production (extrapolated data from natural pools and data from the habitat size experiment respectively). The linear regression for the ephippium traps in natural rock pools was $\log_{10}(\text{ephippia day}^{-1}) = 1.57 + 0.15 * \log_{10}(\text{volume})$ and for the habitat size experiment irrespective of the treatment was $\log_{10}(\text{ephippia day}^{-1}) = 0.62 + 0.29 * \log_{10}(\text{volume})$ (Fig. 4). As one may expect, the data from natural rock pools were more variable than those from the experimental mesocosms.

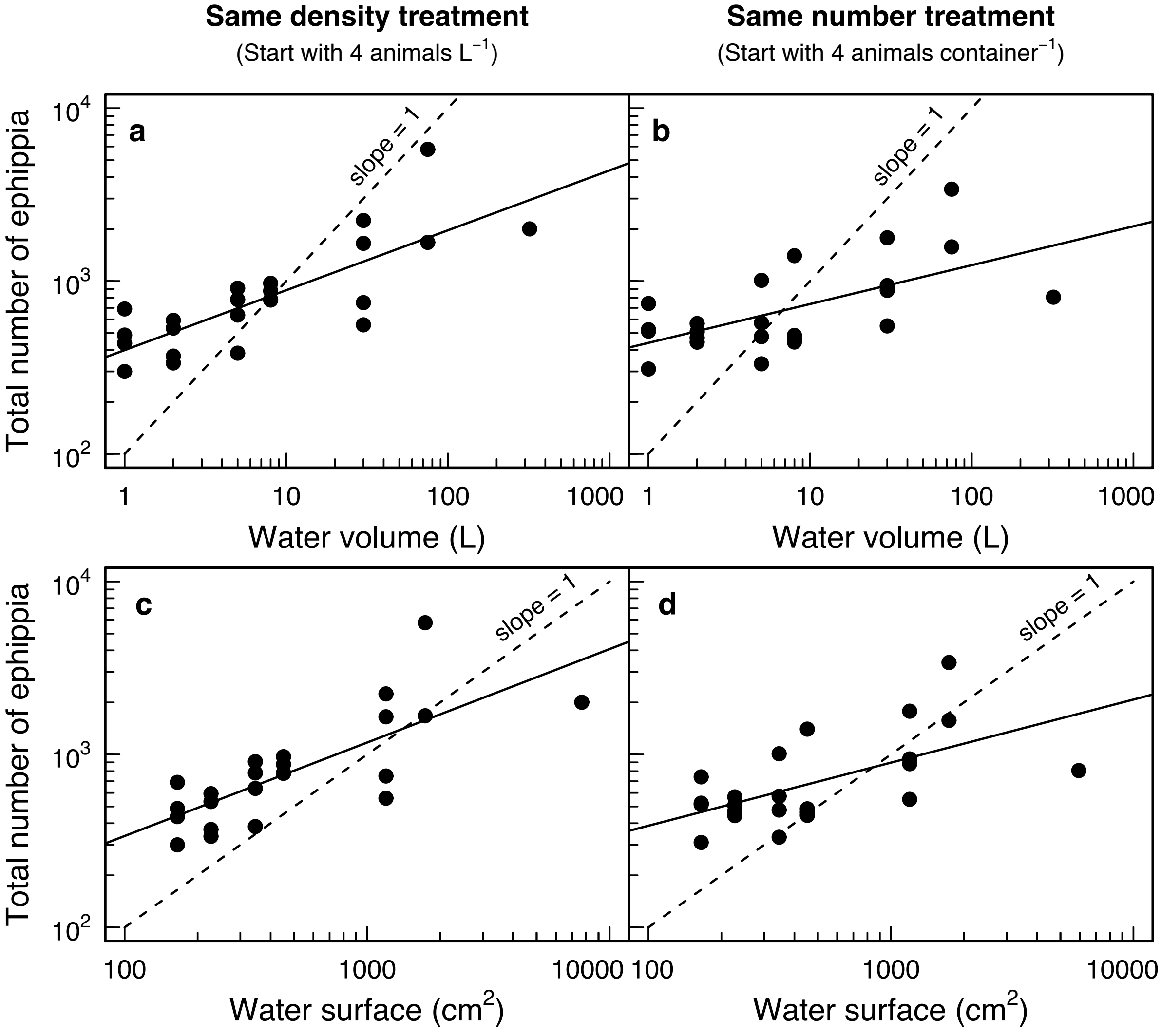

Fig. 3. Total number of ephippia produced by *Daphnia magna* populations in mesocosms under semi-natural outdoor conditions in function of water volume (a, b) or water surface (c, d). In the two treatments "same density" and "same number" initial population size was manipulated. Populations in the same density treatment had an initial population density of 4 individuals L^{-1} while populations in the same number treatment had a total initial population size of 4 individuals irrespective of the container's volume. The populations' ephippium production correlated significantly with the water volume and the water surface of the containers (solid lines) but there was no significant difference in the number of ephippia produced between the two different treatments. The slopes were significantly smaller than the slope of 1 (dashed). Both axes on logarithmic scale.

Desiccation experiment

The *D. magna* in the containers produced ephippia prior to (Fig. 5, A) and during desiccation (B_1) as well as after post-drought reestablishment (B_2). Only ephippia survived the drought in the desiccation treatment. At the end of the drought, there were less ephippia in the desiccation treatment than in the control treatment (Fig. 5, mean number of ephippia produced per population: $B_1 = 77.6$ vs. $C_1 = 108.3$, measured just before refilling the container on 31 July; Wilcoxon rank test, $W_{16,16} = 74.5$, $p = 0.046$). This difference was likely to be due to the difference in the production time. The difference was no longer significant when the daily ephippium production was used (mean number of ephippia produced per day with desiccation = 1.33 and control = 1.86; Wilcoxon rank test, $W_{16,16} = 110$, $p = 0.51$). Thus, the lower ephippium production in the desiccation treatment can be ascribed at least in part to the twelve days of complete drought (19 to 31 July), during which no ephippia were produced. The length of complete drought was arbitrarily chosen, and therefore it is better to use ephippia produced per day rather than the total number.

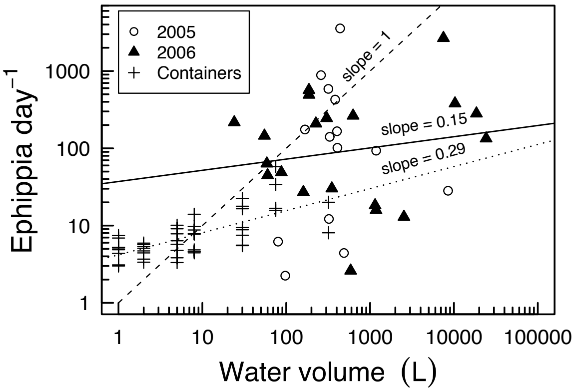

Fig. 4. Comparison of the correlation between water volume of the habitat (in L) and number of ephippia produced by *Daphnia magna* populations in natural rock pools pooled over two years (solid line, data pooled over two years) and in mesocosms in containers (dotted line, same data used as in Fig. 2 and 3). The dashed line depicts the 1:1 line for visualization. Both axes on logarithmic scale.

Refilling the containers with water after the drought initiated hatching and viable populations were built up within the same season in all but one replicate. The hatching synchronously occurred five days after refilling in all replicates and the animals produced the first asexual eggs within five to eight days after hatching. The number of hatchlings was positively correlated with the number of ephippia (linear regression with the intercept forced through zero, $t = 7.92$, df = 15, $p < 0.00001$; Fig. 6). The females that hatched from the ephippia reproduced asexually and produced ephippia before the end of the experiment in autumn. Production of ephippia per day did not differ between the desiccation treatment, the density-reduction treatment and the control during the time when the desiccation treatment had been refilled until the end of the season (this corresponds to the slopes of the three lines in the most right part of Fig. 5; Kruskal-Wallis test, $X^2 = 4.26$, df = 2, $p = 0.12$). In the desiccation treatment the absolute number of ephippia present at the end of the season was about half than in the control ($B_2 = 77.63$ vs. $C_2 = 144.25$; Wilcoxon signed rank test, $W_{16,16} = 54.5$, $p = 0.006$). Coincidently, in the desiccation treatment the mean number of ephippia at the end of the experiment (B_2) reached about the same level as before desiccation (B_1). Consistent with the phenology in natural pools (Fig. 1), the production of ephippia in August was much smaller than earlier in the season and the number of ephippia produced after desiccation just substituted the "loss" due to hatching.

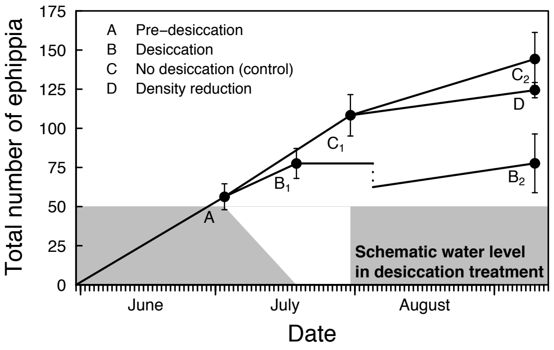

Fig. 5. Cumulative number of ephippia produced by *Daphnia magna* populations in the desiccation experiment (mean ± SE) at different moments in time. Ephippium production was measured after an initial pre-desiccation phase (A), after a desiccation phase (B_1 and in parallel in the control C_1) and at the end of the season (C_2, D, B_2). All measurements are from independent replicates, each of which was only once destructively sampled. The light grey area schematically depicts the water level change in the desiccation treatment. In the desiccation treatment there was a reduction in ephippia due to hatching of *Daphnia* few days after refilling the water (subtracted from the total, dotted line). In the control, the water level stayed constant. In the density reduction treatment (D), *Daphnia* population density was reduced to a density equivalent to the hatchling density in the desiccation treatment.

Discussion

We studied the production of ephippia in a natural metapopulation of *Daphnia magna*. The number of ephippia reflects a population's contribution of potential migrants in the metapopulation, as dispersal occurs passively by wind (Maguire, 1963) and possibly via waterfowl (Proctor & Malone, 1965, Figuerola & Green, 2002). In the studied metapopulation, the inhabited rock pool differ in size over several orders of magnitude, and a high colonization-extinction turnover of populations has been found (Ebert et al., 2001, Pajunen & Pajunen, 2003). Short-lived populations in small pools have been suggested to act as sinks that do not contribute migrants, while long-lived populations in large pools were classified as the source of migrants (Pajunen, 1986, Pajunen & Pajunen, 2003, Bengtsson, 1989). We were especially interested in populations' ephippium production in qualitatively different habitats and focused on the effects of habitat size, initial population size and summer desiccation thereon. We showed that populations in small, desiccation-prone pools produced more potential migrants than previously thought, and thus might contribute substantially to the global stability of the metapopulation.

Tab. 1. a) ANCOVA for the effects of water volume of the containers (L), treatment ("same density" or "same number") and their interaction on a populations' ephippium production per season and replicate. b) ANCOVA for the effects of water surface of the containers (cm^2), treatment ("same density" or "same number") and their interaction on a populations' ephippium production per season and replicate.

a)	**Source of variation**	**df**	**MS**	***F***	***P***
	Volume	1	1.731	44.03	<0.0001
	Treatment	1	0.045	1.15	0.29
	Volume x treatment	1	0.079	2.00	0.16
	Error	42	0.039		
b)	Surface	1	1.639	39.007	<0.0001
	Treatment	1	0.042	1.005	0.32
	Surface x treatment	1	0.061	1.444	0.24
	Error	42	0.042		

Phenology of ephippium production

Daphnia hatched in the first half of May. The *D. magna* populations then started to produce ephippia within four weeks (Fig. 1). They continued to produce ephippia until September with peak production during June and July. The peak of ephippium production occurred during the warmest month (July; data not shown), which could be due to a dependence of productivity on water temperature (Brown, 1929), but may also be a result of the typically high population densities during summer (personal observations). The rapid start of ephippium production in spring and the peak in summer might also reflect an adaptation to avoid extinction during summer droughts. Desiccation can be fast and unpredictable and is occurring frequently in small pools in this metapopulation (personal observations). Therefore the early and continuous production of ephippia is an essential bet-hedging trait of the present *Daphnia* populations that enables them to survive in unpredictable habitats (Cáceres & Tessier, 2003, Hopper, 1999). In contrast, lake *Daphnia* usually produce much fewer ephippia and usually only at distinct times, as they can survive year-round in the planktonic phase (Cáceres, 1998, Cáceres & Tessier, 2004). The rapid start and continued production of ephippia over an extended period also indicates that ephippium production is not limited to large and long-lasting "mainland" populations (Pajunen & Pajunen, 2003), but is equal in small, desiccation prone pools.

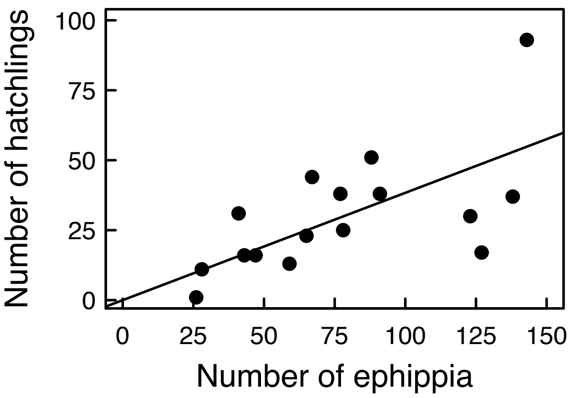

Fig. 6. Correlation between the number of ephippia and number of *Daphnia magna* hatchlings after experimental desiccation and subsequent refilling of the containers (see desiccation treatment in Fig. 5). Linear regression line forced through the origin (slope = 0.38). As there are usually two eggs per ephippium, the estimated average hatching rate can be calculated to be about 19 %.

Influence of habitat size and initial population size on ephippium production

The fundamental difference between the Levins' type metapopulation suggested by Hanski and Ranta (1983) and the mainland-island model suggested by Pajunen and Pajunen (2003, 2007) is the number of migrants produced by populations in different patch types, whereby patches differ both in size and risk of desiccation. We consistently found an increased ephippium production of populations in larger rock pools and larger mesocosms. However, the increase in habitat size resulted in a much smaller increase in a population's ephippium production: a 1000-fold increase in the pool volume resulted only in a 2.8 to 7.2-fold increase in the ephippium production (Fig. 4). This means that, relative to their size, populations in small pools contributed over-proportionally to the pool of dispersal stages. The same results were found when using pool surface to describe the size of a habitat: a 100-fold larger surface area resulted in a 5 to 12-fold increase in ephippium production (Fig. 3c and 3d).

There are several possible explanations why populations in large pool produce less ephippia than one could expect from the pool volume. In this metapopulation many *D. magna* populations occur in small, desiccation-prone pools (Pajunen & Pajunen, 2007). To survive in such an environment it may therefore be adaptive to invest a lot into the production of ephippia, especially by populations in the smallest pools. Environmental factors triggering the production of ephippia such as shortage of food, crowding and rapid changes in temperature (Carvalho & Hughes, 1983, Stross & Hill, 1965, Kleiven et al., 1992) may not be buffered in small pools and populations are more susceptible to stochastic processes. These environmental factors may thus be ultimate reason why populations in small pools produce many ephippia. An analogue example where populations in small habitat patches contribute most migrants in a metapopulation is given by Crone et al. (2001). They studied a vole metapopulation on skerry islands where the environment was unpredictable on small islands. Population densities were less stable on small islands, and dispersal rates increased before a subpopulation's extinction. In our data from the natural metapopulation (Fig. 2), one could argue that animals from populations in small pools differ in their genetic predisposition to produce ephippia compared to animals from populations in large pools. Natural populations in small pools are often descending from recent colonizations, and colonizers may carry alleles that favor the production of dispersal stages. This is for example the case in a butterfly metapopulation, in which individuals of newly founded populations have a higher genetic predisposition to disperse (Haag et al., 2005, Hanski et al., 2006). However, in our experiment the *Daphnia* populations in the different sized mesocosms consisted of the same genotypes (Fig. 3). Therefore the effect was not due to a genetic difference of the populations in small versus large pools but solely due to differences in habitat size.

Larger pools usually harbor long-lived populations with potentially many hatchlings out of the ephippia from the preceding year (Bengtsson, 1989, Pajunen & Pajunen, 2003, Altermatt et al., in Prep.). In contrast, populations in newly colonized or small rock pools have a higher risk of extinction (Bengtsson, 1989), a shorter time of persistence and harbor no or small ephippium banks (Pajunen & Pajunen, 2007). Due to the instability of the habitat with respect to desiccation, populations in small pools may also go more often through bottlenecks. Therefore, we expect in these pools less ephippia and only few hatchlings in spring. To incorporate differences in the number of *Daphnia* at the beginning of the season on the seasonal ephippium production, the habitat size experiment was started with different initial population sizes. The same density treatment reflected the situation in long-lived populations, while the same number treatment reflected conditions in newly colonized rock pools with only few hatchlings. The initial difference of population sizes did not have an effect on the total number of ephippia produced over the whole season (Tab. 1, Fig. 3). In a metapopulation context this means that populations differing in their initial population size may still be equivalent in the production of ephippia.

The observed increase in populations' ephippium production with habitat size was much more shallow than expected under a mainland-island model, in which the vast majority of migrants come from a few mainland populations and hardly or no migrant come from populations in the small island (Harrison, 1991). We showed that populations in small habitats, which are usually short-lived (Pajunen & Pajunen, 2007), produced substantial amounts of ephippia. As an implication of this, populations in small habitats are more important in this metapopulation than previously thought (Pajunen & Pajunen, 2003). In this *D. magna* metapopulation, most populations occur in small rock pools (Altermatt et al., in Prep.). Overall, these populations may even produce in total more ephippia than those in the large pools. However, this arguments needs to be worked out in a more quantitative way. Furthermore, small pools are more prone to desiccation. During droughts ephippia are more exposed to wind and birds in the dry sediments (Maguire, 1963, Proctor & Malone, 1965), which may result in even more dispersal from small, frequently desiccating pools than from large and permanent pools (Vanschoenwinkel et al., 2008). It should be noted that we did not measure dispersal itself, but only the production of potential migrants. It is generally difficult to measure dispersal rate and dispersal success (Hanski et al., 2000) and the measuring of migrants usually requires either individual marking or recognition of migrants. In our system all ephippia are potential migrants. Dispersal occurs passively either by wind or birds (Maguire, 1963, Proctor & Malone, 1965). We therefore assume a positive correlation between the number of ephippia and the number of effective migrants.

Desiccation of the habitat

Annual extinction rates and population turnover are high in the studied *D. magna* metapopulation (Pajunen & Pajunen, 2003, Pajunen & Pajunen, 2007) and mesocosm experiments in containers showed that populations in smaller habitats have a higher extinction risk (Bengtsson, 1989). Instability of pools was used as a main argument to explain why populations in small pools do not contribute migrants (Pajunen & Pajunen, 2003). Desiccation of the habitat is a possible instability. While pools desiccate, the *Daphnia* population density and the salinity of the water increase. As both high population density and salinity are triggering ephippium production (Banta & Brown, 1929, Carvalho & Hughes, 1983), we speculated that these factors might boost the ephippium production. In contrary to our expectations, desiccation did not induce a higher ephippium production in our experiment (Fig. 5). The productivity (ephippia day^{-1}) was not different between the desiccation treatment and the control. Though, ephippium production continued in the control populations during the complete drought in the desiccation treatment, resulting in a higher absolute ephippium production. During the second half of the season (31 July until 10 September) the rate of ephippia produced per day was smaller than early in the season (Fig. 6). This is consistent with the seasonal pattern of ephippium production in natural pools (Fig. 1). The rate of late season ephippium production did not differ between previously desiccated and non-desiccated treatments (Fig. 5). This is consistent with the container size experiment (Fig. 3), where the ephippium production was independent of the initial population size.

Only ephippia can survive a drought. It was unclear if and how fast a new viable population can establish after desiccated pools have gained water again. In our experiments hatchlings emerged and formed a viable planktonic population a few days after refilling the containers within the same season. Thus, *D. magna* can survive a summer drought even in the absence of an ephippium bank from previous years. Winter conditions are not obligatory for hatching after the drought. It also suggests that ephippia can be produced, dispersed and colonize a new habitat patch within the very same year. Taken together, a drought seems not to boost ephippium production, but could give the *Daphnia* a dispersal advantage as the ephippia get exposed on the dry sediments of the pool. Also, desiccation does not put populations in small pools at an immediate risk to go extinct. Nevertheless, it reduces the end-of-season number of ephippia, which may reduce the likelihood to survive the next winter. This may contribute to the observed high extinction rates of populations in small pools (Pajunen & Pajunen, 2007).

Conclusions

We found in the studied *D. magna* metapopulation that populations in small habitat patches are able to produced substantial numbers of dispersal stages. The instability associated with these smaller patches does not compromise their ability to produce migrants and it would be even an evolutionary stable strategy of animals to produce more migrants in an unpredictable environment. Thus, when focusing on the production of dispersal stages, our data do not support the hypothesis of Pajunen and Pajunen (2007) who suggested that a few long-lasting populations in large pools are the key populations in this metapopulation. As droughts, which mainly affect small pools, may increase the exposure of ephippia to passive dispersal, it may even be the short-lived populations in small pools that contribute most effective migrants. If true, it is the locally instable part of the metapopulation, which contributes most to the global stability. Small and instable patches may also be in other systems more important for the functioning of metapopulations than previously thought (Crone et al., 2001). This may change predictions about the future development of metapopulations. For example, when a protection scheme in conservation biology can only be applied to a subset of populations, the key populations with respect to the origin of migrants must be known to guarantee an ongoing of metapopulation processes.

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Chapter 2

Desiccation of rock pool habitats and its consequences for a *Daphnia* metacommunity

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Manuscript

Abstract: Different environmental factors influence the stability and quality of fresh water habitats and determine a species' occurrence and distribution. An important factor is the availability of water itself, especially for small to medium sized water bodies such as rock pools. These habitats may desiccate temporarily, which may influence the suitability of the pools as a habitat. Desiccation is detrimental for planktonic populations but may also have beneficial aspects, such as the exclusion of certain predators or parasites. The ecological significance of habitat instability and especially the rarely studied desiccation risk is underlined by the various drought resistant resting stages of many aquatic organisms. We studied evaporation in 50 rock pools within a metacommunity of the three *Daphnia* species *D. magna*, *D. longispina* and *D. pulex* over one summer. Evaporation and ultimately desiccation was significantly correlated with pool surface area, the presence of vegetation, ambient temperature, wind and standardised evaporation measures. With the estimates from the regression model we developed a simulation model to predict changes in the water level in each pool on a daily basis. Eventually, desiccation events and drought lengths could be predicted for all 530 natural rock pools over a 25 years period for which the *Daphnia* communities in all these pools have been recorded. A whole-season survey of all rock pools in 2006 confirmed that the predicted desiccation events from the model were consistent with the actually observed droughts. We correlated the predicted number of desiccation events in each pool with pool-specific occupation lengths of the three species. Both the number of desiccation events as well as the lengths of droughts was important, as they influenced occupation lengths of all *Daphnia* species in this metacommunity.

Introduction

The presence of a species is strongly influenced by habitat size and quality (Ellenberg, 1974, Schwoerbel, 1971, Begon et al., 2005) and most species' occurrence is predetermined by climatic constraints such as weather extremes, by physical properties of the habitat, by the presence of food resources or by inter-specific competition. The so-called ecological niches are well studied for many species and can be indexed using abiotic parameters (Ellenberg, 1974, Begon et al., 2005, Hutchinson, 1958). Nevertheless, a suitable habitat may not always be inhabited and the occurrence of a species may change over time. For example, the absence of a population can be due to a time lack in colonisation after the emergence of suitable habitats, due to isolation of such habitats or due to recurrent extinction.

An often neglected, temporal aspect of a species' occurrence in a specific locality is habitat instability. Instability can be a key characteristic of a habitat and may occur due to seasonality (Andrewartha & Birch, 1954), climate change (Walther et al., 2002) or disturbances such as catastrophic events (Connell, 1978). Changes in the habitat due to environmental instability may be detrimental for the local populations. Forest fires, floods or droughts are well known examples that lead to local extinction of populations by killing all individuals or strongly disrupting their life cycles (Begon et al., 2005, Fuentes et al., 1995). As a result, many species are restricted to stable habitats. However, habitat instabilities are common, and some species evolved mechanisms to deal with them. Prerequisites such as fire-resistant seeds in pine trees, dispersing morphs in insects, or alternative life-history strategies such as vegetative growth versus sexual reproduction in alpine plants enable tolerance or escape of the instability. Species that can outlast unsuitable periods in dormancy may in fact profit from the habitat instability, as other species may become excluded from the temporarily unsuitable habitat. Eventually, the resources are used by fewer species and allospecific competition is reduced. Also, populations may be purged from parasites during instability induced changes in the habitat, as the parasite's life cycle or transmission may become disrupted (Ebert, 2005). Therefore, instabilities are important characteristics of a habitat and should be known to understand species communities.

Metapopulations provide a further possibility to survive in an unstable environment. They consist of a network of empty and inhabited habitat patches. At any moment, populations only occur in a subset of all possible patches (Hanski & Gaggiotti, 2004, Levins, 1968). Local extinctions are buffered by migrants that colonise new patches and thereby ensure global stability of the metapopulation over time (Hanski, 1999, Hanski & Gaggiotti, 2004, Levins, 1970). The metapopulation dynamics are influenced both by extinctions as well as colonisations. In early approximations, all patches were assumed to be of equal quality and connectivity (Levins, 1968, Levins, 1970). However, different empirical studies showed that habitat patches differ in their connectivity (Saccheri et al., 1998, Fleishman et al., 2002), quality (Franken & Hik, 2004), in habitat or population size (Hanski & Gaggiotti, 2004), and populations in some patches may be less affected by local instabilities (Pulliam, 1988). Theoretical studies also showed that large perturbations and instabilities prolong the transient time for a population to return to population-dynamic equilibrium (Saccheri et al., 1998). When populations in individual patches are differently exposed to instabilities, this may not only influence the local survival, but also metapopulation dynamics in general. It is essential to parametrise and quantify these habitat instabilities to understand metapopulations.

Dispersal and the origin of migrants may be related with habitat instability. It may be good to be prepared for a leave in an unstable habitat patch. Theoretical studies showed that dispersal rate evolution is affected by unequal local extinction rates of populations in different patches (Olivieri et al., 1995) and that dispersal is favoured in unstable environments (Gandon & Michalakis, 1999, Holt, 1997, Clobert et al., 2001). Some studies have found that individuals from populations in variable habitats have a higher dispersal propensity (Hill et al., 1999, Denno et al., 1996), and that dispersal propensity may evolve in unstable habitats (Friedenberg, 2003).

We studied natural desiccation of rock pools in a metacommunity of the three *Daphnia* species *D. magna*, *D. longispina* and *D. pulex*. The three *Daphnia* species occur in rock pool habitats that vary in size over several orders of magnitude (Pajunen, 1986, Ranta, 1979). In such systems, both local and regional processes play an important role in structuring invertebrate communities (Vanschoenwinkel et al., 2007). Here, we were interested in the stability of different sized rock pools in respect to the occurrence of water and in relation to pool characteristics. We then related desiccation of pools to the occurrence of *Daphnia* populations.

Many other biological phenomena have already been studied in rock pool systems, including abiotic niche differentiation (Lagerspetz, 1955, Ranta, 1979, Pajunen & Pajunen, 2007), effects of inter- and intra-specific competition (Hanski & Ranta, 1983, Bengtsson, 1986), metapopulation dynamics (Pajunen, 1986, Pajunen & Pajunen, 2003), parasite occurrence (Bengtsson & Ebert, 1998, Ebert et al., 2001), genetic effects of inbreeding and local adaptation (Altermatt et al., 2007, Ebert et al., 2002) and effects of climate change on migration (Altermatt et al., 2008). The high instability due to desiccation is a peculiar characteristic of rock pools, as already pointed out by Ranta (1979). Personal observations showed that desiccation occurs often and especially small pools may dry up several times per season and that strong precipitation events fill up all pools to their maximal level. However, even if desiccation of rock pools is a typical and common process (Ebert, 2005, Pajunen & Pajunen, 2003), it has never been quantified and investigated for its biological implications.

For *Daphnia* populations, desiccation may have both beneficial as well as detrimental aspects. Droughts kill planktonic populations and disrupt their life cycle. To frequent droughts may even prevent the successful occupation of a pool and droughts shorten the time of habitat usability. On contrary, *Daphnia* can survive droughts as resting stages, while many of their allospecific competitors and predators such as fish, copepods and water insects can not survive droughts (Ranta, 1982). The resting stages, which usually lie on the sediment surface, are exposed during droughts (Ebert, 2005). This may increase migration by means of wind or birds (Maguire, 1963, Vanschoenwinkel et al., 2008). Thus, as desiccation influences the ecology of *Daphnia* in several ways, it may be biologically more relevant than parameters such as pool size or water chemistry alone (Ranta, 1979, Pajunen & Pajunen, 2007). Therefore, its quantification is in the center of this study.

We measured evaporation in 50 rock pools in a metacommunity over one summer and related it to both meteorological and pool variables. We then modelled with a simulation model the number of desiccation events and drought lengths in all individual rock pool habitats of our study area on a daily basis over a period of 25 years. Desiccation was correlated with pool occupation of *Daphnia*. *Daphnia* populations of the three different species occurred in all type of pools, including the highly unstable ones. Long-lasting populations were mostly found in pools with on average less than one desiccation event per year.

Material and Methods

We studied rock pools in a metacommunity of the three planktonic crustaceans *Daphnia magna* Straus, *D. longispina* O. F. Müller, and *D. pulex* De Geer (Crustacea: Cladocera) focusing on the number of desiccation events and drought lengths. The three *Daphnia* species are widely distributed along the coast of the Baltic Sea. They inhabit freshwater rock pools on the skerry islands which suffer frequent desiccation (Bengtsson & Ebert, 1998, Ebert et al., 2001, Pajunen & Pajunen, 2003, Ranta, 1979, Ranta, 1982, Lagerspetz, 1955). For a part of this metacommunity extensive data exist, including *Daphnia* population distribution over a period of 25 years (1982–2006) in 530 pools and description of pool characteristics (Pajunen & Pajunen, 2003, Ranta, 1979, Pajunen & Pajunen, 2007).

Rock pool Daphnia

Daphnia populations in the rock pools represent metapopulation systems, with frequent extinction and colonisation (Pajunen & Pajunen, 2003). The three species studied here either occur singly or coexist in the same rock pool, though they have slightly different ecological preferences (Pajunen & Pajunen, 2003, Ranta, 1979, Pajunen & Pajunen, 2007). They differ in competitive abilities, parasite susceptibilities, and life strategies (Bengtsson, 1989, Ebert, 2005, Hanski & Ranta, 1983). On average, *D. magna* occurs in smaller pools, *D. pulex* in intermediate-sized pools and *D. longispina* in larger pools (Bengtsson, 1988, Lagerspetz, 1955, Ranta, 1979, Pajunen & Pajunen, 2007), though the overlap in used habitats is large. As these species interact with each other, occur in discrete patches and are connected by migration, they are a good example of a metacommunity (Gilpin & Hanski, 1991, Leibold et al., 2004).

All three species reproduce by cyclical parthenogenesis, except for some populations of *D. pulex*, that are obligate parthenogenetic. Cyclical parthenogenesis means that phases of asexual production are intermitted by sexual reproduction. As a result of sexual reproduction, ephippia (= resting eggs) are produced. These ephippia guarantee survival in an unstable environment, because they can outlast unfavourable conditions such as freezing during winter or desiccation of pools during summer (Ebert, 2005, Altermatt & Ebert, 2008). In these shallow pools, long-lasting resting egg banks are absent (Pajunen & Pajunen, 2003). The ephippia also serve as dispersal stages that migrate passively either by wind or birds (Ranta, 1979, Maguire, 1963, Vanschoenwinkel et al., 2008). The ephippia on the sediment surface at the bottom of the pool are particularly exposed to wind and bird in desiccated rock pools (see figure 2.19 in Ebert (2005)), and may eventually be dispersed.

Ephippia can migrate into rock pools that are already inhabited by a conspecific *Daphnia* population (= invasion) or into rock pools that have no conspecific *Daphnia* populations (= colonisation). Due to asexual reproduction, large planktonic populations can develop within a few weeks from a single foundress. This may be followed by an influx of further immigrants over time. The survival of a *Daphnia* population in a rock pool ranges from less than a year to more than 20 years (Pajunen & Pajunen, 2003). Populations go extinct for various reasons. The most common causes are when rock pools are washed out by waves from the surrounding Baltic Sea (Pajunen & Pajunen, 2003), parasite epidemics (Ebert, 2005), competition with other *Daphnia* species (Bengtsson, 1989) and changes in habitat qualities. Desiccation of a pool is detrimental for the planktonic animals, while ephippia can survive (Altermatt & Ebert, 2008, Pajunen & Pajunen, 2003, Ranta, 1979).

Occupation and persistence of populations in different habitats

One of us (V. I. Pajunen) recorded *Daphnia* populations of all three species in 507 rock pools twice a year from 1982 to 2006. For our analysis we included a further 23 pools that could be characterised as marginal habitats, but did not contain *Daphnia* (for detailed methodology see Pajunen (1986) and Pajunen & Pajunen (2003, 2007)). The two yearly samplings took place during periods when all rock pools contained water and when planktonic populations could be detected. In between the samplings, pools may have become desiccated and refilled with water again. During each visit, the presence or absence of each of the three *Daphnia* species was determined for each pool.

Description of habitat characteristics, meteorological data and the evaporation model

Our study area included 530 freshwater rock pools on 18 islands in the archipelago of southwest Finland at Tvärminne Zoological Station (59° 50' N, 23° 15' E). All available rock pools were mapped at the beginning of the study (Pajunen & Pajunen, 2003). For each rock pool we measured the maximal pool surface area (m^2) and the maximal depth (cm) (see also Pajunen & Pajunen (2007)). To estimate the catchment area of a pool, we first visually determined the watershed around the pool. The localisation was verified by two independent persons. The determined watershed was marked with chalk. We then measured the total catchment area (including the pool surface area) with a global positioning system receiver (model Garmin® GPSMAP 76CS) by following the watershed line. This method allowed us to measure catchment areas as small as one square meter. The precision of the GPS measurement was tested for a defined area of exactly 1 m^2. Repeated measurements resulted in deviations of maximal ± 0.1 m^2 (= 10 %). By subtracting pool surface area from the total catchment area we obtained the catchment area around the pool.

We recorded the presence or absence of vegetation in all pools in 2006. Vegetation included moss (*Sphagnum* sp., *Amblystegium* sp.), reed (*Phalaris arundinacea*, *Phragmites australis, Typha* sp.), tussocks or other grass or herb species growing at the edge of a pool and reaching into its water body. We calculated maximal pool volume (liter, assuming that the pool is an inverted pyramid, thus area * depth / 3; see Ebert et al. (2001)). Pool volume was not used in the multiple regression because it is auto-correlated with surface area and depth.

We measured daily evaporation (in mm) in 50 rock pools on two islands at 23 time intervals, each 24 hours long. The 23 intervals were spread over the period from 31 May to 20 August 2006. The 50 rock pools were a representative sub-set of the 530 rock pools. In each rock pool we placed a brick as a constant reference point. Water level was measured manually, and evaporation could be calculated as the difference between two consecutive measurements to the nearest 0.5 mm.

We then related daily evaporation in these 50 rock pools with a multiple regression on the pool variables surface area, depth, presence of vegetation and the weather variables ambient temperature, wind and evaporation. Mean ambient daily temperature (°C), mean daily wind speed (ms^{-1}) and total daily precipitation (mm) data were available from a standard weather station of the Finnish Meteorological Institute at Tvärminne Zoological Station (international identification number WMO 05493, national identification number LPNN 0202). The *Daphnia* populations on the studied islands were about 1.5 to 3.5 km south of that weather station. Local daily evaporation data (mm day^{-1}) were not available. Instead, we used Class A pan (USWB) evaporation data measured at Jokioinen Observatorio of the Finnish Meteorological Institute (WMO 02963, LPNN 1201; 60° 48' N, 23° 30' E). Jokioinen Observatorio is about 100 km north of Tvärminne Zoological Station. All these weather data were available for the whole study period (1982 to 2006).

The estimates of the final regression model were used to parametrise the simulation model. The simulation model predicted evaporation (mm day^{-1}) on a daily basis for all 530 pools during May to September 1982–2006. Desiccation of pools was predicted in the following way: we assumed that each pool was filled with water at 1 May of each year, and this maximal capacity was the upper boundary of the water level. For every day t starting at 1 May, the daily net difference between the water level decrease due to evaporation and the water level increase due to precipitation (including the influx from the catchment area) was calculated. This gave the new water level for day t+1. This was stepwise repeated until 30 September. We knew for each pool the maximal depth, which was the lower boundary. By that, we could predict when a pool would be dry. The model only used the measured maximal surface area of a pool and did not consider changes in the surface area due to evaporation. The latter would be strongly influenced by the shape of the pool, which could not be recorded. By following the water level changes in every individual pool during each summer, we predicted the percentage of pools that were dry at any moment or the percentage of time each single pool was desiccated. We visited all rock pools at five dates in 2006 (15 May, 13 June, 5 July, 12 July and 17 July) and recorded if they contained water or not. These data were used to compare the predicted proportion of dry pools with real data.

Our model considered only the period from May to September. This covered the biological relevant period, as the *Daphnia* growing season was from the beginning of May to the end of September/mid-October. *Daphnia* populations appeared in May and subsisted throughout the summer. The production of resting stages (ephippia) peaked in June and July (Altermatt & Ebert, 2008). By the end of September, the average temperatures dropped below 5 °C and the planktonic phase of the *Daphnia* populations eventually went extinct. Discontinuous ice and snow covers occurred from mid-October until end of April (unpublished data from the local weather station). During winter, the rock pools were frozen and no planktonic *Daphnia* were found. The populations survived as resting eggs.

Statistical analysis

Statistical analyses were performed with R (R Development Core Team, 2007) using the libraries base, date and Hmisc. We used a multiple regression to model daily evaporation in rock pools. We started with a full model containing all explanatory variables as well as their quadratic terms (Crawley, 2002). Model simplification was done in a stepwise procedure and non-significant terms were excluded (Crawley, 2002). We used a linear model to compare model predictions with the actual measurements, non-linear least square fits for relating the predicted time a pool was dry with its volume and generalized linear models for the occupation length analyses. We tested the assumptions of all our models and compared them with alternative models: We first fitted a generalized linear model with different slopes for each of the three *Daphnia* species and compared it with a simplified model with only one slope; the comparison was based on a Chi-square (χ^2) distribution (Venables & Ripley, 2002). If necessary, variables were $\log_{10}$-transformed. The chosen models were best in fulfilling the assumptions. Statistical comparison of estimated and theoretical slopes from linear models were performed according to Scherrer (1984). Density estimates were done according to Venables & Ripley (2002). We used the default kernel and default bandwidth functions implemented in R.

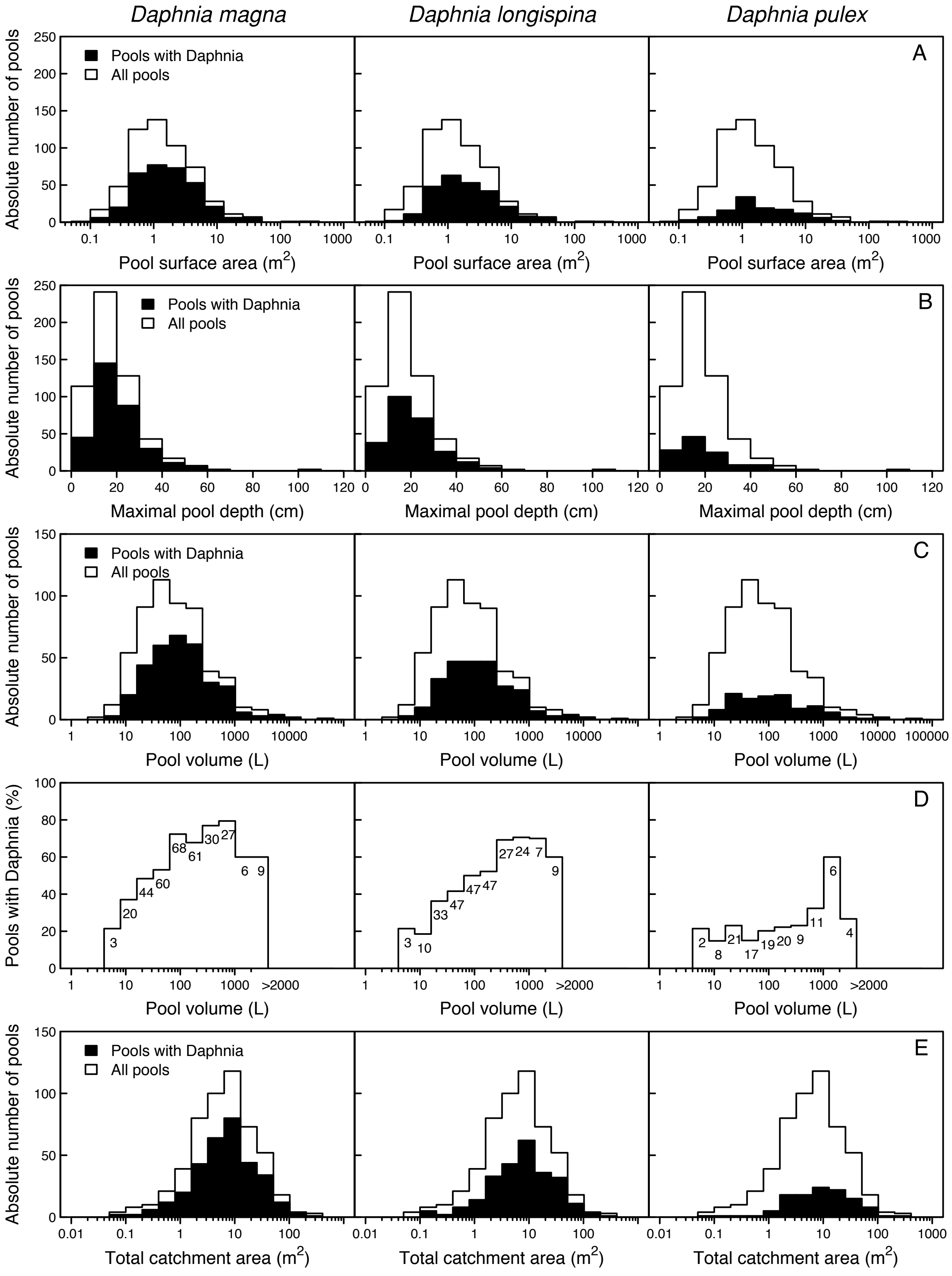

Fig. 1. Distribution of the rock pool characteristics. Distributions are given for all pools (white bars) and for those for pools which contained any of the three *Daphnia* species at least once over the 25 years (black bars; from left to right *D. magna*, *D. longispina* and *D. pulex*). A) Pool surface area; B) maximal pool depth; C) pool volume; D) percentage of pools in each size class that contained at least once a *Daphnia* population. To avoid percentage values that were strongly influenced due to a small total number, we pooled size classes at both ends of the x-axis such that each class contained at least 10 pools. This gave a class that contained all pools smaller than 8 liter volume, and a class that contained all pools larger than 2048 liter. The number in each size class gives the observed number of inhabited pools; E) total catchment area including pool surface area.

Tab. 1. Multiple regression analysis on measured daily evaporation in a subset of 50 rock pools relative to daily evaporation at Jokioinen weather station, pool surface area, mean ambient temperature, daily mean wind speed and the presence or the absence of vegetation. Non-significant terms as pool depth and quadratic terms were removed from the full model in a stepwise manner. The model estimates were used to predict daily evaporation in all rock pools.

Independent variable	*Estimate*	*Standard Error*	*t-value*	*p-value*
Intercept	0.46	0.041	11.4	<0.00001
Evaporation Jokioinen	0.013	0.0039	3.4	0.0008
$\log_{10}$(pool surface area)	–0.11	0.011	–9.9	<0.00001
Temperature	0.0098	0.0021	4.6	<0.00001
Wind	0.13	0.031	4.1	0.00004
Presence of vegetation	0.11	0.013	8.9	<0.00001
Wind^2	–0.023	0.006	–3.9	0.0001

Results

Rock pool characterisation

The 530 rock pools included in our study ranged in surface size and volume over more than four orders of magnitude (Fig. 1a and 1c). Most pools were rather shallow, with depths between 5 and 30 cm (Fig. 1b). *Daphnia* populations were found in rock pools of all sizes, but intermediate to large sized rock pools had been inhabited most often (Fig. 1d). Interestingly, not every very large rock pool has ever contained *Daphnia* (Fig. 1d). Out of all 530 available rock pools, 334 were inhabited at least once by *D. magna*, 258 by *D. longispina* and 120 by *D. pulex* over the study period from 1982 to 2006. 107 of the pools had never been inhabited by any *Daphnia* species.

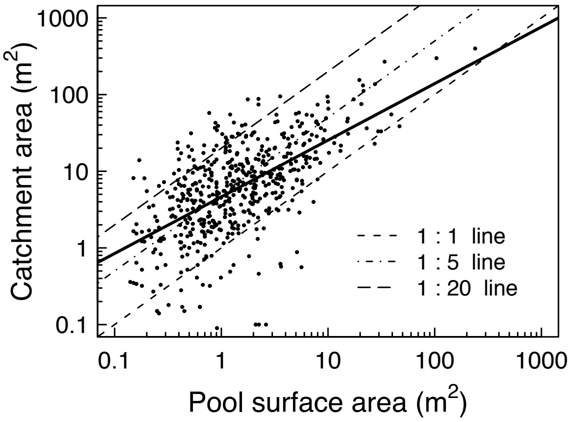

Fig. 2. Catchment area around each rock pool plotted against the pool's surface area. The straight line is the least square fit, which is significantly smaller than a slope of 1. Thus, larger pools have on average a smaller catchment area relative to their surface area. The dashed and dotted lines are for visualisation: the catchment area is as large as the pool surface for pools on the 1 : 1 line, it is five-fold as large as the pool surface on the 1 : 5 line, and twentyfold as large on the 1 : 20 line.

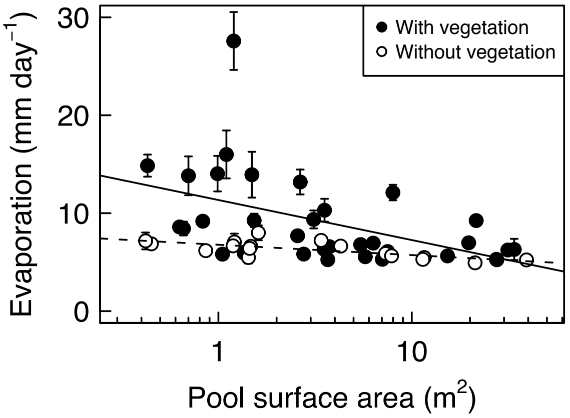

Fig. 3. Mean (± SE) daily evaporation in 50 pools at 23 time intervals in summer 2006. Each interval was 24 hours long. Evaporation significantly negatively correlated with pool surface area and was significantly higher in pools with vegetation compared to pools without vegetation (straight line versus dashed line).

Total catchment area of the pools varied over four orders of magnitude (Fig. 1e). More important than the total catchment area itself is the ratio of the catchment area relative to the pool surface area: after 5 mm of precipitation, the water level in a pool without catchment area will raise 5 mm. When a pool has an additional catchment area tenfold the size of its surface area, the water level will rise 55 mm. The ratio catchment area to pool surface area ranged from less than 1 to 82, with most pools having a ratio between 1 to 20 (Fig. 2). Not surprisingly, there was a significant positive relationship between the pool surface area and the catchment area ($\log_{10}$[catchment area] = 0.67 + 0.74 * $\log_{10}$[pool surface area]; linear model, $R^2 = 0.37$, $F_{1,528} = 315$, $p < 0.00001$). The slope of this relationship was significantly smaller than one (slope test, $t_{528} = 6.27$, $p < 0.00001$), meaning that larger pools had on average relatively smaller catchment areas.

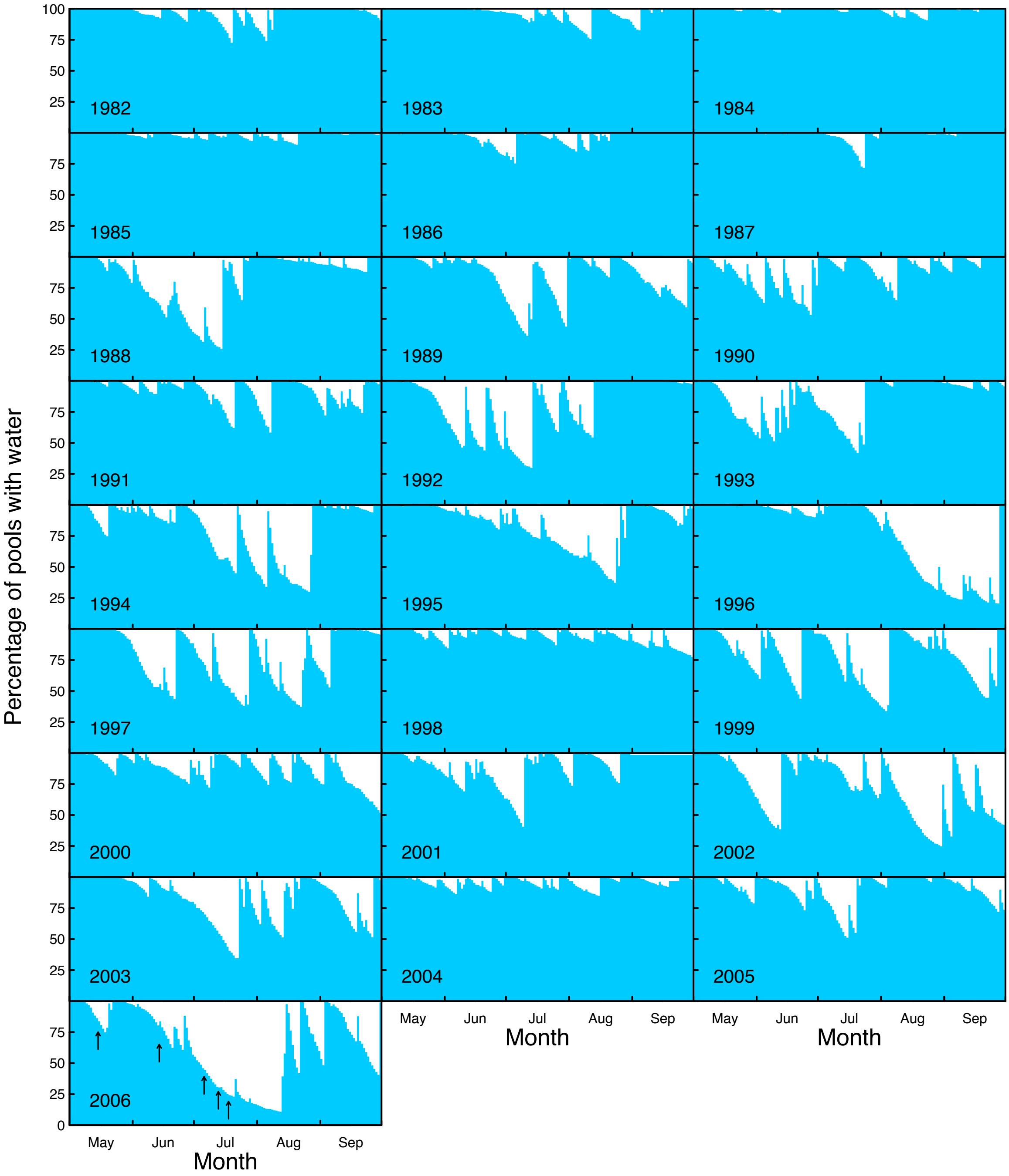

Fig. 4. Predicted daily percentage of pools that contained water within the period between 1 May to 30 September from the years 1982 to 2006. The blue area depicts the percentage of pools containing water at each specific day. In the model, pools were assumed to be brim-full at 1 May. Thereafter, the modelled daily difference between evaporation and influx by precipitation was used to calculate the water level in each pool. Evaporation and desiccation was predicted for each of the 530 pools individually. The arrows indicate the dates when all pools were visited in 2006 to record if they were desiccated or not. These data were used to validate the model.

Evaporation model and desiccation predictions

Evaporation per day per pool varied between 1 to 37.5 mm within the 50 monitored rock pools at the twenty-three 24-hours intervals in 2006 (mean 7.4 mm). Evaporation was significantly related to the following variables and these variables were retained in the multiple regression model: pool surface, presence of vegetation, ambient daily mean temperature, wind and evaporation measured at a weather station (Tab. 1). Evaporation correlated negatively with pool surface area and evaporation was lower in pools without vegetation compared to pools with vegetation (Fig. 3). Evaporation was not significantly influenced by pool depth.

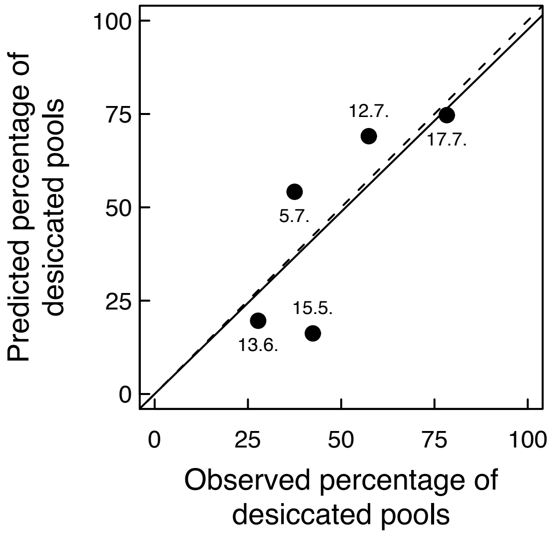

Fig. 5. Comparison between the observed percentage of all 530 pools that were dry at five dates during summer 2006 and the percentage predicted by the model for that time. The straight line is the least square fit (y = x * 0.98; $R^2 = 0.92$, $p = 0.003$). The slope of this curve did not differ from the 1 : 1 line (dashed) and the predicted droughts were in accordance with the observed droughts.

We used the following formula with the estimates from the multiple regression (Tab. 1) to predict evaporation for any pool at any day: $\log_{10}$[evaporation (mm day^{-1})] = 0.46 + 0.013 * evaporation Jokioinen (mm day^{-1}) – 0.11 * $\log_{10}$[pool surface area (m^2)] + 0.0098 * mean daily temperature (°C) + 0.13 * mean daily wind (ms^{-1}) + 0.11 * presence of vegetation – 0.023 * (mean daily wind)^2. With that formula we individually calculated the water level in all pools, and predicted the percentage of the 530 pools that contained water at any day during May to September over the period 1982 to 2006 (Fig. 4). There were large differences in the percentage of pools containing water, the duration of droughts and the timing of droughts between different years. In some years, almost all pools contained water over the whole summer season (for example in 1984, 1985, or 2004; Fig. 4), while in other years, long drought periods occurred where up to 80 % of all pools dried up (for example in 1994, 2002 or 2006; Fig. 4). The local climate changed in the study area over the last 25 years in accordance to global change models (Altermatt et al., 2008), and the year 2006 was exceptionally warm and dry.

In summer 2006, we had recorded all dry pools in the study area on five dates. Our model was able to predict the recorded number of desiccated rock pools consistent to the observed number of droughts (Fig. 5). The linear regression of these five points was significant ($R^2 = 0.92$, $F_{1,4} = 43.7$, $p = 0.003$, regression set through the origin; Fig. 5). The slope did not differ from a slope of 1 (slope test, $t_3 = 0.19$, $p = 0.86$).

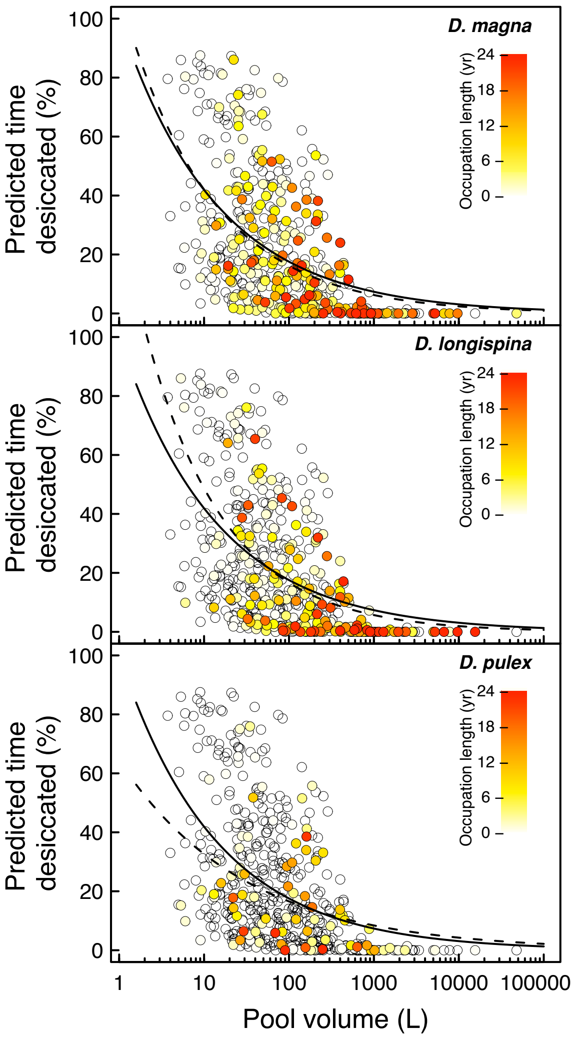

Fig. 6. Predicted percentage of time (between 1 May and 30 September) a pool was desiccated in relation to its volume. Additionally, for each pool the occupation length for *D. magna*, *D. longispina* and *D. pulex* populations is given separately in a colour gradient. The predicted percentage of time a pool was desiccated was non-linearly correlated with pool volume. Pools larger than about 500 liter had a low desiccation probability and did rarely or never dry up. Furthermore, within a volume range of 5 to 300 liter, the percentage of time a pool was desiccated ranged from about 10 to 80 % for pools of similar volume. Pool volume alone is therefore not a good estimate for pool stability in respect with desiccation risk. Pools with a higher percentage of drought periods were occupied by *Daphnia* populations over shorter time, indicated by white to yellow colours. Long-lasting populations (orange to red colours) were mostly found in pools that had both a large volume and did rarely desiccate. Nonlinear least square fit between pool volume and predicted percentage of days desiccated are given for all pools (straight line) and separately for the subset of pools that was at least once inhabited by the specific species (dashed line).

Our model did not only predict correctly the percentage of dry pools at a specific time, but also gave consistent predictions for the individual pools (data not shown). The predicted percentage of time a pool was desiccated (= desiccation frequency) correlated in a non-linear way with pool volume (Fig. 6; non-linear least square fit, $y = 100 * \exp[-0.87 * x]$, $p < 0.00001$). Above a threshold of about 500 liter, the likelihood of a pool to become dry was close to zero. In smaller pools, the desiccation frequency was negatively correlated with volume. However, there was a very large variation in desiccation frequency for pools of similar size (Fig. 6). Within the same size-classes of pools, almost the whole range of desiccation frequencies were found.

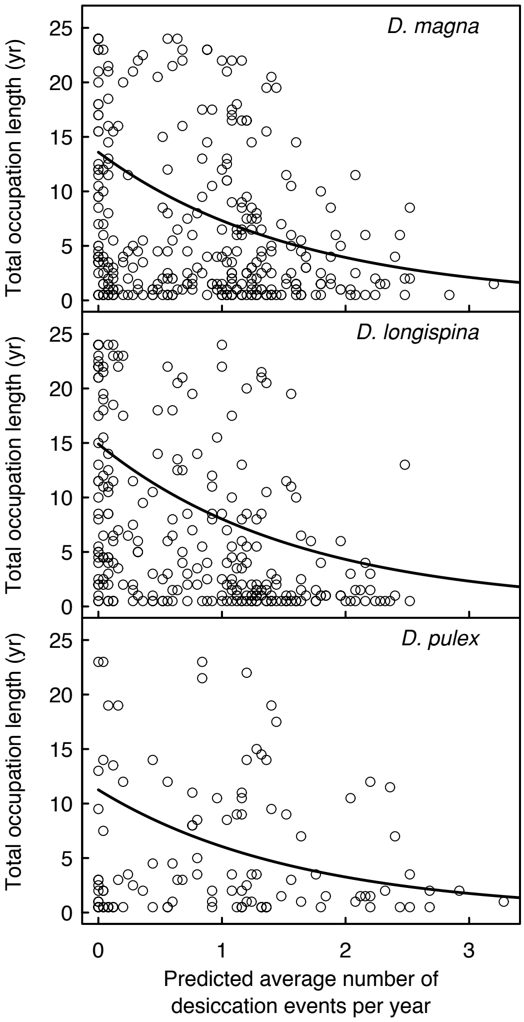

Fig. 7. Total occupation length of *Daphnia magna*, *D. longispina* and *D. pulex* populations relative to the predicted average number of desiccation events per summer. In all species, occupation length correlated negatively with the number of desiccation events. The lines are the predicted values from the generalised linear model. There was no significant difference between the three species.

Occupation and persistence of Daphnia *populations*

Planktonic *Daphnia* populations go extinct at each desiccation event, and the dry pools remain unsuitable for the total length of the droughts. We thus modelled occupation time of *Daphnia* populations using both the predicted number of desiccation events and the predicted lengths of droughts in the full statistical model. The occupation length of *Daphnia* populations in pools was significantly negatively related to the pool's average number of predicted desiccation events per summer (generalized linear model, $Z = -7.4$, $p < 0.0001$, Fig. 7). Pool volume and predicted lengths of droughts were both non-significant ($Z = 0.95$, $p = 0.34$ and $Z = -0.81$, $p = 0.41$) and therefore excluded during model simplification (Crawley, 2002). There was no significant difference between the three *Daphnia* species (model with one slope compared with model with three different slopes, D df = 2, D deviance = 27, $p = 0.2$).

Finally, we compared pools that contained *Daphnia* with pools that did not contain *Daphnia* and related it to the percentage of time a pool was desiccated. We used probability density estimates from all pools and separately from the subset of occupied pools. Density estimates are based on mathematical models that give continuous estimates of a probability distribution, and can be informally seen as "smoothed" versions of a histogram. We calculated the percentage of occupied pools relative to all pools over the range of the predicted percentage of time desiccated (Fig. 8). First, we did our estimates between pools that contained the specific *Daphnia* at least once relative to all pools (Fig. 8A). Then, we did our estimates between pools where the specific *Daphnia* species established a population for longer than one year relative to all pools (Fig. 8B). In the latter case, short-term colonisations (≤1 year) are not considered. Inhabitation estimates for all three *Daphnia* species decreased with the percentage of time the pools were desiccated (Fig. 8).

Discussion

We were interested in the stability of local habitat patches in a metacommunity of three *Daphnia* species. These planktonic animals occur in rock pools of various sizes and desiccation of the pool is a drastic event. A drought kills all planktonic animals and only resting stages (so-called ephippia) survive. However, desiccation may also have beneficial aspects, as it excludes predators and allospecific competitors that may not survive droughts (Ranta, 1982). Ephippia are also the dispersal propagules, and in the dry sediments they are exposed to passive migration (Ebert, 2005, Maguire, 1963, Ranta, 1979, Vanschoenwinkel et al., 2008). Thus, desiccation has a direct influence on many biological aspects of the *Daphnia* life-cycle. Furthermore, frequent desiccation is not only a habitat instability that might influence the local persistence of populations, but has also the potential to influence metapopulation processes. It is thus interesting to predict desiccation in individual habitat patches and to relate it to the occurrence of *Daphnia* populations.

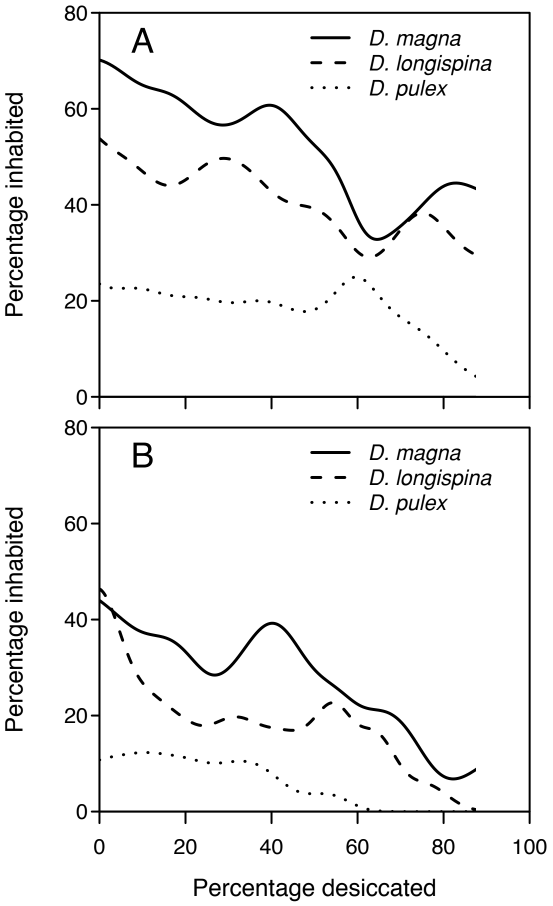

Fig. 8. A) Estimated percentage of pools that contained the specific *Daphnia* species at least once over the 25 years (independent of occupation length) in relation to the predicted percentage of time these pools were desiccated. Percentages are calculated from kernel density estimates of inhabited versus all pools. B) As A, but short-term colonisations (≤1 year) were not considered as successful inhabitation and were therefore excluded.

Rock pool characterisation and the occurrence of Daphnia

As already pointed out by Ranta (1979) and Pajunen & Pajunen (2007), the ecological niches of the three *Daphnia* species in rock pools are largely overlapping, and habitat separation is only weak. The patchy distribution of the pools may allow spatial coexistence of the three ecologically similar species (Hanski & Ranta, 1983). Consistent with Ranta's study (1979), we found that *D. magna* was the most common species, followed by *D. longispina* and *D. pulex*. The occupancy data are from a 25 years survey in more than 500 pools, already described in detail and analysed by Pajunen (1986) and Pajunen & Pajunen (2003). The three *Daphnia* species occurred in rock pools that covered the whole range of surface areas and pool depths of pools found on the studies islands (Fig. 1A & 1B; see also Pajunen & Pajunen (1986, 2003, 2007) and Ranta (1979)). Even though *Daphnia* could be found in pools of all sizes (Fig. 1C), there were large differences in the percentage of occupied pools at different sizes (Fig. 1D). Pajunen & Pajunen (2003, 2007) suggested that the stability of the system depended on the existence of more favourable patches that provide the migrants. They classified long-lived populations in very large pools as such "mainland-type" populations. We found the highest occupation frequencies in medium sized to large pools, of which 70 to 80 % had been inhabited at least once by any *Daphnia* species (Fig. 1D). The occupation frequency appears to decrease somewhat for the very large pools (>1000–2000 liter), which are rare however. The very large pools may often contain predators such as fish (e. g. sticklebacks) or water beetles as well as allospecific competitors of *Daphnia* (Ranta, 1982), which may make them less suitable. This is the case with some very large pools on some islands outside our study region. They do not contain any of the three *Daphnia* species (personal observation).

Besides the size of the pools we also measured total catchment area (Fig. 1D), as it influenced the rate of refilling: pools with a large catchment area (relative to pool surface area) will be faster filled with rain water than comparable pools with a small catchment area. The catchment area around small pools was on average proportionally larger than around large pools, indicating that small pools will be refilled faster (Fig. 2). Pools with a small surface area are usually shallow and may be filled up to their maximum after even a little amount of precipitation (personal observation). Therefore, small pools may get filled with water and dry up in several consecutive rounds per summer. Large pools get only filled after heavy rainfalls.

Evaporation model and desiccation predictions

In a next step, we made a model for predicting desiccation events and drought lengths for all monitored rock pools, and related them to the occurrence of *Daphnia*. We first measured daily evaporation in 50 representative rock pools over the summer 2006. We then fitted a multiple regression with daily evaporation as the dependent variable, explained by both pool and environmental variables (Tab. 1). Evaporation was higher in pools with directly adjacent or incorporated vegetation (Fig. 3). The plants were mostly growing at the pool's edge. They take up the water from the pool, transpire it and thus increase evaporation. We found a rather large scatter in evaporation rates for pools with vegetation. This may be due our use of presence or absence of vegetation as binary data type. The extent of vegetation cover varied greatly between pools (personal observation). In some pools only few tussocks were present, while other pools were surrounded by dense reed. Though, we had deliberately chosen the binary data type, as we used the vegetation recordings from 2006 to extrapolate 25 years backwards. While it is reasonable to assume that pool volume stayed constant over that time, the extension of vegetation has definitely changed. By using a binary measure, we avoided an unjustified accuracy.

Evaporation was higher in pools with a smaller surface area (Fig. 3). These pools had usually a smaller volume, subsequently the water body warmed up faster during the day and evaporated more. Furthermore, everything else being equal, the ratio circumfence to area is larger for small areas compared to large areas. This results in a relative long water-land contact zone at the edge of pools with a small surface area, which may favour evaporation. Evaporation in pools correlated with the environmental variables ambient temperature, wind and standardized evaporation measured at a close-by weather station. These correlations are not surprising, as the influence of temperature and wind on evaporation is common knowledge (Linacre, 1977).

The different explanatory variables used were certainly not independent from each other. This, however, is not a problem here, as we did not interpret significance tests for rejecting hypotheses but were interested in the estimates of the multiple regression to be used for predictions. Following the principle of parsimony, we used the estimates from the minimal adequate model for our simulation model. With the simulation model, we could make a backward prediction of evaporation per day in all 530 habitat patches over the last 25 years (Fig. 4).

At five dates during summer 2006, we monitored all 530 rock pools and recorded all dry pools. We compared the observed percentage of desiccated pools with the percentage predicted by the model and got a consistent result (Fig. 5). The model's predictions were accurate on a daily base, and the predicted percentage of pools did on average not differ from the observed percentage. The largest discrepancy between the predicted and observed number of droughts was on 15 May. In 2006, it was exceptionally dry and no rain occurred between 20 and 30 April. While the model assumed that all pools are brim-full on 1 May, in that specific year many pools had already a lower water level at that time. Thus, more pools were actually desiccated at that time than predicted by the model. Nevertheless, in most previous years the model's assumption of brim-full pools on 1 May was realistic, as at that time either snowmelt or rain occurred. The exceptional drought at the end of April 2006 may be a concomitant phenomenon of global change (Jylhä et al., 2004, Altermatt et al., 2008).

Occupancy and persistence of Daphnia *populations*

In previous studies, the occurrence of *Daphnia* in rock pools was correlated to pool volume, organic carbon content of the water, pH or salinity (Ranta, 1979, Pajunen & Pajunen, 2007). Desiccation as a common phenomenon was mentioned (Pajunen, 1986, Pajunen & Pajunen, 2003, Ranta, 1979, Ebert, 2005), but never quantified. These earlier studies asserted the significance of populations in a metapopulation context by using pool volume as a measure of stability (Pajunen & Pajunen, 2007). Experimental studies indeed showed that *Daphnia* populations in larger vessels had a higher probability to survive (Bengtsson, 1989, Bengtsson, 1993). But stochasticity due to desiccation was not considered in the vessels, and water level was kept constant.

Desiccation events directly influence the life cycle of *Daphnia* by killing the planktonic population. The populations establish from the ephippia after refilling of the pool with water (at summer temperature, Altermatt & Ebert, 2008). To survive another drought, the populations must again produce ephippia. Ephippia are not produced instantly by the new planktonic population, and therefore a time-span of a few weeks is required between the end of a drought (rain filling the pool) and the start of the next drought: female *Daphnia* must hatch out of the ephippia (this happens about five days after refilling with water (Altermatt & Ebert, 2008)), grow to maturity (7 to 10 days), produce male offspring, these males must mature as well (7 to 10 days) and only then females can mate with males to produce ephippia, which takes another 2 to 3 days (personal observations, at lower temperatures these processes take more time). Thus pools that contain water for a too short time may not be suitable. However, at the same time a drought also reduces predator populations (Ranta, 1982), which may be beneficial for the *Daphnia*. We therefore argue that the complex variable desiccation is biologically relevant as it has a direct influence on *Daphnia* biology.

We compared pool volume and desiccation frequency and found a non-linear relationship between pool volume and predicted drought lengths (Fig. 6). Pools larger than about 500 liter were almost never dry, while smaller pools varied a lot in predicted drought lengths. Important here, pools of similar volume had very different desiccation risks, due to different surface-depths ratios, different catchment areas and due to the presence or absence of vegetation. This resulted in a rather poor relationship between predicted time desiccated and pool volume for pools smaller than 500 liter (Fig. 6). It clearly shows that the use of pool volume alone does not explain the occurrence of *Daphnia*, as the small to medium sized pools are the vast majority of all pools.

Daphnia populations of all three species were found to persist the longest in pools with a low desiccation risk (orange to red circles, Fig. 6 & 8), while occupation lengths were shorter both in small pools and in pools with long predicted droughts (white to yellow circles). However, also some very large and desiccation resistant pools had never a *Daphnia* population in 25 years of observation

(Fig. 1D & 6). It is possible that the continuous water body in these pools enabled the occurrence of predators such as fish (Ranta, 1982, Pajunen & Pajunen, 2003), which was not tested in our study. Thus, the habitat may have been unsuitable for the considered *Daphnia* species.

We then correlated the long-time occurrence of any of the three *Daphnia* species with the variables pool volume, predicted drought lengths and predicted number of desiccation events. After model simplification, only the average number of desiccation events per year remained significant in explaining occupation lengths (Fig. 7). While the number of desiccation events influences the planktonic populations, the length of the droughts may have an influence on the dispersal of resting stages. Ephippia survive in the dry sediments, and are thereby exposed to passive migration by wind and birds (see figure 2.19 in Ebert (2005)). It seams reasonable that the longer these dry sediments are exposed, the more likely ephippia are dispersing (see also Vanschoenwinkel et al., 2008).

The recurrent desiccation events may also influence intra- and interspecific dynamics. A previous study found a correlation between population age and parasite richness (Ebert et al., 2001). This finding was explained by an accumulation of parasites over time. A further, non-exclusive explanation could be that young and short-lived populations are mostly found in desiccation-prone pools. During droughts, parasite prevalence and possibly richness is reduced (Lass & Ebert, 2006), making these populations less parasitized. Uninfected ephippia may be more successful as migrants (Altermatt et al., 2007). Indeed, we showed elsewhere that dispersal and consecutive colonisation rates were higher after warm and dry summers, where long drought periods can be expected (Altermatt et al., 2008). This unpredictability might enable the coexistence of the three *Daphnia* species (Hanski & Ranta, 1983), which outcompete each other under constant conditions (Bengtsson, 1986, Bengtsson, 1989, Bengtsson, 1993). In summary, the number of desiccation events and the lengths of drought are important in this metacommunity, as they influence occupation lengths and eventually also coexistence and dispersal rates.

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Chapter 3

Climate change affects colonisation dynamics in a metacommunity of three *Daphnia* species

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Abstract: Climate change is expected to alter the range and abundance of many species by influencing habitat qualities. For species living in fragmented populations, not only the quality of the present patches but also access to new habitat patches may be affected. Here we show that colonisation in a metacommunity can be directly influenced by weather changes, and that these observed weather changes are consistent with global climate change models. Using a long-term data set from a rock pool metacommunity of the three species *Daphnia magna*, *D. longispina* and *D. pulex* with 507 monitored habitat patches, we correlated a four-fold increase in colonisation rate with warmer, drier weather for the period from 1982 to 2006. The higher colonisation rate after warm and dry summers led to an increase in metacommunity dynamics over time. A mechanistic explanation for the increased colonisation rate is that the resting stages have a higher exposure to animal and wind dispersal in desiccated rock pools. Although colonisation rates reacted in the same direction in all three species, there were significant species-specific effects that resulted in an overall change in the metacommunity composition. Increased local instability and colonisation dynamics may even lead to higher global stability of the metacommunity. Thus, whereas climate change has been reported to cause a unidirectional change in species range for many other species, it changes the dynamic and composition of an entire community in this metacommunity, with winners and losers difficult to predict.

Introduction

The Earth's climate has changed during the past 100 years on a regional as well as global scale, and models predict continued changes (Dore, 2005, IPCC, 2001, IPCC, 2007). The observed and predicted changes are attributable to human activities (IPCC, 2001, IPCC, 2007). Recorded weather trends over the past 100 years and models for the future generally show an increase in temperature and localized precipitation and a higher year-to-year variability in the weather (Dore, 2005, IPCC, 2001). Due to complex physio-geographic interactions and depending on the season, the effects can also be inversed on a temporal or regional scale (IPCC, 2001, Jylhä et al., 2004). Many studies document unidirectional correlations between climate change and biological phenomena such as altered phenologies (usually a shift towards earlier times in the year), shifts in species ranges (usually polewards and/or to higher altitudes), extinctions (due to changes in the habitat or increased stochasticity), altered life histories, community compositions, ecosystem functions, or genetic changes (Balanya et al., 2006, Parmesan, 1996, Parmesan et al., 1999, Parmesan & Yohe, 2003, Roy & Sparks, 2000, Thomas et al., 2004, Walther et al., 2002, McLaughlin et al., 2002). Changes in habitat quality have particularly received a lot of attention (Walther et al., 2002), as they have large implications on the future composition of the fauna and flora. Depending on the system and its location, a change in climate can cause improvements, shifts or degradations of habitats, with consequent effects on the species occurring in these habitats. Species may become locally extinct or colonize new localities (Franco et al., 2006, Parmesan et al., 1999, Wilson et al., 2006). The ability of a species to shift to new locations will depend on its dispersal potential, on the speed of the habitat change, and on the availability of new habitats for colonisation (Holt & Keitt, 2000). It is assumed that the availability of new habitats is affected by climate change (Walther et al., 2002), though not the process of dispersal or colonisation itself. However, climate change can potentially have a large influence on colonisation rate and colonisation success itself, especially in species whose dispersal depends on the weather (Clobert et al., 2001). A combined influence of climate change on both habitat availability and dispersal can either increase or decrease the effects of climate change on biological systems (reinforcement) or make the effects less predictable due to interactions. Furthermore, a direct influence of climate change on dispersal and colonisation can also make the systems potentially more variable.

Effects of climate change on habitats were most often seen at the borders of species or population ranges, where the highest sensitivity can be expected (Bohning-Gaese & Lemoine, 2004, Parmesan, 1996). On contrary, for species that occur in spatially structured systems such as metacommunities or metapopulations, climate change might not only affect the borders of a population's range, but also entire populations and their interaction between other populations and species (Thomas & Hanski, 2004). Metapopulations are dynamic *per se*; extinction and colonisation on a local scale are implicit features of them, as is dispersal (Hanski & Gaggiotti, 2004, Gonzalez et al., 1998). Therefore, metapopulations offer the possibility of studying the effects of climate change on these processes themselves. In metacommunities, colonisation and extinction rates are also functions of the presence of other species, creating an additional level of complexity (Leibold & Miller, 2004, Holyoak et al., 2005). Metacommunity theory is mainly directed toward explaining species coexistence. Species-specific changes in dispersal rates may influence community composition through species sorting in local patches and may alter the overall structure of a metacommunity (Leibold & Miller, 2004).

We studied effective dispersal rates and colonisation dynamics in a metacommunity of three *Daphnia* species in southwest Finland. There, these species occur in small freshwater rock pools and exhibit such classical metapopulation dynamics as frequent extinctions and colonisations (Ebert et al., 2001, Hanski & Ranta, 1983, Levins, 1970, Pajunen & Pajunen, 2003). Competition takes places between conspecifics but also between allospecifics (Bengtsson, 1989, Hanski & Ranta, 1983). In this system, particularly the small and shallow rock pools tend to desiccate during warm, dry periods in summer (Ranta, 1979). When pools dry up, all juvenile and adult *Daphnia* in the population die, and only the resting stages survive. These are potential migrants. Resting eggs are exposed to passive dispersal in the sediments of desiccated rock pools (Ebert, 2005, Maguire, 1963, Ranta, 1979, Vanschoenwinkel et al., 2007) and may colonise new habitat patches.

We wanted to test, if climate change has an effect on metapopulation processes within the metacommunity of three *Daphnia* species. We were interested in general trends and in species' specific reactions that could be explained by the species' ecology. Furthermore, we were interested how changes in colonisation dynamics influence the metacommunity composition and diversity.

To answer our questions, we studied the colonisation dynamics in 507 potential habitat patches over 24 years during which the weather became significantly warmer and drier. We showed that evaporation in rock pools is significantly higher when ambient temperatures are high. During the same 24 years, we found a significant increase in colonisation rates in this *Daphnia* metacommunity. We then correlated colonisation rates with annual desiccation risk, using temperature and precipitation as approximation for desiccation risk (Linacre, 1977), to provide a mechanistic explanation for the observed changes. We found increased colonisation rates after warm and dry summers for all species in this metacommunity. The increase in colonisation rates differed significantly between the three species, and species abundance and composition within this metacommunity changed in accordance with colonisation rates. With larger year-to-year changes, the system became more dynamic. As climate change is expected to continue (Jylhä et al., 2004, Walther et al., 2002), its influence will continue and may become even more important. To our knowledge, this is the first time that changes in metapopulation processes as well as metacommunity dynamics and compositions are directly linked to climate change.

Material and Methods

We studied the influence of the weather and climate change on colonisation rates in a metacommunity of the three planktonic crustaceans: *Daphnia magna* Straus, *D. longispina* O. F. Müller, and *D. pulex* De Geer (Crustacea: Cladocera) over a period of 24 years (1982–2006). The three species are widely distributed along the coast of the Baltic Sea, inhabiting freshwater rock pools on the Skerry Islands (Bengtsson & Ebert, 1998, Ebert et al., 2001, Pajunen & Pajunen, 2003, Ranta, 1979, Ranta, 1982, Lagerspetz, 1955).

Study area

Our study area included 507 freshwater rock pools on 18 islands in the archipelago of southwest Finland at Tvärminne Zoological Station (59° 50' N, 23° 15' E). The rock pools are discrete habitat patches for the three species we studied here, and range in size from 10 to 24000 liter (data not shown, but see Pajunen & Pajunen (2003) and Altermatt & Ebert (in press)). The typical depth of rock pools ranges between 10 to 30 cm.

All available rock pools were mapped at the beginning of the study and the number of potential habitats stayed constant during the study period. Only very few rock pools might have become unsuitable due to succession, and no new rock pools were created by the postglacial land uplifting during the study period. Practically all islands and the surrounding Baltic Sea in our study area belong to the Tvärminne Zoological Station and are privately protected since 1901. Although some of the islands may have been used for sheep grazing and lumbering until the mid 1940s, neither activity is likely to have interfered with the rock pools, which are located in the shore belt of the islands. From the 1950s onwards, all use of natural resources was ceased, and only natural succession occurred. Therefore there have been no changes due to human land use since well before our study time.

We measured daily evaporation (in mm) in 17 rock pools on two islands at 23 24-hour intervals. The intervals were representatively spread over the period from 31 May to 20 August 2006. All of these rock pools were free of vegetation. In each rock pool we placed a brick as a constant reference point. Water level was measured manually, and evaporation could be calculated as the difference between two consecutive measurements to the nearest 0.5 mm. We then related the measured daily evaporation in these rock pools with daily ambient temperature data from the local weather station.

Weather and characteristics of Daphnia *sp.*

We used weather data from a standard weather station of the Finnish Meteorological Institute measured at Tvärminne Zoological Station (international identification number WMO 05493, national identification number LPNN 0202). The *Daphnia* populations on the studied islands were about 1.5 to 3.5 km south of the weather station. Daily mean temperature (°C) and daily precipitation (mm rain) were available for the whole study period (1982 to 2006). A discontinuous snow cover can occur from mid-October until end of April (unpublished data from the local weather station). The growing season is from the beginning of May to the end of September/mid-October (unpublished data from the local weather station). Local standardized daily evaporation data (mm day^{-1}) were not available. Instead, we used Class A pan (USWB) evaporation data measured at Jokioinen Observatorio of the Finnish Meteorological Institute (WMO 02963, LPNN 1201; 60° 48' N, 23° 30' E). Jokioinen Observatorio is about 100 km north of Tvärminne Zoological Station. These data were used to compare standardized daily evaporation relative to ambient mean temperature.

Daphnia populations appear in May and can subsist throughout the summer. The production of resting stages (ephippia) peaks in June and July (Altermatt & Ebert, in press). After the end of September, the average temperatures drop below 5 °C; *Daphnia* slow down or stop reproduction, and the planktonic phase of the population eventually goes extinct. During winter, the rock pools are frozen, and no planktonic *Daphnia* are found. The populations survive in form of resting eggs. Thus, for this analysis we only used meteorological data from the biologically-relevant summer period (May to September). For each year we calculated the average daily temperature from 1 May until 30 September, and the total summer precipitation (summed over the same period).

Because rock pool *Daphnia* differ both in their biology as well as in habitat processes from conspecific populations in lakes, we thus highlight intrinsic characteristics of these populations. All *Daphnia* populations in the rock pools represent metapopulation systems, with frequent extinction and colonisation (Pajunen & Pajunen, 2003). The three species studied here either occur singly or coexist in the same rock pool, though they have slightly different ecological preferences (Pajunen & Pajunen, 2003). They differ in competitive abilities, parasite susceptibilities, and life strategies (Bengtsson, 1989, Ebert, 2005, Hanski & Ranta, 1983). On average, *D. magna* occurs in small pools, *D. pulex* in intermediate-sized pools and *D. longispina* in larger pools (Bengtsson, 1988, Lagerspetz, 1955, Ranta, 1979). As these species interact with each other, occur in discrete patches and are linked by migration, they are a good example of a metacommunity (Gilpin & Hanski, 1991, Leibold et al., 2004). All three species reproduce by cyclical parthenogenesis, except for some populations of *D. pulex*, that are obligate parthenogenetic. Cyclical parthenogenesis means that phases of asexual production are intermitted by sexual reproduction. Resting eggs (= ephippia) are usually produced as a result of sexual reproduction. These ephippia can outlast unfavourable conditions such as freezing during winter or desiccation of pools during summer (Ebert, 2005). Due to several hatching stimuli per year, long-lasting resting egg banks are absent (Pajunen & Pajunen, 2003). The ephippia also serve as dispersal stages that migrate passively either by wind or birds (Ranta, 1979, Maguire, 1963), and they are particularly exposed to migration in desiccated rock pools (see figure 2.19 in Ebert (2005) and Vanschoenwinkel et al. (2007)). Desiccation of individual rock pools has not been monitored. However, evaporation and subsequent desiccation of pools is directly influenced by temperature and precipitation (Linacre, 1977). Also in the herein studied rock pools, evaporation is higher at warmer temperatures (Fig. 2). Especially shallow pools (10–15 cm deep) may dry up within 2 to 4 weeks, and will be only refilled after sufficient precipitation (Altermatt & Ebert, in prep.).

Ephippia can migrate into rock pools that are already inhabited by a conspecific *Daphnia* population (= invasion) or into rock pools that have no conspecific *Daphnia* populations (= colonisation). Only the latter is considered in this study. Generally, migration and the absolute number of migrants are difficult to estimate (Stenseth & Lidicker, 1992, Turchin et al., 1991), especially when migrants invade existing populations. However, migration and successful establishment into empty habitat patches (= free of conspecifics) can be easily documented by monitoring available habitat patches. Due to asexual reproduction, large planktonic populations can develop within a few weeks, even in rock pools that were colonized by only one ephippium. Genetic data of populations in newly colonized rock pools suggest that most rock pools are colonized by a single or very few individuals (Haag et al., 2005). This may be followed by an influx of further immigrants over time. The survival of a population in a rock pool ranges from less than a year to more than 20 years

(Pajunen & Pajunen, 2003). Populations go extinct for various reasons. The most common causes are when rock pools get washed out by waves from the surrounding Baltic Sea (Pajunen & Pajunen, 2003), parasite epidemics (Ebert, 2005), competition with other *Daphnia* species (Bengtsson, 1989) and changes in habitat qualities. Contrary to colonisations, which we expect to increase in parallel to the increase in pool desiccation, we have no predictions about extinctions in the context of a warmer and drier climate.

Data collection and analysis

One study author (V. I. Pajunen) visited all 507 rock pools in the study area twice a year from 1982 to 2006 (for detailed methodology see Pajunen (1986) and Pajunen & Pajunen (2003)). Dry pools as well as pools filled with water can be colonised, though colonisation can only be detected with our method when water is present. Thus, the two yearly samplings did only take place during periods when all rock pools contained water and when planktonic populations could be detected. In between the samplings pools may have become desiccated and refilled with water again, especially in warm and dry summers. During each visit, presence or absence of each of the three *Daphnia* species was determined for each pool, yielding the number of populations per year. Yearly population numbers for these three species differ somewhat from the values in Pajunen & Pajunen (2003) due to some corrections in the raw data. The number of populations in a specific year consists of the number of populations in the previous year minus extinctions plus colonisations. From these data, we calculated the number of colonisations and colonisation odds. Colonisation was defined as the occurrence of a *Daphnia* population in a rock pool where this species had not been observed on the two previous samplings (as defined in Pajunen (1986) and Ebert et al. (2001)). Thus, colonisations could only be calculated from the third sampling onwards (meaning from 1983 onwards; no calculations concerning colonisations could be made for the starting year 1982, see also Pajunen & Pajunen (2003)). As a conservative practice to avoid false positive records, a single negative observation in a series of positive records was not considered as extinction followed by colonisation (analogous to Pajunen (1986), Ebert et al. (2001) and Pajunen & Pajunen (2003)). Colonisation odds are the number of available empty habitats that were colonized divided by those that were not colonized. For example, if there were 100 available habitats and 20 of these got colonized, colonisation odds were 20 divided by 80 = 0.25. This represents a relative measurement of dispersal (also referred to as colonisation rates) and not an absolute number of colonisations, as colonisation odds are independent of the number of inhabited patches. Available habitats consisted of all monitored rock pools that had not been inhabited by conspecifics on the two previous samplings. Colonisation odds reflect the magnitude of dispersal better than the absolute numbers of colonisations, as the latter number might level off when only few empty patches are available. For all three species, we used the colonisation odds of rock pools free of these species as a response variable in the later models and in the figures.

Community diversity in the metacommunity was calculated with the Shannon diversity index H for each year (Zar, 1999). The Shannon diversity index gives the distribution of observations among nominal categories (Zar, 1999). We categorized each inhabited pool on a nominal scale into one of the seven categories of all possible community compositions (the seven categories were the three-species community *D. magna/D pulex/D. longispina*, the two species communities *D. magna/D. pulex*, *D. magna/D. longispina* and *D. pulex/D. longispina* or the one-species communities *D. magna* alone, *D. pulex* alone and *D. longispina* alone). Within these seven categories the proportion of observations found in each category were used to calculate H. It should be noted that we calculated the diversity on the community level and not on the species level. A low H value represents a high probability of finding a specific community, while a high value of H does not and is therefore a sign of a high heterogeneity in this metacommunity (Zar, 1999). For example if in a given year all 70 pools were inhabited by three-species communities *D. magna/D. pulex/ D. longispina*, the diversity measure H would be 0. If equal proportions of pools would be inhabited by each of the seven communities (i. e. always 10 pools with each of the possible communities), the diversity measure H would be 1.95, indicating the much higher diversity.

Statistical analyses were performed with R (R Development Core Team, 2007) using the libraries base, date, Hmisc and asuR. We used linear models to analyse the weather data and a nonparametric Spearman rank correlation for the diversity analysis. We compared colonisation rates using generalized linear models with a binomial and quasibinomial error distribution respectively and the logit link function. We tested the assumptions of all our models and compared them with alternative models using other error distributions and other link functions. The chosen model was the best one in respect of fulfilling all assumptions. For analysing changes in population numbers over time, we used a generalized linear model with a Poisson error distribution. The response variable was the absolute value difference in number of populations in two consecutive years calculated for each species separately. It is an integrative measurement of the year-to-year dynamic. The intercepts in all generalized linear models were compared at the arithmetic mean value of the explanatory variable to get a meaningful interpretation. We always fitted a generalized linear model with different slopes for each of the three

Daphnia species and compared it with a simplified model with only one slope; the comparison was based on a Chi-square (χ^2) distribution (Venables & Ripley, 2002). If model simplification was not appropriate, we used the former, more complex model. The populations over time are time series, and the measurements are not independent. Therefore we only fit running medians of the odd span as a robust scatter plot smoothing. We calculated the integer width of the median window using the algorithm AS 296 of Härdle & Steiger (1995). It is implemented in the R function runmed(), to calculate robust median smoothing (R Development Core Team, 2007).

Results

Weather

At Tvärminne, in the archipelago of southwest Finland, the average summer temperature increased significantly between 1982 and 2006 (linear model, $R^2 = 0.27$, $F_{1,23} = 8.71$, $p = 0.007$, N = 25 years, Fig. 1a). The increase was about 1.6 °C, from 12.8 °C to 14.4 °C over the 25 years. In parallel, summer total precipitation decreased about 30 % (linear model, $R^2 = 0.12$, $F_{1,23} = 3.16$, $p = 0.08$, N = 25 years, Fig. 1b), but showed also a large year-to-year variation. These changes are consistent with global climate change expectations (Jylhä et al., 2004, Walther et al., 2002). Both temperature and precipitation varied highly between different years. Summer average temperature and summer total precipitation were negatively correlated (linear model, $R^2 = 0.42$, $F_{1,23} = 16.5$, $p = 0.0005$, N = 25 years).

In natural rock pools, evaporation per day was significantly positively correlated with the ambient temperature at 23 representative days during summer 2006 (linear model, $R^2 = 0.27$, $F_{1,21} = 7.7$, $p = 0.01$; Fig. 2a). Thus, temperature has a direct impact on evaporation and eventually desiccation. This relation is consistent with standardized evaporation measurements at the weather station: evaporation was also significantly positively correlated with ambient daily temperature over the time period from 1 May to 30 September 1982–2006 (ANCOVA, $F_{1,3799} = 709$, $p < 0.0001$; additionally, there was a significant year-effect, $p < 0.0001$; Fig. 2b). The slope of the correlation from the rock pools (slope = 0.24) did not significantly differ from the slope from the weather station (slope = 0.19; slope-test $t_{21} = 0.6$, $p = 0.54$).

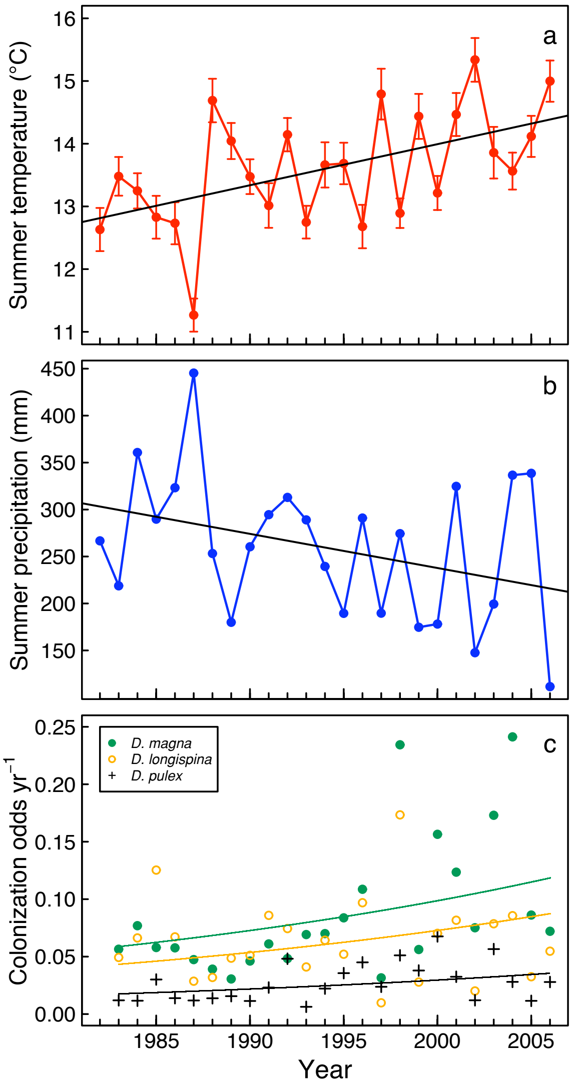

Fig. 1. Weather data from southwest Finland during the study period from 1982 to 2006 and parallel changes in colonisation rates in a metacommunity of three *Daphnia* species. For the weather data, daily weather data from 1 May until 30 September were used for each year. a) Summer average daily temperature (± SE) increased significantly during this period (black line: least squares fit, $p = 0.007$). b) In parallel, there was a marginal significant decrease in summer total precipitation (black line: least squares fit, $p = 0.08$). c) Colonisation odds in a metacommunity of three *Daphnia* species increased significantly during the same time period ($p < 0.002$). Colonisations odds are the number of available empty habitats that were colonized divided by those that were not colonized (see methods). The lines show estimated values from the generalized linear model fitted for each species.

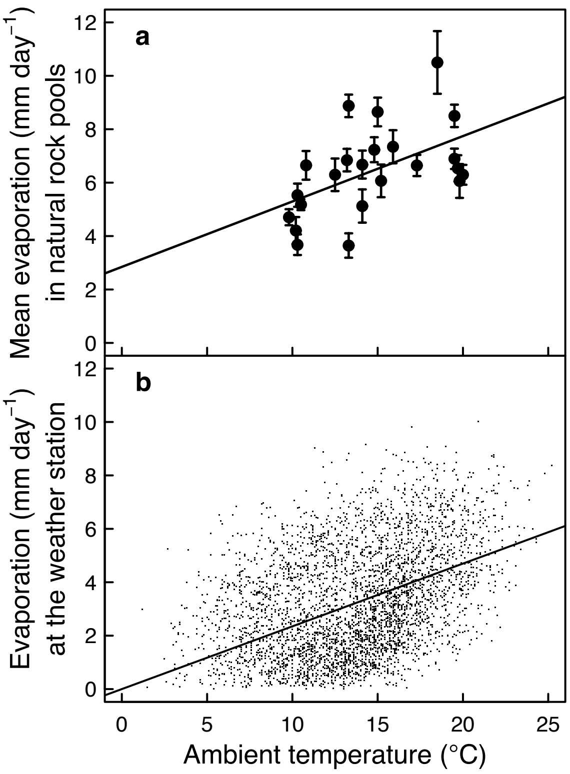

Fig 2. Evaporation relative to ambient daily mean temperature. a) Mean evaporation (mm day^{-1}) in 17 rock pools at 23 representative days during summer 2007. Evaporation was significantly higher at warmer temperatures ($p = 0.01$). The straight line is the mean squares line. b) Mean evaporation (mm day^{-1}) relative to ambient temperature measured at a standardised weather station. All days from 1 May to 30 September 1982–2006 are included. Evaporation was significantly higher at warmer temperatures ($p < 0.0001$). The straight line is the mean squares line.

Colonisation

During the study period, 440 out of the 507 available rock pools were inhabited at least once by any of the species (= 87 %). In this metacommunity, we observed 763 colonisations of empty habitat patches by *Daphnia magna*, 604 colonisations by *D. longispina* and 284 colonisations by *D. pulex*. In the same time period, colonisation rates of the three *Daphnia* species significantly increased (generalized linear model, $Z = 3.15$, $p = 0.002$, Fig. 1c), indicating a relation to climate change. The change of colonisation rates did not differ between the three species. Though both the weather and colonisation rates changed significantly during the period of 1982 to 2006, they do not necessarily have to be related. Thus, we additionally tested if colonisation rates do not only change over time, but are furthermore depending directly on the weather. We found that the annual colonisation odds of the three *Daphnia* species correlated significantly positively with average summer temperature and negatively with summer total precipitation in the preceding year (Fig. 3 and 3). The correlation with precipitation was less pronounced, probably due to the large year-to-year variability. There are not only significant changes in both the weather and colonisation rates over time, but furthermore we highlight a significant direct correlation between the weather and colonisation rates. Summers with low average daily temperatures were followed by a year with fewer colonisations of all three *Daphnia* species than summers with a high average daily temperature (generalized linear model, $Z = 4.48$, $p < 0.00001$, Fig. 3). The slopes of the curves differed significantly for the three *Daphnia* species (model with separate slopes for each of the *Daphnia* species compared with a model with only one common slope: Δ df = 2, Δ deviance = 7.64, $p = 0.02$). Similar results were found when comparing colonisation odds and summer total precipitation. Dry summers (low precipitation) were followed by a year with more colonisations of all three *Daphnia* species than wet summers (generalized linear model, $Z = -5.61$, $p < 0.00001$, Fig. 4). Again, the three *Daphnia* species reacted differently, and the slopes of the species' curves differed (model with separate slopes for each of the *Daphnia* species compared with a model with one common slope: Δ df = 2, Δ deviance = 7.62, $p = 0.02$). Thus, *Daphnia* colonisation rates increased after both dry and warm summers, and this effect varied across species. Summers are getting warmer and drier due to climate change (Fig. 1a and b). Therefore climate change has a direct influence on colonisation dynamics (Fig. 1c, 2 and 3) in this metacommunity.

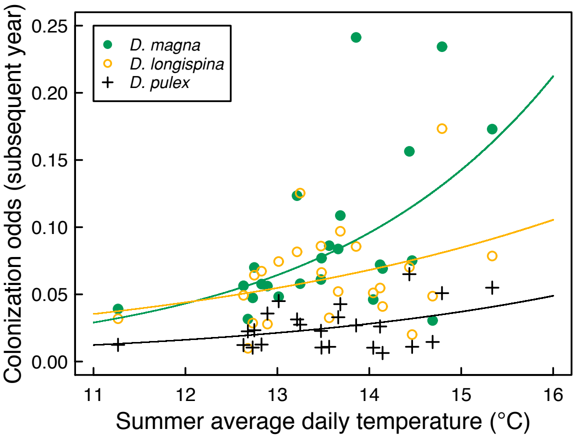

Fig. 3. Colonisation odds of three *Daphnia* species relative to the temperature in the preceding year. There was a significant positive effect of the summer average daily temperature on the colonisation odds in the subsequent year ($p < 0.00001$). This effect was significantly different for the three *Daphnia* species ($p = 0.02$). The lines show estimated values from the generalized linear model fitted for each species.

Community composition

Looking at the weather changes from 1982 to 2006 together with the correlation between colonisation rates and weather phenomena in three *Daphnia* species, one can expect to see temporal effects in the composition of this metacommunity. First, the number of populations per year changed for each of the species over the study period (Fig. 5a). Interestingly, while all species had higher colonisation rates consistent with local climate change, the number of populations developed differently for each of the three species. In this metacommunity, the number of *D. magna* and *D. pulex* populations doubled over the study period, while the number of *D. longispina* populations decreased over time (Fig. 5a). Second, our data suggest that this metacommunity became more dynamic in recent years (Fig. 5b). There was a significant increase in absolute value changes for all species among consecutive years over time (1982 to 2006; generalized linear model, $Z = 3.6$, $p = 0.0003$). However, the magnitude of this effect differed significantly between the three species and the increase was most pronounced in *D. magna* (model with separate slopes for each of the *Daphnia* species compared with a model with one common slope: Δ df = 2, Δ deviance = 32.6, $p < 0.0001$, Fig. 5b). It is therefore possible that climate change did not only influence colonisation rates, but especially in *D. longispina* also extinction rate, since losses in the number of populations are included in the absolute changes. When considering community composition as species interactions (namely the possibility of multi-species communities), we found that diversity in the metacommunity increased significantly over time ($R_S = 0.50$, $n = 25$, $p = 0.012$, Fig. 6). Overall, the whole metacommunity became more dynamic and more diverse.

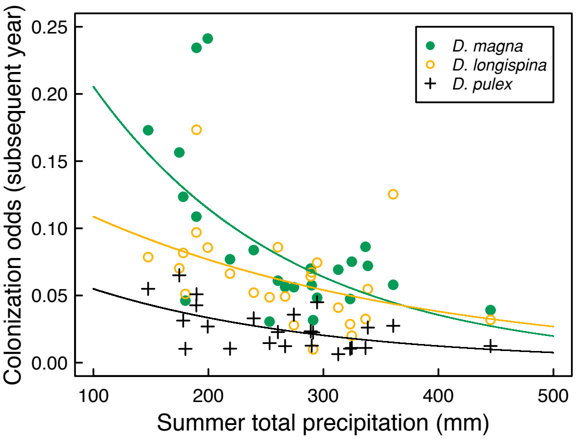

Fig. 4. Colonisation odds of three *Daphnia* species relative to the total precipitation in the preceding year. There was a significant negative effect of the summer total precipitation on the colonisation odds in the subsequent year ($p < 0.00001$). The effect was significantly different for the three *Daphnia* species ($p = 0.02$). The lines show estimated values from the generalized linear model fitted for each species.

Discussion

In southwest Finland, the summer temperature significantly increased and precipitation decreased between 1982 and 2006, which is in accordance with the global climate change (Fig. 1a and b). Changes in precipitation are known to be more variable and less pronounced than the changes in temperature in all climate change models (Jylhä et al., 2004, Walther et al., 2002). In Northern Europe, annual precipitation is predicted to increase (Jylhä et al., 2004, Zhang et al., 2007) due to an increase during the winter. However, precipitation is predicted to decrease or stay constant over the summer (Jylhä et al., 2004) and also evident in our data (Fig. 1b). Over the summers, we found a highly significant increase in mean temperature, but only a marginal significant decrease in precipitation, with a large year-to-year variation in precipitation. In the same time period, an increase in colonisation rates for all *Daphnia* species (Fig. 1c), species-specific changes in colonisation rates and changes in the metacommunity composition were found (Fig. 5 and 5). Colonisation rates in *Daphnia* metapopulations were significantly higher after warm and dry summers and the magnitude of the effect differed for the three species. A mechanistic explanation for these findings is that evaporation in rock pools is positively correlated with the ambient temperature (Fig. 2a). Pools dry up more rapidly during warm, dry periods, and ephippia get exposed to dispersal by wind and bird (Ebert, 2005, Maguire, 1963, Ranta, 1979). The species-specific reaction changed the absolute numbers of populations and made the whole metacommunity more dynamic. Along with many documented changes in habitat availability due to climate change (Walther et al., 2002), this is the first time that changes in metapopulation processes, metacommunity composition and dynamics themselves can be related to climate change.

Temporal trends of the weather

It is generally accepted that annual mean temperature and precipitation are influenced by climate change in Northern Europe (IPCC, 2001, Jylhä et al., 2004). Whereas temperature is predicted to increase in all seasons, precipitation trends differ among seasons. We therefore restricted our comparisons to the time period of *Daphnia* growth (i.e., May to September), when precipitation is either predicted to stay constant (Jylhä *et al.*, 2004) or decrease (Walther et al., 2002). The significant increase in temperature and the marginal significant decrease in precipitation in our weather data from the Tvärminne Zoological Station are in accordance with these predictions (Fig. 1a and b).

Evidence for weather driven changes in colonisation rates

Metapopulations and metacommunities are predisposed for the study of colonisation and extinction (Hanski & Gaggiotti, 2004). Colonisation is an intrinsic process in these systems, and metapopulations also explicitly integrate the fact that the distribution of suitable habitats does not remain constant through time (Hanski & Gaggiotti, 2004, Thomas & Hanski, 2004). Species in metapopulations have prerequisites to deal with the dynamic availability of habitats, and colonisation-extinction processes are thought to be under selection (Hanski & Gaggiotti, 2004).

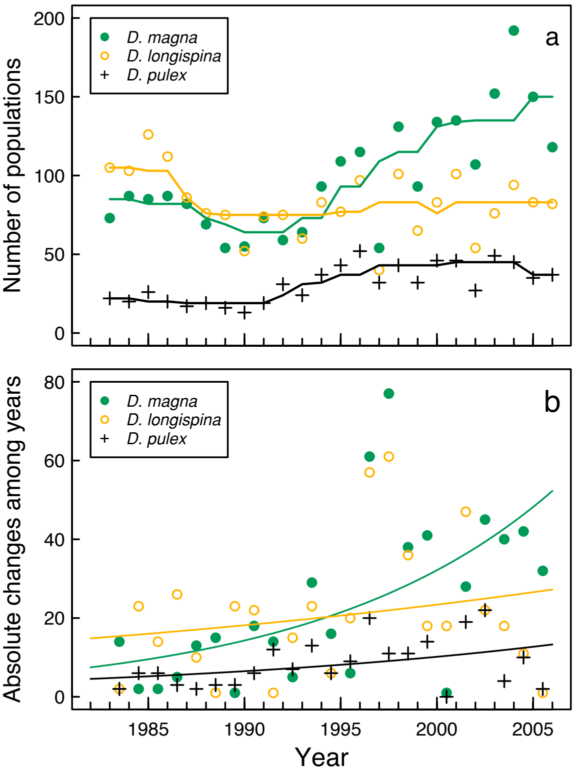

Fig. 5. a) Number of populations of three different *Daphnia* species within a metacommunity with 507 available habitats during 1982 and 2006. The species both occurred separately or coexisted in the same rock pools. The lines show separate running medians for the three species. b) The same data, but now absolute changes in populations' number between two consecutive years are shown for each species. There was a significant increase in the year-to-year variation in number of populations, and thus the metacommunities became more dynamic over time ($p < 0.0003$; generalized linear model, see methods). The lines show estimated values from the generalized linear model fitted for each species.

In a metacommunity of three different *Daphnia* species, we found a correlation between colonisation rates and weather in the previous season. The occurrence and timing of rock pool desiccation varied widely between the years and among pools (Ebert, 2005, Pajunen & Pajunen, 2003). Daily evaporation is correlated with ambient temperature (Fig. 2), and desiccation depends on the depth of rock pools (Ebert et al., 2001). In desiccated pools, ephippia are more exposed to dispersal by wind and birds (see figure 2.19 in Ebert (2005)). Thus, the strength of a drought determines which pools will be potential sources of migrants, as medium-sized pools, for example, may only contribute migrants during longer droughts. In the context of climate change, summer weather will change in a direction that increases migration in this metacommunity.

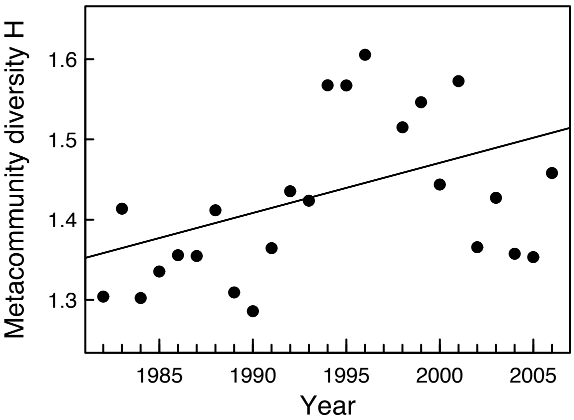

Fig. 6. The metacommunity diversity (Shannon index) increased significantly over time ($p = 0.012$). Annual composition of the metacommunity was analysed by categorizing each inhabited pool into seven categories on a nominal scale (three one-species categories, three two-species categories and one three-species community). Thereof the overall community diversity was calculated. Black line: least squares fit for illustration.

In all three species, not only the dispersal but also the production of ephippia might be influenced in the same direction by the weather. Ephippia are produced continuously during the summer, and populations in small pools produce large numbers of ephippia (Altermatt & Ebert, submitted). The continuous production of large numbers of ephippia in small pools may even be an adaptation in such dynamic habitats, as it guarantees long term survival in semi-stable habitats. Warm water temperatures stimulate population growth, and the production of ephippia peaks in mid-summer when droughts are also occurring (Altermatt & Ebert, submitted). Genetic data suggest that there are usually one to two colonizers in a newly colonized rock pools (Haag et al., 2005, Haag et al., 2006). It is therefore likely that populations in the most-newly colonised rock pools resulted from one successfully colonized ephippium, containing one or two eggs. Due to asexual reproduction, even a single coloniser might have established a planktonic population in the same year or in the next spring and was detected by our sampling scheme. Given a causal relationship between temperature/precipitation and colonisation, we expect even more dispersal and colonisations in the future due to climate change, and that the additional desiccated pools will become sources of migrants. Parallel to new colonisations, migration into existing populations (= invasion) is expected to increase. This will influence the genetic structure of the resident population (Ebert et al., 2002, Haag et al., 2005), affect local adaptation of parasites (Altermatt et al., 2007) and parasite dispersal itself (Ebert et al., 2001): newly colonized and short-lived host populations may be more inbred and have less parasites, and there may also be disturbed dynamics in host-parasite coevolution within populations.

We already have good indications that between-population dynamics in this metacommunity increased, as we found that absolute changes in annual population numbers increased in parallel to the ongoing climate change (Fig. 5b). This effect was most pronounced in *D. magna*. One possible explanation could be that a higher colonisation rate after warm and dry summers leads to the colonisation of habitats where either suboptimal abiotic factors or increased allospecific competition are present. This occupancy of suboptimal habitats could result in short-term occupancy followed by local population extinctions. The different trends in number of populations per year (Fig. 5a) must be due to changes in the colonisation-extinction balance or colonisation-extinction trade-offs (Calcagno et al., 2006, Hanski & Ranta, 1983). While *Daphnia magna* and to some extent also *D. pulex* can actually increase the number of populations in parallel with the increase in colonisations, *D. longispina* does not become more frequent over time even though its colonisation rate is increasing as well. It suggests that in *D. longispina* a trade-off exists. Warmer and drier weather will benefit colonisation, but could reduce survival of populations. This could be due to aspects of that species' biology, as preferences for large pools with low salinity (Ranta, 1979).

In our study area we can exclude changes in habitat patch numbers and human activities as alternative explanation for the changes in colonisation dynamics (see Material and Methods for details). This is an advantage of our data, as in many other studies the influence of climate change is strongly confounded by anthropogenic habitat changes (Walther et al., 2002, Warren et al., 2001), complicating the separation of these forces. Also, within all monitored rock pools, the number of available empty rock pools (= free of a specific species' population) always outnumbered the inhabited rock pools by a factor of 2 to 10, depending on the species and the year (Fig. 5a). Thus, the availability of empty rock pools seemed not to be a limiting factor.

Species' specific effects alter the metacommunity composition

Colonisation rates relative to weather in the previous year differed significantly among the three *Daphnia* species (Fig. 3 and 4). At the same time, we observed a change in species abundances within the metacommunity whereby *D. magna* became more abundant (Fig. 5a and b) and an increase in the metacommunity diversity (Fig. 6). Increased local instability and colonisation dynamics may even lead to higher global stability of the metacommunity. The link between the individual changes in the three species populations and the increase in the metacommunity diversity is not compulsory (Holyoak et al., 2005): the three species could increase their population numbers in separate pools. Pool occupancy is still far from saturation.

Metacommunity diversity includes the occurrence of all seven possible species communities in each rock pool at any time. Although the three *Daphnia* species are competitors (Bengtsson, 1989), the increased colonisation dynamics resulted in a higher number and diversity of multi-species communities (Holyoak et al., 2005) and not in monopolisations of habitats (Fig. 6). This supports the hypothesis of Hanski & Ranta (1983, 1979), that coexistence of the three ecologically similar species is possible due to the patchy distribution and metapopulation dynamics.

Immigration into already existing populations is generally more complex than colonisation of empty habitat patches. The existing populations numerically dominate the immigrants (De Meester et al., 2002), which makes immigration difficult. Though, the presence of parasites (Altermatt *et al.*, 2007) or predators (Ranta, 1982) may increase immigration success. Even though predators as dytiscids or corixids may have an influence on population densities, they are not capable of driving populations to extinction (Pajunen & Pajunen, 2003).

Hanski & Ranta (1983) suggested that *D. magna* is the best coloniser, but worst competitor, *D. longispina* the best competitor but worst coloniser and *D. pulex* intermediate. This hypothesis could not be confirmed on the basis of genetic data (Haag et al., 2006). We do not make direct statements on the colonisation and competitive abilities for these three species, but rather on relative changes in colonisation processes over time. However, our data are consistent with the idea that *D. magna* is a good coloniser (Hanski & Ranta, 1983), as its colonisation rates were highest and it profited most from the warmer weather. Nevertheless, it does not replace the other two species, indicated by an increasing diversity of pool-communities (Fig. 6). This shows that it either colonises only previously empty habitats, or pools already inhabited by allospecific *Daphnia* without driving them to extinction.

The species-specific colonisation rates can in part be explained by the dispersal strategies and the known habitat preferences of these species. First, the range of possible habitats is large, though only *D. magna* favours or can handle more saline habitats (Bengtsson, 1988, Lagerspetz, 1955). *Daphnia* *magna* also occurs more often in smaller pools, while *D. pulex* is more often found in intermediate-sized pools and *D. longispina* in large pools (Bengtsson, 1988, Lagerspetz, 1955, Ranta, 1979). Warm, dry summers create the type of habitat that is favoured by *D. magna*: water volume is smaller and evaporation leads to higher salinity. Ephippia in small pools get exposed more often during dry periods. Indeed, the colonisation rate of this species not only increased after warm summers, but its population numbers also doubled within the metapopulation over the 24 years. On the contrary, *D. longispina* prefers larger rock pools with low salinity (Bengtsson, 1988, Lagerspetz, 1955, Ranta, 1979) and *D. pulex* has intermediate preferences. Large rock pools with lower salinity may be more available during cooler and wet summers (also less evaporation), and are also less vulnerable to desiccation (Ranta, 1979). Second, the ephippia of the three species differ morphologically (Hanski & Ranta, 1983). Only ephippia from *D. magna* have long pennate appendages that might facilitate their dispersal either by wind or birds (Hanski & Ranta, 1983). We speculate that *D. magna* had the largest response in colonisations after warm and dry years because this species combines these two characteristics that could favour dispersal from desiccated pools. Furthermore, an increased colonisation rate in one species could influence allospecific colonisations, as priority effects or changes in competition-colonisation balances may occur (Hanski & Ranta, 1983).

Conclusions

A rapid and directed climate change is occurring due to human activities. We found correlative evidence that colonisation rates in a metacommunity of three *Daphnia* species are higher after warm, dry summers, and that these weather conditions are becoming more frequent due to climate change. A mechanistic explanation for the increased colonisation rates is that resting stages in dry pools are more exposed to wind and animal dispersal. Some species profited more, in respect to colonisation, from warm, dry weather and their number of populations increased. When individual species colonised habitat patches that were already inhabited by allospecific *Daphnia*, the whole metacommunity got more diverse. Thus, climate change not only increased the dynamics in the system, but also altered metacommunity composition itself.

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Chapter 4

Quantification of the total production of migration stages in a *Daphnia magna* metapopulation

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Manuscript

Abstract: Migration is the key process in metapopulations. Migrants colonise new habitat patches and create gene flow between existing populations. Therefore, the number and origin of migrants is important to understand metapopulations. Most metapopulation models assume a positive correlation between habitat size and the number of migrants, but little empirical data exist. Here, we estimated the total production of migration stages (ephippia) in a *Daphnia magna* metapopulation. We considered 500 potential habitat patches that ranged in volume between 6 to 48,000 liter and have been monitored over 25 years. Up to now, long-lived populations in the largest rock pools were seen as most important for metapopulation processes. We predicted the production of ephippia by populations occurring in rock pools of different sizes and stability. We used pool-volume-based predictions of ephippium production and extended it for the whole metapopulation. We corrected the estimates for reduced suitability when individual pools were dry and estimated the proportion of ephippia that became exposed to passive dispersal during desiccation events. We estimated that about 50 % of all ephippia were produced by populations in pools smaller than 300 liter. These populations contributed about 90 % to the exposed ephippia. We predicted that almost no ephippia became exposed to passive dispersal in pools larger than 1000 liter. We suggest that populations in small ephemeral habitat patches are driving most of the metapopulation dynamics in this system.

Introduction

Many animal and plant species occur in metapopulations that are assemblages of spatially delimited local populations coupled by migration (Levins, 1970, Hanski & Gaggiotti, 2004). Migration is the process that genetically connects separated populations and enables colonization of empty habitat patches (Clobert et al., 2001, Townsend et al., 2000, Ovaskainen & Hanski, 2004), counteracting local extinction. In the original metapopulation model (Levins, 1970) and in subsequent extensions, each population contributed equal number of migrants. Later models (Hanski, 1999) considered migration as a function of the population size or density in the patch of departure or the patch size. Populations were also classified differently in source-sink (Pulliam, 1988, Brown & Kodrick-Brown, 1977, Kawecki, 2004) and mainland-island models (Harrison, 1991, Boorman & Levitt, 1973). In the former, reproduction in sink populations does not balance mortality, and sink populations are only maintained by immigration from source populations. The latter focuses on local population size rather than demography. Large populations in the usually large "mainland" patches have a negligible risk of extinction, while the remaining small populations in small "island" patches run a high risk of extinction. But the mainland-island model also implicitly makes a statement about the origin of migrants: migrants are predominantly or exclusively originating from the permanent "mainland" populations, while "islands" are only receiving immigrants (Hanski, 1999, Kawecki, 2004).

In all these cases, the long-term survival of a metapopulation depends on the balance between local extinction and colonization and thus on the ability to produce migrants (Levins, 1970, Ovaskainen & Hanski, 2002). The total number of migrants as well as populations of origin are of interest because both may influence the genetic structure of a metapopulation. Populations in newly colonised patches reflect their populations of origin in several ways. When all migrants are originating from a single population, the newly colonised populations will be genetically similar to each other. On the other hand, a diverse origin of migrants results in genetically more diverse colonisations. Migration may be a way to escape parasites (Torchin et al., 2003, Altermatt et al., 2007), but at the same time parasites may also co-migrate with its hosts. Depending on the populations of origin, parasites may be spread over the metapopulation or be more challenged by the influx of new host genotypes into local populations.

The identification of key populations with respect to the origin of migrants is also essential in the context of habitat fragmentation and global climate change (Clobert et al., 2004, Thomas & Hanski, 2004). Conservation efforts are often only directed to a few large populations and small populations are neglected (Runge et al., 2006). However, population size may not be the best criterion to identify key populations, and in the worst case the wrong populations with respect to metapopulation dynamics are protected. A notable example in which the protection of the large populations would not guarantee long-term survival is given by Crone et al. (2001, coincidently studied in the same archipelago whereas we did our studies). They show in a vole metapopulation that the small ephemeral populations rather than the more permanent populations in large habitat patches are the important sources of migrants. They conclude that the stable long-term equilibrium in that metapopulation is only allowed by the small ephemeral high-emigration subpopulations (Crone et al., 2001).

The total number and origin of migrants in metapopulations has been estimated in relatively few empirical studies, even though this knowledge is essential for an understanding of metapopulations (Clobert et al., 2001, Bullock et al., 2002). Hanski et al. (2000) use mark-recapture data from a *Melitaea diamina* metapopulation to estimate rates of migration among populations and scale them with habitat patch area and isolation. In their model, emigration is proportional to patch area and populations in large patches contribute more migrants than populations in small patches. In the well-studied Glanville fritillary (*Melitaea cinxia*) metapopulations on the Åland Islands, the number of migrants is estimated by monitoring the occupation of patches as well as mark-recapture measurements (reviewed in Ehrlich & Hanski, 2004). Again, large and long-lasting populations contribute most of the migrants. Wind-dispersed migration stages of aquatic organisms living in ephemeral pools are studied by Brendonck et al. (1999) and Vanschoenwinkel et al. (2008). They find that the number of migration stages in the traps correlates positively with the occurrence of droughts. However, these studies do not give estimates on the contribution of migration stages by individual populations or overall estimates. One of the most detailed studies in which the origin and number of migrants is monitored has been done in the above mentioned vole metapopulation (Crone et al., 2001, Pokki, 1981) by individually marking all individuals in the metapopulation over several years. They find that migrants are especially coming from small populations in small patches, and that emigration is promoted by deteriorating conditions in the patch of origin.

Here, we estimated the production of the migration and resting stage (ephippium) in over 500 patches in a *Daphnia magna* metapopulation. We considered populations in 530 potential patches and estimated the contribution by populations in pools of different volume over 25 years. *Daphnia magna* commonly occurs in freshwater rock pools along the coast of the Baltic Sea that vary in size over five orders of magnitude (pools of about 10 to 40000 liter Ebert et al., 2001, Hanski & Ranta, 1983, Pajunen, 1986, Pajunen & Pajunen, 2003). In this metapopulation, extinction and colonization in local patches occurs at a high annual rate (Pajunen, 1986, Pajunen & Pajunen, 2003), stressing the importance of migration for the persistence of the metapopulation. *Daphnia* migrate as resting stages, so-called ephippia, which are passively dispersed by wind or birds (Maguire, 1963). Furthermore, ephippia are also essential to endure freezing in winter and droughts in summer. Due to the obligate freezing of the rock pools, only populations that produce ephippia can survive the winter (Pajunen, 1986, Pajunen & Pajunen, 2003). Thus, the number of ephippia may influence a population's contribution of migrants as well as a population's long-term survival ability.

Earlier, we had shown that a population in a large pool produces more ephippia than a population in a small pool (Altermatt & Ebert, 2008). However, the increase was several orders of magnitude smaller than the increase in volume. Neither the overall number of ephippia nor their proportional exposition to migration had ever been quantified in this metapopulation. We estimated the annual production of ephippia that were produced by integrating the production of ephippia in single populations in various sized habitats and the occurrence of populations over time (Pajunen & Pajunen, 2003, Pajunen & Pajunen, 2007). Furthermore, we predicted desiccation of individual inhabited rock pools (Altermatt et al., in Prep.) to adjust for the time when planktonic populations experienced unsuitable conditions. With a pool-based model (Altermatt et al., in Prep.), we predicted the length of droughts and the number of desiccation events for each pool separately. We predicted the total number of ephippia produced as well as their likelihood to become exposed to passive dispersal during desiccation events. Our overall estimates suggested that about 50 % of all ephippia were produced by populations in pools smaller than 300 liter. Considering the whole metapopulation these populations contributed about 90 % to the exposed ephippia. We predicted that almost no ephippia become exposed in pools larger than 1000 liter and therefore populations in these pools may not contribute migrants.

Material and Methods

Probability density estimates and absolute frequencies

We used the long-term dataset of Pajunen & Pajunen (2003, 2007) on the occurrence of *Daphnia magna* in a natural metapopulation in southwest Finland. They had monitored 507 rock pools for the presence or absence of *D. magna* over 25 years (1982–2006; for details see Pajunen & Pajunen (1986 & 2003)). All pools had been visited twice a year. Some additional, permanently unoccupied pools were included only later (for details see Pajunen & Pajunen, 2007), giving in total 530 pools. With a simulation model we predicted daily evaporation, daily inflow of water due to precipitation and eventually desiccation for each pool separately (for details see Altermatt et al., in Prep.).

The distribution of all pool volumes is shown in a conventional histogram (Fig. 1). For the histogram we categorized all pools depending on their volume using classes on a logarithmic scale with the following breakpoints: $<10^{0.5}$, 10^{1}, $10^{1.5}$, 10^{2}, $10^{2.5}$, 10^{3}, $10^{3.5}$, 10^{4} and $>10^{4.5}$ liter. To calculate the overall production of ephippia, however, we used another method. The method is based on probability density estimates and allowed continuous estimates. Probability density estimates are mathematical models that give continuous estimates of a probability distribution based on observed data (Venables & Ripley, 2002). Our observed data were the pool volumes. Kernel density estimates (Venables & Ripley, 2002, Parzen, 1962) were used to get the continuous probability density distribution of all pools (Fig. 1) as well as of the subset of yearly inhabited pools (Fig. 2). Informally, these estimates can be seen as "smoothed" versions of histograms, where the area under the curve is one. The analogy is shown in figure 1, in which both the histogram as well as the frequency distribution are plotted together. The frequency distribution is the probability density distribution multiplied with the total number of observed data to get absolute values on the y-axis. The continuous estimates over all pool volumes of the probability density method are their main advantage to discontinuous methods such as histograms. Furthermore, yearly predictions and the overall mean could be conveniently shown in one figure (it is graphically not possible with histograms). However, only continuous mean predictions of other parameters such as desiccation could be integrated with the probability density method.

Occupation of pools relative to volume

To begin with, we calculated the absolute frequency distribution of pools that were inhabited by *D. magna* separately for each year between 1982–2006. Therefore, we made probability density estimates for the subset of pools that was inhabited by *D. magna* in each year. We then used these probability density estimates to calculate the absolute frequency distribution of occupied rock pools. This was done by multiplying the probability density estimate of each year with the total number of pools that were occupied by *D. magna* in that year (Fig. 2A). These 25 curves as well as their mean curve were used to calculate the total number of ephippia that were produced.

Estimation of the total ephippium production

Altermatt & Ebert (2008) give linear regression models to calculate the daily production of ephippia relative to pool volume and mesocosms volume respectively. We used the mean slope and mean intercept of their regression models. We multiplied the estimates form the linear regression with the yearly frequency distributions of occupied pools over the range of volumes (shown in Fig. 2A). This gave a continuous curve on the absolute frequency distribution of ephippia per day in the whole metapopulation (Fig. 2B).

In a next step we extended the estimates on the ephippium production per day to the total production per year. We considered the time period between 1 May and 30 September, which is relevant for the *Daphnia* (Altermatt & Ebert, 2008). Ephippia are only produced when pools contain water and we therefore adjusted the production only for the length of time when pools contained water (Altermatt & Ebert, 2008). With the help of an evaporation model (Altermatt et al., in Prep.), we predicted the yearly length of time when pools were dry relative to their volume for all pools that contained a *D. magna* population. We modelled the lengths of droughts and desiccation events for all pools separately on a daily basis (Altermatt et al., in Prep.). Non-linear least squares models were fitted for each year (see Fig. 3). These curves give the percentage of time when pools contained water relative to their volume. We used these curves to adjust the production of ephippia (Fig. 2C) by multiplying the value from Fig. 2C with the corresponding yearly curve from Fig. 3 over the range of all pool volumes. For the predicted percentage of time when a pool contained water, we used simple non-linear least square fits. These fits were good in most years, but sometimes underestimated the percentage of time a pool contained water for the very large pools.

Finally, we predicted for each pool volume the percental contribution to the overall number of ephippia (Fig. 2D). The number of desiccation events relative to pool volume was predicted by non-linear least squares models for each year separately (Fig. 4). We assumed that ephippia get exposed to wind and birds proportionally to the number of desiccation events (Vanschoenwinkel et al., 2008). Thereby, we could predict the percental contribution of exposed ephippia relative to pool volume (Fig. 2D). We assumed that exposure occurred at each desiccation event and thus only used the number of separate desiccation events in this analysis.

Statistical analysis

Statistical analyses were performed with R (R Development Core Team, 2007). Probability density estimates were done according to Venables & Ripley (2002). We used $\log_{10}$-transformed pool volume data, the default kernel and default bandwidth functions implemented in R (gaussian kernel and bandwidth being the standard deviation of the kernel). We used non-linear least square fits for relating the yearly predicted time a pool was dry (period 1 May to 30 September) with its volume. These predictions were used to scale the percentage of time when ephippia could be produced relative to pool volume. We also used non-linear least square fits for relating the number of desiccation events per year with pool volume. Again, these predictions were used to scale the percental contribution of exposed ephippia.

We calculated the number of colonisations and colonisation odds in the *D. magna* metapopulation from the long-term monitoring data (Pajunen & Pajunen, 2003). Colonisation was defined as the occurrence of a *D. magna* population in a rock pool where it had not been observed on the two previous samplings (as defined in Pajunen (1986) and Ebert et al. (2001)). Thus, colonisations could only be calculated from the third sampling onwards (meaning from 1983 onwards; no calculations concerning colonisations could be made for the starting year 1982, see also Pajunen & Pajunen (2003)). Colonisation odds are the number of available empty habitats that were colonized divided by those that were not colonized. For example, if there were 100 available habitats and 20 of these got colonized, colonisation odds were 20 divided by 80 = 0.25. This represents a relative measurement of dispersal (also referred to as colonisation rates) and not an absolute number of colonisations, as colonisation odds are independent of the number of inhabited patches. Available habitats consisted of all monitored rock pools that had not been inhabited by conspecifics on the two previous samplings. Colonisation odds reflect the magnitude of dispersal better than the absolute numbers of colonisations, as the latter number might level off when only few empty patches are available. We used the colonisation odds as a measure of the overall migration rate in the metapopulation.

Results and Discussion

Rock pool volume

The rock pools in the herein studied *D. magna* metapopulation ranged in volume from 4 to 48,000 liter with most pools having a volume between 10 to 500 liter (Fig. 1). *Daphnia magna* was found in pools of practically all sizes. The smallest pool ever inhabited had a volume of 6 liter, while the largest inhabited pool had a volume of 48,000 liter. Most populations occurred in pools of a volume between 20 to 1000 liters (Fig. 2A). The probability density estimates that considered the volume of all pools had a peak at about 30 to 40 liter (Fig. 1), whereas the mean peak was slightly shifted to the right when only occupied pools were considered (100 to 120 l; Fig. 2A).

The actual number of occupied pools varied remarkably between different years (Fig. 2A), and so did the occupation length in different pools (see Pajunen & Pajunen, 2003). In general, larger pools were more often and longer occupied (Pajunen & Pajunen, 2007). This had led to the idea that populations in these pools may act as mainland populations). The populations in smaller rock pools had a shorter survival length and a higher turnover (Pajunen & Pajunen, 2003, Pajunen & Pajunen, 2007), but extinctions were balanced by new colonisations.

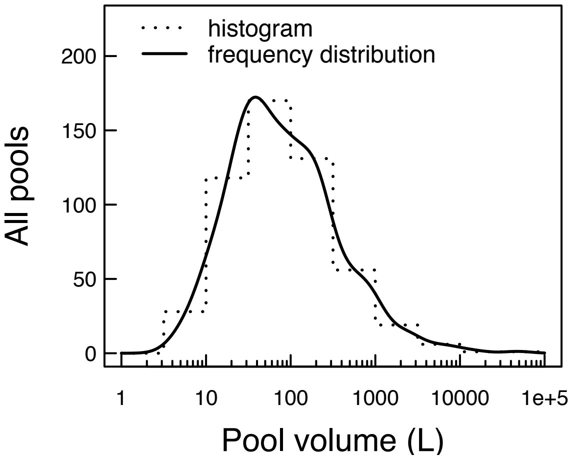

Fig. 1. Distribution of the volume of all 530 monitored rock pools sown as histogram (dotted line) and as absolute frequency distribution (continuous line). Absolute frequency distributions are analogue to histograms, but give continuous estimates.

Production of ephippia

We were interested in the production of ephippia. We estimated the production of ephippia relative to the volume of occupied pools and relative to the frequency of desiccation events and the time-length when pools were dry. We used continuous absolute frequency estimates relative to pool volume to calculate directly the production of ephippia with estimates from a linear regression. The estimates came from the linear regression model (Altermatt & Ebert, 2008) that was used to predict the production of ephippia per day relative to pool volume. We directly applied this linear regression model to the absolute frequency distribution of all pools in every year (see Fig. 2A) and calculated the total number of ephippia that were produced per day in the whole metapopulation for every year from 1982 to 2006 (Fig. 2B). The continuous absolute frequency distribution of all ephippia suggested that most ephippia were produced by populations in pools between 20 to about 1000 liter volume (Fig. 2B). The curves in figure 2B are slightly shifted to the right compared with the curves in figure 2A, as populations in larger pools produced more ephippia. However, this shift is not very pronounced. Thus, the estimated daily production of ephippia was mostly influenced by the numerical dominance of populations in small to medium sized pools. This was already predicted by Altermatt & Ebert (2008), because an increase of the pool volume over several orders of magnitude resulted in a much smaller increase in the production of ephippia.

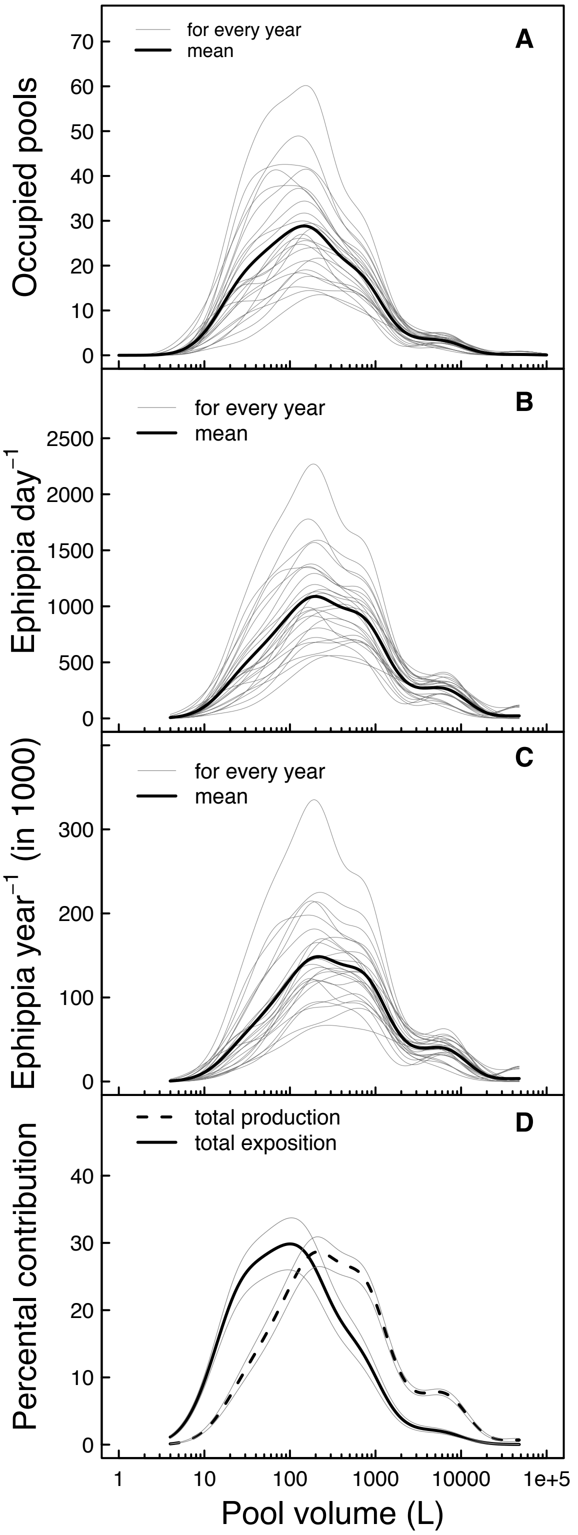

Fig. 2. A) Absolute frequency distributions of the subset of rock pools that were occupied by *D. magna*. The frequency distribution is given for each year from 1982–2006 separately (thin grey lines), and as the mean over all years (thick line). The yearly frequency distributions were calculated by multiplying the probability density estimates with the absolute number of occupied pools in each specific year. B) Estimated absolute frequency distribution of the ephippium production in the whole metapopulation (thin lines separately for each year 1982–2006). For each specific year, the daily production of ephippia was calculated relative to pool volume and the absolute frequency distribution of occupied pools. The thick line gives the mean over all years. C) Estimated absolute frequency distribution of the total production of ephippia per year. Thin lines give estimates separately for each year (1982–2006), while the thick line gives the mean over all years. The production of ephippia was calculated by extending the daily estimates from B, but correcting them for the mean length of time pools were desiccated relative to their volume. D) Predicted mean (± SE) percental contribution to the yearly total production of ephippia (dashed line, data as in C and total exposition (solid line) in the whole *D. magna* metapopulation relative to pool volume. Exposition of ephippia was assumed to be proportional to the predicted number of pool-specific desiccation events. About 50 % of all ephippia were produced by populations occurring in pools smaller than 300 liter. These populations contributed about 90 % of all ephippia that were exposed in the metapopulation. The difference between the numeric production of ephippia and their exposition to migration was especially pronounced for populations occurring in pools between 10 to 100 liter.

Generally, small rock pools dry up faster and are dry for longer times than large rock pools, but with considerable differences in different years due to the yearly weather (Altermatt et al., in Prep.). No ephippia are produced when a pool is dry. The shorter time-span during which small pools are suitable for planktonic populations was used to argue against their significance in a metapopulation context. For the yearly production of ephippia we included the reduction in time during which small pools contained no water. Individual pool-based estimates on the percentage of days when pools contained water were available for all years from 1982 to 2006 (Altermatt et al., in Prep.). However, it was not possible to use these pool-specific estimates directly for the absolute frequency distributions, as singularities cannot be combined with this continuous curve. We modelled non-linear least squares fits on the percentage of time when a pool contained water relative to its volume for each specific year (Fig. 4, see also Altermatt et al., in Prep.). These yearly curves were then multiplied with the absolute frequency distributions of the number of ephippia per day and extended to the whole year (again considering the relevant time between 1 May and 30 September, Fig. 2C). This correction for desiccated pools did however change the overall picture only marginally: the vast majority of the total number of ephippia per year was produced in pools smaller than 1000 liter. Between the different years, the overall number of ephippia ranged between several hundred thousand to about one million.

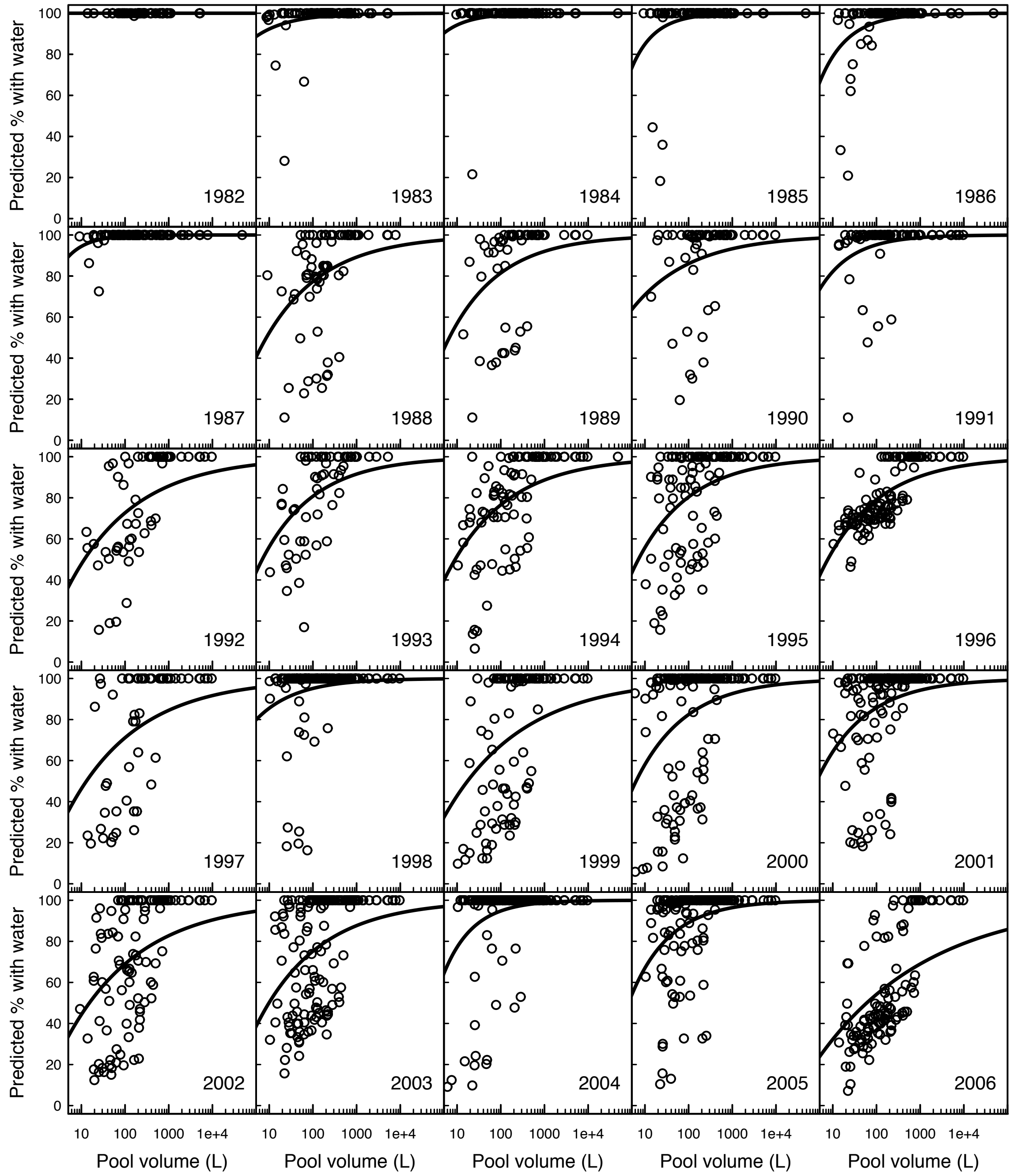

Fig. 3. Predicted yearly percentage of time when pools contained water relative to their volume. For every year, non-linear least square fits were calculated based on all inhabited pools. These yearly curves were used to correct the total number of ephippia per season (Fig. 2C).

Our model predicted that the majority of the ephippia was produced by populations in pools between 100 to 1000 liter (about 60 %, Fig. 2C). Only about 20 % of all ephippia were produced by the populations in the largest pools (>1000 liter). We were also interested in the exposition of the ephippia. We assumed that ephippia are exposed to wind and birds each time when a pool dries up, and that exposition is proportional to the number of desiccation events (Vanschoenwinkel et al., 2008). We calculated the percental contribution of populations in the different classes of pool volume to the total number of exposed ephippia (Fig. 2D). We found a remarkable shift in the percental contribution of exposed ephippia towards pools of smaller size classes. Almost 90 % of all exposed ephippia originated from pools that were smaller than about 300 liter (Fig. 2H) even though only about 50 % of all ephippia were produced by populations in these pools. The remaining 10 % of exposed ephippia originated from populations in pools between 300 and 1000 liter.

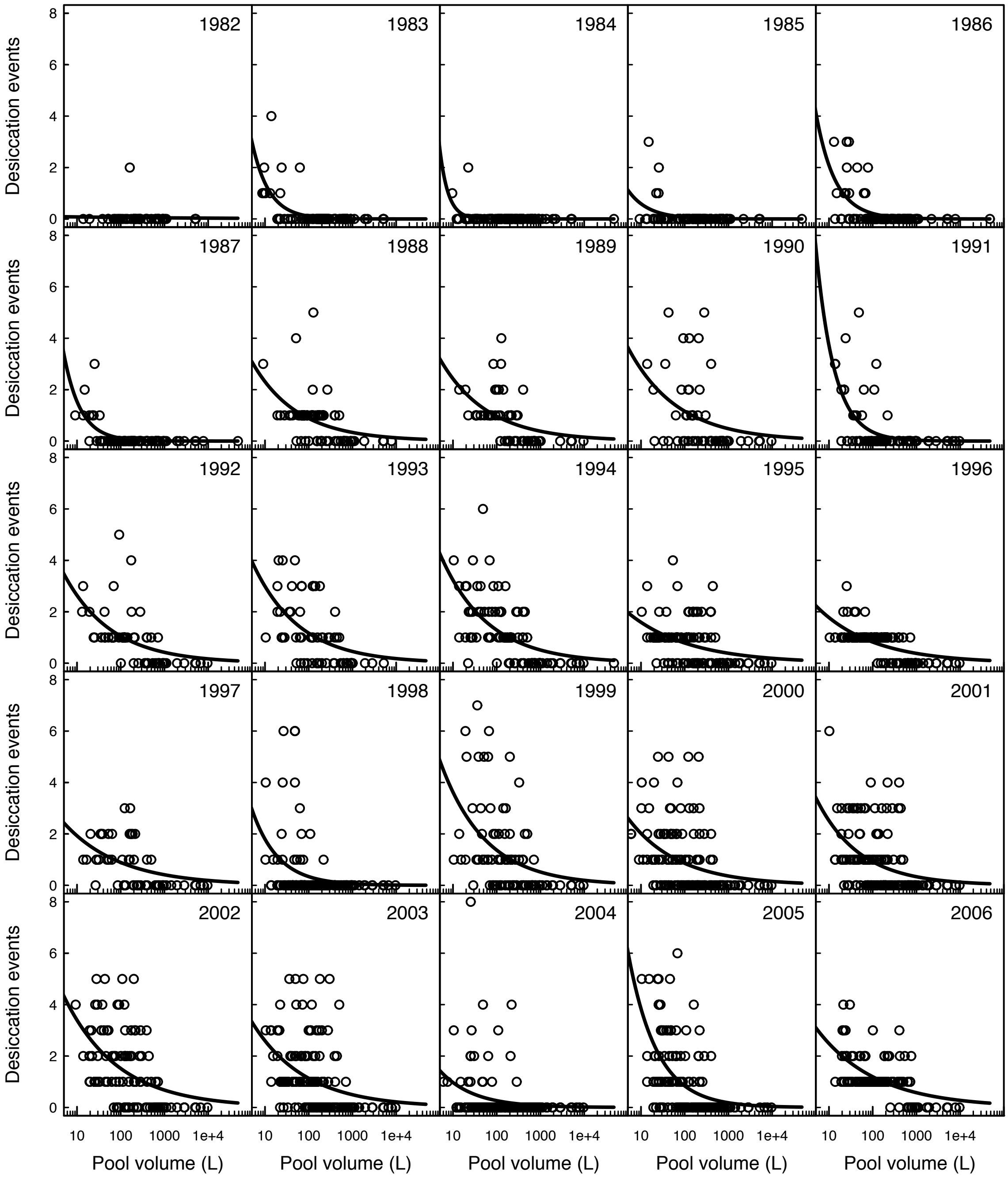

Fig. 4. Predicted yearly number of desiccation events relative to pool volume. For every year, non-linear least square fits were calculated based on all inhabited pools. These yearly curves were used to calculate the percental contribution to the exposed ephippia (Fig. 2D).

Ephippia are both the resting and migration stage, and their absolute number is of interest for the survival of individual populations and metapopulation dynamics. Even though ephippia have a twofold function, migration occurs especially when pools are desiccated (Vanschoenwinkel et al., 2008). We found that populations in small pools contributed most migrants (about 90 % of all exposed ephippia). Even though populations in these pools were very short lived and have a high turnover, they may be essential for the maintenance of metapopulation dynamics. This is analogue to a vole metapopulation, where small and short-lived populations contribute most of the migrants, and thereby have an increased extinction risk (Crone et al., 2001, Pokki, 1981). Contrary, the substantial numbers of ephippia produced in large and stable pools are rarely exposed to migration, as these pools almost never dry up (Fig. 4, Vanschoenwinkel et al., 2008). While populations in these large pools may not contribute much to the overall metapopulation dynamics, the large absolute number of ephippia may guarantee long-term survival.

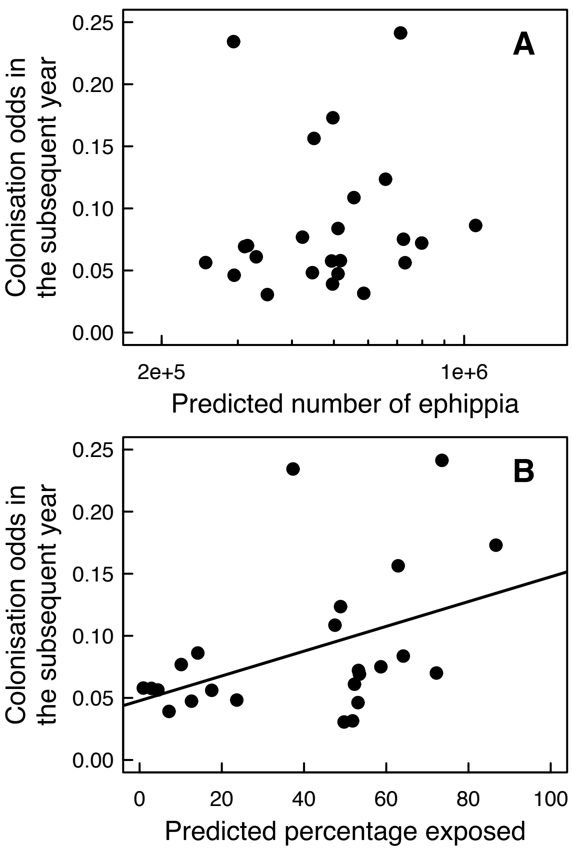

Fig. 5. A) Colonisation odds relative to the predicted production of ephippia. There was no significant relationship between the total production of ephippia and the colonisation odds in the subsequent season (Spearman rank correlation, $S = 1840$, $R_S = 0.2$, $p = 0.35$). B) Colonisation odds relative to the percentage of exposed ephippia. Exposition was proportional to the number of pool-specific desiccation events. Colonisation odds were significantly higher after years when large proportions of ephippia were exposed (Spearman rank correlation, $S = 1318$, $R_S = 0.43$, $p = 0.038$). The mean least squares line is shown for illustration.

Correlations between ephippium production and colonisations

With our models we predicted the absolute number of ephippia that were produced per year (1982–2006) but did not know if these ephippia were dispersed. Dispersal of the ephippia is passive (Maguire, 1963) and occur predominantly when pools are dry (Vanschoenwinkel et al., 2008). We do not have direct estimates on dispersal related to desiccation. We tested the idea that migrants are originating from dry pools by using our predictions on the total number and percental exposition of ephippia. In a first step we assumed that migration is directly proportional to the number of ephippia that were produced in the metapopulation. This assumption does not depend on exposition after desiccation events. If correct, we should find a positive correlation between the predicted number of ephippia in a given year and the colonisation odds in the subsequent year (as colonisations were detected by our sampling regime only in the subsequent year). However, this was not the case (Spearman rank correlation, $S = 1840$, $R_S = 0.2$, $p = 0.35$; Fig. 5A). The absence of a correlation suggest that migration rates did not directly depend on the absolute number of ephippia but migration is influenced by further factors. We then tested if migration occurs especially at times when pools are desiccated (as shown by Vanschoenwinkel et al., 2008). We therefore used the percentage of exposed ephippia per year in the analysis. Indeed, we found a significant correlation between the percentage of exposed ephippia and the colonisation odds in the subsequent year (Spearman rank correlation, $S = 1318$, $R_S = 0.43$, $p = 0.038$; Fig. 5B). Migration rates were significantly higher in years when large proportions of all ephippia in the metapopulation were exposed due to desiccation. This supports the idea that exposition of ephippia in the sediments of dry pools increases dispersal rates. (Vanschoenwinkel et al., 2008)

To our knowledge, our study is one of the few examples that estimated the total number of migration stages in a metapopulation over several years. The majority of ephippia was produced by populations occurring in medium sized pools. Especially small pools dry up frequently and ephippia are exposed to wind and birds. We showed that migration rates were influenced by the percentage of exposed ephippia and not by their absolute number. We concluded that populations in the small, ephemeral pools contributed predominantly to the migrants and may be most important for the metapopulation dynamics. On contrary, the ephippia from populations in large and stable pools were numerical less important, and may not migrate at all.

Acknowledgements

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Chapter 5

The genotype specific competitive ability does not correlate with infection in natural *Daphnia magna* populations

Florian Altermatt & Dieter Ebert

Altermatt & Ebert (2007) *PLoS One* **2**(12): e1280

Abstract: *Background* Different evolutionary hypotheses predict a correlation between the fitness of a genotype in the absence of infection and the likelihood to become infected. The cost of resistance hypothesis predicts that resistant genotypes pay a cost of being resistant and are less fit in the absence of parasites. The inbreeding-infection hypothesis predicts that the susceptible individuals are less fit due to inbreeding depression. *Methods and Results* Here we tested if a host's natural infection status was associated with its fitness. First, we experimentally confirmed that cured but formerly infected *Daphnia magna* are genetically more susceptible to reinfections with *Octosporea bayeri* than naturally uninfected *D. magna*. We then collected from each of 22 populations both uninfected and infected *D. magna* genotypes. All were treated against parasites and kept in their asexual phase. We estimated their relative fitness in an experiment against a tester genotype and in another experiment in direct competition. Consistently, we found no difference in competitive abilities between uninfected and cured but formerly infected genotypes. This was the case both in the presence as well as in the absence of sympatric parasites during the competition trials. *Conclusions* Our data do not support the inbreeding-infection hypothesis. They also do not support a cost of resistance, however ignoring other parasite strains or parasite species. We suggest as a possible explanation for our results that resistance genes might segregate largely independently of other fitness associated genes in this system.

Introduction

Parasites and parasitoids are ubiquitous in nature (Ebert, 2005, Poulin, 1998, Hudson & Greenman, 1998, Minchella & Scott, 1991). They harm the infected hosts to various degrees and thereby impose selection. Usually only a subset of individuals within a host population is infected (Duncan et al., 2006, Little & Ebert, 2000, Rauch & Weisser, 2006), either due to genetic or due to epidemiological reasons. For example, hosts of one sex, of a certain age, in certain phenotypic conditions, with a higher inbreeding level or of specific genotypes may be more often found to be infected by parasites (Brown et al., 2000, Bandi et al., 2001, Little et al., 2002, Keller & Waller, 2002). Furthermore, genetic variation for parasite infectivity and virulence can be found among parasites (Lively, 1989), among hosts (Nee, 1989, Sasaki, 2000) or as an interaction of both (Carius et al., 2001). As a consequence of this, hosts may evolve defenses to resist parasites (Carton et al., 2005, Little et al., 2003) and these might correlate with other host fitness components. Different evolutionary hypotheses predict a correlation between a genotype's susceptibility to infection and other fitness components. Due to the evolutionary and ecological significance of parasites, such correlations are of general interest.

The cost of resistance hypothesis predicts that resistant hosts pay a cost for being resistant in the absence of the parasite (Kraaijeveld & Godfray, 1997, Carton et al., 2005). In the presence of parasites, resistant genotypes profit from their resistance and are expected to be good competitors, while the susceptible genotypes are more likely to get infected (Duncan et al., 2006, Little & Ebert, 2000), and are therefore worse competitors. Contrary, in the absence of parasites hosts carrying resistance genes would have a lower competitive ability than susceptible hosts.

Another hypothesis linking resistance to fitness is the inbreeding-infection hypothesis (Coltman et al., 1999, Keller & Waller, 2002, Reid et al., 2003). Inbreeding causes a decrease in heterozygosity due to mating with relatives and is associated with a reduction in fitness, referred to as inbreeding depression (Hartl & Clark, 1997). This fitness reduction is expected both in the absence and in the presence of parasites. Several studies reported that inbreeding might also elevate the susceptibility to diseases (Luong et al., 2007, Reid et al., 2003, Coltman et al., 1999) and thus suggested a correlation between susceptibility and low fitness due to inbreeding. Under this hypothesis, genotypes are both infected and also generally less fit due to inbreeding. It is possible that the negative effects of inbreeding are even more pronounced in the presence of parasites: inbred animals could over-proportionally suffer from parasites (Coltman et al., 1999). However, this form of interaction between parasitism and inbreeding is not always observed (Salathé & Ebert, 2003).

Both the cost of resistance hypothesis and the inbreeding-infection hypothesis assume that resistance has a genetic basis. Under that assumption, the naturally infected genotypes are infected due to their lack of genetic resistance against parasites and not only due to epidemiology. Here, we first test the assumption of a genetic contribution to resistance in natural populations. We then test the two afore mentioned hypotheses. Testing the cost-of-resistance and the inbreeding-infection hypotheses requires the curing of naturally infected genotypes, which is possible in our system. After curing, the relative fitness of formerly infected, susceptible genotypes can be compared with naturally uninfected and possibly resistant genotypes. The direction of the correlation between resistance and fitness can be determined both in the absence or in the presence of parasites. The two hypotheses make different predictions. The cost-of-resistance hypothesis predicts that the relative fitness of naturally uninfected (and possibly resistant) animals should be lower compared to cured but formerly infected (and thus susceptible) animals in the absence of parasites. In contrast, the inbreeding-infection hypothesis predicts that formerly infected genotypes are those that are also more inbreed and thus should have a lower fitness compared to the naturally uninfected genotypes. When the negative effects of inbreeding are more pronounced in the presence of parasites, inbred animals should even over-proportionally suffer from parasites.

We tested an association of a host's natural infection status and its genotype specific fitness in two experiments (thereafter referred to as infection history experiments). We compared the competitive abilities of naturally uninfected and naturally infected but subsequently cured *Daphnia magna.* The parasite of interest was the microsporidium *Octosporea bayeri*. This parasite has a host genotype specific infectivity and virulence (Salathé & Ebert, 2003, Vizoso & Ebert, 2004). We measured host fitness in competition experiments that took place both in the presence and absence of sympatric parasite isolates. By that, we could test for an association between the natural infection status of a host and other fitness aspects such as a cost of resistance or a higher inbreeding depression in naturally infected animals. A strength of our study is the use of replicated host genotypes that were collected in 22 natural populations and differed naturally in their infection status. We then assayed their fitness in controlled experiments. We could standardize host age, exclude sex-specific effects and – most importantly – cure hosts from infections while keeping their genotype constant by using only asexually reproducing female *D. magna*.

Material and Methods

The study system

The freshwater crustacean *Daphnia magna* Straus, 1820 (Crustacea: Cladocera) is widely distributed along the coast of the Baltic Sea, inhabiting fresh water rock pools (Pajunen & Pajunen, 2003, Bengtsson, 1989, Ranta, 1979). These pools are spatially separated and genetic differentiation between the rock pool populations is strong (Haag et al., 2005). Periods of asexual reproduction during summer are intermitted by sexual reproduction when resting eggs are produced. The resting eggs allow over-wintering and also serve as dispersal stages (Ranta, 1979). Rock pool *D. magna* populations often harbour a wide spectrum of parasites (Bengtsson & Ebert, 1998, Ebert et al., 2001, Ebert, 2005). The most common parasite in our study area is the microsporidium *Octosporea bayeri* Jírovec, 1936, which can be found in about 45 % of all *D. magna* populations (Ebert et al., 2001). It is vertically as well as horizontally transmitted and reduces host fecundity and survival (Vizoso & Ebert, 2004). *Octosporea bayeri* is specific to *D. magna*. Local adaptation of the parasite occurs on a population level and the locally adapted parasite harms sympatric hosts more than allopatric hosts. This promotes immigration of the allopatric immigrants (Altermatt et al., 2007). Only resting eggs survive during winter and in spring all hatched *Daphnia* are genetically unique recombinants. Spring hatchlings may be *O. bayeri* infected or not. Vertical transmission of *O. bayeri* through the resting egg is highly efficient and infected spring hatchlings descend from infected mothers (Vizoso & Ebert, 2004). The uninfected hatchlings descend either from an uninfected mother or from an infected mother, but lost the parasite during the resting egg stage. The likelihood of vertical transmission through sexual eggs depends on host genotypes and is lower in outcrossed *Daphnia* relative to inbred *Daphnia* (Ebert et al., 2007). Outcrossing is also beneficial with respect to general competitive abilities, as in this metapopulation a high inbreeding depression was found (Ebert et al., 2002). No totally resistant genotypes have been found yet, and all *Daphnia* may encounter spores from the sediments or from dead infected hosts and acquire a horizontal infection during the summer season.

Experimental set-up of the infection experiment

This experiment tested whether the natural infection status correlates with the susceptibility to infections under controlled laboratory conditions. In spring 2003, two female *D. magna* per population were collected from natural populations. All genotypes were kept individually in 100 ml artificial medium (Klüttgen et al., 1994) at room temperature with a dark/light cycle of 10/14 hours and were fed ad libitum with the green alga *Scenedesmus obliquus*.

We checked each genotype for infections of the microsporidium *O. bayeri*. In six populations, one genotype was naturally uninfected and the other infected. All genotypes were treated with the antibiotic fumagillin to cure infections (Zbinden et al., 2005). The success of curing was confirmed afterwards in all genotypes. We then homogenized additional infected individuals from these six populations to prepare spore solutions and inoculated 2- to 3-day-old second-generation offspring of all genotypes with 50'000 sympatric spores per animal (Vizoso & Ebert, 2005). Per genotype, twelve animals were inoculated, kept in pairs in 2.5 ml of artificial medium for five days and fed daily 3 x 10^5 algae cells. Animals were transferred to 100 ml medium after five days. Thereafter, they were transferred to fresh medium every third day and fed with 2 x 10^6 algae cells per day. After 12 to 16 days they were checked for infections.

Experimental set-up of the infection history experiments

We experimentally tested if there are fitness differences between naturally uninfected and cured but formerly infected host genotypes. Genotypes from different populations differ strongly in their relative fitness (Altermatt et al., 2007, Ebert et al., 2002). Therefore a pairwise within-population comparison was used with 22 genotype pairs, each from a different population near Tvärminne Zoological Station, southwest Finland (59° 50' N, 23° 15' E). Fitness was measured relative to a tester genotype and for eight populations additionally in direct competition within the genotype pair. Relative fitness was measured in population competition experiments both in the presence and in the absence of sympatric isolates of *O. bayeri*. The experiments were performed during the asexual phase of *D. magna.*

In May 2004 we collected *D. magna* females hatched from over-wintering eggs from 22 natural rock pool populations on 12 islands near Tvärminne Zoological Station. As all over-wintering eggs (ephippia) are the result of sexual reproduction, each hatchling is a unique genotype (= clone). We collected all *Daphnia* shortly after their hatching at a time when they did not yet have started to reproduce. Thereby we were sure that each of them was genetically different (Haag & Ebert, 2007). All populations were at least two years old and showed some polymorphism at allozyme loci. However, polymorphism at the allozyme loci is generally rather small in that metapopulation and allozymes do not allow to identify each genotype separately. All populations were known to be infected with *O. bayeri* in the year previous to our studies. Spores of the parasite can be easily seen when an infected *Daphnia* is dissected and investigated with phase-contrast microscopy (400-fold magnification). From each population, we randomly collected one infected and one uninfected female and established clonal isofemale lines (= asexually reproducing genotypes). We then typed them for their allozyme genotypes at five different loci. These five loci were aspartate amino transferase (*Aat*, enzyme commission number EC 2.6.1.1), fumarate hydratase (*Fum*, EC 4.2.1.2), glucose-6-phosphate isomerase (*Gpi*, EC 5.3.1.9), phosphoglucomutase (*Pgm*, EC 5.4.2.2), and mannose-6-phosphate isomerase (*Mpi*, EC 5.3.1.8) (Hebert & Beaton, 1993). All genotypes were kept individually in 100 ml artificial medium (Klüttgen et al., 1994) at room temperature with a dark/light cycle of 10/14 hours and were fed *ad libitum* with the green alga *Scenedesmus obliquus*. To cure the 22 infected genotypes from the *O. bayeri* infections, we treated these genotypes as well as the 22 uninfected genotypes with the antibiotic fumagillin (Zbinden et al., 2005). The success of curing was confirmed afterwards in all genotypes. To avoid maternal effects due to the former infection or the curing procedure, the experiment was started with second-generation offspring of all genotypes. From each genotype about 60 animals were kept in two 250 ml jars, and fed daily with 100 million algae. In parallel, mass cultures of a single uninfected genotype starting from one single female were established. This genotype had been collected in 2003 and kept in the laboratory in its asexual phase ever since then. This genotype differed in at least one of the five allozyme markers to each of the 44 other genotypes and was assigned to be the tester genotype for the competition experiment.

From 28 June to 2 July 2004 the experiment was started. The animals were released into plastic buckets (volume 6 L) containing water filtered through a 20-µm filter from a rock pool free of *D. magna* and free of parasites. The pool from which we took the water was representative for pools found in that metapopulation, though its water quality tends to be in the suboptimal range for *D. magna* (Pajunen & Pajunen, 2007). Per 6 liter of filtered pool water, 250 ml sea-water was added to increase salinity, calcium concentration and nutrients. Thereby the water quality was improved for *Daphnia*. However, we did not try to make the water quality optimal, as under optimal conditions a cost of resistance might be obscured. 20 animals of each of the 44 genotypes were released in two buckets respectively, giving in total 88 replicates. Into all of these buckets also 20 animals of the tester genotype were added. For the eight pairs where the two genotypes of a pair could be distinguished with allozyme markers, 20 animals of each genotype were released together in four (for three populations only two) additional buckets respectively. Into half of all the replicates the parasite *O. bayeri* was added, to measure fitness effects in the presence of parasites, while the other half of the replicates stayed free of parasites. The parasite was added to the buckets by placing 30 dead infected *D. magna* females (freshly killed with CO_2) to each of these replicates. We used sympatric parasites, collected from the 22 corresponding rock pool populations. In five rock pools there were not enough *D. magna* to obtain 30 parasitized animals. In these cases allopatric parasites were used additionally. Parasite spores were passively released from the decaying cadavers of these dead infected *Daphnia*, allowing the infection to spread naturally in the experimental populations. Thirty dead females from parasite free laboratory cultures were added to all parasite free replicates. Also the tester genotype could get infected and its competitive ability may vary in the presence or absence of parasites. Therefore, relative fitness differences between the two treatments (= parasite treatment effect) do not only incorporate a parasite's effect, but also a tester genotype reaction to the parasite. The two components cannot be separated with our design.

The experiment ran until mid of August 2004, which is equivalent to about five to six asexual *Daphnia* generations. Then, from each replicate a random sample was collected. These animals were genotyped to estimate the frequencies of the two genotypes per replicate (on average 69 animals were genotyped per replicate). Additionally, 20 animals per replicate were homogenized and screened for parasite spores to check the success of the parasite treatment.

Fitness measurement and analysis

We estimated the relative fitness *w* of a genotype relative to the tester (e. g. A to B) or fitness of naturally uninfected genotypes relative to cured genotypes using the formula $\ln(w)*t = \ln(A_t/B_t) - \ln(A_0/B_0)$ (Hartl & Clark, 1997), where A_t, A_0, B_t, and B_0 are the frequencies of the two genotypes A and B at time *t* (measured in days) and time 0. As the experiments took place during asexual reproduction of *D. magna*, only clonal competition occurred and changes in allele frequencies are equal to changes in genotype frequencies. Therefore, $\ln(w)$ is a comprehensive measurement of relative fitness (Hartl & Clark, 1997) and was used in the statistical analysis as dependent variable. We did a pairwise comparison between naturally uninfected and cured genotypes (Wilcoxon signed rank test). One replicate was lost in the parasite treatment, as the experimental population went extinct due to unknown reasons. This reduced the number of populations from 22 to 21 in several comparisons. In ten replicates with parasite exposure, the infections did not successfully establish and thus did not allow a pairwise analysis anymore. Therefore we additionally fitted two generalized linear models using both the planned treatments (i. e. exposed to parasites) and the effective treatments (i. e. parasite establishment and spread was observed) as explanatory variables. In the generalized linear models we used genotype frequencies and quasibinomial error structure instead of relative fitness (Crawley, 2002). In all comparisons the biological relevant unit of replication was population. Statistical analyses were performed with R (R Development Core Team, 2007).

Results

Infection experiment

The reasoning of our study hinges on the assumption that the natural infection status is an indicator of resistance. We tested this assumption with pairs of clones from six populations. Within populations, the likelihood to become infected with sympatric parasites is significantly higher for cured but formerly infected genotypes than for naturally uninfected genotypes (paired Wilcoxon signed rank test, $V = 0$, $p = 0.03$, n = 6 pairs; Fig. 1). Mortality during the infection assay did not differ between naturally uninfected and cured genotypes (paired Wilcoxon signed rank test, $V = 5$, $p = 0.59$, n = 6 pairs).

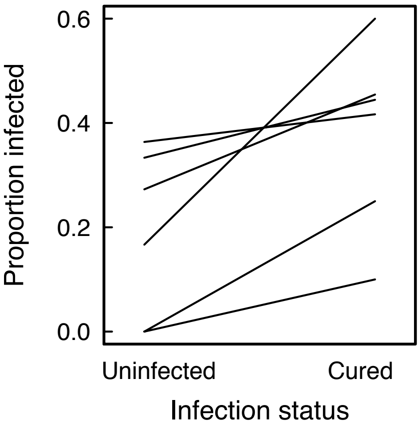

Fig. 1. Susceptibility of naturally uninfected and cured *Daphnia* to infection with the parasite *O. bayeri*. We experimentally compared the susceptibility to infection of naturally uninfected *D. magna* and cured but formerly infected *D. magna* (infection status). Each line is the mean of a genotype pair from a different population. Within a population, cured but formerly infected genotypes had a significantly higher likelihood to become infected than naturally uninfected genotypes.

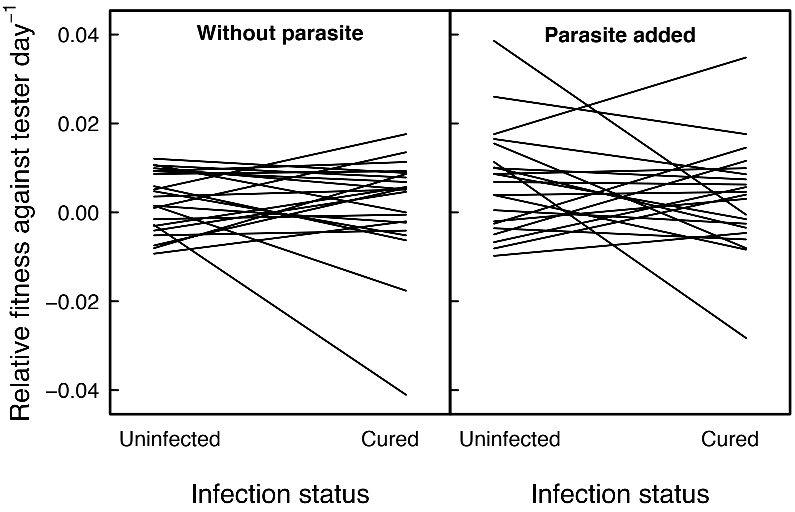

Fig. 2. Relative fitness of naturally uninfected and cured *Daphnia* genotypes. Relative fitness within genotype pairs from 22 populations in the absence (left panel) or in the presence of a sympatric parasite (right panel). Within each pair, one genotype was naturally uninfected and the other cured but formerly infected. There was no significant effect of the infection status on a genotypes' competitive ability. The genotype pairs of each population are connected with a line. Fitness was measured for each of the 44 genotypes individually in a competition experiment against a common tester genotype.

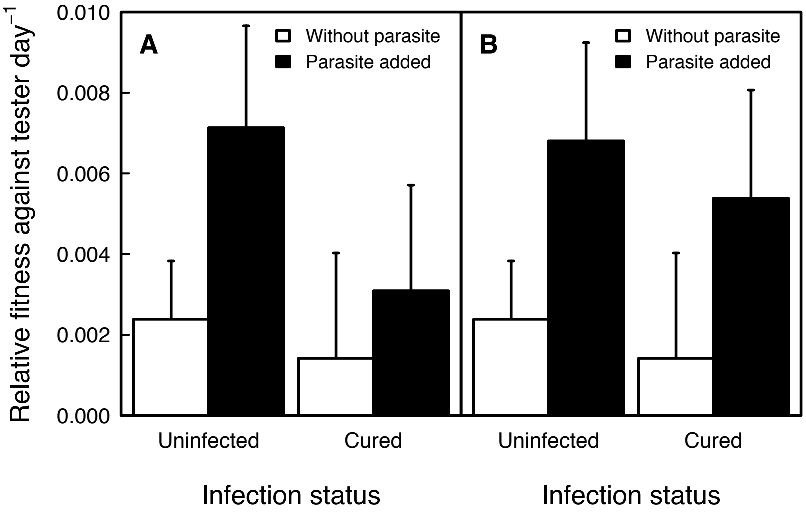

Fig. 3. Relative fitness of naturally uninfected and cured *Daphnia* genotypes. Relative fitness within genotype pairs from 22 populations in the absence or in the presence of a sympatric parasite. These are the same data as in Fig. 2, but now mean values (± SE) of relative fitness are given. We furthermore distinguished between the planned treatments where all replicates were analysed (A) and the effective treatments (B). In the effective treatments we excluded replicates in which a successful establishment of the parasite could not be verified.

Infection history experiments

We did not find a fitness difference between naturally uninfected and cured but formerly infected genotypes both in the absence (paired Wilcoxon signed rank, $V = 128$, $p = 0.98$, $n = 22$ pairs; Fig. 2a) or in the presence of a sympatric parasite (paired Wilcoxon signed rank test, $V = 134$, $p = 0.54$, $n = 21$ pairs; Fig. 2b). A generalized linear model with quasibinomial error distribution of genotype frequency as the dependent variable and the explanatory variables infection status, population of origin and parasite treatment was consistent with this analysis: infection status $F_{1,85} = 1.3$, $p = 0.30$; population of origin $F_{21,63} = 2.8$, $p = 0.001$ and parasite treatment $F_{1,84} = 2.5$, $p = 0.12$ (Fig. 3a). The parasite did not successfully establish in 10 out of 43 parasite treatment replicates. Excluding these replicates did not change the results qualitatively: infection status $F_{1,75} = 0.36$, $p = 0.55$; population of origin $F_{21,53} = 2.8$, $p = 0.001$ and effective parasite treatment $F_{1,74} = 4.6$, $p = 0.04$ (Fig. 3b). No replicates of the parasite-free treatment became infected. The mean relative fitness of all genotypes was higher in the presence of parasites than in the absence of parasites. Apparently, the tester genotype was generally a worse competitor in the presence of the parasite.

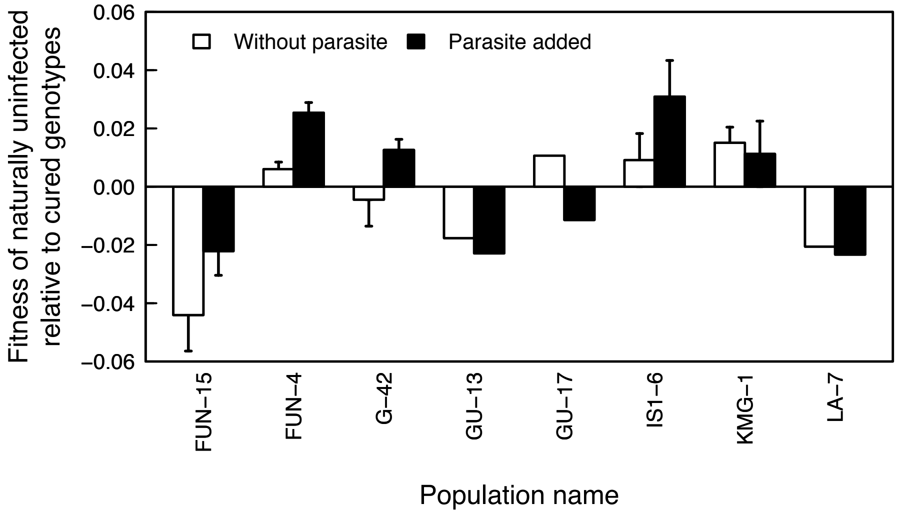

Fig. 4. Fitness of naturally uninfected relative to cured *Daphnia* genotypes. Relative fitness difference (mean ± SE) per day within genotype pairs from eight populations. In each pair, one genotype was naturally uninfected while the other was cured but formerly infected. Relative fitness was measured in direct competition. There was no effect of the infection status on competitive abilities, as the overall mean was not significantly different from zero. All genotypes were uninfected at the onset of the experiment and in half of the replicates we added sympatric parasites (black bars).

Within pairs from eight populations, genotypes differed by chance in their genetic markers and could therefore be used to measure competitive abilities in direct competition between these genotypes. In this case, fitness differentials were calculated in the perspective of the naturally uninfected genotype relative to the cured genotype. In accordance to the previous experiment, there was no effect of the infection status on relative fitness (generalized linear model with quasibinomial error structure, overall mean estimate = −0.13, $t = -1.1$, $p = 0.3$, population of origin $F_{1,7} = 8.4$, $p = 0.0002$ and parasite treatment $F_{1,24} = 2.3$, $p = 0.11$ (Fig. 4).

Finally, we compared the relative fitness of *Daphnia* genotypes in the presence and in the absence of parasites. We again used the same fitness values as above that were estimated in competition against a common tester genotype (Fig. 2 & 3). For this analysis we included in total 42 genotypes from 21 populations (the genotypes of one population were excluded, as in one replicate all animals went extinct). We first plotted the fitness of each genotype in the presence of parasites relative to its fitness in the absence of parasites (one dot for each of the 42 genotypes; in Fig. 5). Open dots represent the 21 naturally uninfected genotypes, while filled dots represent the 21 cured but formerly infected genotypes. We connected the values of the two genotypes from each population with a line. 16 out of these 21 lines have a positive slope. Thus, when comparing two genotypes, the one that has the higher fitness in the absence of parasites has on average also the higher fitness in the presence of parasites, and vice versa (Wilcoxon test, $V = 181$, $p = 0.02$; Fig. 5). A negative slope would indicate a trade off when a genotype is either only good in the presence or in the absence of parasites. This was not the case and therefore the results do not support a cost of resistance. The positive relationship we found is in accordance with different levels of inbreeding within populations. In that scenario, some genotypes are generally superior (irrespective of the presence or absence of parasites), while others may be inferior. The latter might be more inbred. Within each pair, one genotype was naturally uninfected and the other one was cured. However, as already shown above, the natural infection status did not correlate with the genotype's relative fitness, which is contrary to the inbreeding-infection hypothesis.

The use of a tester genotype

Our experiments were mainly based on fitness measurements relative to one tester genotype (Fig. 2. & 3). We corroborated the conclusions of this approach by additionally using direct fitness estimates between genotypes from eight populations (Fig. 4). The results of the two approaches were consistent when comparing the natural infection status and the genotypes' relative fitness (see above). This consistency of the two approaches was also seen when directly comparing the fitness estimates: the fitness estimates against a tester genotype correlated significantly with the fitness estimates from direct competition between the genotypes (linear model; $y = 1.46x + 0.001$; $R^2 = 0.61$, $p = 0.02$, n = 8 pairs of genotypes, Fig. 6).

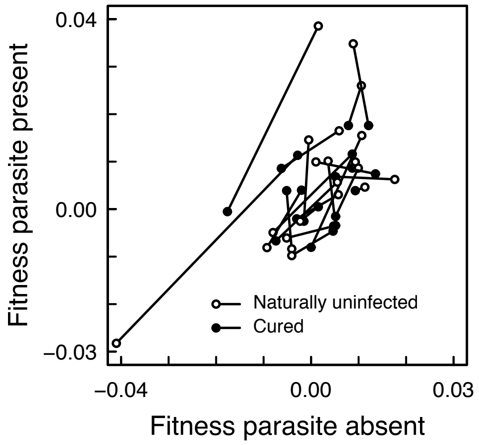

Fig. 5. Relative fitness of *Daphnia* genotypes in the presence and in the absence of parasites. Each dot represents the relative fitness of a genotype in the presence of parasites (y-axis) compared with its fitness in the absence of parasites (x-axis). In total, 42 genotypes from 21 populations have been tested. Open dots represent naturally uninfected genotypes, while filled dots represent cured but formerly infected genotypes. We connected the dots of the two genotypes from each population with a line. 16 out of these 21 lines have a positive slope. Thus, within a pair of genotypes, the one that has a high fitness in the absence of parasites has also a high fitness in the presence of parasites, and vice versa ($p = 0.02$). Within each pair, one genotype was naturally uninfected and the other cured but formerly infected (open and filled dots). However, there was no correlation of the natural infection status with the genotype's relative fitness.

Discussion

We compared the competitive abilities of naturally uninfected and cured but formerly infected *D. magna* from 22 natural populations. By this, we tested for a correlation between the fitness of a genotype and its natural infection status within populations. All *Daphnia* were cloned and treated against parasites before the experiments. The natural infection status (naturally uninfected vs. cured) did not correlate with a genotype's competitive success, neither in competition against a tester genotype (Fig. 2 & 3) nor in direct competition between the genotypes (Fig. 4). Furthermore, the presence or the absence of a sympatric parasite during the experimental trials did not change the competitive abilities (Fig. 5). Thus, we do not have an indication that other fitness components are related with the natural infection status of *D. magna*. There is no indication for a cost of resistance in our experiment. Likewise, there is no evidence that naturally infected genotypes are generally poor competitors as predicted by the inbreeding-infection hypothesis.

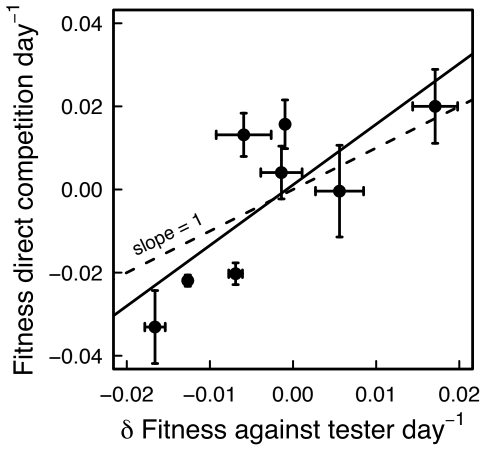

Fig. 6. Fitness estimates from direct competition compared with estimates from competition against a common tester genotype. Correlation of mean (± SE) fitness estimates from eight genotype pairs estimated with two different approaches. The first approach used relative fitness of each genotype against a common tester and the difference between naturally uninfected and cured genotypes is calculated (x-axis). The second approach used direct competition within each genotype pair, and fitness of naturally uninfected relative to cured genotypes is given (y-axis). There is a significant positive correlation between these two estimates. Each genotype pair was from a different population. For comparison, we show the slope of one (dashed line), which would be a perfect fit of the two approaches.

When postulating a correlation between a genotype's natural infection status and its fitness, a genetic basis of susceptibility and thus being infected is assumed. Indeed, cured but formerly infected genotypes had a higher likelihood to become infected compared with naturally uninfected genotypes (Fig. 1). This shows that susceptibility to infection has a genetic basis within host populations and that this genetic effect influences the likelihood to be infected under natural conditions. Such an increased susceptibility of formerly infected genotypes to infections has also been shown in the *Daphnia magna-Pasteuria ramosa* system (Little & Ebert, 2000, Duncan et al., 2006).

In a cost of resistance scenario, a trade-off is assumed between resistance and other fitness components (Kraaijeveld & Godfray, 1997, Groeters et al., 1994). However, neither in our system nor in the *D. magna-P. ramosa* system was evidence for a cost of resistance found (Little & Ebert, 2000, Little et al., 2002, Duncan et al., 2006). Naturally uninfected *Daphnia* did not have a lower fitness compared with cured but formerly infected genotypes in the absence of parasites (Fig. 2–4). Some genotypes were better competitors irrespective of the presence or the absence of parasites and irrespective of their natural infection status (Fig. 5). The absence of a cost of resistance is somewhat surprising. The parasite *O. bayeri* decreases host fitness significantly (Vizoso & Ebert, 2004, Vizoso & Ebert, 2005) and is highly abundant in the studied metapopulation (Ebert et al., 2001). Also *P. ramosa* is highly virulent and common in its host populations (Little & Ebert, 2000, Jensen et al., 2006, Duncan et al., 2006). Therefore, even rather costly mechanisms should be selected for if they reduce susceptibility to disease. A possible explanation could be trade-offs in resistance against different parasites (and costs paid for the resistance). In our experiments we did not test for a cost that comes in the currency of resistance to other parasite species or to other than sympatric *O. bayeri* isolates. Such a cost may be sufficient to maintain variation for resistance and could theoretically explain our results. We restricted ourselves to *Octosporea bayeri*, as it is by far the most common parasite in this metapopulation and all other parasites are rarer. Thus *Daphnia* might only profit from a scenario that includes other parasites in a limited number of cases. However, for future studies it would be worth to include different parasites.

Little et al. (2002) compared in the laboratory generally resistant genotypes with susceptible genotypes. It may be argued that the resistance seen in the laboratory did not reflect the natural situation because the outcome of host-parasite interactions may depend on environmental conditions such as temperature (Mitchell et al., 2005). Here we used the natural infection status as explanatory variable and performed our experiments under natural outdoor conditions, but still did not find a cost of resistance. This suggests that such a relation may not be important in our system. An alternative, which we cannot rule out, is that the costs are seen only for fitness components not assessed in our experiments. Zbinden et al. (2008) provide evidence that resistant *D. magna* genotype showed a reduced clonal growth rate, but only under stress free conditions (i. e. without interspecific competition). However, this might not be a frequent situation, as both inter- and intraspecific competition is common in this system (Bengtsson, 1993).

Inbreeding is another fitness aspect to which a genotype's infection status might be related (Coltman et al., 1999, Keller & Waller, 2002, Reid et al., 2003). Inbreeding generally causes a decrease in fitness (Hartl & Clark, 1997) and is common in the herein studied metapopulation due to frequent bottlenecks during colonization of empty rock pools (Ebert et al., 2002, Haag et al., 2002, Haag et al., 2006). However, it is unclear if inbreeding influences the likelihood to be infected within a natural population. The inbreeding-infection hypothesis postulates that cured but formerly infected genotypes should have a lower fitness compared to naturally uninfected genotypes. The sign of this correlation did not change in the absence compared to the presence of parasites in the competition trials, which is contrary to the predictions from the cost of resistance scenario. This arises, because inbred animals are expected to be consistently worse competitors (Haag et al., 2003, Salathé & Ebert, 2003). Parasites may intensify the negative effects of inbreeding, but are not expected to change the sign of the association.

In our experiments we found a significant population effect (Fig. 2 and 3), possibly due to different degrees of inbreeding and inbreeding depression between populations. Furthermore, within populations some genotypes were generally better competitors than others irrespective of the presence or the absence of parasites (Fig. 5). These might be genotypes that suffered less from inbreeding depression. However, their relative fitness was not related to their natural infection status, and within a population cured but formerly infected animals were not generally worse competitors as suggested by the inbreeding infection hypothesis (Fig. 2–4). Thus, we conclude that different degrees of inbreeding within populations did not explain variation in resistance to *O. bayeri*.

The natural infection status can be important in a metapopulation context. In a previous study (Altermatt et al., 2007) we showed that uninfected migrants are more successful than infected migrants. The current study emphasizes the importance for migrants to be free of parasites: there is no general fitness difference between susceptible and resistant genotypes, and thus being actually infected is a supplementary negative fitness component. Migrants entering populations with or without parasites would do best in any case being uninfected and resistant, as resistant immigrants will not have a disadvantage in uninfected resident populations.

In all experiments we used competitive ability as an integrative measurement of the overall fitness rather than individual fitness components such as carrying capacity or reproductive success (Baganz et al., 1998, Capaul & Ebert, 2003, Fellowes et al., 1998). Fitness values are implicitly taken as additive and being ordered, meaning that if fitness of genotype A > fitness of genotype B, and B > C, then A > C (Hartl & Clark, 1997). We did not test directly this assumption, but our two approaches, namely a comparison of fitness measurements from direct competition between genotypes and second using fitness differences from competition against tester genotypes gave consistent results (Fig. 6). Thus, we assume that our competition trials give meaningful fitness estimates.

In conclusion, we did not find a correlation between the natural infection status (naturally uninfected vs. cured) of a genotype and its competitive success. This was the case both in the presence as well as in the absence of sympatric parasites during the competition experiments. Therefore, our data do not support the inbreeding-infection hypothesis. They also do not support a cost of resistance, however ignoring potential costs caused by other parasite strains or parasite species. We suggest as a possible explanation for our results that resistance genes might segregate largely independently of other fitness associated genes in this system.

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Chapter 6

Genetic diversity of *Daphnia magna* populations enhances resistance to parasites

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Abstract: The diversity-disease hypothesis states that decreased genetic diversity in host populations increases the incidence of diseases caused by pathogens (= monoculture effect) and eventually influences ecosystem functioning. The monoculture effect is well known from crop studies and may be partially specific to the artificial situation in agriculture. The effect receives little attention in animal populations of different diversities. Compared to plants, animals are mobile and exhibiting social interactions. We followed the spread of a microsporidian parasite in semi-natural outdoor *Daphnia magna* populations of low and high genetic diversity. We used randomly selected, naturally occurring host genotypes. Host populations of low diversity were initially monoclonal, while the host populations of high diversity started with ten genotypes per replicate. We found that the parasite spread significantly better in host populations of low diversity compared to host populations of high diversity, independent of parasite diversity. The difference was visible over a three-year period. Host genotypic diversity did not affect host population density. Our experiment demonstrated a monoculture effect in independently replicated semi-natural zooplankton populations, indicating that the monoculture effect may be relevant beyond agriculture.

Introduction

The diversity of species or genotypes influences the functioning and dynamics of natural ecosystems (Loreau et al., 2001). Several studies found a positive correlation between a higher diversity of species or functional groups and productivity and density within plant communities (Engelhardt & Ritchie, 2001, Hector et al., 1999, Tilman et al., 1996). A higher genotypic diversity within species produces the same effect (Hughes & Stachowicz, 2004, Reusch et al., 2005). Furthermore, a high diversity increases resilience, and more diverse ecosystems resist better to negative influences and recover faster after disturbances (Reusch et al., 2005). Parasites pose such a negative influence (Poulin, 1998, Anderson & May, 1979), and are therefore an important factor to consider when studying effects of host genetic diversity. Parasites are of general importance in all natural systems, and epidemics can lead to population extinctions (Anderson & May, 1979, Ebert, 2005, Pounds et al., 2006, Rauch & Weisser, 2006, Weisser, 2000). Parasites and parasitoids can reduce host density, host growth or productivity and may thus affect ecosystem functioning (Anderson & May, 1979, Ebert, 2005, Pounds et al., 2006, Hudson et al., 1998). However, the diversity of a system itself may influence parasites as well (Leonard, 1969, Browning & Frey, 1969, Garrett & Mundt, 1999, Elton, 1958, van der Plank, 1963, Keesing, 2006 #1385). The question of how host genetic diversity influences parasite spread is the topic of this study.

The interest of genotypic or species diversity affecting the spread of parasites originated in agricultural research (Leonard, 1969, Elton, 1958). The rapid and devastating spread of diseases in agricultural monocultures is a well known phenomenon (e. g. potato blight or rice blast Zhu et al., 2000, Pilet et al., 2006). The diversity-disease hypothesis states that decreased genetic diversity of hosts increases the incidence of diseases caused by specialist pathogens, a phenomenon called the monoculture effect (Leonard, 1969, Browning & Frey, 1969, Garrett & Mundt, 1999, Elton, 1958, van der Plank, 1963). Several agricultural or silvicultural studies empirically support the monoculture effect (Zhu et al., 2000, Cox et al., 2004, Pilet et al., 2006, Wolfe, 1985, Hagle & Goheen, 1988) and the effect is found for bacterial pathogens, fungal pathogens (Cox et al., 2004, Mitchell et al., 2002), and plant herbivores (Unsicker et al., 2006). However, crop monocultures or managed grasslands represent artificial situations and usually only one or a few cultivars are compared (Garrett & Mundt, 1999, Zhu et al., 2000, Mitchell et al., 2002). The cultivars are selected lines for certain traits such as yield quantity or quality and not a random samples of genotypes (Zhu et al., 2000, Cox et al., 2004, Pilet et al., 2006). We wanted to know if the monoculture effect occurred also in natural and spatially less structured animal systems.

Little is known on the actual mechanism beyond a monoculture effect (reviewed in Keesing et al., 2006). Hosts in monocultures are not *per se* less resistant, but all individuals have a similar susceptibility to diseases. Pathogens transmit from one host individual to another without encountering large changes in the host genotype. Once a host genotype is infected, subsequent spread is facilitated because no genetic or physiological adaptations are required to infect another individual (Anderson & May, 1986). In contrast, in genetically diverse host populations, a pathogen may encounter different host genotypes when it is transmitted from one host individual to another (Keesing et al., 2006). Genetic or physiological adaptations require some time and transmissions to resistant host genotypes are dead ends for the parasites (Anderson & May, 1986). This slows down the spread of parasites. In agriculture, the strong spatial structure of plant genotypes grown in alternating rows may influence pathogen spread. In plots of higher diversity, each species or genotype has a lower density, and pathogen transmission within the susceptible individuals may be reduced by the less probable encounter of susceptible hosts (called dilution effect Schmidt & Ostfeld, 2001, LoGiudice et al., 2003, Mitchell et al., 2002). In the long-term, a low genetic diversity is disadvantageous during coevolution, and parasites may adapt faster to less diverse host populations (Ebert, 1998b). Thus, the monoculture effect may promote the maintenance of genetic diversity and influence host-parasite coevolution.

Here, we experimentally tested the monoculture effect in a natural animal host-parasite system with realistic differences in genetic diversity. Up to now, the study of parasite spread within groups of animals differing in genetic diversity mostly focussed on social insects (van Baalen & Beekman, 2006, Schmid-Hempel & Crozier, 1999, Shykoff & Schmid-Hempel, 1991, Hughes & Boomsma, 2006). These animals live in close proximity within a colony, exhibit social interactions, and are closely genetically related, which all favours the spread of parasites (Altizer et al., 2003). In bumblebees, colonies with a higher genetic diversity due to polyandry contain fewer parasites and show greater reproductive success, than low-diversity colonies (Baer & Schmid-Hempel, 1999). Besides social insects, experimental data on the epidemiological effects of genetic diversity in animal populations are scarce. Compared to plant populations, we strongly reduced spatial structuring by using planktonic animal populations. We thereby diminished the effect of spatial structuring as a mechanistic explanation of the monoculture effect.

We followed the epidemiology of the microsporidian parasite *Octosporea bayeri* in *Daphnia magna* host populations of low and high genetic diversity under outdoor conditions. Both hosts and parasites were collected in a natural metapopulation (Ebert et al., 2001, Pajunen & Pajunen, 2003). In that metapopulation, host populations differ naturally in their genetic diversity (Haag et al., 2005). The *D. magna* populations were placed in mesocosms under outdoor conditions, and parasites were allowed to spread in a natural way. We also used two diversity levels for the parasite to test if a monoculture effect depends on parasite diversity. We monitored the spread of the parasite over three years, which corresponds to about 20–30 host generations and represents the average survival length of natural *Daphnia* populations in our metapopulation (Pajunen & Pajunen, 2003).

Parasites spread significantly better in host populations of low genetic diversity compared to host populations of high genetic diversity. As planktonic populations spatially commix and no totally resistant hosts are known, the herein demonstrated monoculture effect may not be explained by spatial structure or abundance of susceptible hosts (Mitchell et al., 2002, Keesing et al., 2006), but by the different levels of genetic diversity in the host populations. Surprisingly, the genetic diversity of the parasite population had no significant effect on parasite spread.

Material and Methods

Daphnia magna and Octosporea bayeri

The freshwater crustacean *Daphnia magna* Straus, 1820 (Crustacea: Cladocera) is widely distributed along the coast of the Baltic Sea, inhabiting water-filled rock pools (Pajunen & Pajunen, 2003). These pools are spatially separated and genetic differentiation between the rock pool populations is strong (Haag et al., 2005) due to recurrent colonisations, immigrations and extinctions (Pajunen, 1986, Pajunen & Pajunen, 2003). Natural populations strongly differ in their age (Pajunen & Pajunen, 2003). Populations founded by one or a few individuals are highly inbred and possess little genetic diversity (Ebert et al., 2002, Haag et al., 2002). Periods of asexual reproduction during summer are intermitted by sexual reproduction when resting eggs (so-called ephippia) are produced. Only ephippia allow over-wintering (Ranta, 1979).

The rock pool *Daphnia* populations harbour a wide spectrum of parasites, but differ naturally in their parasite community and prevalence (Green, 1957, Bengtsson & Ebert, 1998, Ebert et al., 2001, Ebert, 2005). The microsporidium *Octosporea bayeri* is the most common parasite in our study area. *Octosporea bayeri* is specific to *D. magna* and is found in about 45 % of all populations (Ebert et al., 2001). *Octosporea bayeri* replicates inside the host and infected animals contain up to $5*10^6$ spores of the parasite (Ebert, 2005, Vizoso et al., 2005). Parasite transmission is vertical or horizontal, and infected hosts have reduced fecundity, survival, and competitive ability (Vizoso & Ebert, 2004, Lass & Ebert, 2006). Horizontal infection occurs by ingesting spores from dead infected hosts. Physical contact alone between infected and uninfected living animals does not allow transmission.

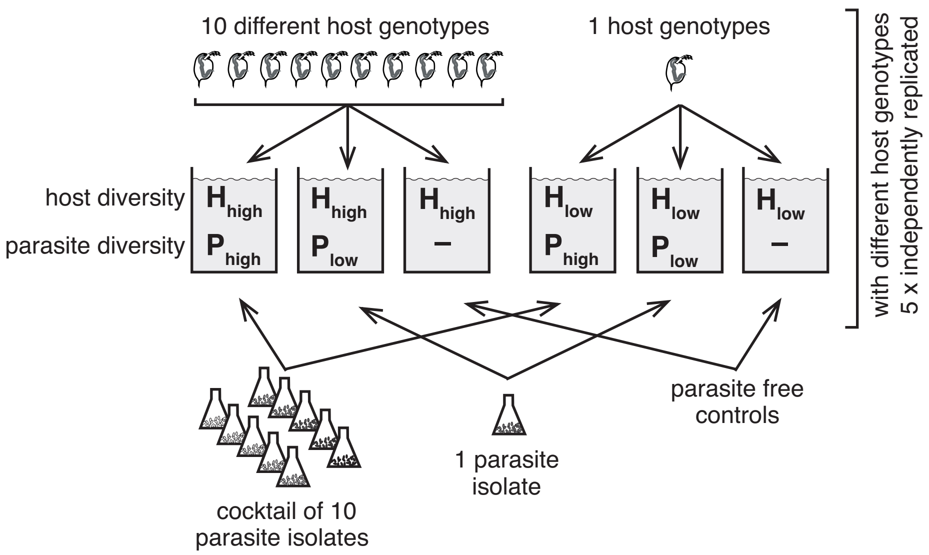

Fig. 1. Set-up of the experimental mesocosms in buckets. The experiment was started with uninfected *Daphnia magna* host populations of high and low genetic diversity (H_{high} and H_{low}). Each of the five different host populations of high genetic diversity started with ten different genotypes at equal proportions. Each of the five different host populations of low genetic diversity started with one different genotype. All populations were set-up in triplicate ([5 + 5] * 3 = 30). Thereafter, we introduced the microsporidium *Octosporea bayeri*. Parasite isolates originated from eleven different natural populations. Within each triplicated host population, one population received a cocktail of ten parasite isolates (P_{high}), one population received only one parasite isolate (P_{low}), and one population remained a parasite free control (–). The buckets were placed outdoors next to natural *D. magna* populations.

Set-up

In spring 2003 and 2004 we took random samples of *D. magna* from 22 rock pool populations on 12 islands near Tvärminne Zoological Station, Finland (59° 50' N, 23° 15' E). We used single females to create clonal cultures in the lab. Each female represented a unique genotype that hatched from an over-wintering egg. Per population, either two (12 populations), three (9 populations) or four (1 population) genotypes were isolated, giving in total 55 genotypes. We treated each genotype against possible infections of the microsporidium *O. bayeri* following the protocol of Zbinden (2005). Infections of *O. bayeri* are easily seen when the host is dissected and investigated with phase-contrast microscopy (400-fold magnification). The success of the treatment was confirmed afterwards. No other parasites were found in the cultures. The natural infection status does not affect the competitive abilities of a genotype (Altermatt & Ebert, 2007), and therefore the cured genotypes were not inferior to the naturally uninfected genotypes. We typed all *Daphnia*-lines for their allozyme genotypes at five loci. These loci were aspartate amino transferase (Aat, enzyme commission number EC 2.6.1.1), fumarate hydratase (Fum, EC 4.2.1.2), glucose-6-phosphate isomerase (Gpi, EC 5.3.1.9), phosphoglucomutase (Pgm, EC 5.4.2.2), and mannose-6-phosphate isomerase (Mpi, EC 5.3.1.8) (Hebert & Beaton, 1993). The genotypes were kept in 400 ml artificial medium (Klüttgen et al., 1994) at room temperature with a dark/light cycle of 10/14 hours and were fed ad libitum with the green alga *Scenedesmus obliquus*.

To breed different parasite isolates, we collected from each of eleven rock pools one *D. magna* female that was naturally infected with *O. bayeri* in May 2005. To avoid a coevolutionary history, we collected the *D. magna* used as hosts in the experiment in different pools than the infected *D. magna* used for parasite cultures. Because of the lack of genetic markers in *O. bayeri*, we could not test if single *D. magna* were infected by multiple parasite strains or not. To cultivate the parasite isolates, we placed the infected *Daphnia* singly in buckets containing eight liter of artificial medium. The buckets were put on an island next to natural pools and the *Daphnia* fed *ad libitum*. Vertical transmission of the parasite is 100 % during the asexual reproduction of *D. magna* (Vizoso et al., 2005). Because of asexual reproduction, we could breed large numbers of *Daphnia* (>1000) infected with the same parasite (herein called "isolate") within a few weeks.

On 18 June 2005 we started the experiment. We released the uninfected *D. magna* into plastic buckets (volume 40 liter) containing 20 liter water from a rock pool free of *D. magna* and free of parasites filtered through a 20-µm filter. We added one liter of sea-water, 700 ml artificial medium and 150 ml of a horse manure suspension (10 kg horse manure suspended in 60 liter sea-water) to increase salinity, increase the nutrient content and ameliorate water quality.

We established *D. magna* populations with a low genetic diversity (H_{low} = 1 genotype per host population) and with a high genetic diversity (H_{high} = 10 genotypes per host population). In both of these treatments we had five completely independent combinations in respect of their host genotype composition (Fig. 1). Every host genotype was only used once (5 times 1 plus 5 times 10 = 55 *D. magna* genotypes). The genotypes in the high genetic diversity treatment were randomly chosen, but such that all genotypes per combination of ten came from different pools of origin. Due to the random assignment of the host genotypes to the treatments, the genotypes did not differ systematically in any aspect between the two different diversity treatments. In the low genetic diversity treatment, 100 animals per genotype were released in buckets (replicated in triplicate), in the high-genetic diversity treatment ten animals of each of the ten genotypes per combination were released together (replicated in triplicate; this gave 2 treatments x 5 population x 3 replicates = 30 mesocosms). The mesocosm buckets were placed outdoor on an island next to natural rock pools.

The host diversity levels were completely crossed with three parasite treatments: no parasite (control), low and high parasite diversity (Fig. 1). The parasite was added to the buckets by placing dead infected *D. magna* females (freshly killed with CO_2) from the parasite cultures to the appropriate replicates. We used only one parasite isolate in the low parasite diversity treatment and one cocktail of ten parasite isolates in the high genetic diversity treatment (Fig. 1). We randomly chose one of the eleven cultured parasite isolates for the low genetic diversity treatment (P_{low}), while we added the other ten together at equal proportions to produce a cocktail of high genetic diversity (P_{high}). We added dead infected females to each replicate on 23 June 2005 (60 dead females per replicate) and again on 6 August 2005 (30 dead females per replicate). Parasite spores were passively released from the decaying cadavers of these hosts, allowing the infection to spread in the mesocosm populations (Vizoso et al., 2005). This represents the natural way of horizontal infection. To the controls, we added the same number of dead *Daphnia* from parasite free laboratory cultures.

Over the summers, water volume in the buckets varied from 15 to 35 liter due to evaporation and precipitation. We added deionised water during phases of long droughts to compensate for excessive evaporation-loss. In each autumn, we lowered the water level to about five liter. This prevented breaking of the buckets during freezing in winter. We covered the buckets with a lid and left them outdoors. Only ephippia and parasite spores survived the freezing in winter. In the subsequent year at the beginning of May, we filled the buckets with deionised water up to the previous level.

We estimated prevalence in random samples of the host populations on 1 August 2005 (summer 2005), 13 September 2005 (autumn 2005), 26 May 2006 (spring 2006), 11 July 2006 (summer 2006), 19 August 2006 (autumn 2006), 26 May 2007 (spring 2007) and 16 August 2007 (autumn 2007). In the year 2005 we estimated prevalence by dissecting 20 *D. magna* females per replicate and sampling date, while in the years 2006 and 2007 always about 30 *D. magna* females per replicate and sampling date were dissected. On 11 July 2006 we took a random sample of all replicates to quantify the frequency of alleles and multi-locus genotypes at the five allozyme loci mentioned above. Per replicate, 66 *Daphnia* females were genotyped. We estimated *Daphnia* density on 30 July and 18 August 2006 by taking a volumetric sample. For the analysis, we used the mean of these two estimates per replicate to balance short temporal fluctuations. Furthermore, we roughly estimated the number of hatchlings at 26 May 2006 using the categories <100 hatchlings, 100–500 hatchlings, 500–1000 hatchlings and >1000 hatchlings.

As mentioned above, *D. magna* reproduces asexually during May to September but only survives the winter (beginning of October to end of April) in the sexually produced ephippia. Therefore, in 2005 only the initial sets of genotypes were present in the mesocosms, while in 2006 and 2007 all animals were recombinants. The monoclonal populations were all selfed from 2006 onwards, which is a common phenomenon in this metapopulation (Haag et al., 2005).

Analysis

We performed statistical analyses with R (R Development Core Team, 2007) using the libraries Matrix (version 0.98-7), lme4 (version 0.98-1), Hmisc and base. We analysed prevalence in autumn 2005 with generalized linear mixed effect models and tested for an effect of host genetic diversity. At that time, no recombinant hosts were present. Host populations of low genetic diversity were still monoclonal, while host populations of high genetic diversity consisted of up to ten genotypes. Clonal selection may have reduced the genotype richness in the high genetic diversity treatments (Capaul & Ebert, 2003). We analysed prevalence changes over the whole three years time period using repeated measure ANOVAs with arcsin transformed prevalence data. We used generalized linear mixed effect models to analyse the total number of alleles and the density data. In all models, contrasts were chosen *a priori* to compare host populations of low and high genetic diversity and the parasite treatment of low and high genetic diversity.

Results

The regular samples revealed that the parasite established, spread and persisted over three years in all mesocosm populations where it had been added. All but one replicates of the parasite free control treatments stayed free of parasites. In one of the low genetic diversity controls we found an *O. bayeri* infection in summer 2006, indicating an immigration of either an infected *Daphnia* or of the parasite. This replicate was excluded from the analyses.

We found a significant monoculture effect. Parasites were more successful and reached a higher prevalence in the host populations of low genetic diversity compared to the host populations of high genetic diversity in autumn 2005 (lmer contrasts, $t_{16} = -2.6$, $p = 0.02$; Fig. 2). At that time, host populations had not yet recombined and still reflected the initial composition of genotypes. At the same time, the parasite diversity treatments had no effect on parasite prevalence (lmer contrasts, $t_{16} = -1.1$, $p = 0.30$). The interaction between the host and parasite diversity treatment is marginal non-significant (lmer contrasts, $t_{16} = 1.9$, $p = 0.06$).

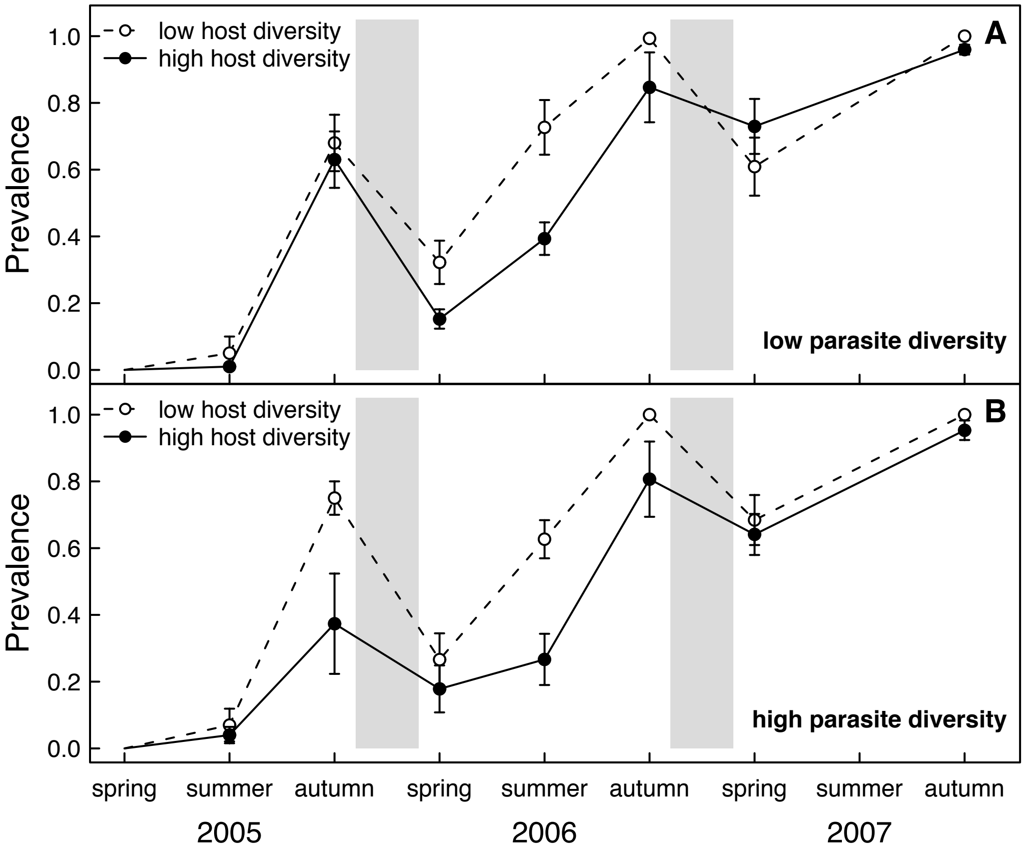

Fig. 2. Parasite prevalence over time in host populations of either low or high genetic diversity. Prevalence of the parasite *O. bayeri* in *D. magna* host populations over time. *Daphnia magna* populations differed in their genetic diversity (low and high) and were all uninfected at the beginning of the experiment. The parasite spread significantly better in host populations of low genetic diversity (open dots) compared to host populations of high genetic diversity (filled dots). This difference was found both in the parasite treatment of low genetic diversity (A) and in the parasite treatment of high genetic diversity (B). Prevalence significantly changed over time. During winter diapause, indicated by the grey area, the hosts only survived in sexually produced resting stages.

We then analysed prevalence over the whole three years. From the second year onwards, host populations were recombined and inbreeding effects may have occurred as well. Again, genetic diversity of the host population significantly influenced the spread of the parasite. The parasite *O. bayeri* spread significantly better in *D. magna* host populations of low genetic diversity compared to host populations of high genetic diversity over the three years' period ($F_{1,16} = 12.7$, $p = 0.0026$; Fig. 2). Parasite prevalence was higher in the host populations of low genetic diversity in all but one sample. In accordance to an earlier study, parasite prevalence showed a cyclic pattern with an increase during the asexual phase from spring to autumn, and a decrease during winter diapause (Lass & Ebert, 2006), resulting in a significant time effect in our analysis ($F_{6,96} = 116$, $p < 0.0001$). The interaction between the effect of host genetic diversity and time is significant ($F_{6,96} = 4.2$, $p = 0.0009$). Prevalence between the parasite treatments was not significantly different ($F_{1,16} = 0.3$, $p = 0.59$; Fig. 2).

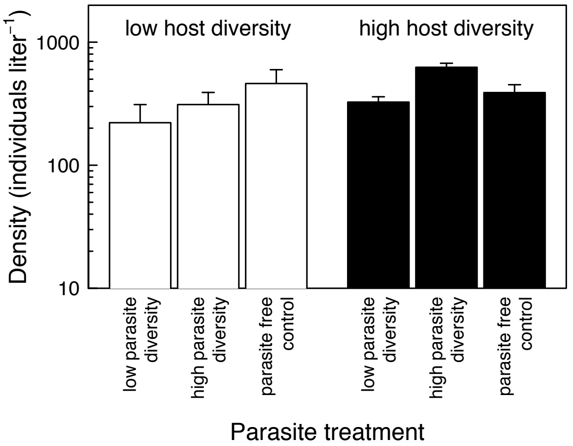

Fig. 3. Host population density in the different treatments. Mean density (± SE) of *D. magna* in summer 2006. Density varied considerably between the different replicates, though there was no significant effect of host or parasite diversity. The mesocosms contained about 25 liter of water at the time of density estimation.

In all replicates, more than 300 hatchlings were present in spring 2006 (data not shown). Most populations had about 500–1,000 hatchlings and a few populations had even more than 1,000 hatchlings. Each hatchling is a unique recombinant. The number of hatchlings between host populations of low genetic diversity and host populations of high genetic diversity was not significantly different (Wilcoxon signed rank test, $W = 87$, $p = 0.24$). Population size increased by subsequent asexual reproduction. We then measured population density at mid-summer. We did not find a significant difference between the density of host populations of low genetic diversity and host populations of high genetic diversity (lmer contrasts, $t_{24} = 1.16$, $p = 0.26$, Fig. 3). Also, the density of the uninfected host populations do not significantly differ from the density of infected host populations (lmer contrasts, $t_{24} = -1.5$, $p = 0.14$, Fig. 3). All population contained at least 1,000 individuals (and up to 30,000 individuals) and only towards the end of the summer population sizes decreased. In some replicated as few as 50 individuals occurred in September/October (personal observation). However, sexual reproduction had already taken place at that time and the majority of ephippia is produced before end of July (Altermatt & Ebert, 2008). Thus, the number of hatchlings and the subsequent population sizes were always large enough to prevent a substantial genetic drift effect over our experimental period of 20–30 generations (Hartl & Clark, 1997). Prevalence and density, both depending variables, significantly correlate with each other ($R_S = 2030$, rho = –0.53, $p = 0.017$, the parasite free controls were excluded in this analysis; Fig. 4).

In the first year of the experiment (2005), the host populations of low genetic diversity were monoclonal and had a mean number of 5.6 alleles at the five loci assessed (SE ± 0.6). The host populations of high genetic diversity contained ten different genotypes at start (Fig. 1). At the same five loci, they had a mean of 10 alleles (SE ± 0.51). As expected, the heterozygosity between the host populations of low and high genetic diversity did not differ at start (Fisher exact test, $p = 0.74$). In the subsequent years, all populations consisted of recombinants, and we could only distinguish a fraction of the genotypes with allozymes. In summer 2006, we confirmed that the diversities in the two different host population treatments still differed according to the initial set-up. As expected, the difference in the number of alleles remains highly significant between the two diversity treatments (lmer contrasts, $t_{24} = 6.2$, $p < 0.0001$; Fig. 5a). New, host populations of low genetic diversity have a significantly lower heterozygosities (lmer contrasts, $t_{24} = 3.3$, $p = 0.003$; Fig. 5b) than host populations of high genetic diversity. We did not find new alleles occurring in any of the replicates and both allele number and heterozygosity were in accordance to the expected values under a model without drift and selection. The occurrence of new alleles would have indicated immigration of hosts from other populations. The parasite treatment did neither affect the number of alleles (lmer contrasts, $t_{24} = 0.82$, $p = 0.42$; Fig. 5a) nor the heterozygosity of the host populations (lmer contrasts, $t_{24} = 0.35$, $p = 0.73$; Fig. 5b).

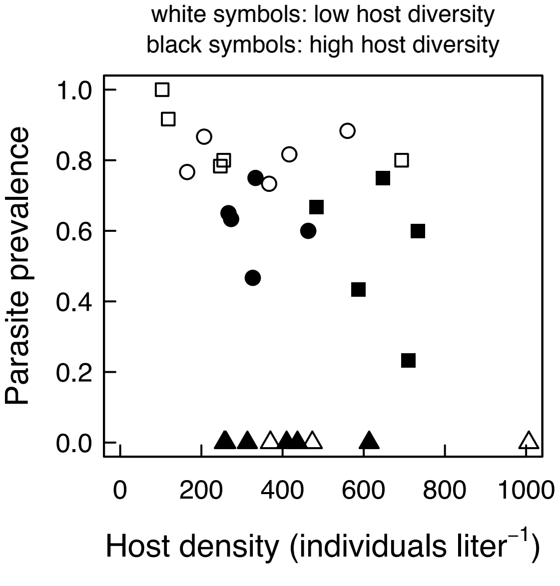

Fig. 4. Correlation between the two depending variables host density and parasite prevalence. The significant negative correlation does not imply causality. Squares represent replicates infected with a parasite isolate of low genetic diversity and circles represent replicates infected with a parasite isolate of high genetic diversity. Triangles represent parasite free controls, and are only shown only for comparison and were excluded from the analysis.

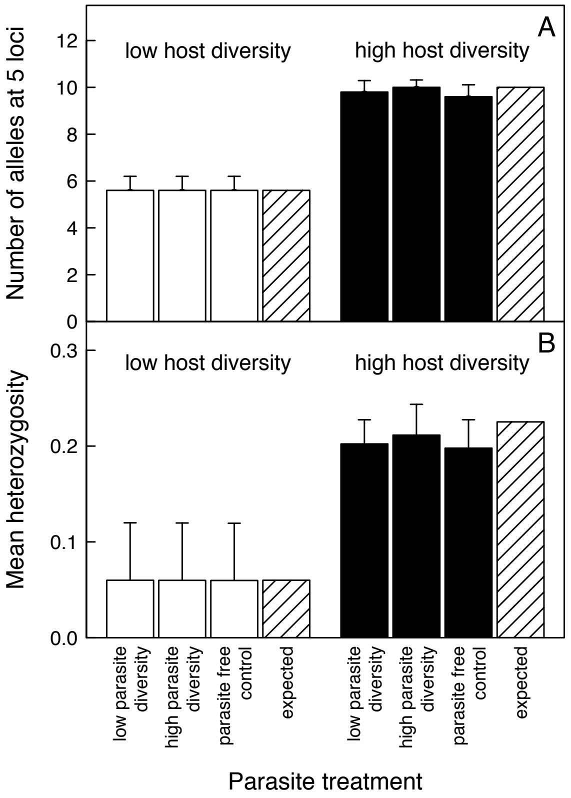

Fig. 5. Allele number and heterozygosity between the different treatments. A) Mean number of alleles (± SE) at five loci in the experimental *D. magna* populations in the second year (summer 2006). Accordingly to the initial set-up, host populations of low genetic diversity (white bars) still contained significantly fewer alleles than host populations of high genetic diversity (black bars). There was no effect of the parasite treatment. We did not find new alleles that would indicate immigration of hosts into the mesocoms. The shaded bars show the initial and thus expected mean number of alleles. B) Mean heterozygosity (± SE) of *D. magna* populations in the second year (summer 2006). Accordingly to the initial set-up, host populations of low genetic diversity (white bars) still had a significantly lower heterozygosity than host populations of high genetic diversity (black bars). There was no effect of the parasite treatment. The shaded bars show the expected heterozygosity assuming no genetic drift or selection.

Discussion

We set up a multi-generation experiment (Fig. 1) to test if the monoculture effect typically seen in spatially structured agricultural systems also occurs in spatially commixing animal populations. Over three years, we studied the spread of the microsporidian parasite *O. bayeri* in replicated *D. magna* host populations of either low or high genetic diversity. The experiment included about 20–30 asexual host generations, two sexual host generations, multiple parasite generations, and two natural diapause events after which all hosts were new recombinants. The parasite spread better in host populations of low genetic diversity (Fig. 2) compared to host populations of high genetic diversity, and the effect was independent of the level of parasite diversity. In the first year, the monoculture effect was only due to different levels of diversity, whereas in the second and third year also the difference in inbreeding among the treatments may have played a role. The level of parasite diversity did not influence parasite spread (Fig. 2) and host density was not different in host populations of low and high genetic diversity (Fig. 3).

We first discuss the monoculture effect found at the end of the first summer (summer 2005, Fig. 2). Parasites spread significantly better in host populations of low genetic diversity compared to host populations of high genetic diversity. At that time, we could exclude inbreeding effects and attribute the monoculture effect to different levels of genetic diversity alone. All host genotypes used were randomly selected, natural isolates. They did not differ systematically between the two treatments, and they were also independently replicated within the treatments. Thereby, we also excluded that the effect was due to a specific feature of the chosen genotypes itself. By using different genotypes of only one host species, we showed that the monoculture effect occurred at the genotypic level (see also Reusch et al., 2005).

The different genetic diversities of the host populations give a mechanistic explanation for our findings. Spatial structure alone can be safely excluded as mechanistic explanation (Mitchell et al., 2002, Keesing et al., 2006), because our plankton populations are spatially commix. Keesing et al. (2006) suggested five mechanisms by which genetic diversity reduces the disease risk in a specialist host-pathogen system and results in a monoculture effect. In their models, diversity is increased by the presence of an additional non-host species. We defined diversity more stringently by using different host genotypes within one host species. Our set-up is comparable to models using multiple host species (Keesing et al., 2006). From previous experiments with the *Octosporea-Daphnia* system we know that *Daphnia* genotypes from this metapopulation differ in susceptibility to the parasite, but full resistance has never been reported (Lass & Ebert, 2006, Vizoso et al., 2005, Altermatt & Ebert, 2007). Furthermore, *Octosporea bayeri* is not transmitted by contact between living hosts alone. Thereby, encounter reduction, transmission reduction and susceptible host regulation are excluded as mechanisms by which diversity could reduce disease risk (Keesing et al., 2006), because all three require the presence of totally resistant host genotypes. We could also exclude recovery augmentation (Keesing et al., 2006) in our system, because hosts do not recover from infections during the asexual phase (Lass & Ebert, 2006, Vizoso et al., 2005, Ebert et al., 2007). From the five suggested mechanisms (Keesing et al., 2006), only differential mortality and reproduction of infected hosts in genetically diverse compared with less diverse populations may be used as mechanistic explanation of how parasite incidence is reduced. In that scenario, infected individuals are less able to tolerate competitive interactions (Keesing et al., 2006) in diverse populations compared to single-host populations. Infected hosts die faster or do not reproduce. Another mechanism which may lead to a reduced spread in diverse host populations would be when horizontal host-to-host transmission is more likely among similar hosts than dissimilar hosts. Then, a parasite may adapt phenotypically, but not necessarily genetically, to the current host genotype. This adaptation results then in a higher transmission success to hosts of the same or a similar genotype. The mechanism would be independent of parasite genetic diversity, and would thus be consistent with our findings. Further, not yet specified mechanisms may arise in systems involving multiple host species or genotypes (Keesing et al., 2006).

We now discuss the effect seen over all three years (2005–2007, Fig. 2). After two years, we found that the genetic structure of our host populations was still in accordance with respect to low and high host genetic diversity at start (Fig. 5). We can thereby eliminate population level effects caused by strong drift or selection as an alternative explanation for the difference in prevalence. From the second year onwards, all host populations in the low diversity treatment were inbred as a result of selfing. Inbreeding is an inherent and naturally occurring situation in *D. magna* populations of low genetic diversity (Haag et al., 2006). Vertical transmission of *O. bayeri* through resting stages is more successful in inbred populations compared to outcrossing populations (Ebert et al., 2007). Previous work suggests that inbreeding depression alone does result in increased parasitism (Altermatt & Ebert, 2007). Rather, inbreeding in a population increases the similarity between host individuals, and parasites may adapt faster to the host populations (Ebert, 1998a, Ebert et al., 2007). From the second year onwards, inbreeding and monoculture effect together may have consistently influenced parasite prevalence and may have allowed for accelerated parasite adaptation. In the natural system, the two mechanisms are intrinsically tied to each other.

The spread of parasites has an evolutionary component when hosts encounter pre-adapted parasites. Parasites infect and spread better in their sympatric host population compared to allopatric populations (Gandon et al., 1996, Lively, 1989, Ebert, 1994, Refardt & Ebert, 2007), also seen in herein studied metapopulation (Altermatt et al., 2007). We started the experiment with uninfected host populations and used allopatric parasites to avoid the confounding effects of local adaptation and coevolutionary history. The experiment thus represented the situation after a new colonisation of a rock pool. Compared to sympatric combinations, parasite fitness strongly varies on allopatric hosts and depends on the specific host-parasite combination (Ebert, 1994). By chance, the rapid spread of the parasite in host populations of low genetic diversity may be due to an *a priori* presence of parasite genotypes that could easily infect the host genotype in the monoculture (Carius et al., 2001). As we used five independent replicated host populations, it seems unlikely that pre-adapted parasites were present in several replicates of either low or high host genetic diversity. However, the general increase of parasite prevalence over time in both treatments (Fig. 2) may reflect some local adaptation of the parasite over time.

In our study, the prevalence of *O. bayeri* was about 20 % lower in host populations of high genetic diversity compared to host populations of low genetic diversity (Fig. 2). Thus, the monoculture effect was considerable smaller than in crop studies. For example, Zhu et al. (2000) found a 94 % blast reduction in mixtures compared to monocultures (but see Cox et al., 2004). Part of the monoculture effect size in the plant studies is mechanistically explained by the spatial structure in plant cultivations (Mitchell et al., 2002, Zhu et al., 2000, Cox et al., 2004). Plant pathogens are transmitted stepwise and spread in circular waves within a field. In diverse cultures, susceptible plants may grow at distances that are too large for the pathogen to spread successfully. The same effect occurs by growing the plants at larger distances within a monoculture. The reduction in relative density of the susceptible host is mechanistically equivalent to encounter reduction (Keesing et al., 2006). Our planktonic hosts are mobile and commix spatially, which favours the transmission and spread of parasites (Altizer et al., 2003, Anderson, 1995). Consequently, the monoculture effect can be expected to be smaller, as spatial structuring does not hamper parasite spread.

Contrary to other studies (Engelhardt & Ritchie, 2001, Hughes & Stachowicz, 2004, Reusch et al., 2005 1431), we did not find a positive effect of the genetic diversity on host density (Fig. 3). Possibly, a high genetic diversity in the host populations suppressed parasite prevalence but interacted with other parameter such as productivity (Fig. 4). In our experiment we could not control for these two dependent variables.

We expected a higher prevalence in infections with diverse parasite mixtures. A high genetic diversity in the parasites may both have increased the probability of matching parasites to be present (Carius et al., 2001) as well as facilitated subsequent parasite adaptation (Gandon & Michalakis, 2002). However, the effect was not found (Fig. 2). There are several, non-excluding explanations for the lack of a significant difference in our experiment. First, even though each of the eleven parasite strains was bottlenecked by starting with only one infected *D. magna* (as successfully applied in the same system by Vizoso & Ebert, 2005), each isolate could still have been genetically diverse. In case of a high within isolate diversity, mixing different isolates in the cocktail would have little effect and the parasite treatment of either low or high genetic diversity would differ less than expected. Second, contrary to the host populations, we did not replicate the parasite treatment of low and high genetic diversity. For the parasite, we had only one cocktail of high genetic diversity and one isolate of low genetic diversity. If the single isolate used in the low genetic diversity treatment was by chance exceptionally virulent or infectious, the difference may vanish. However, this is unlikely and would require the parasite to be generally virulent on all independently replicated host genotypes. A generally superior parasite is contrary to all current evidence in *Daphnia*-parasite systems, including the herein studied metapopulation (Carius et al., 2001, Ebert, 1994, Refardt & Ebert, 2007, Altermatt & Ebert, 2007).

In summary, our study contributed realistic data on both the generality and effect size of the monoculture effect in animal populations. Compared to agricultural studies we also included randomly selected host genotypes and a multigenerational setting under outdoor conditions. Host populations of high genetic diversity had an advantage over a period of three years, including multiple generations and diapauses under realistic conditions. Thus, our study empirically shows that the monoculture effect may be important beyond the known agricultural situation. The epidemiological advantage of a high genetic diversity in host populations will in the long run also hamper parasite adaptation (Hughes & Boomsma, 2006, Ebert et al., 1998).

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Chapter 7

Résumé, conclusions and further directions of research

I studied rock pool *Daphnia* metapopulations with a focus on the production of migration stages and consequences related to migration. For the first time, I quantified the production of migration stages in rock pools of different sizes. Populations in small and desiccation-prone pools produced almost as many migration stages as populations in large and stable pools. This is contrary to metapopulations following a mainland-island or all-equal Levins' type model (Harrison, 1991, Levins, 1968, Hanski, 1999, Kawecki, 2004). I also showed that one of the most common habitat instabilities, namely desiccation, is not detrimental for *D. magna* populations, as resting stages survived and built-up viable populations within the same season. In a further step, I developed an individual-pool-based model, to retrospectively predict desiccation events and drought lengths in more than 500 monitored rock pools over the last 25 years. Droughts were common, and *D. magna* populations were also found in pools with a high risk of desiccation. Desiccation may not only be involved in triggering ephippium production (Stross & Hill, 1965, Banta & Brown, 1929), but will also expose the ephippia in the dry sediments to passive dispersal by wind or birds (Brendonck & Riddoch, 1999, Ranta, 1979, Maguire, 1963). Populations in small and instable patches contributed over-proportional number of migrants. Even though occupancy was shorter in these pools, they have a key role in this *D magna* metapopulation.

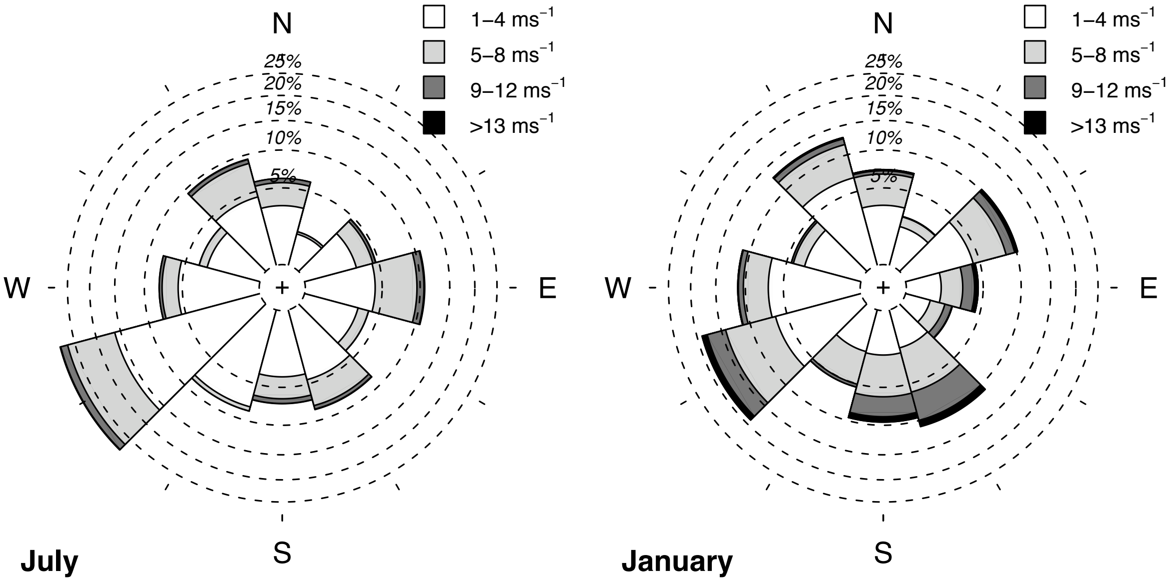

Fig. 1. Windrose diagram showing mean wind direction and average wind speed at Tvärminne Zoological Station over the last 25 years. Exemplary, data are given for July (left panel) and January (right panel). In July, pools are often desiccated (chapter 3), and ephippia exposed to passive dispersal by wind. During that time, the prevailing wind direction is west-southwest, where the wind is coming from in more than 25 % of the time. Thus, migrants could be transported predominantly in the axis from southwest to northeast. I hypothesize that colonisations and invasions will follow this gradient. In summer, average wind speed is moderate to strong (1–8 ms^{-1}). Contrary to July, there is no predominant wind direction in January, and average wind speed is generally higher (5–13 ms^{-1}). However, during winter pools are frozen and often covered with snow, making migration unlikely.

The herein studied *D. magna* metapopulation is a further example of a metapopulation in which deteriorating environmental conditions may lead to high emigration (see also Crone et al., 2001, Pokki, 1981, Vanschoenwinkel et al., 2007). It is the second documentation of a metapopulation in which ephemeral populations seem to be the important sources of migrants (Crone et al., 2001). Short-lived populations in desiccation-prone pools are less parasitized (Ebert et al., 2001), and will consequently produce more parasite-free migration stages. This would give migrants an additional advantage (Altermatt et al., 2007).

The significance of migration may generally increase, as in many species more populations get isolated by the ongoing fragmentation of the landscape. Isolated populations may only survive due to immigrants, and otherwise go extinct because of demographic or genetic reasons. In my work I showed that populations in small and ephemeral habitats are essential for metapopulation processes. Until recently, small populations were often neglected, and conservation efforts focussed on a few large populations. However, the classification "small is negligible and large is important" may not be a generality. Theoretical work showed that under some circumstances several small populations are more effective than a few large ones (single large or several small = SLOSS, Ovaskainen, 2002), mostly depending on the relationship between habitat patch area and rates of immigration, emigration and local extinction. It is predicted that under some circumstances populations in small and ephemeral patches have an over-proportional significance in a metapopulation, even though many of the well-documented metapopulations may depend on a few large populations (Hanski, 1999, Thomas & Hanski, 2004). However, this could be due to the chosen study organisms, which were often butterfly metapopulations (Ehrlich & Hanski, 2004, Hanski & Gaggiotti, 2004, Thomas & Hanski, 2004). Butterflies seem often to occur in metapopulations. But they mostly do not posses resting stages that can tolerate bad environmental conditions and they disperse in the adult stage. Furthermore, butterflies are relatively sensitive to changes in the environment and population size is positively correlated with survival probability (McLaughlin et al., 2002, Harrison et al., 1988) and migration may be density dependent. Therefore, a large population may guarantee long-term survival and also contribute most of the migrants.

Daphnia have a resting stage, which is also the migration stage. Populations in small ephemeral habitat patches thus produce ephippia to survive the environmental hazards, but at the same time this also increases the number of potential migrants. These migrants are exposed during unfavourable conditions. I think that the herein documented situation may be more general in systems that fulfil the same criteria (where the resting stage is also the migration stage, but see Crone et al., 2001 that found the same patter in a vole metapopulation). Vanschoenwinkel et al. (2007) have measured drought resistant migrants of planktonic organisms in a rock pool system. They found increased migration rates during times when pools were dry. Even though they could not allocate the migration stages to the ephemeral pools, it is consistent with the herein studied metapopulation. I conclude that populations in small and ephemeral patches may be more significant for metapopulations, and that the herein studied *Daphnia* metapopulations are not a very special case.

Still, open questions remain with respect to the origin of migrants. One of the most challenging aspects is the direct observation and tracking of migrants (Hanski et al., 2000). Here, I used the number of migration stages as a surrogate of migration. I think that it is a safe assumption to relate migration proportionally to migration stages, as migration is passive. However, the direct measurement of migrants with the help of ephippium traps (Maguire, 1963, Brendonck & Riddoch, 1999, Wolters et al., 2004, Henry & Molau, 1997, Vanschoenwinkel et al., 2007) will not only give quantitative but also qualitative data (e. g. genotype and parasitism level of migrants) and temporal data of migration on a fine scale. Tracking of migrants on a fine spatial and temporal scale might be combined with models including the spatial distribution of rock pool habitats (Vanschoenwinkel et al., 2007). Further research directions may include the influence of wind and wind-direction on dispersal (Fig. 1) as well as the study of spatial gradients of gene- and parasite flow within the metapopulation.

Unpublished observations suggest a much higher engagement and faster induction of ephippium production of *Daphnia* in this metapopulation compared to conspecific *Daphnia* from lake populations. This may be explained by selection for genotypes in rock pools to have a higher propensity of producing resting stages than lake *Daphnia*. I suggest that it could be fruitful to investigate genetic components of ephippium production. What are the genetic components for ephippium production and is there a “migrant-producing”-gene? Also trade-offs between asexual reproduction and the engagement in the sexual production of ephippia have been largely ignored. Such trade-offs may not only be influenced by inter- or intraspecific competition, but also by parasitism (Duncan et al., 2006). Finally, little is known on the size and density of planktonic *Daphnia* populations in the natural rock pools. As population density influences the production of ephippia (Carvalho & Hughes, 1983, Stross & Hill, 1965), consistent differences between populations in various sized habitats are of interest.

In my work I showed an influence of climate change on both colonisation dynamics and metacommunity composition. Climate change is ongoing and may even accelerate (IPCC, 2007). Up to now, there are several good examples that show an influence of climate change on phenology or shifts of single populations to a higher latitude or altitude (Parmesan et al., 1999, Parmesan et al., 2003, Walther et al., 2002), while almost no studies exist on the effect of climate change on metapopulations (Thomas & Hanski, 2004). Thus, the herein presented results add two important aspects on effects of climate change on biological systems. First, we showed for the first time that climate change has an influence on metapopulation dynamics. Dynamics such as migration are the central processes in metapopulations and an influence of climate change may also be important in other fragmented populations. Second, we showed that also the metacommunity composition was affected by climate change. Both our findings are novel, and may open up a new field of research.

In the chapters five and six of my thesis, I investigated correlations between the fitness of *D. magna* genotypes and their natural infection status (Coltman et al., 1999, Keller & Waller, 2002, Reid et al., 2003, Kraaijeveld & Godfray, 1997, Carton et al., 2005). In a previous study I showed that uninfected immigrants have an advantage (Altermatt et al., 2007). Here, I tested if there were further correlations between a genotype's fitness and its infection status. However, this was not the case. Probably, resistance genes segregate independently of other fitness associated genes in this system. I only measured relative fitness during the asexual phase and I cannot rule out that the costs are seen only for fitness components not assessed in my experiments. It would be appealing to expand the conclusions to the sexually produced migration stages. Zbinden et al. (in prep.) provide evidence that resistant *D. magna* genotypes have reduced growth rates under stress-free conditions, as they might occur during exponential growth. This could be tested as well.

Finally, I studied the epidemiology of *O. bayeri* in replicated host populations of both low and high genetic diversity. It is a well known phenomenon that pathogens spread less efficiently in host populations of high diversity compared to low diversity, called the monoculture effect van der Plank, 1963, Leonard, 1969). However, most evidence on that phenomenon comes from artificial systems in agriculture such as crop cultures or managed grasslands (Zhu et al., 2000, Mitchell et al., 2002). In these cases, a few pre-selected genotypes were used, and the conclusions were restricted to plants. I used randomly chosen *D. magna* genotypes and set-up populations of realistic genetic diversity. I found that the parasite spread less successful in *D. magna* populations of high genetic diversity compared to low genetic diversity. I thereby found good evidence for the generality of the monoculture effect in animal populations under realistic natural conditions.

The experiment, though, is not only ideal to study the epidemiology of the parasite (as also done by Lass & Ebert, 2006), but also ongoing host-parasite coevolution. Genetic diversity is a key factor for local adaptation (Gandon et al., 1996, Lively & Dybdahl, 2000, Gandon & Michalakis, 2002, Gandon, 2002, Kawecki & Ebert, 2004). In *Daphnia*, local adaptation of parasites to their sympatric host populations has been shown repeatedly (Ebert, 1994, Refardt & Ebert, 2007), including in the herein studied metapopulation (Altermatt et al., 2007). Until now, experimental coevolution has been mostly addressed in microorganisms such as bacteria-phage systems (Buckling & Rainey, 2002, Morgan et al., 2005, Brockhurst et al., 2007), but see the exception of a snail-schistosome study (Webster et al., 2004). Usually, the parasites are thought to be ahead in the arms race with their hosts and are therefore locally adapted (Ebert, 1994, Ebert & Hamilton, 1996). The argumentation includes that parasites have larger population sizes, shorter generation times and sometimes elevated mutation rates, all of which help them to evolve fast. Using my experimental populations, questions on the ongoing process of coevolution can be tested in the future.

The main scientific contributions of my thesis are the quantification and localisation of the migration stage production in a natural *D. magna* metapopulation, the introduction and quantification of the new variable "desiccation" to describe habitat instability in a *Daphnia* metacommunity and the confirmation of the monoculture effect in animal populations. I contributed in each of them new scientific aspects and data. This not only gives a better understanding of the herein studied *Daphnia* metacommunity but also for metapopulations in general.

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