Daphnia metacommunity dynamics
The roles of inbreeding, parasitism, competition, and dispersal

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PhD thesis
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Switzerland

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An expert is a man who has made all the mistakes
which can be made in a very narrow field.

Niels Henrik David Bohr, as quoted by Edward Teller (10 October 1972),
and Alan L. Mackay (1991) A dictionary of scientific quotations, p. 35.

Daphniain laulu

Koko maailmamme lätäkkö pienoinen on,
Mitä tuosta, on elomme suruton,
Ja tulkohon mikä kohtalo vain,
Me silmä pyöreenä potkimme ain'.

Kun aurinko paistavi yllämme kirkas,
Ja vesi on lämmin, on elomme vilkas,
Eestakaisin pyrstömme heiluu vain,
Ja me silmä pyöreenä potkimme ain'.

Jos syö joku meidät, mi hätänä ois',
Pian muutenkin nukumme elosta pois,
Elo kestävi muutaman päivän vain,
Silloin silmä pyöreenä potkimme ain'.

Pian saapuvi kuivuus tai hallayö,
Ja jo meille viimeinen hetki lyö,
Mut iloiten elämme, huoleti vain,
Ja silmä pyöreenä potkimme ain'.

Ja kun yllämme on ikihiljaisuus,
Tomustammepa nousevi polvi uus,
Es'isien haudoilla riemuiten vain
Se silmä pyöreenä potkivi ain'.

Y. K. Suominen? Guest book entry 6 August 1921, Tvärminne Zoological Station.
Edited by Laila Keynäs 15 November 1994.

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Contents

Summary

The habitats of many species are not continuous but fragmented, either naturally or increasingly due to human land use. Communities of species inhabiting fragmented habitat patches are connected by dispersal and form a metacommunity, a spatial overlay of the metapopulations of the individual species. The factors enabling coexistence of different species in communities may shed light on the core questions of ecology, which is on the search for explanations for the abundance of species and their interactions with the biotic and abiotic environment. With this thesis, I aimed at contributing to a better understanding of the interplay of potential determinants of species coexistence at the local and regional scales in metacommunities.

I studied a metacommunity of the three generalist filter-feeding planktonic water flea species *Daphnia longispina*, *D. magna*, and *D. pulex*, which form local communities in ephemeral freshwater rock pools although they have largely overlapping niches. To corroborate my observational and experimental findings, I combined them with a long-term demographic data set by V.I. Pajunen on the presence of the three species in over 500 rock pools since 1982.

The largest and most abundant species *D. magna* is known to harbour a high diversity of ecto- and endoparasites, and the diversity of parasites in local *D. magna* populations was shown to correlate primarily with population age. I screened rock pool populations of the congeneric species *D. longispina* over a period of three years and inspected which epidemiological, spatial, temporal, and physicochemical factors explained most of the variance in the number of parasites. Contrary to the situation in *D. magna*, parasite richness in *D. longispina* was generally substantially lower, and surprisingly, the best predictor was the presence of *D. magna*, indicating that the co-occurring congeneric species might serve as a reservoir of parasites from which *D. longispina* is constantly inflicted. At the same time, the presence of parasites could be the decisive factor mediating coexistence between the two *Daphnia* species.

Daphnia magna does not only carry the largest parasite burden but is as well the most dynamic species in terms of extinction and colonisation events in the metacommunity. To understand the factors of invasion success of *D. magna*, I conducted a fully factorial outdoor mesocosm experiment where *D. magna* clones that were outbred, unparasitised or did not face competition by resident *D. longispina* had a higher invasion success. The three factors inbreeding, parasitism, and interspecific competition are suggested to affect colonisation dynamics in the metacommunity and to determine whether the sequential arrival of *Daphnia* species in rock pool habitats can result in local coexistence.

Not only invasion success proved to be diminished by competition, the competitive hierarchy of species is generally considered an important determinant for coexistence. Therefore I studied the interspecific competitive abilities of the three *Daphnia* rock pool species in two further outdoor mesocosm experiments. The interspecific competitive abilities of forty *D. magna* clones competing against *D. longispina* and *D. pulex* correlated positively and were higher when the clones originated from genetically more diverse populations, which is suggested to be a consequence of reduced inbreeding depression. The competitive abilities of the three *Daphnia* species competing in pairs against one another followed almost invariably a transitive competition hierarchy, i.e. the largest species *D. magna* was competitively superior to the intermediately sized *D. pulex* which in turn was competitively superior to the smallest species *D. longispina*. Such transitive competitive hierarchies are supportive of the size efficiency hypothesis which states that larger species dominate smaller species in the absence of predation because of their higher feeding efficiency. Conversely, I propose that the rare but naturally possibly more prevalent intransitive competitive relationships are a key factor for the maintenance of clonal and species diversity in *Daphnia* rock pool communities.

Since rock pools are highly stochastic habitats, a requirement of local coexistence is the sufficient provisioning of immigrants by means of dispersal. *Daphnia* depend on passive dispersal by resting stages, so-called ephippia. To obtain a direct estimate of the absolute dispersal effort, I trapped ephippia over three years in areas inhabited predominantly by *D. longispina* and *D. magna*. The majority of caught ephippia stemmed from *D. magna*, which supports earlier conclusions that *D. magna* ephippia are more often exposed to the supposedly dominant dispersal agent wind since the species prefers smaller, more desiccation-prone rock pools. In contrast, I speculate that *D. longispina* may be dispersed predominantly by means of insects which may be charged by this species' buoyant ephippia.

Eventually, for a contribution to another study, I conducted an outdoor mesocosm experiment to investigate whether inbreeding and interspecific competition augment the prevalence of a microsporidian parasite of *D. magna*. Experimental *D. magna* populations whose ancestors were faced with competition by *D. longispina* suffered from considerably elevated levels of infection, possibly because parasite multiplication and transmission had been facilitated by stressed hosts. This is a further indication that parasites may mediate coexistence between rock pool *Daphnia*.

In a nutshell, my thesis provides observational and experimental evidence for key factors shaping *Daphnia* rock pool communities. *Daphnia magna* might be the competitively superior species, yet inbreeding and parasitism in combination with intransitive competitive relationships have the potential to mediate species coexistence in rock pool communities which were shown to be connected by passive dispersal of ephippia.

Zusammenfassung

Die Lebensräume vieler Arten sind nicht kontinuierlich sondern entweder natürlicherweise oder aufgrund menschlicher Landnutzung fragmentiert. Artengemeinschaften, welche fragmentierte Lebensräume bewohnen, sind durch Ausbreitung miteinander verbunden und bilden zusammen eine Meta-Artengemeinschaft, eine räumliche Überlagerung der Metapopulationen der einzelnen Arten. Die Faktoren, welche das Zusammenleben verschiedener Arten in Gemeinschaften ermöglichen, könnten helfen, die Kernfragen der Ökologie zu beantworten, welche sich mit der Häufigkeit von Arten und deren Interaktionen mit der belebten und unbelebten Umwelt beschäftigen. In diesem Sinne war es das Ziel dieser Dissertation, zu einem besseren Verständnis des Zusammenspiels von möglichen Einflussgrössen der Koexistenz von Arten auf lokaler und regionaler Ebene in Meta-Artengemeinschaften beizutragen.

Ich studierte eine Meta-Artengemeinschaft der drei sich filtrierend ernährenden planktonischen Flohkrebse *Daphnia longispina*, *D. magna* und *D. pulex*, welche lokale Artengemeinschaften in kurzlebigen Süsswasserteichen bilden, obwohl sie stark überlappende Nischen besitzen. Um meine Ergebnisse aus Beobachtungen und Experimenten zu erhärten, kombinierte ich sie mit einem Langzeit-Datensatz von V.I. Pajunen, welcher die Präsenz der drei Arten seit dem Jahr 1982 in über 500 Teichen überprüft.

Die grösste und am weitesten verbreitete Art *D. magna* besitzt eine grosse Vielfalt an Ekto- und Endoparasiten, und es wurde gezeigt, dass die Vielfalt der Parasiten in lokalen Population von *D. magna* am besten mit dem Populationsalter korreliert. Ich besammelte Populationen der verwandten Art *D. longispina* während dreier Jahre und untersuchte, welche epidemiologischen, räumlichen, zeitlichen und physikalisch-chemischen Faktoren am meisten Varianz in der Anzahl der gefundenen Parasiten erklärten. Im Gegensatz zu *D. magna* war die Parasitendiversität von *D. longispina* im allgemeinen deutlich niedriger und der beste Prädiktor war die Anwesenheit von *D. magna*, was darauf hindeutet, dass *D. magna* als Parasiten-Reservoir dienen könnte, aus welchem *D. longispina* fortwährend infiziert wird. Zugleich könnte jedoch gerade die Anwesenheit von Parasiten der ausschlaggebende Faktor für die Vermittlung von Koexistenz zwischen den *Daphnia*-Arten sein.

Daphnia magna trägt nicht nur die grösste Parasitenlast, sondern ist auch die dynamischste Art bezüglich Aussterbe- und Kolonisierungs-Ereignissen in der Meta-Artengemeinschaft. Um die Einflussgrössen des Invasionserfolgs von *D. magna* zu verstehen, führte ich ein komplett faktorielles Mesokosmos-Experiment im Freiland durch, bei welchem Klone von *D. magna*, welche ausgezüchtet oder nicht parasitiert waren oder keine zwischenartliche Konkurrenz erleiden mussten, einen höheren Invasionserfolg aufwiesen. Die drei Faktoren Inzucht, Parasitismus und interspezifische Konkurrenz könnten die Kolonisierungs-Dynamik in der Meta-Artengemeinschaft beeinflussen und bestimmen, ob die Abfolge des Eintreffens von *Daphnia*-Arten in Teichen zu lokaler Koexistenz führt.

Nicht nur der Invasionserfolg wird durch zwischenartliche Konkurrenz geschmälert, die Hierarchie der Konkurrenzfähigkeiten von Arten wird auch im allgemeinen als wichtiger Faktor für die Koexistenz betrachtet. Deshalb untersuchte ich die zwischenartlichen Konkurrenzfähigkeiten der drei *Daphnia*-Arten in zwei weiteren Mesokosmos-Experimenten. Die Konkurrenzfähigkeiten von vierzig Klonen von *D. magna*, welche gegen Klone von *D. longispina* und *D. pulex* antraten, korrelierten positiv und waren grösser, wenn die Klone aus genetisch diversen Populationen stammten, was eine Folge von verringerter Inzuchtdepression sein könnte. Die Konkurrenzfähigkeiten der drei Arten in paarweisen Begegnungen folgten fast durchgehend einer transitiven Konkurrenz-Hierarchie, d.h. die grösste Art *D. magna* war kompetitiv überlegen, währenddessen die mittelgrosse Art *D. pulex* wiederum kompetitiv der kleinsten Art *D. longispina* übergeordnet war. Solche transitiven Konkurrenz-Hierarchien bekräftigen die sog. Grösseneffizienz-Hypothese (*size efficiency hypothesis*), welche besagt, dass grössere Arten kleinere Arten in der Abwesenheit von Prädation aufgrund ihrer höheren Effizienz bei der Nahrungsaufnahme dominieren. Im Gegensatz dazu schlage ich vor, dass die seltenen, aber natürlicherweise vielleicht häufigeren nicht transitiven Konkurrenzverhältnisse ein Schlüsselfaktor für die Aufrechterhaltung von Klon- und Arten-Diversität in Teich-Gemeinschaften von *Daphnia* sein könnten.

Da die untersuchten Teiche höchst stochastische Lebensräume sind, ist eine Grundvoraussetzung für die lokale Koexistenz die ausreichende Versorgung mit Migranten mittels Verbreitung. Die Gattung *Daphnia* ist abhängig von passiver Verbreitung von Dauerstadien, sogenannten Ephippien. Um eine direkte Schätzung des absoluten Verbreitungsaufwands zu erhalten, fing ich während dreier Jahre Ephippien in Gebieten, welche vornehmlich von *D. longispina* und *D. magna* bewohnt werden. Der Grossteil der gesammelten Ephippien stammte von *D. magna*, was frühere Schlussfolgerungen untermauert, dass Ephippien von *D. magna* häufiger dem vermutlich wichtigsten Vektor Wind ausgesetzt sind, da die Art kleinere, häufiger von Austrocknung betroffene Teiche bevorzugt. Zudem spekuliere ich, dass *D. longispina* vornehmlich durch Insekten verbreitet wird, welche durch die schwimmfähigen Ephippien dieser Art beladen werden könnten.

Schliesslich führte ich für einen Beitrag an eine andere Studie ein Mesokosmos-Experiment durch, bei welchem der Einfluss von Inzucht und zwischenartlicher Konkurrenz auf die Prävalenz eines Endoparasiten von *D. magna* getestet wurde. Experimentelle Populationen von *D. magna*, deren Vorfahren Konkurrenz durch *D. longispina* ausgesetzt waren, litten unter deutlich höheren Infektionsraten, welche womöglich durch Stress im Wirt und folglich höherem Wachstum und höherer Transmission des Parasiten zustande kamen. Dies ist ein weiterer Hinweis darauf, dass Parasiten Koexistenz zwischen *Daphnia*-Arten vermitteln könnten.

Zusammenfassend kann gesagt werden, dass meine Dissertation Belege beobachtender und experimenteller Natur für die Schlüsselfaktoren der Koexistenz in *Daphnia*-Artengemeinschaften liefert. *Daphnia magna* mag zwar die kompetitiv überlegene Art sein, jedoch haben Inzucht und Parasitismus in Kombination mit nicht transitiven Konkurrenzverhältnissen das Potential, Koexistenz in Teich-Artengemeinschaften zu vermitteln, für welche ausserdem gezeigt werden konnte, dass sie durch passive Verbreitung von Ephippien miteinander verbunden sind.

Introduction

Coexistence in metacommunities

A lot of species live in a patchy environment because their habitat is fragmented naturally or increasingly due to human land use (reviewed by Andren 1994). Suitable habitat patches are colonised by migrants that found local populations which eventually go extinct, but at a regional spatial scale, a species is able to persist in the longer term as a metapopulation, in an interplay of local extinction and colonisation events balancing each other (Levins 1970, Hanski 1994, Hanski & Gaggiotti 2004). Different species commonly share the same habitat patches and live in communities, and if the species' respective metapopulations overlap, they collectively form a metacommunity (Wilson 1992, Leibold et al. 2004, Holyoak et al. 2005). Coexistence of species in metacommunities can be defined at a local scale but again as well at a regional scale owing to the interconnectedness of habitat patches by dispersal (Clobert et al. 2004, Holyoak et al. 2005). Thus even though from a metacommunity perspective, coexistence is already given when species' local populations are spatially separated but occupy habitat patches which are potentially inhabitable by the involved species, the factors mediating coexistence in local communities feed back to regional dynamics and vice versa (Leibold et al. 2004, Holyoak et al. 2005). An understanding of the determinants of local species coexistence is also increasingly warranted in extending the insights gained from metapopulation ecology for the conservation of fragmented populations (Hanski & Simberloff 1997) since the inclusion of more than one species in considerations on reserve and corridor design may result in aberrant recommendations (Koelle & Vandermeer 2005).

When considering multiple species sharing a common habitat, interspecific competition suggests itself as a structuring force. Interspecific competition has been perpetually demonstrated to be at work in nature (Begon et al. 2005), even though alternative explanations, such as the neutral theory which postulates community assembly without any influence of interspecific competition, are warranted (Lawlor 1980). An old but still attractive because simple principle of how communities are being structured is the competitive exclusion principle (Gause 1932). The principle neglects the influences of environmental heterogeneity, but since environmental heterogeneity is the norm rather than the exception, the rules for species coexistence derived from the principle have been criticised as all-too simplistic (Levins 1979). In fact, environmental heterogeneity per se has been postulated as a decisive factor enabling coexistence (Levins 1979, Chesson & Warner 1981). For species to be able to coexist, niche differentiation is a requirement of the competitive exclusion principle and is illustrated by intriguing evidence from field studies (e.g. Brown & Davidson 1977, Davidson 1977). However, niche differentiation does not necessitate interspecific competition since environmental conditions might for instance just be intolerable for some of the putatively competing species (Connell 1961). On the other hand, interspecific competition does not coercively need to end up in niche differentiation to enable coexistence since the later can be ensured by trade-offs between competitive and colonisation abilities of species in spatially structured habitats (Tilman 1994). Moreover, niche differentiation might in many cases be the result of interspecific competition, yet often not of current but of competition in evolutionary history, which is commonly known as the ghost of competition past (Connell 1980). While this may in many circumstances be a sensible explanation for coexistence due to niche differentiation, past interspecific competition is difficult to prove, and thus present vs. past and ecological vs. evolutionary effects, respectively, are difficult to disentangle (Abramsky & Sellah 1982, Schluter & McPhail 1993).

While interspecific competition or more generally interspecific interactions may be an immediate force structuring communities, for instance by excluding co-residents (Bengtsson 1989) or by preventing immigration of invaders (Tilman 1997, Stachowicz et al. 1999, Shurin 2000, Kennedy et al. 2002), there are many other factors affecting coexistence of species. Inbreeding of founders of a local population may increase the risk of population extinction due to effects of inbreeding depression (Newman & Pilson 1997, Nieminen et al. 2001). On the other hand, inbreeding in a local population may facilitate immigration by giving immigrants a selective advantage by means of hybrid vigour of outbred offspring (Ebert et al. 2002). Parasites may mediate coexistence of multiple potential host species by apparent competition (Bonsall & Hassell 1997, Hudson & Greenman 1998, Tompkins et al. 2000, 2003). But parasites may also prevent successful immigration of their hosts into communities (Keane & Crawley 2002, Mitchell & Power 2003, Torchin et al. 2003, Callaway & Ridenour 2004, Colautti et al. 2004). Dispersal connects local communities and is crucial for the persistence of metacommunities (Leibold et al. 2004, Holyoak et al. 2005). Conversely, dispersal may also drive the assortment of species assemblages directly (Leibold et al. 2004, Vanschoenwinkel et al. 2008). These exemplary factors may thus all enable or prevent the cohesion of communities within metacommunities both locally and regionally (Holyoak et al.

2005). The aim of this thesis was to specifically evaluate the potential of the four factors inbreeding, parasitism, interspecific competition, and dispersal as mediators of coexistence in a metacommunity of three species of *Daphnia* inhabiting a network of ephemeral rock pools.

The study system

According to Holyoak et al. (2005), a metacommunity is easiest to conceptualise when it possesses the following properties: all interacting species utilise the same habitat patches, these habitat patches have discrete boundaries, and the local populations use resources at the same within-patch scale. Metacommunities whose properties fulfil the mentioned claims well can be found along the skerry island coast of the Baltic Sea in the form of overlapping rock pool metapopulations of *Daphnia* species (Crustacea: Cladocera) (Hanski & Ranta 1983, Bengtsson 1986, Pajunen 1986). On the skerry islands of the Baltic Sea, *Daphnia* inhabit freshwater filled rock depressions, so-called rock pools (Fig. 1a), thus the first claim is fully fulfilled. The rock pools have discrete boundaries which are set by the water level and by the topography (Altermatt et al. 2009), therefore the second claim is fully fulfilled (Fig. 1b). *Daphnia* are generalist filter-feeders and show niche separation but their niches largely overlap (Ranta 1979), and furthermore, each *Daphnia* individual is able to reach each location in the rock pool water body, consequently the third claim is at least partially fulfilled.

A particularly well studied *Daphnia* rock pool metacommunity is situated south of Tvärminne Zoological Station in southwest Finland (Hanski & Ranta 1983, Pajunen 1986, Pajunen & Pajunen 2003) which at the same time delineates my study area (Fig. 2). Pajunen & Pajunen (Pajunen 1986, Pajunen & Pajunen 2003) have been compiling an inventory of biannually collected presence/absence data of the three autochtonous species *D. longispina* O. F. Müller, *D. magna* Straus, and *D. pulex* Leydig beginning in the year 1982 for over 500 rock pools on 13 different skerry islands. The three *Daphnia* species have a cyclical parthenogenetic life cycle (Zaffagnini 1987) (Fig. 3), with the exception of some *D. pulex* populations that are obligately parthenogenetic (Haag & Ebert 2004a). Cyclical parthenogenesis involves a cycle in which females reproduce asexually and give rise to genetically identical daughters (Fig. 1c). Under certain conditions, but especially when environmental conditions turn unfavourable (Banta & Brown 1929, Stross & Hill 1965, Carvalho & Hughes 1983, Kleiven et al. 1992), e.g. in the case of rock pool desiccation or freezing, clonal males are produced which then fertilise the females' sexual eggs (Zaffagnini 1987). Usually two of these sexually produced eggs are packed into a chitinous shell and shed the next time the female moults as a so-called ephippium (Fig. 1d). Ephippia serve as the resting

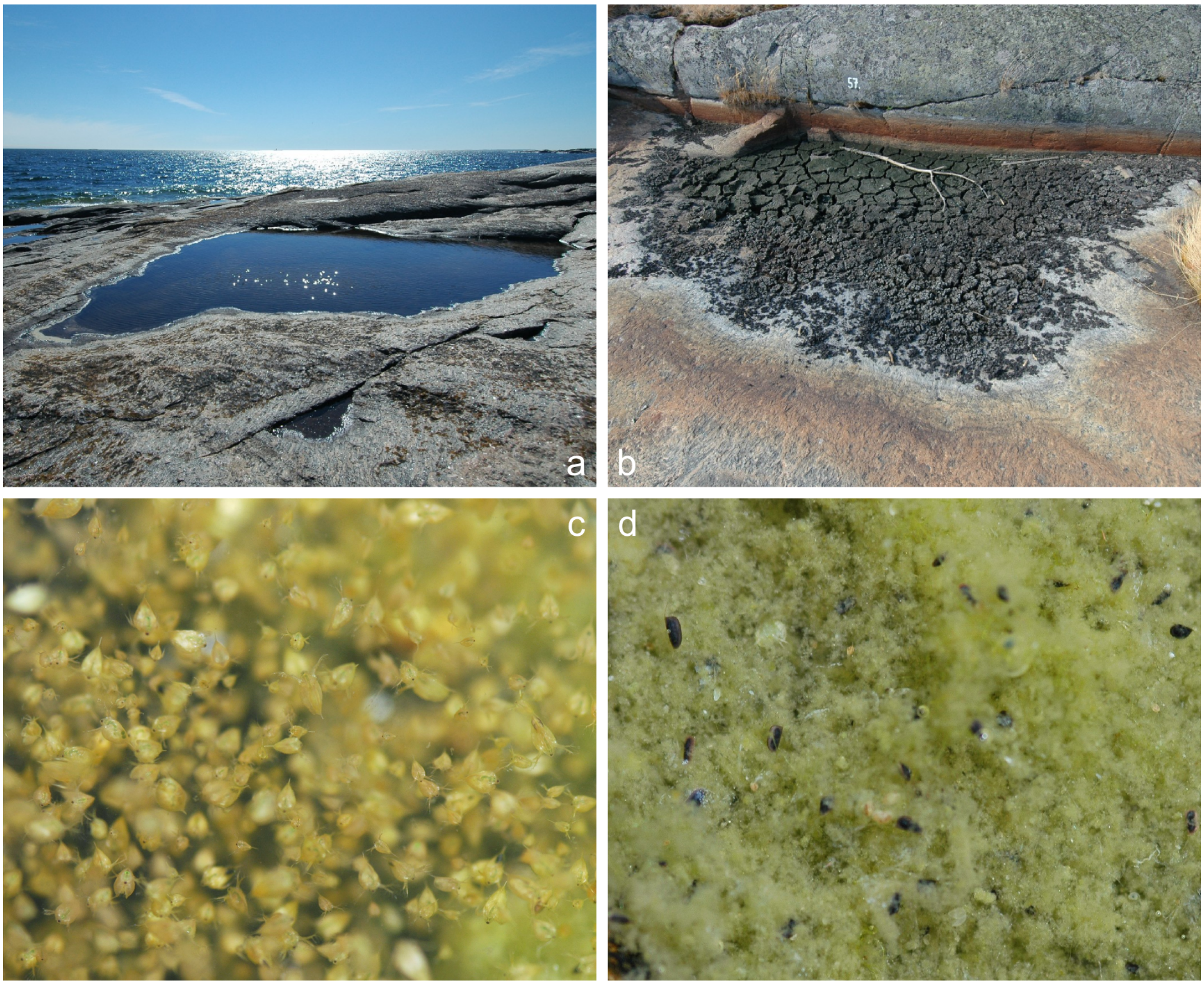

Figure 1 Rock pool habitats of *Daphnia*. (a) Rock pool at the shore of a skerry island in southwest Finland. (b) Desiccated rock pool with dried up sediment and possibly exposed ephippia. (c) Dense swarm of *D. magna*. The individuals with a whitish glow probably suffer from WBD (white bacterial disease). (d) Shed *D. magna* ephippia on the ground of a rock pool.

stages and are able to persist through harsh conditions such as summer drought or winter freezing, but they also serve as dispersal stages which are passively transported between potentially inhabitable rock pools (Pajunen 1986, Pajunen & Pajunen 2003, Haag et al. 2005, 2006). Because of the central roles that ephippia hold, the long term demographic data set of Pajunen & Pajunen (Pajunen 1986, Pajunen & Pajunen 2003) allows to estimate population persistence times and extinction and colonisation events for each of the three *Daphnia* species over currently 27 seasons. I therefore made regular use of the data set to have a relevant empirical base for comparison with observational and experimental findings.

I aimed at shifting the focus from *D. magna*, which has been the main study subject of the metacommunity (for references of main contributors see below), to its direct competitor *D. longispina* and investigating interactions that might explain coexistence of the two species in time and space at a local and regional scale. Thus the two species *D. longispina* and *D. magna* formed the core of my study system (Fig. 4). *Daphnia pulex* is less abundant in the metacommunity but was considered in some of the studies. Rock pool *Daphnia* are often heavily infested with epibionts and endoparasites (Green 1974, Bengtsson & Ebert 1998, Ebert et al. 2001) and thus one has to consider parasites to be integral parts of the community. The endoparasite *Hamiltosporidium tvaerminnensis* Haag (Microsporidia) (formerly misidentified as *Octosporea bayeri* Jirovec, Haag et al. 2011) is specific to *D. magna* (Vizoso & Ebert 2004, Vizoso et al. 2005) and occurs in nearly half of all *D. magna* rock pool populations in the study area (Ebert et al. 2001). In interactions where *D. magna* is involved, it is thus desirable to include its main parasite, which is why *H. tvaerminnensis* was also occasionally considered part of the study system (Fig. 4).

Outline

I conducted observational and experimental studies in which I examined correlations and causal relationships of explanatory variables related to inbreeding, parasitism, competition, and dispersal with response variables related to the performance of *Daphnia* species with the goal to provide explanations for the coexistence of *Daphnia* species in the aforementioned metacommunity.

Chapter 1 presents an observational study on parasite richness in *D. longispina* in the *Daphnia* metacommunity. Ebert et al. (2001) postulated that parasite richness in *D. magna* is primarily explained by the duration rock pools have been colonised by the host, indicating that parasite diversity increases over time. Applying a similar screening protocol for *D. longispina* but extending the sampling procedure over three years,

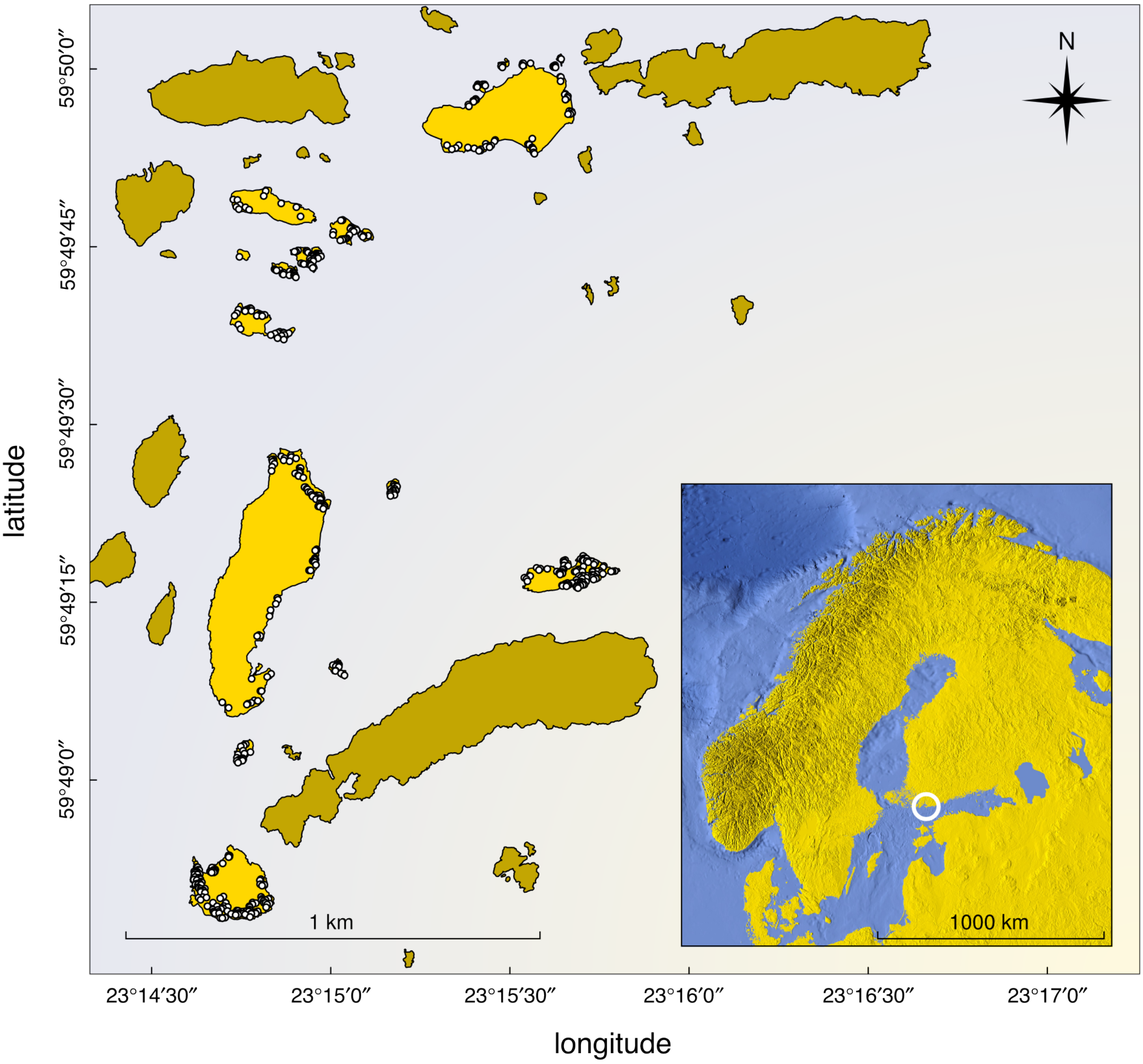

Figure 2 Location of the study site south of Tvärminne Zoological Station on the peninsula of Hanko in southwest Finland (indicated with a circle on the inlet map of Fennoscandia). The islands harbouring the study rock pools are shaded in a brighter hue, the study rock pools are indicated with circles. The inlet map is an equidistant conic projection of the Shuttle Radar Topography Mission V2 elevation data (NASA) and the Micro World Data Bank II vector data (Central Intelligence Agency). The vector vertex coordinates of the islands were projected to KKJ (Kartastokoordinaattijärjestelmä, Finland Uniform Coordinate System).

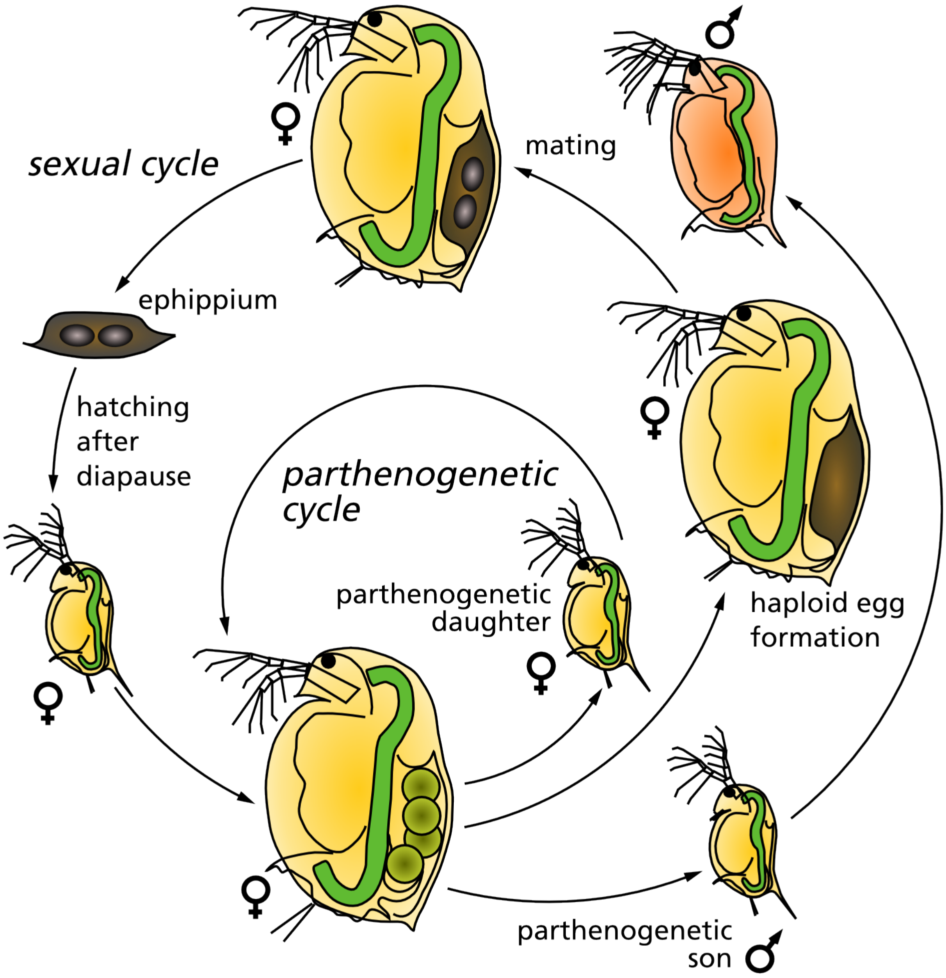

Figure 3 Cyclical parthenogenesis of *Daphnia* species. In the parthenogenetic subcycle, females give birth to clonal female offspring. In the sexual subcycle, clonal males are produced which fertilise sexual eggs. These are then encapsulated and shed as resting stages, so-called ephippia. After breakage of diapause, female offspring hatch from the eggs and initiate either of the two subcycles. (The drawing is courtesy of Dita B. Vizoso.)

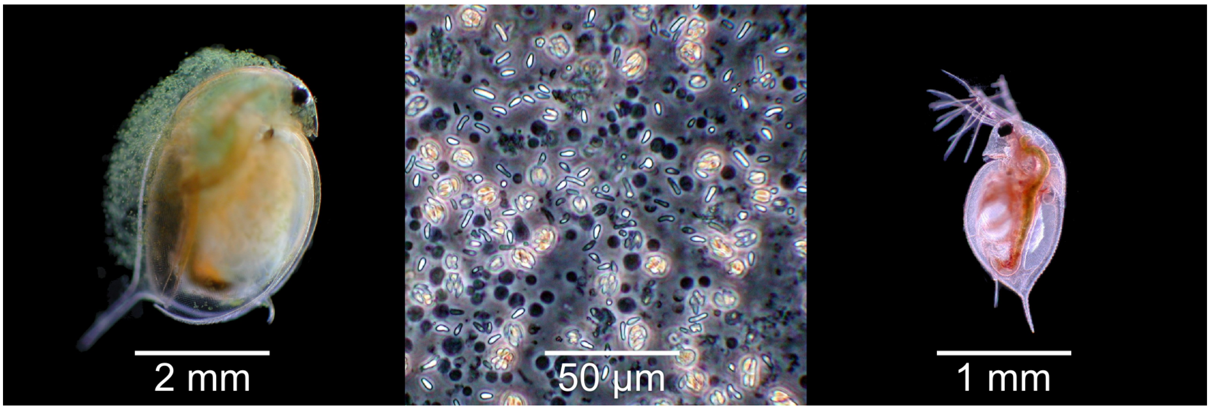

Figure 4 The main study species of most chapters were the two crustaceans *D. magna* (left, back covered with epibiontic algae) and *D. longispina* (right). The microsporidian endoparasite *H. tvaerminnensis* (middle, spores) which is specific to *D. magna* was also considered as an integral part of the study system in selected chapters.

I compiled correlative evidence for the association of parasite richness and a range of epidemiological, spatial, temporal, and physicochemical variables. The results of the screening were contrasted with the original findings of Ebert et al. (2001) to judge whether *D. longispina* harboured generally fewer parasite species than the congeneric *D. magna* as previously suggested by Stirnadel & Ebert (1997) in another pond system. Furthermore, the generality of parasite diversity patterns in the *Daphnia* metacommunity were assessed according to whether the predictors explaining most of the variance in parasite richness and the presence/absence patterns of the most abundant epibiont and parasite species in *D. longispina* were the same as in *D. magna*.

Chapter 2 comprises a full factorial outdoor mesocosm experiment which tested the influence of the three factors inbreeding, infection by the microsporidian parasite *H. tvaerminnensis*, and interspecific competition by the niche sharing congeneric competitor *D. longispina* on the invasion success of the focal species *D. magna*. Field, mesocosm, and lab experiments with *D. magna* from rock pool populations found that inbreeding (Ebert et al. 2002, Haag et al. 2002, Salathé & Ebert 2003), parasitism (Haag et al. 2003, Haag & Ebert 2004b, Altermatt et al. 2007, Ebert et al. 2007, Altermatt & Ebert 2008), and interspecific competition (Hanski & Ranta 1983, Bengtsson 1989, 1993) overall reduce fitness. I inferred that these factors should equally reduce invasibility of mesocosms by *D. magna* independently and possibly synergistically. Provided that the hypothesis holds, and given that the mesocosms closely mimic natural rock pools, the implications for the metacommunity would be straightforward: outbred or uninfected *D. magna* individuals or individuals invading unoccupied rock pools should have a higher establishment success than inbred or infected *D. magna* individuals or individuals invading rock pools inhabited by *D. longispina*. If these selective pressures were strong enough, invasion dynamics in the *Daphnia* metacommunity would be expected to change in the long term.

Chapter 3 approaches the fundamental question of how interspecific competitive abilities of the three *Daphnia* species co-occurring in the metacommunity are related to each other. The size efficiency hypothesis predicts that in the absence of predation, larger species should be better competitors due to higher feeding efficiency (Brooks & Dodson 1965). Consequently, the largest species *D. magna* should be competitively superior, the intermediately sized species *D. pulex* competitively intermediate, and the smallest species *D. longispina* should be competitively inferior. Assessments of competition between the three species are overall supportive of the size efficiency hypothesis, nevertheless exceptions abound (Bengtsson 1987). First, the competitive abilities of forty *D. magna* clones were tested in competition with reference clones of the two congeneric species *D. longispina* and *D. pulex*. Second, clones of all three species competed in all pairwise combinations. The resulting competition hierarchy was expected to be transitive with the competitive abilities following the body size order of the three *Daphnia* species. The degree of deviation from intransitive competitive relationships would be an indicator of how likely coexistence of *Daphnia* clones and species in rock pool communities actually is.

Chapter 4 describes a novel approach of quantifying *Daphnia* resting stages directly in the field, which is generally regarded as more difficult (Hanski et al. 2000, Bilton et al. 2001) than using indirect methods (Neigel 1997, Bossart & Prowell 1998). Astroturf mats, commonly used to measure plant seed rain (Henry & Molau 1997, Molau & Larsson 2000), were fixed in a regular grid to skerry islands harbouring a high density of rock pools to catch *Daphnia* ephippia and other crustaceans' resting stages. The sediments of the mats were collected and incubated in rock pool water periodically over three years to check for the presence of viable resting stages. Extrapolating from the amount of found resting stages, I expected to obtain estimates of the size of the ephippia banks of the *Daphnia* metacommunity inside and outside of rock pools and to find species specific differences in investments into dispersal.

Chapter 5 includes a contribution to another study. In a follow-up to the full factorial experiment of chapter 2, I tested whether *D. magna* hatchlings whose ancestors were infected with the microsporidian parasite *H. tvaerminnensis* and suffered from interspecific competition in the previous season had higher prevalences of *H. tvaerminnensis* than hatchlings whose ancestors were not faced with interspecific competition. Similarly as for inbreeding (Ebert et al. 2007), I speculated that mothers having been stressed by interspecific competition would have less means to control parasite multiplication and survival, eventually resulting in higher prevalences of *H. tvaerminnensis*. Competition between *D. longispina* and *D. magna* would thus not only be mediated by the direct effect of the parasite as discussed in chapter 2 but also indirectly by *D. longispina* which is expected to give the parasite of its congeneric competitor a transmission advantage across seasons. These mechanisms could stabilise coexistence of *Daphnia* species and lead to an overall higher proportion of metacommunity rock pools inhabited by local communities rather than rock pools containing single species populations.

The metacommunity concept is rather young, and thus relatively few empirical studies have been conducted to specifically test its ideas (Holyoak et al. 2005). The chapters making up this thesis strive to provide a couple of empirically collected jigsaw pieces to complement the bigger picture and help theoreticians on their way to develop a comprehensive framework of metacommunity dynamics.

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Chapter 1

Parasite species richness in a *Daphnia* metacommunity: cling together, swing together?

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Abstract

Endoparasite and epibiont species diversity in an extensively studied *Daphnia magna* rock pool metapopulation in southwest Finland had been shown to predominantly correlate with increasing host population age. Although *D. magna* and the co-occurring species *D. longispina* have largely overlapping ecological niches, it was unclear whether they also share the same set of parasites. Here, we present results of a three year survey on endoparasite and epibiont species richness dynamics of *D. longispina* that expand and update previous insights obtained for the congeneric species *D. magna* in the same metacommunity. Surprisingly, the crucial factor for the presence of endoparasite and epibiont species in *D. longispina* did not seem to be host population age but the presence of the alternative host species *D. magna*. *D. longispina* harboured only a single abundant endoparasite species (*Larssonia obtusa*) and on average had a lower total parasite species richness than *D. magna*. Other factors, such as local density of patches and patch quality, only played a marginal role in explaining the presence/absence patterns of parasite species but not of parasite species richness as a whole. We suggest that the presence of *D. magna* itself leads to a higher parasite species richness in *D. longispina*, supposedly by serving as a refugium for parasites during temporal pelagic absence of *D. longispina*. We conclude that, with regard to endoparasites and epibionts, *D. longispina* and *D. magna* possess strongly divergent ecological niches.

key words endoparasite, epibiont, rock pool, spatial dynamics, temporal dynamics, *Daphnia longispina*, *Daphnia magna*, *Larssonia obtusa*

Introduction

Approximately half of the currently described metazoan species have a parasitic life style (Poulin & Morand 2000). Taking parasitic protozoa, fungi, bacteria, and viruses into account, parasite species outnumber host species by far (Dobson et al. 2008). Some host species harbour significantly more parasite species than others (Poulin & Morand 2004), but many host populations harbour only a subset of the potential parasite species assemblage (Price 1980, Anderson & May 1991, Hanski & Simberloff 1997, Poulin 1998). The relationship of parasite species diversity and a wealth of possible factors has been extensively studied, ranging from physiological, immunological, infectiological to temporal and spatial demographical, epidemiological, biogeographical, phylogenetic and also methodological (compare e.g. Arneberg et al. 1998, Lindstrom et al. 2004, Kuris & Lafferty 1994, Guégan & Kennedy 1993, Dritschilo et al. 1975, Morand & Poulin 1998, Simkova et al. 2001, Nunn et al. 2003, Gregory 1990).

Ebert et al. (2001) put forward correlational evidence for the constitution of the parasite community in and on the freshwater crustacean *Daphnia magna* Straus in a rock pool metapopulation off the skerry island coast of southwest Finland. Host population age explained more than half of the variance in the total number of endoparasite and epibiont species, supporting the colonisation time hypothesis tracing back to Southwood (1961).

The *D. magna* metapopulation is superimposed by metapopulations of *D. longispina* O. F. Müller and *D. pulex* Leydig, which together form a metacommunity on the skerry islands of the Baltic Sea (Pajunen 1986, Pajunen & Pajunen 2003). The closely related daphnid species have largely overlapping niches (Ranta 1979). Still, under similar environmental conditions in a British population, *D. longispina* was reported to be much less parasitised than *D. magna* (Stirnadel & Ebert 1997). In order to shed light on this inconsistency, we aimed at (1) unravelling the factors explaining parasite species richness in *D. longispina* and (2) comparing these with the factors explaining parasite species richness in *D. magna*.

Table 1.1 lists the ecological factors studied in Ebert et al. (2001, see also Table 1 therein). In addition, the factors presence of plants, catchment area, desiccation risk, and island area were included. All factors are grouped into four conceptually partly

Table 1.1 Categories of ecological factors that were predicted to influence parasite species richness in *D. longispina* populations in Baltic sea coast rock pools in southwest Finland. A positive predicted association indicates that an increase in the explanatory variable is expected to correlate with an increase in the parasite species richness measures.

	Category	Factor	Variable studied	Predicted association
1	epidemiology	population size	rock pool volume	+
		no. host species	*D. magna* presence	+
2	space	local density of patches	no. *Daphnia* communities within 10 m radius	+
			no. *D. longispina* populations within 10 m radius	+
			no. *D. longispina* populations on same island	+
		distance to neighbour	spatial autocorrelation	+
3	time	age of patch	time since *Daphnia* colonised pool	+
			time since *D. longispina* colonised pool	+
4	patch quality	impact of sea and vegetation	relative distance to sea	+
			water conductivity	–
			water colour	+
			presence of plants	+
		impact of topology	catchment area	+
			desiccation risk	–
			island area	+

overlapping categories, and for each factor there is at least one corresponding variable that is either a measurement of the factor itself or a surrogate variable in case the factor could not be measured directly. Detailed reasoning and extensive references on the type and direction of associations between parasite species richness measures and ecological factors are given in Ebert et al. (2001).

The first category concerns factors related to host population size (Table 1.1, category 1). It has repeatedly been demonstrated that larger host populations harbour more parasite species (Anderson & May 1979, 1978, 1991, Price & Clancy 1983, Gregory 1990, Dobson & Pacala 1992). The underlying reasons might be a higher encounter rate with parasites, a lower risk of passing through population bottlenecks followed by parasite extinction, and a higher likelihood of being above the parasite specific population threshold size. Closely related is the argument that a higher number of potential host species leads to a larger total host population size which in turn decreases the likelihood of parasite extinction due to population crashes of one of the host species.

The second category includes factors connected to spatial dynamics of extinction and colonisation (Table 1.1, category 2). Patches with hosts can be regarded as islands, and spatial isolation of such patches comes along with a smaller likelihood of colonisation both by hosts and parasites (Kuris et al. 1980). It was therefore predicted that spatial isolation should reduce parasite richness (Kennedy & Bush 1994). Similarly, a higher local density of patches is thought to decrease the risk that host populations go locally extinct, thereby increasing the probability of persistence for parasites.

The third category comprises factors dealing with temporal dynamics of host population turnover (Table 1.1, category 3). The idea is that the accumulated probability that parasite species could have invaded is higher the older a host population is. Consequently, the parasite community is not saturated in young host populations because they have not yet encountered all parasites for which they are potential hosts (Dobson & Pacala 1992).

The fourth category is a collection of habitat characteristics (Table 1.1, category 4). Characteristics related to decreasing influence of the Baltic Sea and less ephemeral conditions in the rock pools in general are believed to provide more stable conditions for hosts and consequently parasite species. Furthermore, species richness of free living organisms in rock pools generally declines with increasing salinity since rock pools are are thought to be essentially freshwater habitats (Ranta 1982). One consequence could be that epibionts, which are particularly influenced by their physicochemical environment, would meet less favourable conditions with increasing salinity. General patterns regarding patch quality are however difficult to predict as has been demonstrated by Ebert et al. (2001) who found contradicting patch quality preferences for different parasite species. The predicted associations of the different habitat characteristics are based on the positive or negative influence they have on the stability of the rock pool itself or the suitability of its water for epibionts (compare Table 1.1).

Here we provide evidence for associations between parasite species richness in a *D. longispina* metapopulation and the ecological factors listed in Table 1.1. In order to be able to contrast the sets of endoparasite and epibiont species harboured by the two potential host species, we worked at the same field site, used the same sampling and screening workflow, and applied mostly the same statistical procedures as Ebert et al. (2001). Because the data set was collected over the course of three years, we can also analyse temporal dynamics. We use the term parasites to collectively refer to endoparasite and epibiont species throughout the text, and it is furthermore important to note that the term parasite as well as the associated term parasite richness are meant to be neutral with respect to the the virulence of involved endoparasite or epibiont species. It should be stressed that since our analyses are based on purely observational data, we have to rely on correlational evidence and cannot exclude confounding.

Material and methods

Study system

We studied the endoparasite and epibiont community of the freshwater crustacean *D. longispina* in a freshwater rock pool metapopulation in southwest Finland off the coast of the peninsula of Hanko south of Tvärminne Zoological Station (59° 50′ N, 23° 15′ E; Fig. 1.1; `http://www.helsinki.fi/tvarminne/`). The site was chosen because one of us has been checking over 500 rock pools on 13 islands twice a year (early and late summer) since 1982 to assess the presence/absence of the three *Daphnia* species *D. longispina*, *D. magna*, and *D. pulex* in the very same area (Pajunen 1986). The long term record enables us to relate demographic variables to parasite species richness.

D. longispina is, like *D. magna*, a freshwater crustacean with a cyclical parthenogenetic life cycle. Under standard conditions, *D. longispina* is considerably smaller (adult body size about 1.4–2.2 mm) and has smaller mean clutch sizes (about 8 as opposed to 14 offspring) than *D. magna* but a slightly shorter clutch interval (3.2 vs. 3.7 days) and longer lifespan (40 vs. 35 days) (Bengtsson 1986). *D. longispina* tends to prefer larger, less saline, and more acidic rock pools, albeit has a niche largely overlapping with *D. magna* (Ranta 1979).

Samplings took place in the summers of 2004 (August 6–23), 2005 (July 21–26), and 2007 (August 6–12) in all pools of the study area that were found to contain *D. longispina* (59, 37, and 45 respectively). 31 pools were common to the samples in 2004 and 2005, 30 in 2005 and 2007, 33 in 2004 and 2007, and 26 contained *D. longispina* in all three years. In 32 of the 141 samples, *D. magna* occurred as well, whereas *D. pulex* only co-occurred in 2 samples. None of the samples contained all three *Daphnia* species. In the very dry summer of 2006 most rock pools dried up and too few populations were left in July/August for a meaningful sampling.

Over the three sampling years, *D. magna* was the most abundant species in the metacommunity and *D. longispina* was three times more abundant than *D. pulex* which on average only occupied about 20 pools subsequently over the course of a whole season. Furthermore, there were on average only six to seven rock pools where *D. magna* and *D. pulex* co-occurred. Thus we omitted *D. pulex* from the analyses and also did not attempt to collect data on its parasites although its intermediate status with regard to parasite susceptibility (Stirnadel & Ebert 1997) might be insightful for the understanding of the drivers of parasite richness.

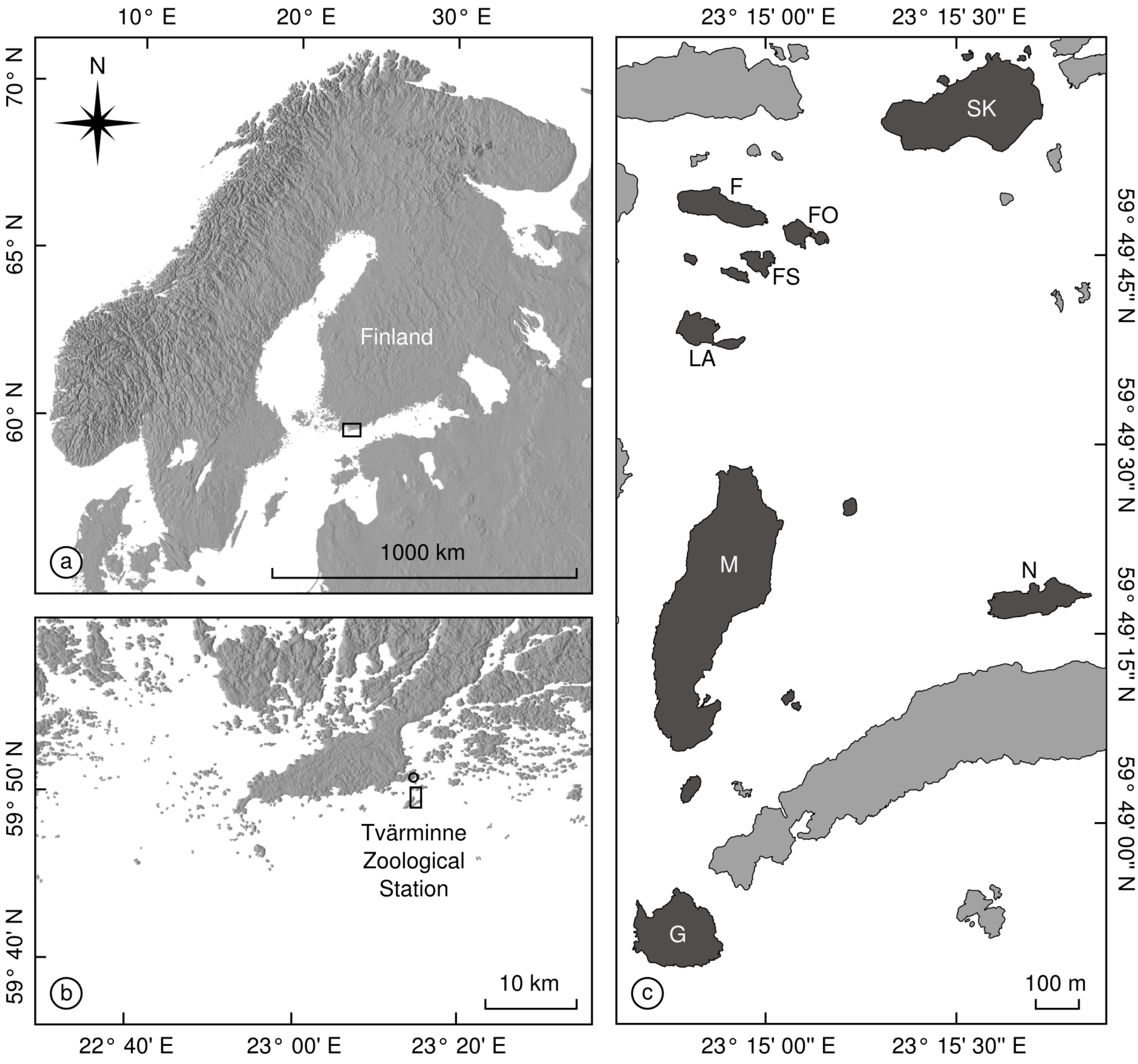

Figure 1.1 Location of the study site. (a) Map of Fennoscandia with a rectangle indicating the region around the Hanko peninsula (b), which in turn shows a rectangle enclosing the study area south of Tvärminne Zoological Station (indicated with a circle) (c). The islands where samples were taken are shaded in dark gray. The maps (a) and (b) are equidistant conic projections of the Shuttle Radar Topography Mission V2 elevation data (NASA) and the Micro World Data Bank II vector data (Central Intelligence Agency). The vector vertex coordinates of (c) were projected to KKJ (Kartastokoordinaattijärjestelmä, Finland Uniform Coordinate System).

Data collection

Parasite screening 50 adult individuals of *D. longispina* and 10 individuals of potentially co-occurring *D. magna* were taken to the lab and kept at 10° C prior to inspection. Screening of the animals was accomplished within 2 days after sampling. To circumvent problems arising from host sex differences, we only sampled females since they dominate the cyclical parthenogenetic life cycle. Following the methods of Ebert et al. (2001), we first checked the 50 *D. longispina* individuals for the presence of visually conspicuous parasite species under a microscope (25–50 × magnification). Then, 10 randomly chosen animals were dissected for better detectability of gut parasites and investigated with phase-contrast microscopy (100–400 × magnification). All visible endoparasite and epibiont species were recorded and classified following Ebert et al. (2001, see Table 3 therein), using the papers of Green (1957, 1974) and Larsson et al. (Larsson 1981, 1988, Larsson et al. 1996a,b, 1997, 1998) for identification of species. On *D. longispina*, the endoparasite species found were the microsporidium *Larssonia* sp. (most probably *L. obtusa*, thus referenced hereafter), the bacterium *Pasteuria ramosa*, an unknown species of a presumably endoparasitic filamentous bacteria, and an unknown species of microsporidia.

L. obtusa and *P. ramosa* infect both *D. longispina* and *D. magna*, though it is unclear whether individual parasite strains are compatible with both host species. In the co-occurring *D. magna* populations, we further found the *D. magna* specific microsporidia *Hamiltosporidium tvaerminnensis* (formerly misidentified as *Octosporea bayeri*, Haag et al. 2011) and *Ordospora colligata* (sometimes in the same individuals), but not *P. ramosa*, the unknown bacterial and microporidian species. The detected epibiont species on *D. longispina* were the algae *Colacium vesiculosum* and *Chlorangiella* sp., the ciliates *Epistylis helenae* and *Vorticella octava*, the fungus *Amoebidium parasiticum*, and an unknown species of filamentous bacteria. The co-occurring *D. magna* harboured the same epibiont species.

In case *D. longispina* was co-occurring with *D. magna*, the epibiont coverage was quantified prior to dissection. Numbers were estimated to the closest integer on a scale of the powers of 3 written in base 9 $(1, 3, 10, 30, \ldots;$ decimal logarithm roughly doubles at each incremental step) in case counting was not feasible. The same procedure of epibiont coverage quantification, dissection and parasite species recording was applied to the 10 individuals of co-occurring *D. magna*.

In 2005 and 2007, for each rock pool that contained *D. magna* alongside *D. longispina*, the nearest neighbouring rock pool only harbouring *D. magna* was sampled in addition. 10 individuals were subjected to the same procedure as described above.

Surrogate variables for ecological factors For each of the ecological factors in Table 1.1, we took measurements of one or more surrogate variables which are known to correlate positively with the corresponding factor and could actually be studied (see also detailed explanations in Ebert et al. 2001).

Population size increases with rock pool volume which was estimated as an inverse pyramid from measurements of surface area and depth. The number of host species is restricted to two and boils down to *D. magna* presence which in turn indicates whether *D. longispina* was the only *Daphnia* species or co-occurring with its direct competitor. The variable was extracted from Pajunen's long-term presence/absence data set of the three autochtonous *Daphnia* species (Pajunen & Pajunen 2003).

Local density of patches, represented by the numbers of *Daphnia* communities or *D. longispina* populations within 10 m distance from a specific *D. longispina* population or on the same island were calculated from Pajunen's data set, too.

For patch age, the same data set was used to infer the number of years since any *Daphnia* species or more specifically *D. longispina* had colonised a rock pool. To estimate community and population age respectively, we counted the number of years since that last extinction event which was defined based on the assumption that extinction occurs at the latest after two consecutive negative samplings.

The variables related to patch quality can roughly be attributed to the impact of the sea, vegetation, or topology. Relative distance to sea is defined as the shortest distance from a rock pool to the sea divided by the distance from the sea to the nearest tree. It is a measure for the exposition of a rock pool, which is a mixture of the proximity to the sea as well as the proximity to climax vegetation. Water samples from each rock pool were used to measure conductivity, largely reflecting input of sea water (measured in the lab with a conductivity meter). Water colour, or rather the brownness of it, which is an indicator of the amount of humic acid content, was ranked by eye on a continuous scale from 0 (clear) to 3 (dark brown). Precipitation and evaporation can bring about substantial changes in water properties over time, however the direction of change is the same for all rock pools, which allows for relative comparisons of estimates. Rock pool water catchment area was measured by walking along the estimated boundary while recording the position continuously with a global positioning system (GPS) device. Desiccation risk is defined as the number of 5 subsequent visits to the rock pool during which it was dry in a drought period in 2007 (Altermatt & Ebert 2008). Island area refers to the area of the island a rock pool is located on and was also estimated by tracking the boundary with the GPS device. Presence of plants indicates whether higher plants were growing in a rock pool. Affiliation of a rock pool with the island it is located on is treated as a categorical variable and not used in all analyses.

Variables

The definition and selection of response and explanatory variables closely follows Ebert et al. (2001).

The response variables are defined as (1) total, (2) endoparasite, and (3) epibiont species richness, expressed as the sum of endoparasite and/or epibiont species per *D. longispina* rock pool population. The only abundant endoparasite of *D. longispina* was the microsporidian *Larssonia obtusa*, and the sole other endoparasites found only occurred in one population each. Endoparasite species richness is therefore treated as a binary variable. The occurrences of the most abundant endoparasite species *L. obtusa* and epibiont species *Amoebidium parasiticum*, *Colacium vesiculosum*, and *Vorticella octava* were analysed separately. Data on parasite infections of individual *Daphnia* females was dichotomised to presence/absence of particular parasite species per rock pool because parasite prevalence estimates in rock pool populations are not time invariant and might therefore be misleading.

The explanatory variables are listed in Table 1.1. *D. magna* presence, presence of plants, desiccation risk, and geographic location are treated as categorical variables, all others as continuous.

Mean and range for all variables are given in Table 1.2.

Statistical analysis

The response variables total parasite and epibiont species richness were assumed to approximately follow a Poisson distribution. Endoparasite species richness and *L. obtusa* occurrence are identical except for single appearances of the unknown bacterial and microporidian species and *P. ramosa*. They were treated as binary variables, likewise the presence/absence patterns of single endoparasite and epibiont species.

For each pair of explanatory variables, Spearman's rank correlation coefficient was calculated. The data were aggregated by averaging over all three sample years prior to correlation analysis in order to exclude an overrepresentation of rock pools that were sampled in more than one year. Geographic location is not an ordered factor and was therefore not included.

To assess the influence of each explanatory variable on each response variable separately, simple generalised linear regression models were fit. Poisson regression was used for total parasite and epibiont species richness, logistic regression for the binary response variables. For the adjustment of p values due to multiple testing we used the correction proposed by Benjamini & Yekutieli (2001), which, unlike the commonly used sequential Bonferroni correction, controls for the false discovery rate rather than for the family wise error rate (García 2004).

Table 1.2 Descriptive summary of variables (mean and range).

Variable	2004 (N = 59)		2005 (N = 37)		2007 (N = 45)	
response						
total parasite species richness	1.80	0–5	0.649	0–3	0.733	0–2
endoparasite species richness	0.237	0–1	0.135	0–1	0.178	0–1
epibiont species richness	1.56	0–4	0.514	0–3	0.556	0–2
epidemiology						
pool volume (m^3)	1.05	0.0191–15.6	0.915	0.0191–9.65	0.843	0.0191–9.65
D. magna presence (proportion of pools)	0.237	0–1	0.297	0–1	0.156	0–1
space						
no. pools with *Daphnia* within 10 m radius	1.97	0–7	1.68	0–4	2.24	0–6
no. pools with *D. longispina* within 10 m radius	1.31	0–4	1.24	0–3	1.93	0–5
no. *D. longispina* pools on the same island	14.7	6–21	13.6	4–22	18.5	4–28
time						
time since *Daphnia* colonisation (years)	8.46	0–22	11.6	2.50–23	12.2	0.500–25
time since *D. longispina* colonisation (years)	5.23	0–22	8.36	1–23	8.40	0–25
patch quality						
relative distance to sea	0.414	0.00629–0.929	0.477	0.0769–0.929	0.509	0.182–0.929
water conductivity ($mS\,m^{-1}$)	18.9	1.86–145	44.5	4.50–280	21.2	4.77–55.4
water colour (rank scale)	1.58	1–3	1.67	1–3	1.76	1–3
presence of plants (proportion of pools)	0.780	0–1	0.757	0–1	0.822	0–1
catchment area (m^2)	36.5	1.40–402	32.2	1.40–175	33.5	1.40–175
desiccation risk (rank scale)	1.08	0–5	0.730	0–3	1.11	0–5
island area (10^4 m^2 = ha)	2.15	0.252–8.92	2.26	0.252–8.92	2.08	0.252–8.92
geographic location						
island on which rock pool is located	–	–	–	–	–	–

With a stepwise forward selection procedure, multiple generalised linear regression models were fit for each response variable by iteratively including explanatory variables that contributed significantly to the total deviance and removing explanatory variables that did not. As a measure for the total amount of variance explained by each model we calculated McFadden's pseudo R^2.

For both the simple and multiple regressions, the parameters were estimated for the whole data set with sampling year as a categorical cofactor to adjust for occurrences of some rock pools in multiple years.

Spatial autocorrelation was estimated by Moran's I over distance classes formed by the powers of 2 from 0 to 4096 m (covers the whole range of distances between rock pools). The two first (0 – 4 m) and two last (1024 – 4096 m) classes were pooled because of too small sample sizes.

Spatiotemporal autocorrelation was estimated with Mantel tests which compared a matrix of geographical distances between host populations and a matrix of asymmetric binary distances (Bray-Curtis dissimilarity index, Bray & Curtis 1957), representing the interannually varying presence of single parasite species. For each Mantel test, 10^6 random permutations were used.

Characteristics measured on both potential host species *D. longispina* and *D. magna* were compared with Wilcoxon signed rank tests. Since *D. longispina* is on average much smaller in body size than *D. magna*, epibiont coverage was related to body surface.

All analyses were performed and all plots were produced with R (R Development Core Team 2011, version 2.13.0). Add-on packages that were used are ecodist (Goslee & Urban 2007, version 1.2.3), lattice (Sarkar 2008, version 0.19-26), MASS (Venables & Ripley 2002, version 7.3-13), sp (Pebesma & Bivand 2005, version 0.9-82), and spdep (Bivand et al. 2008, version 0.5-33).

Results

Correlation As we intended to test the associations between each response and explanatory variable pair separately, it is important to know the associations among the explanatory variables in the first place. Of the 91 Spearman's rank correlation coefficients (Fig. 1.2), 29 are significant ($p < 0.05$) and 9 remain significant after adjustment for multiple testing. 3 out of 26 concern correlations within variable groups and 6 out of 67 correlations between variable groups. The strongest associations exist between catchment area and pool volume, between the number of *Daphnia* and *D. longispina* communities/populations within a radius of 10 m, and between age of the *Daphnia* and the *D. longispina* population. The first is a direct consequence of topological constraints,

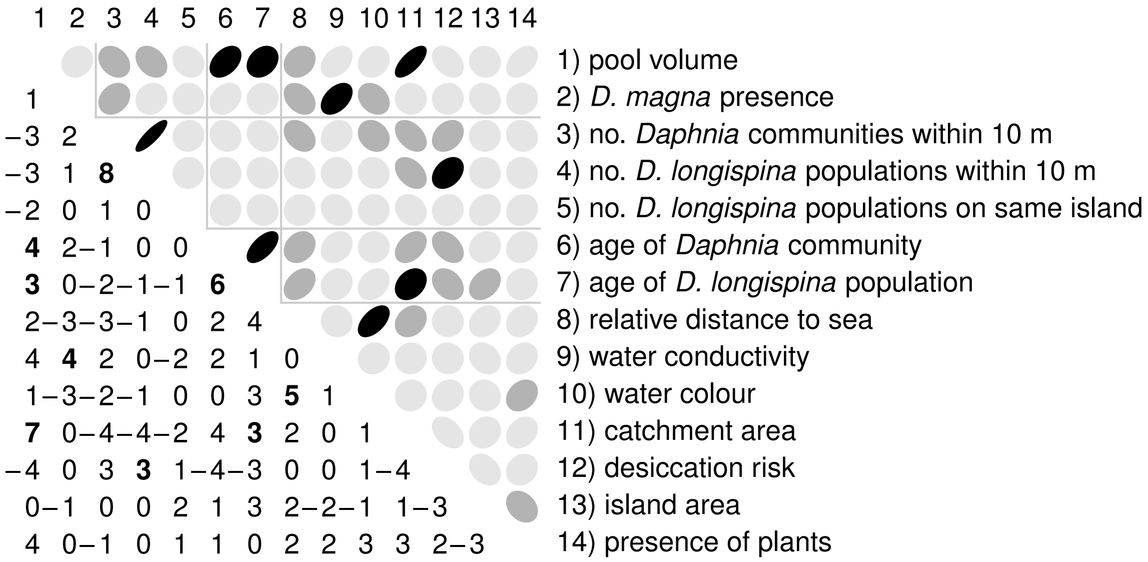

Figure 1.2 Correlation matrix of the explanatory variables aggregated over all three study years (73 aggregated samples) depicted using ellipse-shaped glyphs for each entry. Each ellipse represents a level curve of the density of a bivariate normal with the matching correlation (Murdoch & Chow 1996). The numbers are the Spearman rank correlation coefficients multiplied by 10 and rounded to one digit. Correlations that are significant are coloured in medium gray, and correlations that are significant after adjustment for multiple testing are black or bold-face respectively.

the second and third are due to a large overlap of the variables involved. All strong correlations are positive and mostly autocorrelations.

We did not exclude any of the variables due to high correlation but consulted the correlation matrix while discussing the regression results.

Simple regression For each explanatory variable, Poisson regressions were fit for total parasite and epibiont species richness and logistic regressions were fit for the occurrences of the four most common parasite species (Fig. 1.3).

D. magna presence has a consistently strong positive impact on total parasite species richness, epibiont species richness, and the occurrences of the three common epibiont species on *D. longispina*, but no association with *L. obtusa* presence/absence patterns can be detected (Fig. 1.3). The only effects that are significant after adjusting for multiple testing are also attributable to *D. magna* presence.

With great caution, one can also consider the significant effects of those explanatory variables whose p values are regarded as not significant after adjustment. In the case of epibiont species richness, there is a slightly positive association with *Daphnia* community age and a slightly negative association with island area. *L. obtusa* occur-

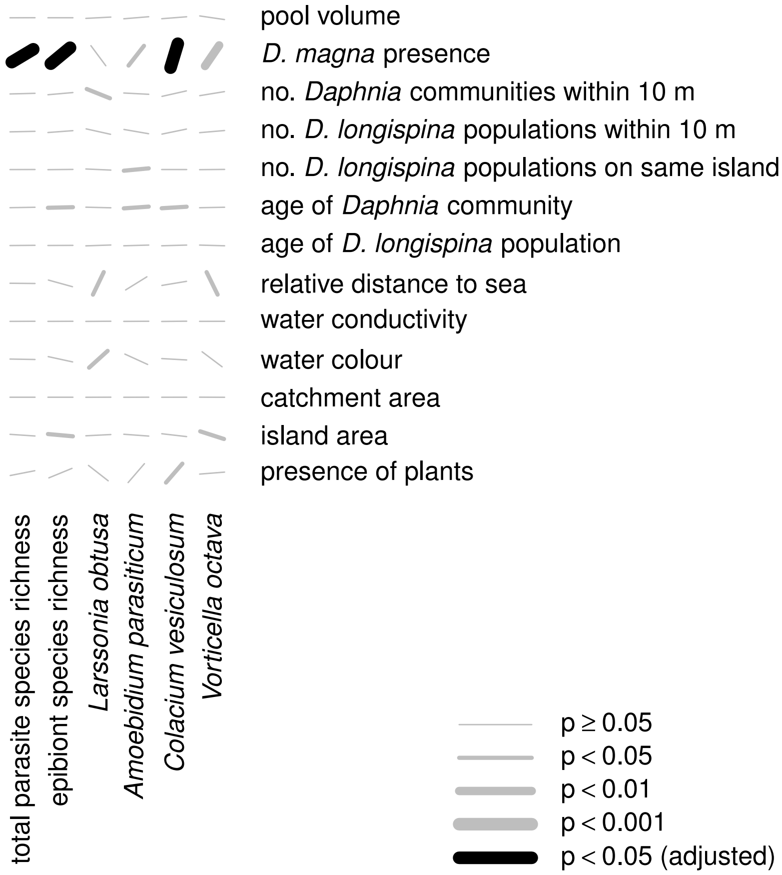

Figure 1.3 Regression parameter estimates of all explanatory variables resulting from Poisson (total parasite species richness and epibiont species richness) or logistic regressions (occurrences of the four most common parasite species) on all response variables. Each estimate is represented by a line whose rotation relative to the abscissa corresponds to the coefficient in the linear predictor of the generalised linear model, i.e. a rotation of 45 ° counter clockwise corresponds to a coefficient of 1. Note that the coefficient estimates for Poisson regressions correspond to log relative risks and the coefficient estimates for logistic regressions correspond to log odds ratios. The line thickness indicates whether the explanatory variable has a significant effect on the corresponding response variable. Black lines indicate associations that remain significant after adjustment for multiple testing. The results for desiccation risk and geographic location are not shown due to too many factor levels to depict.

Table 1.3 Multiple regression models.

Response variable[a]	Explanatory variable[b]	Estimate[c]	z[d]	Pseudo R^{2}[e]
total parasite species richness	*D. magna* presence	1.818	3.6***	0.29
epibiont species richness	*D. magna* presence	2.287	4.7***	0.30
Larssonia obtusa	island FO[f]	0.087	−2.1*	0.23
	island FS[f]	0.044	−2.7**	
	island G[f]	0.198	−2.5*	
	island M[f]	0.163	−1.9	
	island N[f]	0.000	0.0	
	island SK[f]	0.188	−1.8	
	island LA[f]	0.000	0.0	
Amoebidium parasiticum	*D. magna* presence	3.549	2.2*	0.32
	no. *D. longispina* populations on same island	1.102	2.2*	
Colacium vesiculosum	*D. magna* presence	26.282	4.6***	0.32
	presence of plants	3.210	2.0*	
Vorticella octava	*D. magna* presence	3.703	2.8**	0.15
	island area	0.756	−2.0*	

Regression models were fit with stepwise forward selection. Only explanatory variables with significant effects were included in the final model. The covariate sampling year was forced to be included, and apart from the model for response variable *L. obtusa* occurrence it is very highly significant.

[a] The response variables are total parasite species richness, epibiont species richness (Poisson regressions), and presence/absence of the most common endoparasites and epibionts (logistic regressions).

[b] The full model includes all variables listed in Table 1.1 except for spatial autocorrelation.

[c] Coefficient estimates of regression parameters. Note that the coefficients for Poisson regressions (total parasite and epibiont species richness) correspond to relative risks and the coefficients for logistic regressions (occurrences of the four most common parasite species) correspond to odds ratios.

[d] Value of z test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

[e] McFadden's pseudo R^2 are approximations of the total amount of variance explained by the model.

[f] The reference level of the treatment contrasts of the categorical variable "island" is "island F".

rence is negatively associated with the number of *Daphnia* communities within 10 m and positively associated with relative distance to sea and water colour. *A. parasiticum* occurrence is influenced positively by the number of *D. longispina* populations on the same island and *Daphnia* community age. For *C. vesiculosum* occurrence, age of the *Daphnia* community and the presence of plants are positive predictors. *V. octava* occurrence is additionally determined by a negative effect of relative distance to sea and a slightly negative effect of island area.

Multiple regression The multiple regression models for the response variables total parasite species richness, epibiont species richness, and occurrences of the major parasite species reflect largely the results of the simple regressions. Additionally, they give an idea of the relative importance of the explanatory variables (Table 1.3). The covariate sampling year was forced to remain in the model in order to correct for year to year variation and is highly significant in all cases but for the model for *L. obtusa*. *D.*

magna presence occurs in 5 out of 6 models and always has a positive, significant effect, suggesting that the presence of the competitor drastically increases parasitism in *D. longispina*. Apart from the factor sampling year, it is the only additional explanatory variable in the models for total parasite species richness and epibiont species richness and is dominant in the models for the three epibiont species occurrences.

In addition to *D. magna* presence, the models suggest that *A. parasiticum* occurrence is reigned by a small positive effect of the number of *D. longispina* populations on the same island, *C. vesiculosum* occurrence increases with the presence of plants, and *V. octava* occurrence is higher on islands of larger area. Interestingly, the model for *L. obtusa* occurrence is the only one not containing *D. magna* presence and only includes the island a specific rock pool is located on as an additional explanatory variable. The coefficients for all the island variables are negative relative to the reference island F where *L. obtusa* was more prevalent than elsewhere.

Spatial autocorrelation Spatial autocorrelation was assessed with Moran's *I* for total parasite species richness, epibiont species richness, and separately for the occurrence of the four most common parasite species (Fig. 1.4). The general pattern shows that autocorrelation is strong in lower distance classes and close to zero in higher distance classes, meaning that rock pools that lie closer to each other are more similar to one another than one would expect from the null hypothesis of random distribution. All values significantly different from zero are positive, which is an indication for similarities due to spatial neighbourhood.

When comparing the individual year-wise trajectories of Moran's *I* (Fig. 1.4), it is important to notice that total parasite species richness (top left panel) is the sum of *L. obtusa* occurrence (bottom left panel) and epibiont species richness (middle left panel), which in turn is approximately the sum of the occurrences of the three most common epibiont species (three panels on the right).

For total parasite species richness, there is an unusual positive significant value in the distance class $256 - 512\,\mathrm{m}$ in 2004, but otherwise the trajectory follows the one for epibiont species richness very closely. For *L. obtusa*, there are five relatively large positive significant values distributed over an extended range of distance classes from $2 - 128\,\mathrm{m}$. The major epibiont species show a similar picture with some, albeit fewer, large positive values in the lower distance range and two exceptions in the $256 - 512\,\mathrm{m}$ range for *V. octava* and in the $512 - 1024\,\mathrm{m}$ range for *C. vesiculosum* with relatively small values of Moran's *I*.

Spatiotemporal autocorrelation Strong correlations between the distances between rock pools and the interannual presence/absence of single parasite species were almost

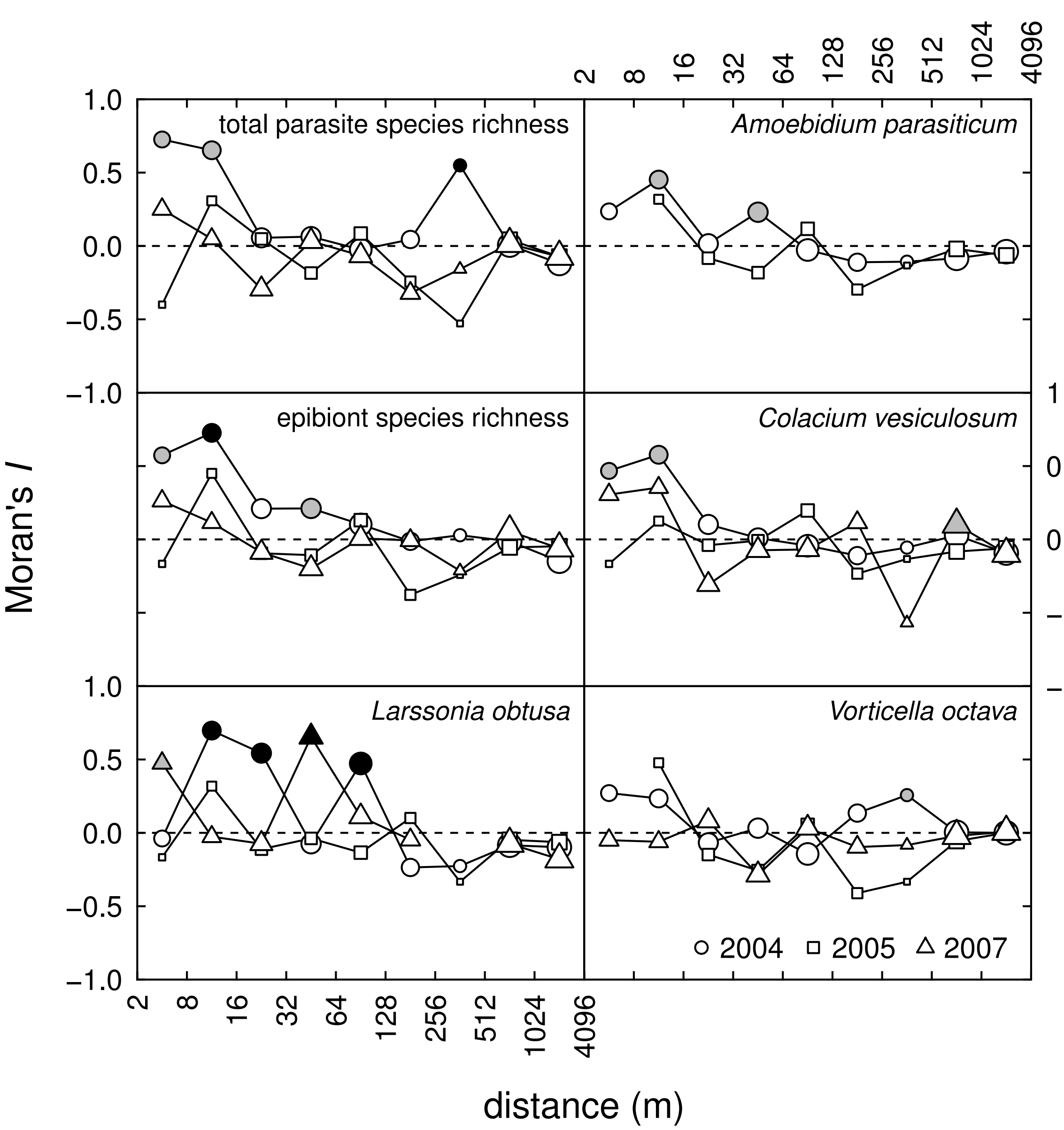

Figure 1.4 Correlogram for the spatial autocorrelation of total parasite species richness, epibiont species richness, and the occurrences of the most common endoparasite and epibiont species. Moran's autocorrelation coefficient *I* is shown in relation to distance classes formed by the powers of 2. The lowest two classes (2–8 m) and the highest two classes (1024–4096 m) were pooled due to too small sample sizes. The plot symbols' areas are proportional to the sample sizes (maximum 59). Significant values are indicated by filled symbols, significant values that are significant after adjustment for multiple testing are indicated by black symbols. For certain years and distance classes, Moran's *I* could not be calculated because the rock pools did not differ from each other regarding the parasite composition or because the parasite species was absent.

exclusively found for *L. obtusa*, namely in the comparisons of the years 2004 vs. 2005 (Mantel $r = 0.112$, $p = 0.02$), 2004 vs. 2007 ($r = 0.159$, $p = 0.007$), and for the comparision of all three sampling years ($r = 0.135$, $p = 0.028$). The only other significant comparison was found in *A. parasiticum* for the years 2004 and 2005 ($r = 0.1$, $p = 0.039$). All other comparisons of geographical distance and temporal presence did not differ significantly from the null hypothesis of random association.

Host species comparison There were 32 samples from rock pools that contained both *D. longispina* and *D. magna*. The total parasite species richness is slightly higher in *D. magna* than in *D. longispina* (Wilcoxon signed rank test, $V = 104$, $p = 0.035$), but there is no difference in epibiont species richness (Wilcoxon signed rank test, $V = 54.5$, $p = 0.21$). For 14 of the two-species rock pools, reference rock pools containing only *D. magna* were sampled. A comparison of total parasite species richness and epibiont species richness between *D. magna* from the two-species pools and *D. magna* from the reference pools yields no significant differences.

Note that we potentially underestimate the parasite species richness in *D. magna* since the sample sizes for this host species is only 10 compared to 50 in *D. longispina*. Mean values for total parasite, endoparasite, and epibiont richness for all sampled *D. magna* populations were 2.3, 0.76, and 1.56 respectively and thus are all in the order of or even higher than in the study of Ebert et al. (2001). The likelihood to underestimate parasite species richness in *D. longispina* should therefore be negligible.

By far the most abundant epibiont species occurring on both host species was *C. vesiculosum*. It occurred in 23 of the two-species pools, and the mean coverage for both *Daphnia* hosts is depicted in Fig. 1.5. A comparison of the *C. vesiculosum* coverages of *D. longispina* and *D. magna* reveals a highly significant difference with *D. longispina* being much less covered (Wilcoxon signed rank test, $V = 268$, $p < 0.001$). Because *D. longispina* is on the average much smaller than *D. magna*, one should relate coverage to mean body surface. The limit case where *D. longispina* is still significantly less covered (Wilcoxon signed rank test, $V = 203$, $p = 0.048$) is reached when assuming that the body surface of *D. magna* is on average 8.3 times bigger than the body surface of *D. longispina*. This corresponds roughly to an average 2.9-fold increase in body length, which is clearly above the relative difference of about 2 commonly observed in the field. Thus relative coverage is still different in the two species.

Of the pure *D. magna* reference rock pools, 11 also contained *C. vesiculosum*. A comparison of *C. vesiculosum* coverage between *D. magna* from pools with and without *D. longispina* yields no difference (Wilcoxon signed rank test, $V = 15$, $p = 0.12$, Fig. 1.5).

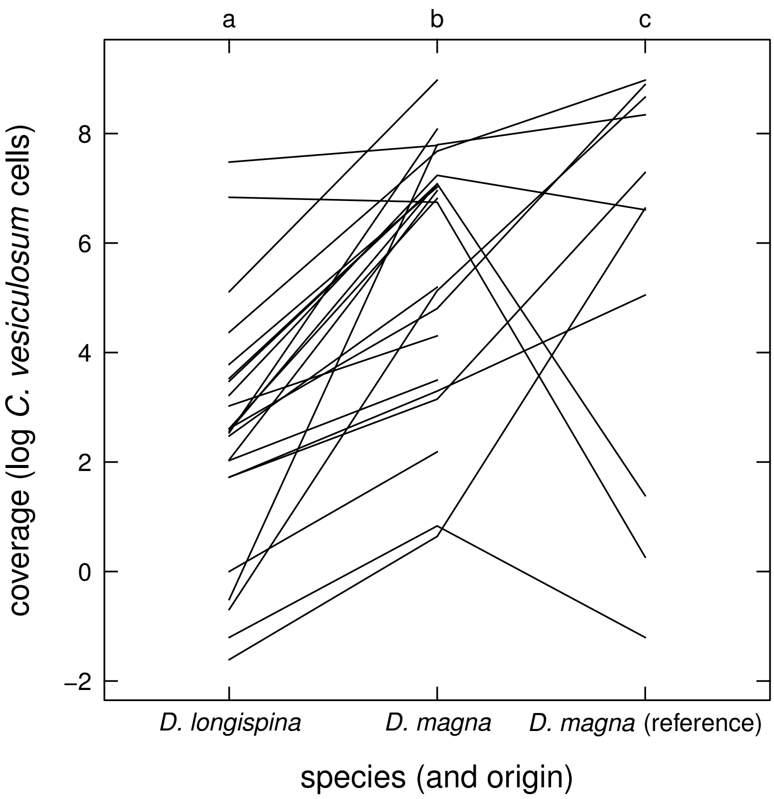

Figure 1.5 Coverage of the epibiontic green alga *C. vesiculosum* in relation to host species and host origin of *Daphnia* populations which contained (a) *D. longispina* as well as (b) *D. magna*. Estimates for *D. longispina* and *D. magna* from the same rock pool are connected with a straight line. If available, estimates from pure *D. magna* reference rock pools that lie nearby are given, too (c).

Discussion

The life histories (Bengtsson 1986) and niches (Ranta 1982) of *D. longispina* and *D. magna* have much in common. On the other hand, a comparison of the results obtained in the study at hand and the findings of Ebert et al. (2001) implies that the two *Daphnia* species differ in many respects regarding parasite species richness. Not only does *D. longispina* only harbour a subset of the endoparasite species found in *D. magna*, but endoparasite and epibiont species diversities at the population level are also comparatively lower.

As was consistently shown throughout all analyses, the single most important factor for parasite species richness in *D. longispina* is the presence of the competitor *D. magna*: in *D. longispina* populations that are not part of a community with *D. magna*, parasite species richness is lower. This finding might be explained by some hidden variables that determine the presence of *D. magna* and at the same time favour high parasite species diversity, or it might be directly related to the presence of *D. magna*. Since we rely on correlational evidence, we cannot disentangle the two explanations.

The correlation matrix indicates that *D. magna* presence correlates in a highly significant manner positively with water conductivity and negatively with relative distance to sea and water colour (Fig. 1.2). These correlations are in accordance with the preference of *D. magna* for pools more influenced by the sea (Ranta 1979). However, not a single one of the three patch quality variables occurs in any of the six multiple regression models (Table 1.3), and even when excluding the correlating factor *D. magna* presence from the analysis (results not shown), still only the model for *V. octava* includes a significant patch quality variable (water colour). Furthermore, Ebert et al. (2001) report that epibiont species richness in *D. magna* was negatively associated with water conductivity. Put differently, while the ecological variables correlate with *D. magna* presence and should favour lower epibiont species richness, *D. magna* presence correlates with higher epibiont species richness in *D. longispina*. Since total parasite species richness in *D. longispina* is almost exclusively determined by epibiont species richness, the above statement can be extended to total parasite species richness. We thus suggest that *D. magna* presence itself is a good predictor of parasite species diversity in *D. longispina* and not just a surrogate for potentially hidden unknown ecological factors.

The presence of alternative hosts has been shown to correlate with parasite species richness in some systems. Caro et al. (1997) found that gregariousness in fish host species is correlated with higher helminth species richness, and the same relationship was detected by Krasnov et al. (2004) for rodents and fleas. It is hypothesised that a positive effect could manifest itself through two non mutually exclusive basic mechanisms. First, concurrently present host species might either differ with regard to susceptibility or might be infected at different times and/or under different environmental conditions. Second, host species might be present sequentially in time and allow parasites to switch between host species and thereby bypass temporal absence of one host species. For the special case of parasites with obligate host change, one of these mechanisms is compulsory. A combination of both mechanisms would be feasible in the case of a sequential occurrence of the host species with a temporary overlap. In *Daphnia* metacommunities, spatial and temporal coexistence of *D. longispina* and *D. magna* can both be observed (Bengtsson 1986, Pajunen 1986, Pajunen & Pajunen 2003). In other words, *D. magna* might serve as a reservoir from where *D. longispina* is constantly afflicted. The lack of reservoirs would consequently lead to the demise of at least some endoparasite and epibiont species to the point where the *D. longispina* population is free of it.

In stark contrast to the current results, Ebert et al. (2001) found host community or population age to be the single most important correlate of parasite species diversity in the congeneric species *D. magna*. Conversely, neither *Daphnia* community age nor *D. longispina* population age had an important influence on parasite species richness measures in *D. longispina*. *Daphnia* community age appears in the simple regressions

(Fig. 1.3) but has by no means the same predictive power as in the *D. magna* study. One reason for this discrepancy may be due to the rareness of endoparasites in *D. longispina* and thus low power of statistical tests. Moreover, in *D. magna*, epibiont species richness increased only for a few years and then levelled off whereas endoparasite species richness continued to increase even after a decade (Ebert et al. 2001). A similar, albeit more blurred picture arises for *D. longispina* epibiont species richness, which reached its maximum very soon after founding of a new population (results not shown). Note that these relationships between parasite richness and *Daphnia* population age are based on the sample data and not on follow-up studies, therefore merely reflect associations but not observed temporal dynamics. Since total parasite species richness in *D. longispina* is only slightly influenced by endoparasite species, it closely follows the temporal comportment of epibiont species richness. Thus contrary to the situation in *D. magna*, the variables *Daphnia* community age and *D. longispina* population age as such do not have a substantial effect on *D. longispina* parasite species richness in this metapopulation.

Even when *D. longispina* co-occurs with *D. magna*, the latter has a larger total parasite species richness, whereas epibiont species richness does not differ significantly. However, quantitatively there seem to be differences, too, exemplified by the differential coverage by the green algal epibiont species *C. vesiculosum* (Fig. 1.5), which is still significant after correction for body surface differences. In this study, endoparasites and epibionts are collectively referred to as parasites, which by definition harm their hosts. While *Daphnia* endoparasites are unanimously described as parasites in the strict sense, *Daphnia* epibionts live in a range of parasitic (e.g. Allen et al. 1993) to mutualistic (e.g. Barea-Arco et al. 2001) relationships with their hosts. According to this strict definition, giving endoparasites a higher weight over epibionts with regard to the amount of harm they cause in the host, parasite species richness would be even lower in *D. longispina* in relative terms.

Daphnia longispina has on average a smaller body size than *D. magna*, and *D. pulex*, the third species in the Tvärminne metacommunity, is intermediate in average body size as well as in parasite prevalences (Stirnadel & Ebert 1997) but possibly also regarding parasite species richness. For mammals, meta analyses found a positive correlation between body size and parasite species richness (e.g. Arneberg et al. 1998, Ezenwa et al. 2006, Lindenfors et al. 2007), though often the effect vanished after correcting for phylogenetic relationships (e.g. Morand & Poulin 1998, Nunn et al. 2003). The three *Daphnia* species lie phylogenetically at the most distant locations within the genus, forming three subgenera (Colbourne & Hebert 1996). Whether host body size would be a good predictor of parasite species richness even after correction for phylogenetic relationships can therefore not be tested without further information on parasite species richness in other *Daphnia* species.

Spatial autocorrelation of parasite species diversity measures are mostly in accordance with expectancy: rock pools that lie close to each other are more similar to one another than rock pools at larger distances apart. *L. obtusa* showed significant positive autocorrelation at larger distances than the epibionts. It occurred in several rock pools on the same island but rarely on others, which could explain why autocorrelation breaks down across islands and why the relevant distances are in the range of the diameters of the islands in the study area. Furthermore, the corresponding distances of distance classes where Moran's I is significant lie mostly within and not between islands. The strong autocorrelations are thus most likely due to island effects. Moreover, *L. obtusa* showed the most consistent and strongest spatiotemporal associations between rock pool distances and presence/absence patterns over two or three sampling years. Taken together, these observations imply that *L. obtusa* might be bound to certain rock pools from where it spreads locally on the islands but rarely to other islands.

A very conspicuous difference between the parasite species diversities in *D. longispina* and *D. magna* is the rareness of endoparasites apart from *L. obtusa*. As stated above, body size could partly explain why endoparasites might be more restricted in the smaller *D. longispina* than the larger *D. magna* although this is not a mechanistic but rather a simple associative explanation. From the perspective of parasites, hosts could be regarded as resource patches, and the bigger a resource patch is, the more parasites and ultimately parasite species it could sustain. Thus we could apply the same reasoning as we did for the spatial factors determining parasite richness on the level of host population. There were only single occurrences of a filamentous bacterium in 2004 and *P. ramosa* in 2007. *L. obtusa* on the other hand is relatively common in both *D. longispina* and *D. magna*, although it does not reach the same degree of metapopulation pervasion as the *D. magna* specific microsporidian *Hamiltosporidium tvaerminnensis*, which can be found in nearly half of the populations and regularly achieves prevalences close to 100 % at the end of the season (Lass & Ebert 2006). Yet *L. obtusa* might be more cryptic and only visible in the terminal phase of infection and thus harder to detect. Moreover, it is suggested that *L. obtusa* has a complex life cycle involving more than one host, as has been found for related microsporidia (Andreadis & Vossbrinck 2002). In case the second host were volant, it could serve as a vector and spread the parasite even between islands. Overwintering might take place in the second host in relatively stable rock pools from where new *Daphnia* populations could be invaded, which is again congruent with the results of spatial autocorrelation.

To recapitulate, based on our correlative evidence, parasite species richness in *D. longispina* seem to be population and community age independent and primarily mediated by the presence of *D. magna*, whose parasite species richness in turn can largely be explained by the age of the host population or community. From an anthropocentric view, the saying "cling together, swing together" accurately encapsulates the consequences that *D. longispina* has to bear due to cohabitation with *D. magna*.

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Chapter 2

Concerted reduction of *Daphnia magna* invasion success by inbreeding, parasitism, and interspecific competition

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Abstract

In metacommunities, localities are often occupied by species with overlapping niches. When local extinction rates of a species are high, the capability to invade localities is thus crucial for its persistence. Apart from interactions with the local resident community, invasibility is determined by characteristics of the invader itself. We provide results of a full factorial outdoor mesocosm experiment that was designed to test the influence of the ability of the freshwater crustacean *Daphnia magna* to invade metacommunity rock pool localities depending on inbreeding, infection by the major host specific microsporidian parasite *Hamiltosporidium tvaerminnensis*, and interspecific competition by the niche sharing congeneric *D. longispina*. Inbreeding, parasitism, and competition were all found to reduce invasion success. Furthermore, a significant interaction between inbreeding and competition was found, with inbred invader clones being detained overproportionally by interspecific competition. Since (1) inbreeding in local populations of *D. magna* rock pool metapopulations is generally very strong, (2) *H. tvaerminnensis* occurs in about half of all populations where it regularly reaches prevalences of 100 %, and (3) *D. longispina* and *D. magna* often occur concurrently in the rock pools of this metacommunity, we predict that observed invasion events are shifted towards cases with outbred or uninfected invaders or into unoccupied localities. Our results show that inbred and parasitised *D. magna* suffer a cost which was previously not incorporated into concepts of inbreeding depression and virulence. We therefore speculate that on a metacommunity level, selection acts against inbreeding and parasitism.

Key words colonisation, rock pool, metacommunity, mesocosm, *Daphnia longispina*, *Hamiltosporidium tvaerminnensis*, Microsporidia

Introduction

The concept of metapopulations is intrinsically tied to the capability to colonise empty habitat patches by means of dispersal counteracting local extinction events (Levins 1970, Hanski & Gaggiotti 2004). Likewise, the concept of metacommunities requires the participating species to be able to invade local communities unless their local populations remain spatially completely separated (Leibold et al. 2004, Holyoak et al. 2005). For a better understanding of extinction-colonisation dynamics and the formation of species assemblages in metacommunities, it is crucial to determine the invasion capability of a species or, viewed from the opposite perspective, the invasibility of a resident community by this species (e.g. Law et al. 2000, Shurin 2000).

The phenomenon of invasion has been primarily studied for alien species introduced into native communities (Williamson 1996) where research has been spurred by the tremendous economical costs that non-indigenous, naturalised species cause (Pimentel 2002, Pimentel et al. 2005). An important and by now well supported explanation why certain alien invasive species are highly successful is the enemy release hypothesis which states that invasive species have an advantage because they might get rid of their associated predators and parasites during the invasion process (e.g. Keane & Crawley 2002, Mitchell & Power 2003, Torchin et al. 2003, Callaway & Ridenour 2004, Colautti et al. 2004). Finding such explanations of general validity, which allow to predict invasive species' establishment success, is an increasingly important challenge in the face of invasive species becoming a major driver of contemporary mass extinctions (Gurevitch & Padilla 2004, Clavero & García 2005).

Extinction is the antagonist of colonisation or invasion in metapopulations and metacommunities (Hanski & Gaggiotti 2004, Holyoak et al. 2005). At the same time, extinction is decisively influenced by the quality of the invaders (Gaggiotti & Hanski 2004). A pivotal quality criterion is the degree of inbreeding exhibited by invaders. In animals (e.g. Nieminen et al. 2001) as well as in plants (e.g. Newman & Pilson 1997) local populations founded by inbred colonisers were shown to have higher extinction rates than populations founded by outbred colonisers. Ultimately, this means that inbreeding can result in a reduction of invasion success.

A third important constituent of invasion success, besides the release of parasites and quality of invaders, is the intensity of competition exerted by local communities (Amarasekare et al. 2004). In this regard, the prominent though controversial diversity resistance hypothesis states that more diverse and thus potentially more competitive communities are less invasible by alien species (Levine & D'Antonio 1999). Experimental studies found mostly negative (Tilman 1997, Stachowicz et al. 1999, Shurin 2000, Kennedy et al. 2002) and observational studies found predominantly positive relationships (Stohlgren et al. 1999, Levine 2000, Sax 2002, Shea & Chesson 2002) between local native diversity and invasion success. Shurin & Srivastava (2005) suggested that the contradiction could by explained by the circumstance that experimental studies are usually conducted at small spatial scales and thus implicitly consider native invasion whereas observational studies commonly embrace spatial scales several orders of magnitude larger and thus implicitly consider alien invasion. While community assembly dynamics and criteria for invasibility have been theoretically elaborated on a local spatial scale (e.g. Morton & Law 1997), they are more difficult to grasp on a larger spatial scale (Connor & Simberloff 1979, Ricklefs 1987), exemplified by the inverse relatedness of invasibility and origin of invaders (e.g. Cornell 1985, Ricklefs 1987, Ricciardi & MacIsaac 2000), indicating that most communities are unsaturated or invasible with respect to the global species pool.

Although parasite release, inbreeding of invaders, and competition by residents are potentially all important elements of invasion success, the portability of the findings of invasion biology to the extinction-colonisation dynamics studied in (meta)community ecology and vice versa is complicated by the varying degree of common evolutionary history that the involved species share (Leibold & Norberg 2004, Urban & Skelly 2006, Urban et al. 2008). Nevertheless, the application of niche concepts developed in community ecology to alien species could lead to a fruitful reconciliation of the fields of community and invasion ecology (Shea & Chesson 2002), with community ecology providing a useful framework for understanding biological invasions (Simberloff 2004). Shea & Chesson (2002) suggested that available resources, natural enemies, and the physical environment determine an invader's growth rate. However, invasibility of a community does not only depend on the particular local conditions but also on characteristics of the invader itself (Kolar & Lodge 2001), notably parasitism and inbreeding as outlined above. But to date, only few empirical data are available on how community and invader traits, on their own and interdependently, influence invasion success (e.g. Kolar & Lodge 2002). In metacommunities, extinctions and colonisations occur by definition on a regular basis at different spatial scales (Holyoak et al. 2005). Scrutinising the factors determining invasibility of local communities in metacommunities might therefore by a promising approach to achieve a better comprehension about invasion processes in general (Simberloff 2004). In this vein, we tried to unravel determinants of invasion success by experimentally manipulating characteristics of the invader as well as the resident community and analysing the outcome of individual invasion events.

According to Holyoak et al. (2005), a metacommunity is easiest to conceptualise when it possesses the following properties: all interacting species utilise the same habitat patches, these habitat patches have discrete boundaries, and furthermore the local populations use resources at the same within-patch scale. Because they fulfil these claims comparatively well, metacommunities of *Daphnia* (Crustacea: Cladocera) along the skerry island coast of the Baltic Sea became a popular system to study metacommunity dynamics (e.g. Ranta 1979, Hanski & Ranta 1983, Bengtsson 1986, 1989, Pajunen 1986, Pajunen & Pajunen 2003). Our study system, a metacommunity in southwest Finland, consists of superposed metapopulations of *D. longispina*, *D. magna*, and *D. pulex* which occupy freshwater filled rock rock pools, which in the general terminology of metacommunity ecology are referred to as localities (Holyoak et al. 2005). Local communities have apparent and discrete boundaries and competitors' ecological niches are largely overlapping (Ranta 1979). Furthermore, local populations frequently go extinct and unoccupied patches are recolonised in a counter balancing way (Pajunen 1986, Pajunen & Pajunen 2003). Thus apart from the close accordance to a prototype metacommunity (Holyoak et al. 2005), this *Daphnia* rock pool metacommunity provides ample opportunity to study invasion.

In spite of largely overlapping niches (Ranta 1979) and substantial environmental differentiation of rock pool habitats (Pajunen & Pajunen 2007), after all roughly 15 % of the rock pools harbouring either *D. longispina* or *D. magna* contain both species concurrently (Pajunen & Pajunen 2003), whereas *D. pulex* occurs in lower frequencies and is therefore not considered here. *Daphnia magna* is the most abundant species and shows higher extinction-colonisation dynamics than *D. longispina* (Hanski & Ranta 1983, Pajunen & Pajunen 2003, Altermatt et al. 2008). Moreover, the by far most abundant endoparasite in the *Daphnia* metacommunity, *Hamiltosporidium tvaerminnensis* (Microsporidia), is specific to *D. magna* (Ebert et al. 2001). Thus, the tentatively most representative local *Daphnia* rock pool community consists of *D. longispina* as the resident and *D. magna* as the invader plus its specific parasite *H. tvaerminnensis*, the later being an integral part of the community.

For our focal species *D. magna*, there is a growing body of evidence showing that its populations are affected by inbreeding (Ebert et al. 2002, Haag et al. 2002, Salathé

& Ebert 2003), parasitism (Ebert et al. 2001), interactions of inbreeding and parasitism (Haag et al. 2003, Altermatt et al. 2007, Altermatt & Ebert 2008a, Ebert et al. 2007), and interspecific competition (Hanski & Ranta 1983, Bengtsson 1989, 1993) in the context of rock pool metacommunities. These three factors have however not been studied in combination with regard to invasion. Being a key process in metacommunity ecology, we here focus on the ability of *D. magna* to invade rock pools under different regimes of inbreeding, parasitism, and interspecific competition.

Inbreeding is here considered as a characteristic attributable solely to the invader. Genetic bottlenecks lead to strong local inbreeding in *Daphnia* rock pool populations (Haag et al. 2005, 2006). And since inbreeding in these populations is known to result in strong inbreeding depression (Ebert et al. 2002, Haag et al. 2002), it was assumed to negatively affect invasion ability. Parasitism by *H. tvaerminnensis* is special in the sense that it can be attributed as a characteristic both to the invader and, once invasion has occurred, to the local community. Due to the harm that parasites cause to their hosts in general and specifically due to reduced fecundity and survival caused by *H. tvaerminnensis* (Vizoso & Ebert 2004, Vizoso et al. 2005), we expected infection by *H. tvaerminnensis* to lower invasion success of *D. magna*. Eventually, interspecific competition by *D. longispina* is clearly a characteristic of the local community. As competitive exclusion has been reported to occur in *Daphnia* rock pool communities (Hanski & Ranta 1983, Bengtsson 1989, 1993), interspecific competition is predicted to decrease invasion success as well. In summary, we therefore hypothesised that inbred, infected, or competition-faced *D. magna* clones would have a lower invasion success than their outbred, uninfected, or competition-free counterparts. We tested the three factors in a fully factorial design to be able not only to draw conclusions on the effects of single factors but also on their potentially concerted impact on invasion success of *D. magna*.

Material and methods

Study system

The study was conducted in a metacommunity which is located on a skerry archipelago at the coast south of Tvärminne Zoological Station in southwest Finland (59° 50′ N, 23° 15′ E, `http://www.helsinki.fi/tvarminne/`). The metacommunity consists of the freshwater crustaceans *D. longispina* O. F. Müller, *D. magna* Straus, and *D. pulex* Leydig (Pajunen 1986, Pajunen & Pajunen 2003). The generalist filter-feeders inhabit freshwater filled rock depressions, the so-called rock pools, and have a cyclical parthenogenetic life cycle (Zaffagnini 1987). The asexual cycle, in which females give rise to clonal daughters, is intermitted by the production of clonal sons and the subsequent fertilisation of sexual eggs induced by a variety of stimuli (Banta & Brown 1929, Stross & Hill 1965, Carvalho & Hughes 1983, Kleiven et al. 1992). The sexual eggs are shed in the form of resting stages, so-called ephippia, which serve on the one hand to persist through harsh conditions such as droughts and frost and on the other hand as the dispersal stage. Every spring, but also after temporary summer droughts (Altermatt & Ebert 2008b), females hatch from ephippial eggs and found new populations by clonal expansion. *Hamiltosporidium tvaerminnensis* HAAG (formerly misidentified as *Octosporea bayeri* JIROVEC, Haag et al. 2011), the abundant endoparasitic microsporidium specific to *D. magna*, is horizontally as well as vertically transmitted (Vizoso & Ebert 2004, Vizoso et al. 2005). Vertical transmission of *H. tvaerminnensis* occurs both via the parthenogenetic eggs and the sexual eggs of the resting stages, the later path possibly being an effective way to invade new host populations (Ebert et al. 2007).

Experimental setup

We conducted an outdoor mesocosm experiment within the perimeter of the *Daphnia* metacommunity. The experiment followed a full factorial design and covered the *D. magna* - *D. longispina* - *H. tvaerminnensis* community outlined above. *Daphnia magna* served as the invading species and varied with respect to the two factors inbreeding status and status of infection by *H. tvaerminnensis*. *Daphnia longispina* served as the optionally present resident species and mediated the third factor, i.e. interspecific competition.

In the first two weeks of June 2006, water and *D. longispina* individuals of 10 different rock pools from the surrounding metacommunity were collected. We included rock pools that continuously harboured both *D. magna* and *D. longispina* over the past 10 years (Pajunen 1986, Pajunen & Pajunen 2003) and were sufficiently large not to suffer from the removal of about 150 l of water for the experiment. The water was filtered with 48 μm mesh size and filled into 10 sets of 24 plastic buckets of a volume of 10 l (referred to as mesocosms) to a filling level of 6 l each and installed at the shore of a skerry island in the vicinity of natural rock pools. Each set of 24 mesocosms was arranged in a grid containing water from the same rock pool. Half of the mesocosms was charged with random samples of 40 *D. longispina* individuals from the 10 corresponding rock pools each. The resident *D. longispina* populations were then given a full month to adjust to the mesocosm conditions and to grow to carrying capacity.

Outbred and inbred (inbreeding coefficient $F = 0.5$) *D. magna* females were obtained from crossings of clones originating from the metapopulation and from an ongoing long term outdoor mesocosm experiment (Altermatt & Ebert 2008a) whose clones originated from the same metapopulation as well. The females were brought to the lab, and as soon as they had produced clonal offspring, the mothers were checked for parasites. Infected clones were discarded. According to Altermatt & Ebert (2007), competitive ability of clones is not associated with their natural infection status, therefore we assumed that our clones are a random sample from the population with regard to competitive ability. Part of the clonal offspring of each uninfected mother was exposed to transmission stages of *H. tvaerminnensis* isolate mixtures randomly sampled from the *D. magna* metapopulation. The offspring was let grown to maturity and checked for successful infections after having given birth to clonal offspring. Clonal offspring of infected mothers was used for the experiment. Because vertical transmission to asexual offspring is likely to be perfect (Vizoso & Ebert 2004, Ebert et al. 2007), all further descendants of the animals were regarded to be infected.

In mid July, i.e. one month after the setup of the mesocosms, each of 30 outbred and 30 inbred *D. magna* clones were introduced into 4 mesocosms in two different infection states: either uninfected or infected with *H. tvaerminnensis* either into an empty mescosom or a mesocosm with a resident population of *D. longispina* (Fig. 2.1). We introduced 10 individuals per *D. magna* clone instead of single individuals to diminish stochastic effects.

In each grid, we had set up an additional replicate mesocosm with *D. longispina* populations to test whether invasion from rock pools in the vicinity of the experimental site would interfere with experimental invasion.

Ephippia are the only means by which *Daphnia* can survive the harsh winter in the metacommunity. The number of ephippia present at the end of the growing season is therefore a good estimator for the success of the invading and resident species. Consequently, the total ephippium production of each invading *D. magna* clone and resident *D. longispina* population was determined by counting in mid September. To take longer term success into account, we stored the ephippia suspended in rock pool water in open plastic tubes and overwintered them outdoors in a weatherproof box. In May 2007, the beginning of the next season, the ephippia were placed in mesocosms filled with filtered water from the corresponding source rock pools. We counted all emerging hatchlings for both *Daphnia* species. Number of ephippia and number of hatchlings were used as surrogate variables to assess invasion success.

Statistical analysis

The response variables "number of ephippia" and "number of hatchlings" were modelled by the factors inbreeding status (referred to as "inbreeding"), infection by *H. tvaerminnensis* ("parasite"), and interspecific competition by *D. longispina* ("competition") and assessed with generalised linear mixed effects models (GLMM). The three factors and the three corresponding two-way interactions were set as fixed, and meso-

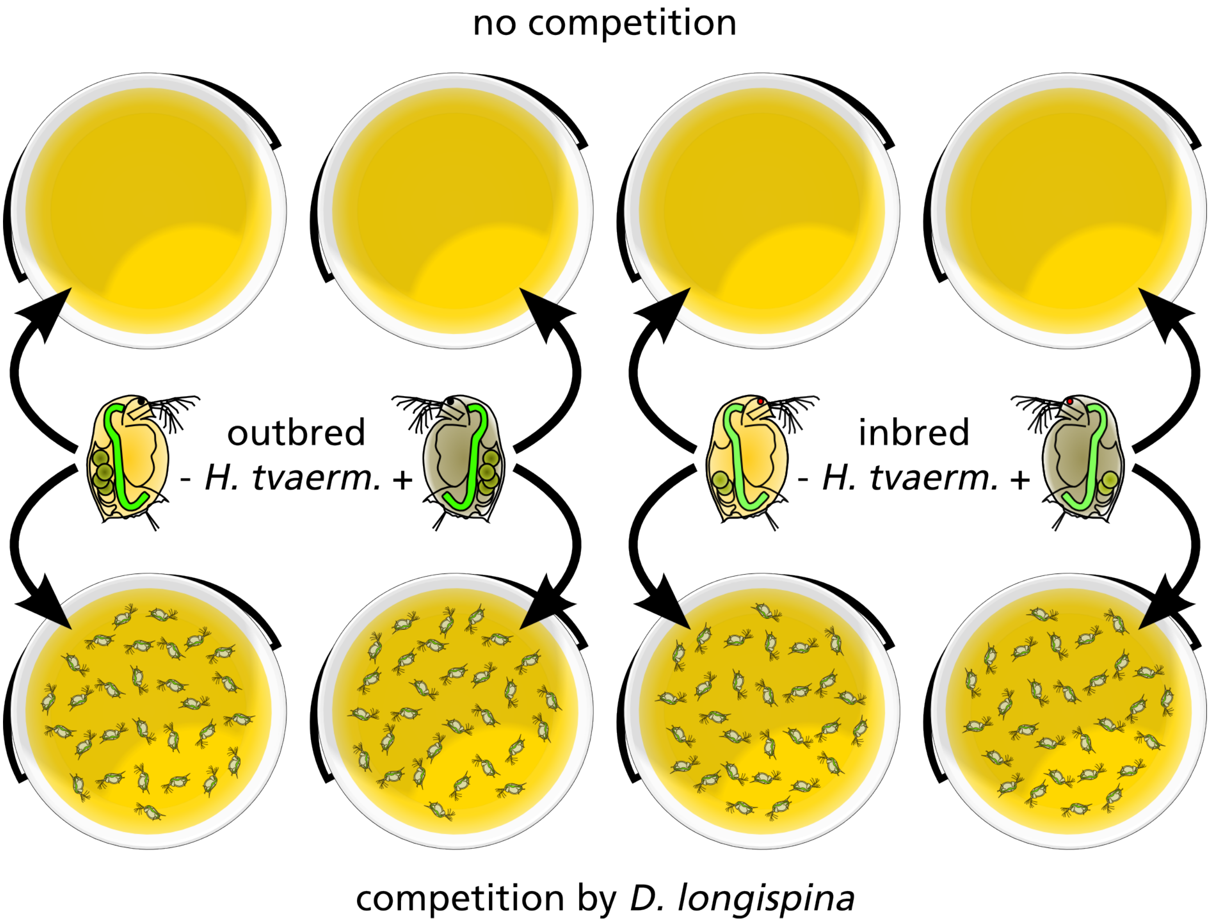

Figure 2.1 Smallest experimental unit of the full 3-factorial experiment. An outbred (two animals on the left) and an inbred (two animals on the right) *D. magna* clone, each of which either uninfected or infected with *H. tvaerminnensis*, were introduced into empty mesocosms (top) and mesocosms with resident *D. longispina* populations (bottom). Three such units of 8 mesocosms contained filtered water and *D. longispina* from the same rock pool and made up one grid. There were 10 grids with 24 mesocosms each. (*Daphnia magna* drawing courtesy of Dita B. Vizoso.)

cosm grid identity ("grid") and the nested *D. magna* clone identity ("clone") were included as random effects by modelling an individual intercept each. For the fixed effects, the baseline factor levels were defined as outbred, uninfected, and competition-free which were compared to the levels inbred, infected, and competition-faced. The response variables showed distributions with a strong positive skew. The traditional approach of modelling count data with a Poisson error distribution was not appropriate because of large overdispersion and an excess of zero values. Instead, we used models with a negative binomial error distribution and the (canonical) log link, which allows for independent modelling of mean and variance (Warton 2005). The values for the negative binomial parameter size parameter were estimated from the data. Since they were found to be very close to 1, we fixed them to 1, which resulted in no qualitative and negligible quantitative differences in the fitted models and allowed us to treat the models as GLMMs in the strict sense (Hilbe 2007).

A biologically meaningful simplification of the response variable "number of hatchlings" consists in a dichotomisation. Only experimental mesocosm populations that were able to produce at least one hatchling in the following season could persist since one single hatchling could found a population by clonal expansion. Translated to natural populations, this is the equivalence of persistence and extinction of local populations within a metapopulation. Therefore in an additional analysis, "number of hatchlings" was dichotomised to a binary variable representing either none or at least one hatchling. For the GLMMs, we used a binomial error distribution and the (canonical) logit link.

In two replicates, *D. magna* showed an infection with *H. tvaerminnensis* although the animals had not been infected at the start of the experiment. Since we performed the experiment under outdoor conditions, parasites could have invaded from neighbouring rock pools or mesocosms (compare Ebert et al. 2001). In order to follow the intention to treat principle, the replicates were not excluded from GLMM analyses which became consequently more conservative and at the same time easier to interpret because the data set was kept fully balanced. Excluding the two replicates did however not alter the results qualitatively.

We further analysed the number of *D. magna* hatchlings per produced ephippium. The ratio was found to be approximately normally distributed and modelled with a linear mixed effects model (LMM). Only replicates in which at least one ephippium and one hatchling had been produced were included in the analysis.

The GLMMs were fit using the Laplace approximation, the LMMs were fit using REML (restricted maximum likelihood). For all models, we used likelihood ratio tests to assess the significance of the interaction terms. Non-significant interaction terms were dropped, which resulted in the final model. For the remaining interactions and for the main effects whose corresponding factors were not involved in a significant interaction, we again used likelihood ratio tests to assess the significance of the individual model terms. For the main effects whose corresponding factors were involved in a significant interaction, we did not apply significance tests since an overall statement in such cases would be futile (e.g. Sokal & Rohlf 1995). Instead we constructed interaction plots based on the raw data which allow evaluating simple main effects, i.e. effects at specific factor level combinations which are not averaged over several factor levels. To assess the significance of simple main effects, we calculated likelihood ratio test statistics for models of subsets of the full data set for each individual level of the factors involved in significant interactions. Following the suggestions by Bolker et al. (2009), we assessed the model assumptions and found the sample sizes and the number of levels of the random effects to be sufficiently large for the chosen approach.

Model fitting and testing was done with R (R Development Core Team 2011, version 2.13.0) and package lme4 (Bates et al. 2008, version 0.999375-39). For the GLMMs with negative binomial error distribution, we used version 0.9975-13 of lme4. Plots were constructed with the package lattice (Sarkar 2008, version 0.19-26).

Results

Number of ephippia and hatchlings

The number of *D. magna* ephippia produced was overall consistently higher in outbred than in inbred, higher in uninfected than in infected, and higher in competition-free than in interspecific competition-faced clones (Fig. 2.2 top left, Table 2.1a). The interaction of inbreeding status with interspecific competition was marginally significant: outbred uninfected and inbred infected *D. magna* showed a decrease in ephippium production under interspecific competition which was stronger than under purely additive conditions (Fig. 2.3 top left, Table 2.1a). Infection status was the only factor not involved in a significant interaction and is therefore strictly speaking the single interpretable main effect in the model (Sokal & Rohlf 1995). It affected the response significantly by reducing the ephippium production of infected *D. magna* clones. Both random effects, mesocosm grid and *D. magna* clone identity, had large highly significant variance estimates. For the number of hatchlings, the situation was qualitatively the same while quantitatively all effects mentioned for the number of ephippia gained substantially in effect size and in significance (Fig. 2.2 top right, Fig. 2.3 top right, Table 2.1b).

The reverse of the pattern for *D. magna* was found for the number of ephippia of resident *D. longispina* clones which was significantly higher when faced with inter-

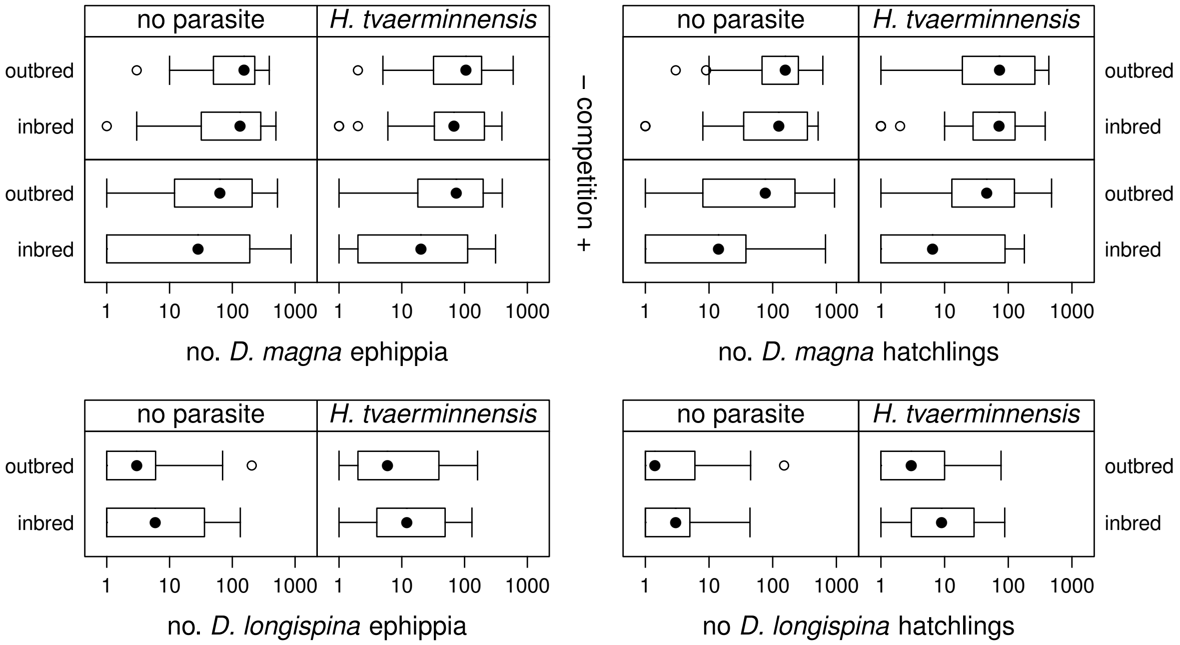

Figure 2.2 Number of ephippia and following-year hatchlings of *D. magna* (invader, top) and *D. longispina* (resident, bottom) cross-classified according to inbreeding status, infection status, and, in the case of *D. magna*, the presence of the interspecific competitor (the count variables have been transformed by $\log_{10}(n+1)$). Each box plot represents 30 data points. The black circle is the median, the box represents the interquartile range, the whiskers indicate the smallest and largest value not lower or higher than 1.5 times the interquartile range from the first or third quartile, and the open circles lie more than 1.5 times the interquartile range from the first or third quartile.

specific competition by *D. magna* clones that were either inbred or infected (Fig. 2.2 bottom left, Table 2.2a). There was no significant interaction between the two factors (Fig. 2.3 bottom left). The results for the number of hatchlings were largely but not entirely consistent with the results for the ephippium production (Fig. 2.2 bottom right, Table 2.2b). A difference was due to the interaction which states that *D. longispina* hatchling numbers differed only slightly in competition with outbred *D. magna* clones but diverged drastically in competition with inbred *D. magna* clones, with hatchling numbers having decreased slightly when the competitor was uninfected and increased substantially when the competitor was infected (Fig. 2.3). In relation to the fixed effect sizes, the random effects for both *D. longispina* response variables were larger than in the corresponding comparison of fixed and random effects of the *D. magna* response variables and were in all cases highly significant (Table 2.2).

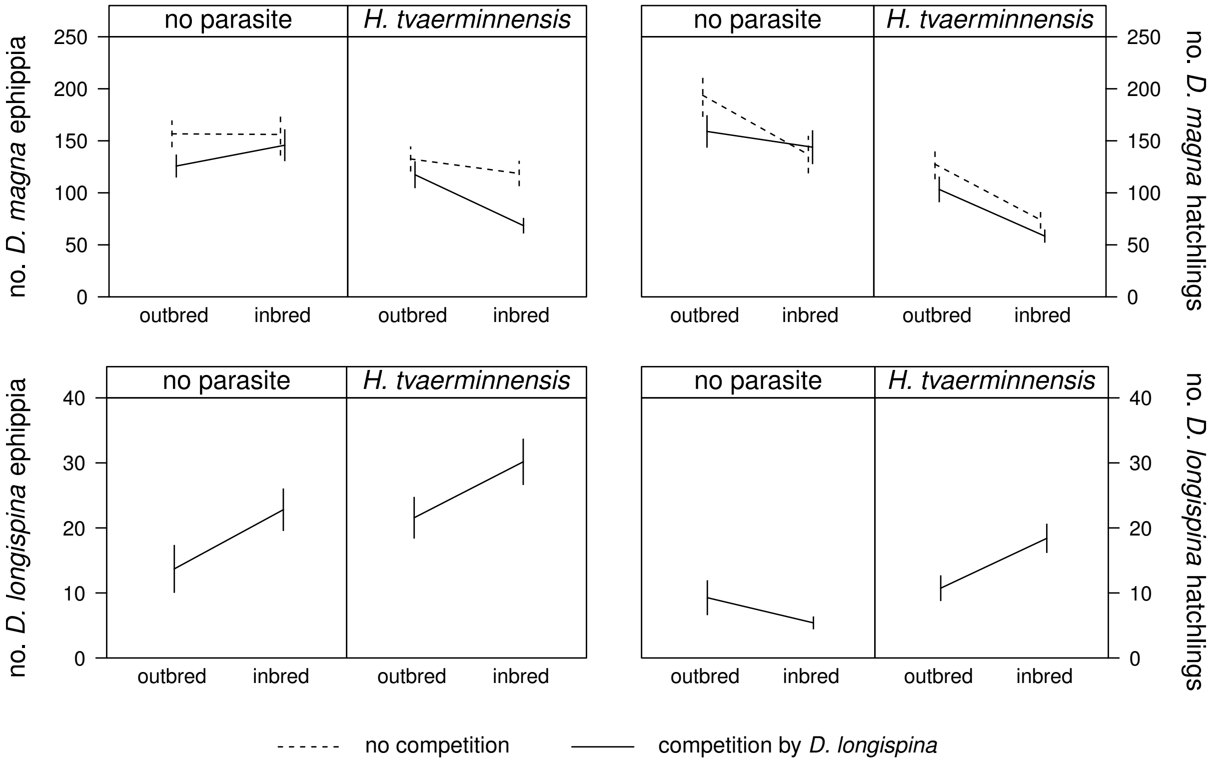

Figure 2.3 Interaction plots for number of *D. magna* ephippia (top left), hatchlings (top right), *D. longispina* ephippia (bottom left), and hatchlings (bottom right). The levels of the factor inbreeding form the *x* axis, the levels of the factor interspecific competition form the traces (lines). The plotted data are conditioned to the two levels of the factor parasitism. Each data point is therefore depicted only once, i.e. the displayed means ± SE represent values of unique factor level combinations and are not averaged over other factors.

Dichotomised number of hatchlings

For an asexual *Daphnia* population to build up, one single reproductive female is enough. Thus, the most stringent criterion to judge population persistence is to test for at least one hatchling. For the GLMM analyses of dichotomised number of hatchlings, no significant two-way interaction was found, possibly because a lot of power was lost due to discarding information during the process of dichotomisation (Fig. 2.4). For *D. magna* clones, inbreeding status and interspecific competition had a highly significant effect on persistence whereas infection by *H. tvaerminnensis* did not show a significant effect (Fig. 2.4 top, Table 2.3a). In contrast, persistence of *D. longispina* due to the occurrence of at least one hatchling was significantly influenced by both inbreeding and infection status of *D. magna* (Fig. 2.4 bottom, Table 2.3b). For both *D. magna* and *D. longispina*, the variance estimate of the grid effect was rather large and significant while the clone effect was negligible.

Of the 10 additional replicates with only *D. longispina* being present, 10 produced ephippia and 9 showed hatchlings in the following year. The one replicate that did not have hatchlings in the following year suffered from a severe infection by the microsporidian parasite *Larssonia obtusa*, which caused a total breakdown of the population at the start of the experiment.

Number of hatchlings per ephippium

The ratio of the two response variables, number of hatchlings to number of ephippia, is shown in Fig. 2.5. The interaction between inbreeding status and interspecific competition, which was also significant in the analyses for the single response variables number of ephippia and number of hatchlings, had a large impact: For *D. magna*, the number of hatchlings per ephippium was about the same for outbred clones regardless of interspecific competition, but for inbred clones, it stayed about the same under interspecific competition while it decreased under competition-free conditions (Table 2.4). Infection by *H. tvaerminnensis*, the single interpretable main effect, decreased the ratio highly significantly below unity. Unlike in the analyses for the single response variables, the random effects were relatively small whereas the clone effect was substantially larger than the grid effect.

Discussion

Our study shows that inbreeding, parasitism, and interspecific competition can decrease invasion success of *D. magna* substantially. Inbreeding and interspecific competition not only do so on their own but also in combination, which leads to an additional

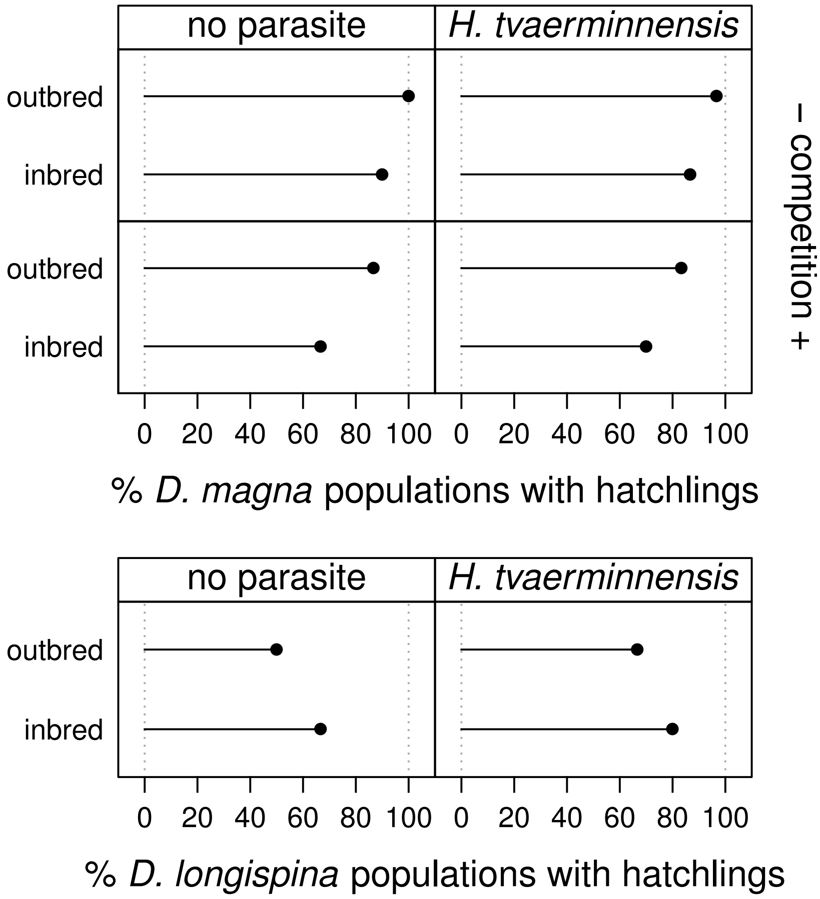

Figure 2.4 Percentage of mesocosms in which at least one hatchling was found in the following year. The percentage corresponds to the proportion of the dichotomised number of hatchlings displayed in Fig. 2.2. 100 % correspond to 30 populations.

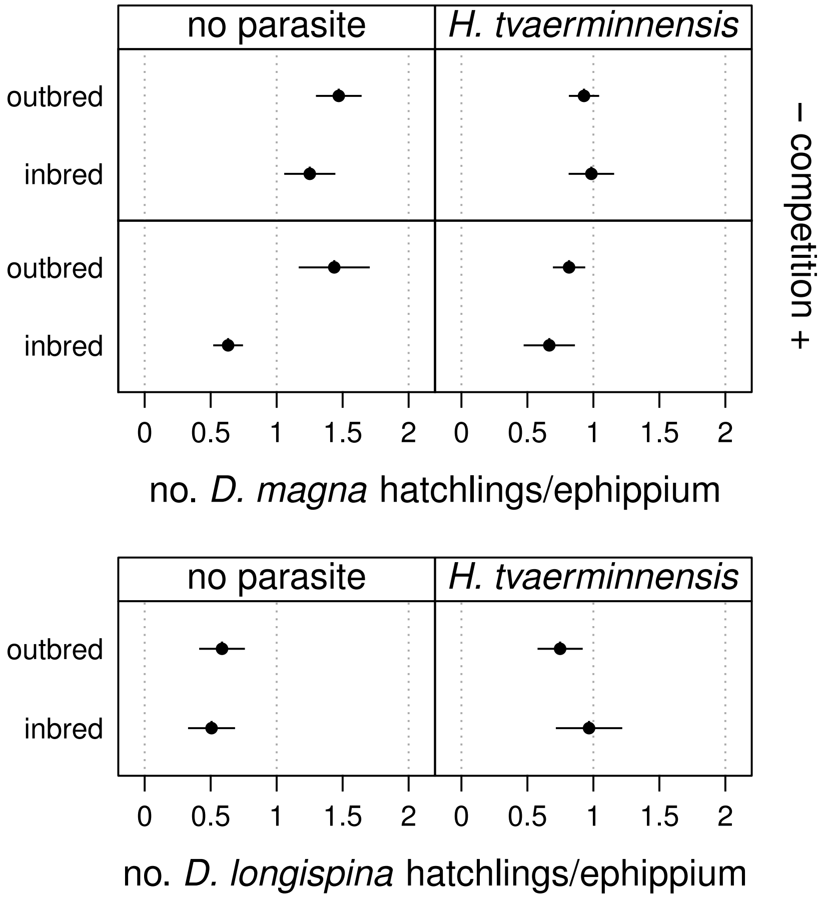

Figure 2.5 Number of *Daphnia* hatchlings per ephippium in the year following the competition experiment (mean ± SE). The dotted vertical lines at 0 and 2 hatchlings per ephippium correspond to the theoretical minimum and maximum given that ephippia usually contain maximally two eggs (very rarely they contain three eggs).

Table 2.1 Results of generalised linear mixed model (GLMM) analyses of number of *D. magna* ephippia and hatchlings as a function of *D. magna* inbreeding status, infection status, competition by resident *D. longispina*, and the interaction of inbreeding status and competition. Grid and *D. magna* clone identity were included as random effects. The errors were modelled according to a negative binomial distribution with size parameter 1 (estimates were $\hat{\theta} = 1.02$ and $\hat{\theta} = 0.90$ for ephippia and hatchlings respectively).
† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$

Variable	Estimate		LR test statistic	
a) no. *D. magna* ephippia				
intercept	risk	140		
inbreeding	relative risk	0.780	$\chi^2_{(1)} = 0.0889$	(no competition)
			$\chi^2_{(1)} = 0.410$	(competition by *D. longispina*)
parasite	relative risk	0.708	$\chi^2_{(1)} = 5.84$ *	
competition	relative risk	0.704	$\chi^2_{(1)} = 1.84$	(outbred)
			$\chi^2_{(1)} = 2.25$	(inbred)
inbreeding × competition	ratio of relative risks	0.571	$\chi^2_{(1)} = 3.71$ †	
grid	variance	0.882	$\chi^2_{(1)} = 23.4$ ****	
clone(grid)	variance	0.565	$\chi^2_{(1)} = 23.7$ ****	
$n = 240$				
b) no. *D. magna* hatchlings				
intercept	risk	149		
inbreeding	relative risk	0.781	$\chi^2_{(1)} = 1.11$	(no competition)
			$\chi^2_{(1)} = 4.26$ *	(competition by *D. longispina*)
parasite	relative risk	0.527	$\chi^2_{(1)} = 19.0$ ****	
competition	relative risk	0.602	$\chi^2_{(1)} = 1.54$	(outbred)
			$\chi^2_{(1)} = 8.82$ **	(inbred)
inbreeding × competition	ratio of relative risks	0.392	$\chi^2_{(1)} = 9.63$ **	
grid	variance	1.37	$\chi^2_{(1)} = 24.5$ ****	
clone(grid)	variance	0.982	$\chi^2_{(1)} = 54.0$ ****	
$n = 240$				

Table 2.2 Results of generalised linear mixed model (GLMM) analyses of number of *D. longispina* ephippia and hatchlings as a function of *D. magna* inbreeding status, infection status, and, in the case of hatchlings, the interaction thereof. Grid and *D. magna* clone identity were included as random effects. The errors were modelled according to a negative binomial distribution with size parameter 1 (estimates were $\hat{\theta} = 1.07$ and $\hat{\theta} = 1.04$ for ephippia and hatchlings respectively).
* $p < 0.05$, **** $p < 0.0001$

Variable	Estimate		LR test statistic	
a) no. *D. longispina* ephippia				
intercept	risk	1.89		
inbreeding	relative risk	1.96	$\chi^2_{(1)} = 3.88$ *	
parasite	relative risk	2.45	$\chi^2_{(1)} = 24.2$ ****	
grid	variance	1.84	$\chi^2_{(1)} = 12.4$ ****	
clone(grid)	variance	2.57	$\chi^2_{(1)} = 99.8$ ****	
$n = 120$				
b) no. *D. longispina* hatchlings				
intercept	risk	1.01		
inbreeding	relative risk	1.22	$\chi^2_{(1)} = 0.567$	(no parasite)
			$\chi^2_{(1)} = 1.52$	(*H. tvaerminnensis*)
parasite	relative risk	2.14	$\chi^2_{(1)} = 0.0631$	(outbred)
			$\chi^2_{(1)} = 6.43$ *	(inbred)
inbreeding × parasite	ratio of relative risks	2.13	$\chi^2_{(1)} = 3.85$ *	
grid	variance	1.98	$\chi^2_{(1)} = 20.9$ ****	
clone(grid)	variance	1.42	$\chi^2_{(1)} = 38.6$ ****	
$n = 120$				

Table 2.3 Results of generalised linear mixed model (GLMM) analyses of the dichotomised number of *D. magna* and *D. longispina* hatchlings as a function of *D. magna* inbreeding status, infection status, and competition by resident *D. longispina*. Grid and *D. magna* clone identity were included as random effects. The errors were modelled according to a binomial distribution. † < 0.1, ** $p < 0.01$, **** $p < 0.0001$

Variable	Estimate		LR test statistic
a) *D. magna* persistence			
intercept	odds	780	
inbreeding	odds ratio	0.119	$\chi^2_{(1)} = 9.40$ **
parasite	odds ratio	0.764	$\chi^2_{(1)} = 0.27$
competition	odds ratio	0.064	$\chi^2_{(1)} = 23.1$ ****
grid	variance	7.48	$\chi^2_{(1)} = 21.2$ ****
clone(grid)	variance	1.21	$\chi^2_{(1)} = 2.13$
$n = 240$			
b) *D. longispina* persistence			
intercept	odds	1.01	
inbreeding	odds ratio	2.28	$\chi^2_{(1)} = 3.67$ †
parasite	odds ratio	2.28	$\chi^2_{(1)} = 3.67$ †
grid	variance	0.971	$\chi^2_{(1)} = 8.14$ **
clone(grid)	variance	0.000	$\chi^2_{(1)} = 0.00$
$n = 120$			

non-additive reduction of *D. magna* invasion success. The findings have strong implications for invasion dynamics in metacommunities, not least because results of outdoor mesocosm experiments are likely to be applicable to natural rock pools.

Mesocosm experiments with rock pool *Daphnia* and their parasites have become an established method to assess fitness of clones and species in the light of a variety of factors of interest, in particular the herein studied factors inbreeding (e.g. Haag et al. 2002, Ebert et al. 2007), parasitism (e.g. Haag & Ebert 2004, Lass & Ebert 2006, Altermatt et al. 2007, Altermatt & Ebert 2008a), and interspecific competition (e.g. Bengtsson 1989, 1993). In previous studies, competitive ability of a clone or species was assessed by placing competitors at the same moment into the mesocosms so that competition started immediately. Our experimental setup differed decisively by the addition of a defined delay before and after which the first and second competitor were introduced into the mesocosms. In this way, one competitor could first establish itself and thereby possibly alter the environmental conditions of the experimental locality e.g. via allelopathic compounds (Seitz 1984, Matveev 1993) or a change in the microbial community (Degans et al. 2002). This change of experimental procedure, contrasting with earlier studies, might have had a profound impact on our estimate of

Table 2.4 Results of linear mixed model (LMM) analysis of number of *D. magna* hatchlings per ephippium as a function of *D. magna* inbreeding status, infection status, competition by resident *D. longispina* and the interaction of inbreeding status and competition. Grid and *D. magna* clone identity were included as random effects. Ratios equal to 0 or larger than 3 (not feasible) were excluded.
† $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Variable	Estimate		LR test statistic	
no. *D. magna* hatchlings/ephippium				
intercept		1.27		
inbreeding	difference	−0.080	$\chi^2_{(1)} = 0.546$	(no competition)
			$\chi^2_{(1)} = 10.2$ **	(competition by *D. longispina*)
parasite	difference	−0.290	$\chi^2_{(1)} = 15.5$ ****	
competition	difference	−0.068	$\chi^2_{(1)} = 0.355$	(outbred)
			$\chi^2_{(1)} = 10.9$ ***	(inbred)
inbreeding × competition	difference of differences	−0.265	$\chi^2_{(1)} = 3.31$ †	
grid	variance	0.002	$\chi^2_{(1)} = 0.000$	
clone(grid)	variance	0.243	$\chi^2_{(1)} = 14.3$ ***	
$n = 193$				

the invasion success of *D. magna*, or equivalently the invasibility of mesocosms, and how it was mediated by inbreeding, parasitism, and interspecific competition.

Ebert et al. (2002) and Haag et al. (2002) demonstrated that local inbreeding is the norm rather than the exception in the *D. magna* metapopulations of the skerry island coast of the Baltic Sea and that outbred offspring of local and immigrant parents have tremendously increased fitness relative to local inbred offspring. In our experiment, outbred *D. magna* clones were not only superior producers of ephippia and especially hatchlings, therefore having an advantage over inbred clones in their capabilities to invade mesocosms (Table 2.1), they also ended up with a higher proportion of experimental mesocosm populations that were able to persist through the winter (Table 2.3a). While inbreeding has been extensively studied as a determinant of extinction of local populations (Lande 1988, 1994, Hedrick 1994, Frankham 1995, Lynch et al. 1995, Newman & Pilson 1997, Frankham 1998, Saccheri et al. 1998, Frankham et al. 2004), we here provide persuasive empirical evidence that it also reduces invasion success. Localities could hence be preferentially colonised by migrants with low inbreeding coefficients in metacommunities in general and in *Daphnia* rock pool metacommunities in particular and potentially alleviate consequences of founder events and genetic differentiation of local populations (Haag et al. 2006, 2005).

The bulk of literature that deals with the influence of parasites on invasion success has been concerned with the problem of intrusion of non-indigenous host species into native communities (for a review see Colautti et al. 2004). Considering parasite-mediated invasion as a general phenomenon, *H. tvaerminnensis* could be both a modifier of invasion success as a hitchhiker in the ephippia of invaders and a modifier of invasibility as a member of the community itself. Testing the first case, we found a strong negative impact of *H. tvaerminnensis* on invasion success over the course of the whole season (Fig. 2.2, Table 2.1). Furthermore, the measurement of ephippium and hatchling production may be more comprehensive and integrative measures of fitness than the instantaneous changes in pelagic densities as used in Altermatt et al. (2007) and many other *Daphnia* mesocosm experiments. In a metacommunity context, localities might therefore predominantly be invaded by uninfected *D. magna* individuals, which is compatible with the observation that only few of the newly founded local *D. magna* populations in the Tvärminne *D. magna* metapopulation harbour *H. tvaerminnensis* while in older populations about 80 % of all populations are infected (Ebert et al. 2001). Thus in analogy to the enemy release hypothesis of invasion ecology in which invasive species are thought to have an advantage over resident species because the invader may be resistant to the local parasites and does not bring its own parasites along (Williamson 1996), we suggest that differential invasion success of infected and uninfected invaders could lead to a release from parasites.

Interspecific competition by *D. longispina* reduced invasion success by *D. magna* in a consistent way (Fig. 2.2, 2.3, 2.4, Table 2.1, 2.3a). Unlike inbreeding and parasitism, competition by a resident species as a characteristic influencing invasion success is attributable to the local community. Such competitive species interactions have traditionally been considered as the main drivers of metacommunity dynamics (Chase et al. 2005), though other types of species interactions as for instance food webs (e.g. Holt & Hoopes 2005), mutualism (e.g. Amarasekare 2004), or parasitism (e.g. Laine & Hanski 2006) gain more and more attention. For the skerry island *Daphnia* rock pool metacommunity, it has been hypothesised that *D. magna* is the best coloniser but the worst competitor (Hanski & Ranta 1983). There is ample support for the best coloniser statement (e.g. Pajunen & Pajunen 2003, Altermatt et al. 2008) but contrary evidence for the worst competitor statement (e.g. Bengtsson 1989). We repeatedly found *D. magna* to be the superior interspecific competitor in outdoor mesocosm experiments, be it in the present study or further assessments of interspecific competitiveness of the three autochtonous *Daphnia* species (Zumbrunn and Ebert, unpublished data). For species coexistence in *Daphnia* rock pool metacommunities, a trade-off between competitive and dispersal ability as it is postulated for our (Hanski & Ranta 1983) but also for other types of metacommunities (Kneitel & Chase 2004, Calcagno et al. 2006) is therefore too simplistic an explanation, although for other rock pool metacommunities this trade-off seems to apply (Pajunen 1979). The species sorting perspective on metacommunities stresses spatial niche separation, and thus for regional coexistence of species environmental heterogeneity of localities is a requirement (Leibold et al. 2004), which certainly applies to the studied *Daphnia* rock pool metacommunity (Pajunen & Pajunen 2007). Like natural rock pools, our experimental mesocosms varied substantially with respect to the origin and composition of rock pool water and resident *D. longispina*. But even though these experimental conditions seemed to have been much more favourable for *D. magna*, the experimental verdict that interspecific competition by *D. longispina* leads to relatively lower invasion success of *D. magna* was clear. Thus the implications for metacommunity dynamics are in congruence with the expectation that the presence of resident competitors decreases invasibility of local communities, which for *Daphnia* metacommunities might supersede the opposite mechanism of increasing local extinction rates caused by interspecific competition (Bengtsson 1989) although an earlier study in a similar *Daphnia* rock pool metacommunity did not find an influence of interspecific competition on colonisation rates (Bengtsson 1991).

The partly synergistic and partly antagonistic effects of inbreeding and interspecific competition became apparent for the number of *D. magna* ephippia and even more so for the number of hatchlings (Table 2.1, Fig. 2.3). In experimental *D. magna* populations, Haag et al. (2002) showed that inbreeding depression is high in competition with outbred clones but low in the absence of intraspecific competition. Such findings cannot easily be extrapolated to communities since invasion success of inbred *D. magna* clones, albeit overall lower than of outbred clones, was not necessarily reduced more and in some situations even less than in outbred clones when interspecific competition was effective (Fig. 2.3). The particular outcome of the interaction depended furthermore on the status of infection of *D. magna* which underlines the initially postulated necessity to study characteristics of the community and characteristics of the invader in combination (Kolar & Lodge 2001). Overall, inbred *D. magna* invaders facing competition by *D. longispina* are thus suggested to have an invasion success which is overproportionally lower than expected by adding the separate effects of inbreeding and interspecific competition.

Even though we focused on the invasion success of *D. magna*, the performance of the resident competitor *D. longispina* provides valuable insights that either corroborate or relativise the findings for *D. magna*. Given that *Daphnia* are generalist grazers of phytoplankton, they interact with each other mainly via exploitation competition (Tessier et al. 2000). If resource utilisation was perfect, one would expect the consumptions of the two species to complement each other. In line with this view, resident *D. longispina* populations produced more ephippia when they competed either with inbred or infected *D. magna* invaders (Fig. 2.2, Table 2.2a). Yet the effects were not simply amplified in the number of hatchlings (Table 2.2b) as was the case for *D. magna*. *Daphnia longispina* performed equally well when faced with an outbred invader regardless of its infection status whereas performance diverged when the invader was infected with *H. tvaerminnensis* (Fig. 2.3). A possible explanation could be that inbred parasitised *D. magna* populations have an altered age structure, and because differently aged individuals supposedly consume differently sized phytoplankton, the food regime may be altered in favour of *D. longispina*. However, resource utilisation of *D. magna* and *D. longispina* indeed seemed to complement each other on average, also with regard to the dichotomised number of hatchlings (Fig. 2.4, Table 2.3).

The reduction of *D. magna* invasion success by inbreeding and parasitism could partially resolve the paradox of the plankton as originally described by Hutchinson (1961) which is concerned with the often observed large diversity of planktonic organisms that a limited pool of resources may sustain. According to the size efficiency hypothesis, larger planktonic grazer species should outcompete smaller competitors under low predation pressure due to their higher resource uptake efficiency, whereas small competitors should dominate under high predation pressure due to predator evasion (Brooks & Dodson 1965). In rock pools, predation pressure on *D. magna* is supposed to be low and consequently the larger bodied *D. magna* should outcompete the smaller congeneric species. The paradox of the plankton is generally considered for large water bodies such as lakes or oceans and is commonly explained with environmental and spatiotemporal heterogeneity (e.g. Descamps-Julien & Gonzalez 2005, Miyazaki et al. 2006) with the consequence that habitat conditions never reach an equilibrium favouring a single species (Scheffer et al. 2003). Nonetheless, the same could apply for *Daphnia* rock pool communities where invasion success of the putatively superior competitor *D. magna* is affected by its inbreeding and infection status as shown by our experiment, which both contribute to environmental and spatiotemporal variability between localities and thus could mediate regional coexistence (Ranta 1979, Pajunen & Pajunen 2007).

The microsporidian parasite *H. tvaerminnensis* was clearly the strongest facilitator for *D. longispina*, making it appear like a biological warfare agent. In apparent competition, interspecific competition is mediated by a shared parasite (Bonsall & Hassell 1997, Hudson & Greenman 1998, Tompkins et al. 2000, 2003). Whereas the 10 *D. longispina* population replicates without invaders performed better than the *D. longispina* populations affected by invading *D. magna* clones, *D. longispina* populations invaded by infected *D. magna* clones showed a significantly higher competitive ability compared to populations invaded by uninfected *D. magna* clones. The parasite *H. tvaerminnensis* is specific to *D. magna* and thus not shared between the two competing *Daphnia* species, but by relaxing this assumption of apparent competition, we were able to demonstrate a more general form of parasite-mediated interspecific competition.

Eventually, although we treated the random effects in our study as nuisance parameters rather than variables of interest, they allow us to generalise conclusions about the estimated fixed effects with higher confidence to new situations (Bolker et al. 2009), e.g. future experiments with water and *D. longispina* populations from different rock pools and different *D. magna* clones. The invasion success of *D. magna* and persistence success of *D. longispina* varied considerably between mesocosm grids, likely because the rock pools from which the filtered water and the *D. longispina* originated differed in quality and quantity of resources. Furthermore, the *D. longispina* populations may have differed in quality, e.g. in their degree of inbreeding. The high dissimilarity of localities is a major prerequisite of the species sorting and mass effects perspectives of metacommunities, but at the same time a criterion for exclusion for the patch dynamics and neutral models (Leibold et al. 2004). In other words, the large significant variance estimates of the grid factor and thus the origin of the mesocosm water and *D. longispina* emphasise that the described effects of inbreeding, parasitism, and competition work over a wide range of conditions.

The factor dependent distributions of the original (Fig. 2.2) as well as the dichotomised (Fig. 2.4) responses of *D. magna* suggest that there was a pronunciation of effect strengths in the transition from the number of ephippia to the number of hatchlings in the next year. Analysis of the ratio of the number of hatchlings per ephippium (Fig. 2.5) confirmed this presumption with the same significant fixed effects as for the individual count variables (Table 2.4). The pronunciation of the inbreeding effect is particularly worth mentioning since all hatchlings are the result of an additional round of inbreeding which one might expect to align the offspring of outbred and inbred parental clones. All *D. magna* populations were monoclonal and thus all hatchlings resulted from selfing. However single clone invasions likely account for the majority of invasion events in natural rock pools (Haag et al. 2006), and therefore subsequent selfing is not an unwanted feature of the experiment. This further underlines the suspected alleviation of consequences of founder events and genetic differentiation of local populations (Haag et al. 2006, 2005) by higher colonisation success of clones with lower inbreeding coefficients. In stark contrast to the fixed effects, the variance estimate for grid tended towards zero whereas the variance estimate for *D. magna* clone was two orders of magnitude higher, suggesting that the number of hatchlings that emerge from an ephippium depends almost entirely on the identity of the ephippium's mother but not on the locality in which the ephippium was produced, overwintered, and incubated for hatching. The pronunciation of effects might have been mediated by differential egg laying, egg mortality, or hatching success, all of which might have the potential to alter longer term invasion success of *D. magna*.

The dichotomous variable indicating whether at least one offspring of the invader could hatch in the season following the experiment may be regarded as a better predictor of long term persistence (Fig. 2.4, Table 2.3). While the binary outcome permits a simplified and therefore easier to grasp view of the data set, one is tempted to overstate its meaning since the probability of long term persistence still depends on the absolute number of hatchlings and is not guaranteed with the hatching of a single individual. If a single hatchling's chances to reproduce are high, then the overall probability of establishment quickly asymptotically reaches unity as a function of the number of hatchlings present, whereas if an individual's chances are dismal, the overall probability of establishment increases almost linearly with the number of hatchlings. Predators, diseases, or segregation of infectivity genes (Haag et al. 2003) can all reduce the chances of hatchlings to reproduce.

We illustrated that inbreeding and infection of invading *D. magna* as well as interspecific competition by *D. longispina* from the local resident community all lead to differential invasion success. Invasion events in the narrow sense between one and another locality are presumably very rare compared to invasion events within localities of the metacommunity. The fitness gain of an individual whose sexual offspring manage to disperse and invade a new locality is potentially immense (Ebert et al. 2002), hence differential invasion success would result in significant differential reproductive success in spite of the rarity of invasion events. We therefore suggest that the three studied factors exert selective pressures on *D. magna* which in turn could entail adaptation. More specifically, our results suggest that inbred and parasitised *D. magna* suffer a cost which was previously not incorporated into concepts of inbreeding depression and virulence. Thus we speculate that on a metacommunity level, selection might act against inbreeding and parasitism. The demonstrated local changes in invasion dynamics have implications for regional metacommunity dynamics and ultimately even metaecosystem dynamics (Loreau et al. 2003). Levin (1998) described communities as complex adaptive systems, and our *Daphnia* metacommunity is no exception, still awaiting a comprehensive theoretical and empirical synthesis.

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Chapter 3

Correlation and transitivity of interspecific competitive abilities in rock pool *Daphnia*

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Abstract

Coexistence of species with largely overlapping niches in communities is codetermined by the hierarchy of interspecific competitive abilities. Based on the size efficiency hypothesis, we propose that in planktonic microcrustacean communities whose members predominantly interact via exploitation competition, (1) a species' competitive abilities against one species correlate positively with the species' competitive abilities against another species and (2) competitive abilities are transitive between different species. The hypotheses were tested with two outdoor mesocosm competition experiments with three species of *Daphnia* naturally co-occurring in a rock pool metacommunity in southwest Finland. First, the competitive abilities of 40 *D. magna* clones were tested in competition with reference clones of the two congeneric species *D. longispina* and *D. pulex*. Second, clones of all three species competed in all pairwise combinations. To assess interspecific competition, we quantified each clone's resting stage production because this is an integrative measure across seasons as opposed to temporary measurements of pelagic densities. The results suggest that within the focal species *D. magna*, the competitive abilities against *D. longispina* indeed strongly positively correlate with the competitive abilities against *D. pulex*. Moreover, interspecific competition between the three species was found to be transitive with *D. magna* as the superior, *D. pulex* as the intermediate, and *D. longispina* as the inferior competitor. The findings do probably not explain the assembly of a *Daphnia* rock pool metacommunity over the full range of environmental conditions but indicate that the outcome of interspecific competition under given conditions can be surprisingly predictable. Conversely, we suggest that the deviations from the general competitive hierarchy in terms of non-linear associations of interspecific competitive abilities and intransitive competitive interactions may be crucial for the maintenance of species coexistence.

Key words interspecific competition, intraspecific competition, size efficiency hypothesis, paradox of the plankton, sexual reproduction, asexual reproduction, metacommunity, mesocosm, *Daphnia longispina*, *Daphnia magna*, *Daphnia pulex*

Introduction

A striking discrepancy between theoretical considerations on the competitive exclusion principle as formulated by Gause (1932) and actually observed patterns of species coexistence is still hotly debated almost half a century after it has been raised as the "paradox of the plankton" (Hutchinson 1961). In its broad sense, the paradox describes the high species diversity observed in planktonic communities despite the species' similar requisites for resources and apparently strong exploitative competition for these resources (Tilman 1982). Though originally aimed at phytoplankton (Hutchinson 1961), the paradox equally applies to zooplankton communities and in particular to communities of cladocerans. Communities of cladocerans are known as one of the best examples of species coexistence in spite of extreme niche overlap in which species diversity is thought to be mediated by temporal heterogeneity similarly as in phytoplankton communities (Hebert 1982). Whereas there are examples of niche differentiation by resource partitioning (Demott & Kerfoot 1982), the majority of laboratory and field experiments on competitive abilities of cladocerans is supportive for the size efficiency hypothesis (Bengtsson 1987). The hypothesis predicts larger animals to be more efficient in food uptake rates and consequently to be superior in exploitative competition in the absence of predation (Brooks & Dodson 1965). In line with the hypothesis, larger daphnid species were found to have a more favourable ratio of assimilation to respiration (Gliwicz 1990) and to suppress algal concentrations they fed on to lower levels, which coincided with a higher competitive ability (Kreutzer & Lampert 1999). Besides exploitation competition, there is growing evidence for interference competition via allelopathic compounds (Seitz 1984, Goser & Ratte 1994), but at any rate, larger cladocerans are commonly found to be the stronger competitors (reviewed in Bengtsson 1987).

In spatially structured habitats, a higher number of species can be supported (Tilman 1994). Similarly, coexistence may be enabled in metacommunities where local communities of competing species are connected by dispersal (Amarasekare et al. 2004). According to Holyoak et al. (2005), a metacommunity is easiest to conceptualise when all interacting species utilise the same habitat patches, these habitat patches have discrete boundaries, and the local populations use resources at the same within-patch scale. Rock pool metacommunities of freshwater *Daphnia* species along the skerry island coast of the Baltic Sea (Bengtsson 1986, Pajunen 1986) fulfil these properties well (Ranta 1979). At the same time, the paradox of the plankton is apparent in local *Daphnia* rock pool communities (Bengtsson 1987). Experiments with three differently sized *Daphnia* species co-occurring in these rock pools and having largely overlapping niches (Ranta 1979) revealed inconsistent patterns of interspecific competition (Bengtsson 1986, 1989, 1993), partly in favour of the size efficiency hypothesis, partly in favour of its opposite, the "small body size hypothesis" (Hanski & Ranta 1983), and partly in favour of alternative explanations (reviewed in Bengtsson 1987). Referring to the paradox of the plankton as a back door and resorting to environmental heterogeneity as an explanation for species coexistence (Bengtsson 1987) is however unsatisfactory as no stringent answers are provided.

Detailed knowledge of the hierarchy of competitive abilities might help to better understand the mechanisms underlying interspecific competition (Tilman 1987), also in the case of *Daphnia* rock pool communities. Large animals need not necessarily be better competitors because of higher feeding efficiency since smaller animals need less resources and size can become a disadvantage in some situations (Persson 1985). Still the size efficiency hypothesis is comparatively well supported experimentally for assemblages of *Daphnia* species (Goulden et al. 1982, Bengtsson 1989, Gliwicz 1990, Kreutzer & Lampert 1999). Whereas transitive competition hierarchies are potentially widespread in plant communities (Keddy & Shipley 1989, Weiner 1990, Shipley 1993), there are numerous examples of intransitive interspecific competition networks in animal communities (Buss & Jackson 1979, Sinervo & Lively 1996). Analogous to the rock-paper-scissors game where no strategy is able to always succeed, intransitive competition can contribute to the maintenance of diversity (Frean 2001), and theory predicts that even modest levels of intransitivity promote coexistence of competing species (Laird & Schamp 2006).

Incomplete transitivity in interspecific competition could also mediate coexistence between the three *Daphnia* species of Baltic Sea rock pool metacommunities (Pajunen 1986, Pajunen & Pajunen 2003). We therefore studied the interspecific competition hierarchy in a set of clones of the three *Daphnia* species in outdoor mesocosm experiments in a twofold approach: first, we assessed the interspecific competitive ability of our focal species *D. magna* in competition with the other two *Daphnia* species, and second, we assessed the interspecific competitive ability of clones of all three species in competition with each other. Based on the size efficiency hypothesis (Brooks & Dodson 1965), we expected *D. magna*, the species with the largest body size, to dominate competition in the first as well as the second assessment. In the first experiment, a higher competitive ability of a *D. magna* clone against a *D. longispina* clone should consequently be associated with a higher competitive ability of the same *D. magna* clone against a *D. pulex* clone. This implies a positive correlation between the interspecific competitive abilities against *D. longispina* and the interspecific competitive abilities against *D. pulex* of the tested *D. magna* clones. The corresponding null hypothesis would be no positive correlation. In the second experiment, the interspecific competitive abilities should be ordered according to the body sizes of the three species. This implies that if species A is competitively superior to species B and B is competitively superior to species C, then it follows that A is also competitively superior to C. More specifically, the three species' competitive abilities are expected to be sorted in the order of their average body sizes, with *D. magna* as the largest species dominating the intermediately sized *D. pulex* which in turn should be competitively superior to the smallest species *D. longispina*. The corresponding null hypothesis would be intransitive competition, i.e. in the example above, C would be competitively superior to A such as in the rock-paper-scissors game (Shipley 1993).

Since *Daphnia* metacommunities mainly consist of small localities with a high risk of detrimental stochastic events followed by extinction, local communities are generally assembled in relatively short periods of time (Pajunen 1986, Pajunen & Pajunen 2003). Besides stochastic events, winter freezing annually and droughts occasionally kill off the whole pelagic community which is rebuilt by recruitment from local resting egg banks (Pajunen 1986, Pajunen & Pajunen 2003) or, to a much lesser degree, by immigration (Haag et al. 2005, 2006). As the local communities in this particular system are characterised by short-term temporal dynamics, we suggest that the correlation and transitivity of competitive abilities may have a profound impact on how species coexistence is prevented or enabled whenever a new community is formed or an existing community is affected by local recruitment or immigration.

Material and methods

Study system

Our study site is located at the coast south of Tvärminne Zoological Station in southwest Finland at the peninsula of Hanko (59° 50′ N, 23° 15′ E, `http://www.helsinki.`

`fi/tvarminne/`). The area harbours a rock pool metacommunity of the freshwater crustaceans *D. longispina* O. F. Müller, *D. magna* Straus, and *D. pulex* Leydig (Pajunen 1986, Pajunen & Pajunen 2003). The generalist filter-feeder of the genus *Daphnia* inhabit freshwater filled rock depressions, the so-called rock pools, on skerry islands and have largely overlapping niches (Ranta 1979). The three species possess a cyclical, certain *D. pulex* populations an obligatory (Haag & Ebert 2004a) parthenogenetic life cycle (Zaffagnini 1987). The asexual cycle in which females give parthenogenetically rise to clonal daughters is accompanied by a sexual cycle in which parthenogenetically produced males fertilise sexual eggs. Sexual eggs are packed in pairs into a chitinous shell (ephippium) which is shed when the female moults. As resting stages, ephippia serve to persist through harsh conditions such as droughts and especially freezing in winter. Females hatch from the overwintered ephippial eggs and found new pelagic populations. At the same time, ephippia serve as the dispersal stage.

In order to test the correlation and transitivity hypotheses, we conducted two competition experiments which we henceforth refer to as the correlation experiment and the transitivity experiment respectively. In the correlation experiment, we contrasted the competitive abilities of clones of the focal species *D. magna* in interspecific competition with each of the two other species to test whether the competitive abilities correlate positively. In the transitivity experiment, we contrasted the competitive abilities of the three species in pairwise interspecific competition to test whether the competitive abilities are transitive. In addition, we attempted to relate inter- and intraspecific abilities in both experiments.

Daphnia clones

In early May 2007, ex-ephippial females of the three species were collected from rock pools and from a long-term field experiment conducted with clones originating from the metacommunity (Altermatt & Ebert 2008a). Ex-ephippial females are the individuals hatching from overwintered ephippial eggs and are thus initially genetically unique in the local population before the onset of clonal expansion. In the lab, the animals were fed *ad libitum* with the green alga *Scenedesmus obliquus* (Turpin) Kützing and kept in artificial medium (Klüttgen et al. 1994) to produce isofemale lines, i.e. to obtain asexually produced female offspring. The mothers were dissected and checked for the presence of parasites according to the procedure of Ebert et al. (2001). Isofemale lines of infected mothers were discarded.

We collected *D. magna* females from experimental mesocosm populations which were known to descend from single clones (monoclonal populations) or 10 different clones (polyclonal populations) with two rounds of sexual reproduction since the start of the experiment two years before (Altermatt & Ebert 2008a), from natural rock pool populations which were known to be relatively young (c. 1–5 years since colonisation) or relatively old (c. 13 to at least 26 years old) according to the long-term data by Pajunen (Pajunen 1986, Pajunen & Pajunen 2003), and lastly from natural rock pools outside the long-term study area in order to cover a larger geographic range of origins. Isofemale lines of 40 *D. magna* clones with at least 60 mature female individuals were produced. The 40 clones were composed of 20 pairs, each of which originated from a different rock pool population. 4 pairs each stemmed from the monoclonal, polyclonal, young, or old populations, or from populations with unknown population history as outlined above. These clones will be referred to as our focal clones and *D. magna* as the focal species.

For 2 *D. longispina*, 2 *D. magna*, and 2 *D. pulex* clones, all collected from natural rock pools, the same parasite screening procedure was applied and mass cultures were set up. These clones served as our tester clones and were labelled as L1 and L2 (*D. longispina*), M1 and M2 (*D. magna*), and P1 and P2 (*D. pulex*). The tester clones served as references for other clones' competitive abilities.

All clones were genotyped with allozyme gel electrophoresis (Hebert & Beaton 1993) at the four loci aspartate amino transferase (Aat, enzyme commission number EC 2.6.1.1), 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). These genetic markers allowed to estimate the clone frequencies in mixtures of two competing conspecific clones.

Mesocosm experiments

We conducted outdoor mesocosm competition experiments to assess the degree of competition between pairs of clones of the three *Daphnia* species. For both experiments, we filled 6 l of 48 μm filtered rock pool water into 10 l plastic buckets (the mesocosms) each and installed them at the shore of a skerry island in vicinity of natural rock pools. In mid July 2007, the outdoor experiments were initiated.

In the correlation experiment, for each of the 40 × 6 = 240 pairwise combinations of the focal *D. magna* and tester clones, 10 mature female individuals of each focal and tester clone were placed together into a mesocosm. The aim was to determine the relative strength of interspecific competition between the 40 focal *D. magna* clones and the four heterospecific *D. longispina* and *D. pulex* tester clones. Besides, the relative strength of intraspecific competition between the 40 focal *D. magna* clones and the two conspecific *D. magna* tester clones was assessed.

In the transitivity experiment, we used the same 6 clones of the three *Daphnia* species serving as tester clones in the correlation experiment. For each of the 2 out of 6 = 15 pairwise combinations of clones, 10 mature female individuals of each clone were placed together into a mesocosm. This set was replicated three times, resulting in 45 mesocosms. The aim was to determine the relative strength of interspecific competition between the 12 heterospecific clone pairs. Besides, the relative strength of intraspecific competition between the 3 conspecific clone pairs was assessed.

In mid September 2007, the contents of all mescosoms were harvested. The pelagic individuals were frozen and stored at −80 °C. The ephippia were dried and stored at room temperature.

Measurements and statistical analysis

For the mesocosms with heterospecific clone pairs, we counted and assigned the ephippia to the corresponding species. While asexual reproduction of a *Daphnia* clone may be an adequate surrogate of competitive ability in permanently pelagic populations such as in temperate zone lakes (Demott & Kerfoot 1982, Hu & Tessier 1995, Cáceres 1998, Cáceres & Tessier 2004), it is less suitable in rock pool communities since all pelagic individuals are killed by freezing each winter. Conversely, ephippia are continuously accumulated during the course of the season and are the sole contribution to the subsequent season's community, thus their number is be regarded as an integrative measure of fitness. Consequently, we used the counts of ephippia for each clone as a surrogate variable for the competitive ability in interspecific competition. For the mesocosms with conspecific clone pairs, we genotyped a random sample of 66 pelagic individuals with allozyme gel electrophoresis and assigned the individuals to their corresponding clone. We used the counts of pelagic individuals to draw indirect conclusions on competitive ability in intraspecific competition. Instead of counts (absolute frequencies), proportions of ephippia (and pelagic individuals) were used for comparisons of competitive abilities to adjust for the variation in mesocosm resource availability.

For the correlation experiment, the association of interspecific competitive abilities of the focal *D. magna* clones in competition with the heterospecific *D. longispina* and *D. pulex* tester clones was estimated by Spearman's rank correlation coefficient since not all tested data pairs strictly follow bivariate normal distributions. Paired sample correlation tests were conducted to test whether correlation coefficients are different from zero. Paired Wilcoxon rank tests were used to contrast competitive abilities of the focal *D. magna* against *D. longispina* or *D. pulex* tester clones. To contrast interspecific competitive ability of focal *D. magna* clones with mono- vs. polyclonal and young vs. old population origin, Wilcoxon signed rank sum tests were applied. For the transitivity experiment, paired Wilcoxon rank tests were calculated to contrast competitive abilities of the three species against each other. Analyses were performed with R (R Development Core Team 2011, version 2.13.0) and packages lga (Harrington 2008, version 1.1-1) for orthogonal regression and gee (Carey et al. 2007, version 4.13-16) for the calculation of robust standard errors, plots were created with package lattice (Sarkar 2008, version 0.19-26).

Results

Correlation experiment

In the correlation experiment, the 40 focal *D. magna* clones competed with the four heterospecific tester clones P1, P2, L1, and L2. The proportion of the number of ephippia produced by the focal clones is positively correlated throughout all six pairwise comparisons (Fig. 3.1), even after adjustment of the p values for multiple testing after the method by Benjamini & Yekutieli (2001) although one p value is only marginally significant. From the distribution of the data points it becomes clear that *D. magna* produced on average more ephippia than the two tester species (213 ± 12.6 SE, robust standard errors determined with generalised estimating equations). *Daphnia pulex* produced on average 96.5 ± 10.8 ephippia and thus more than *D. longispina* with 29.7 ± 7.95 ephippia. Indeed, the paired Wilcoxon signed rank tests contrasting the ephippium proportions of *D. magna* and the tester clones reveal a highly significantly increased ephippium production in competition with *D. pulex* ($V_{80} = 2765$, $p < 0.001$) and *D. longispina* ($V_{80} = 3172$, $p < 0.001$). The supposedly higher ephippium proportions of *D. pulex* competing with *D. longispina* cannot be tested directly, however the comparison is done in the transitivity experiment in which *D. pulex* directly competed with *D. longispina* (see below).

When the ephippium proportions are averaged over the tester clone species, the six plots of Fig. 3.1 collapse to a single plot (Fig. 3.2). The proportions of *D. magna* ephippia in interspecific competition with *D. pulex* and *D. longispina* correlate highly significantly.

Likewise, the ephippium proportions obtained in interspecific competition with the four heterospecific tester clones can be averaged. When they are contrasted with the corresponding averaged proportions of pelagic individuals obtained in intraspecific competition with the two conspecific *D. magna* tester clones, a highly significant positive correlation results (Fig. 3.3). Note that this comparison of inter- and intraspecific competitive abilities of the focal *D. magna* clones is based on sexual reproductive output for the relative strength of interspecific competition and on asexual reproductive output for the relative strength of intraspecific competition, i.e. on two different traits.

A comparison of the individual interspecific competitive abilities exposes a high degree of similarity among the pairs of *D. magna* clones originating from the same population (Fig. 3.4, adjacent panels) even though there is no replication within each

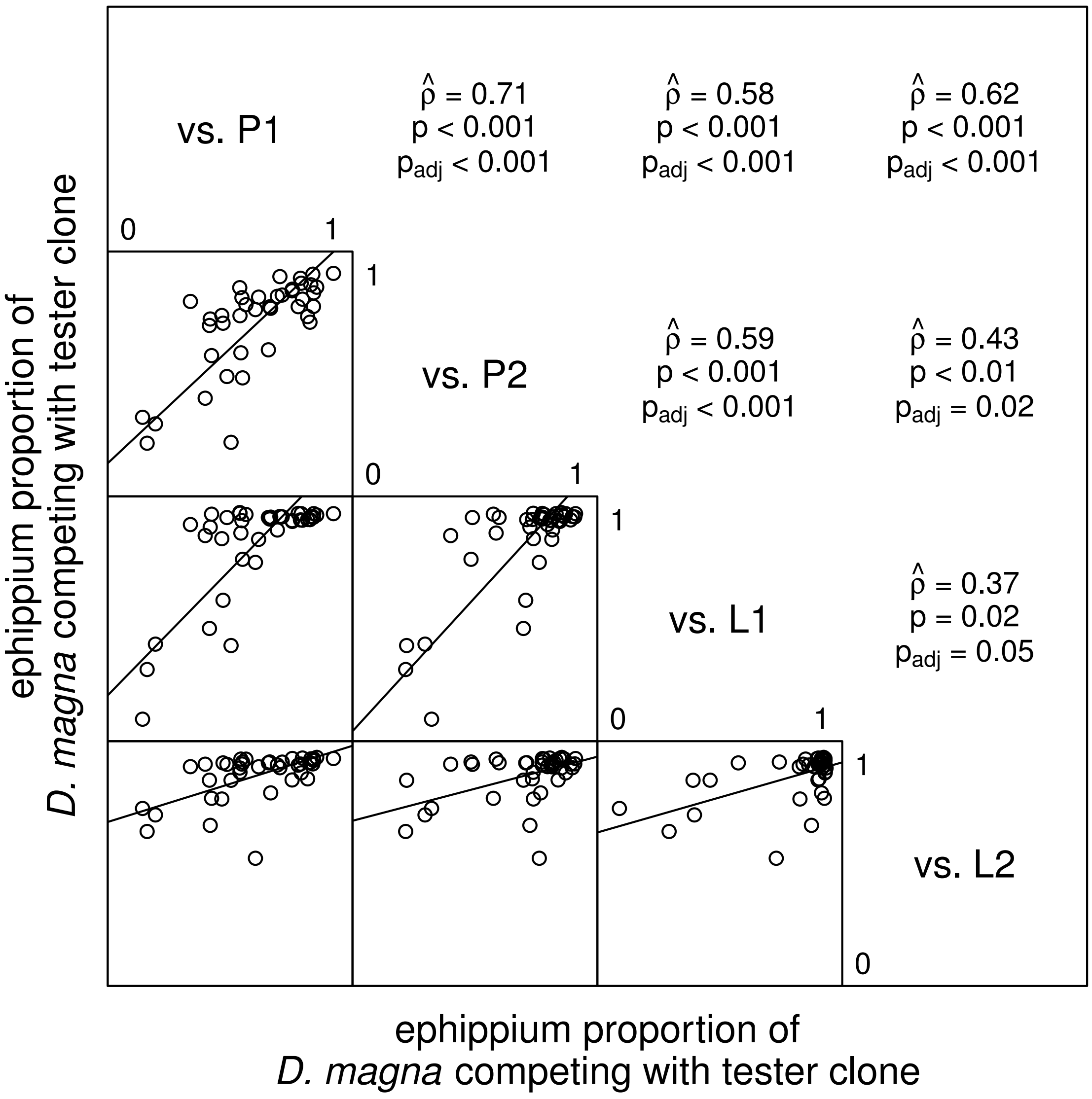

Figure 3.1 Proportion of *D. magna* ephippia produced in competition with the four interspecific tester clones P1, P2, L1, and L2. For each of the six possible pairwise comparisons, a scatter plot with a linear orthogonal regression line is given below the diagonal, the Spearman rank correlation coefficient estimate ($\hat{\rho}$), the p value of the correlation test, and the p value adjusted for multiple testing (p_{adj}) are given above the diagonal.

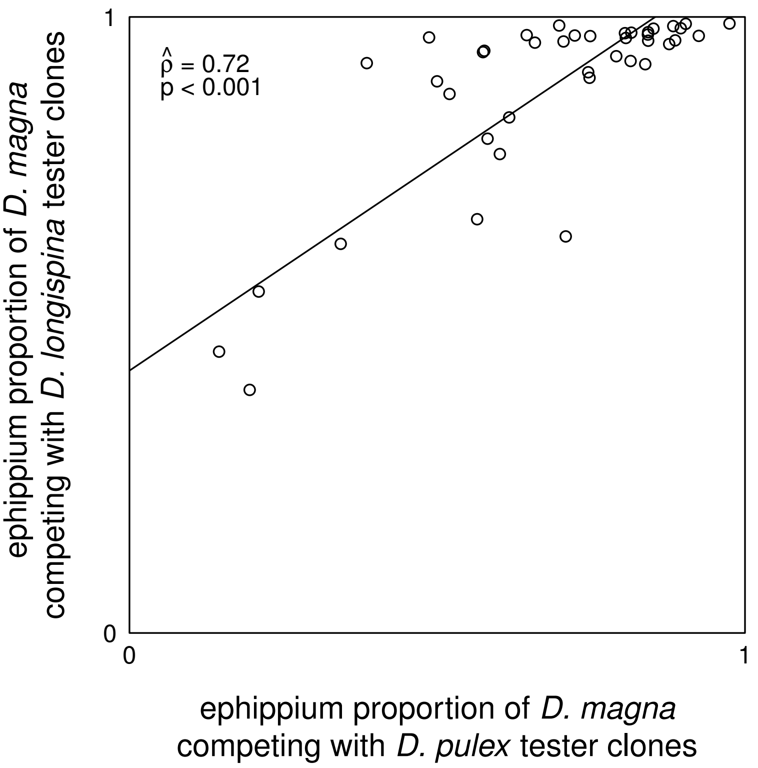

Figure 3.2 Combined proportions of *D. magna* ephippia produced in competition with the 2 *D. pulex* tester clones plotted against the combined proportions of *D. magna* ephippia produced in competition with the 2 *D. longispina* tester clones. The straight line is an linear orthogonal regression line, the estimated Spearman rank correlation coefficient estimate ($\hat{\rho}$) and the p value of the correlation test are given in the plot.

clone and in spite of the genotypic uniqueness of each clone (all clones originated from ex-ephippial females, i.e. from sexually produced eggs). The comparison of ephippium proportions of *D. magna* originating from experimental monoclonal vs. polyclonal populations shows a significantly higher ephippium production in *D. magna* clones from polyclonal populations (Wilcoxon rank sum test, $W_{32,32} = 8$, $p = 0.01$). In the comparison between natural young vs. old rock pool populations, no significant difference was found ($W_{32,32} = 46$, $p = 0.16$), although a trend in the expected direction towards higher proportions in old populations is seen.

Transitivity experiment

In the transitivity experiment, the outcomes of interspecific competition between the 6 clones, which were at the same time used as tester clones in the correlation experiment, led to rather unequivocal results. In 11 out of the 12 pairwise combinations, 3 out of 3 replicates show considerably larger ephippium proportions in one clone than the other

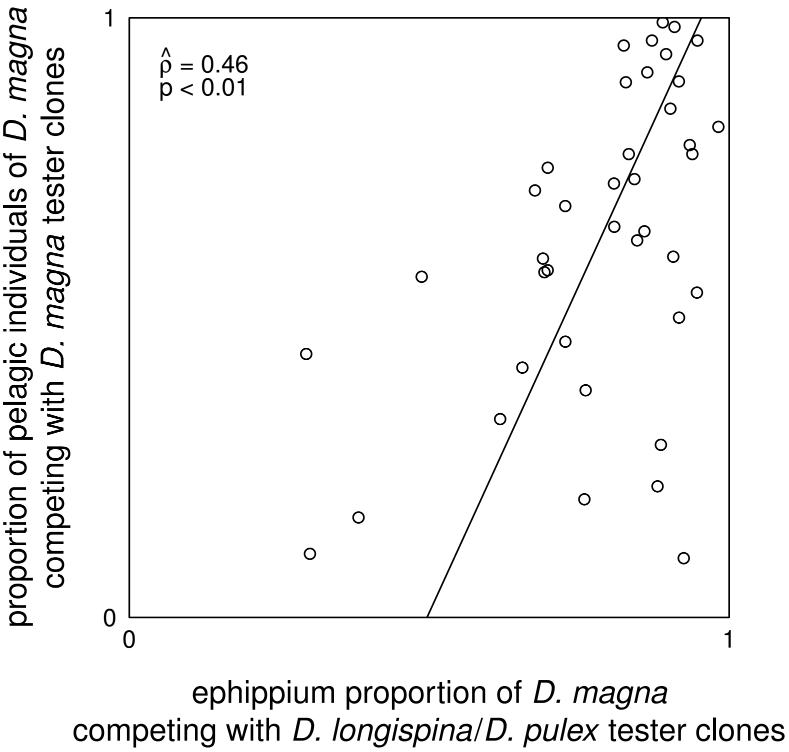

Figure 3.3 Combined proportions of *D. magna* ephippia produced in competition with the heterospecific tester clones plotted against the combined proportions of pelagic *D. magna* individuals produced in competition with conspecific *D. magna* tester clones. Note that the axes show measurements of two different traits. The straight line is a linear orthogonal regression line, the estimated Spearman rank correlation coefficient estimate ($\hat{\rho}$) and the p value of the correlation test are given in the plot.

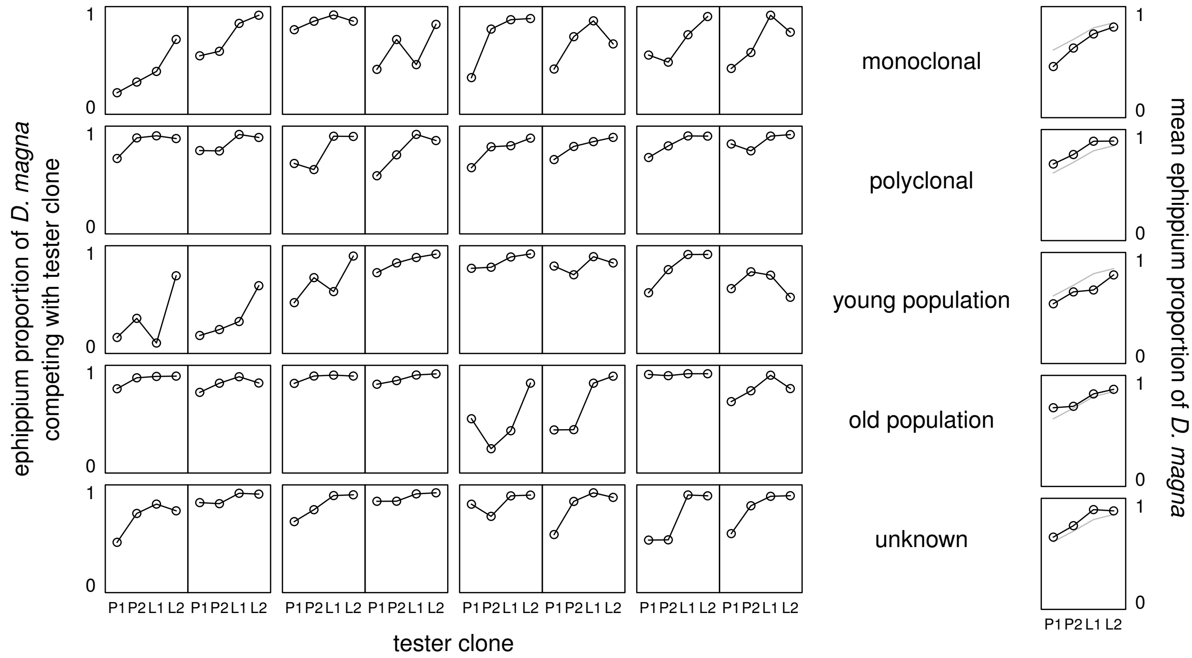

Figure 3.4 Proportion of ephippia produced by each of the focal 40 *D. magna* clones in competition with the 4 heterospecific tester clones P1, P2, L1, and L2. Each panel represents the 4 mesocosm competition outcomes obtained for an individual *D. magna* clone (left). Pairs of clones originating from the same population are grouped by adjacent panels. For each type of genetic background, i.e. 8 *D. magna* clones each, the mean values are given (right). The overall mean values are indicated with gray lines for reference in the background.

(Fig. 3.5). The only exception is the combination M2 vs. P2 in which the three replicates diverged strongly from each other. This was mainly due to a single replicate in which P2 had a higher ephippium proportion. The replicate was also unusual because the absolute number of ephippia produced was the largest of the whole experiment (139 as opposed to the mean of 30.3 for the *D. pulex* clones). The results of the conspecific replicates are not shown in Fig. 3.5 because a comparison of ephippium proportions with pelagic density proportions is not meaningful.

Using the aggregated results per species, the Wilcoxon signed rank tests suggest consistently that *D. magna* was superior to *D. pulex* ($V_{12,12} = 63$, $p < 0.01$), *D. magna* was superior to *D. longispina* ($V_{12,12} = 78$, $p < 0.01$), and *D. pulex* was superior to *D. longispina* ($V_{12,12} = 78$, $p < 0.01$) (Fig. 3.5). Transitivity of interspecific competition as already implied by the results of the correlation experiment is thus confirmed by the results of the transitivity experiment, i.e. interspecific competitive ability follows overall the decreasing order *D. magna*, *D. pulex*, and *D. longispina*.

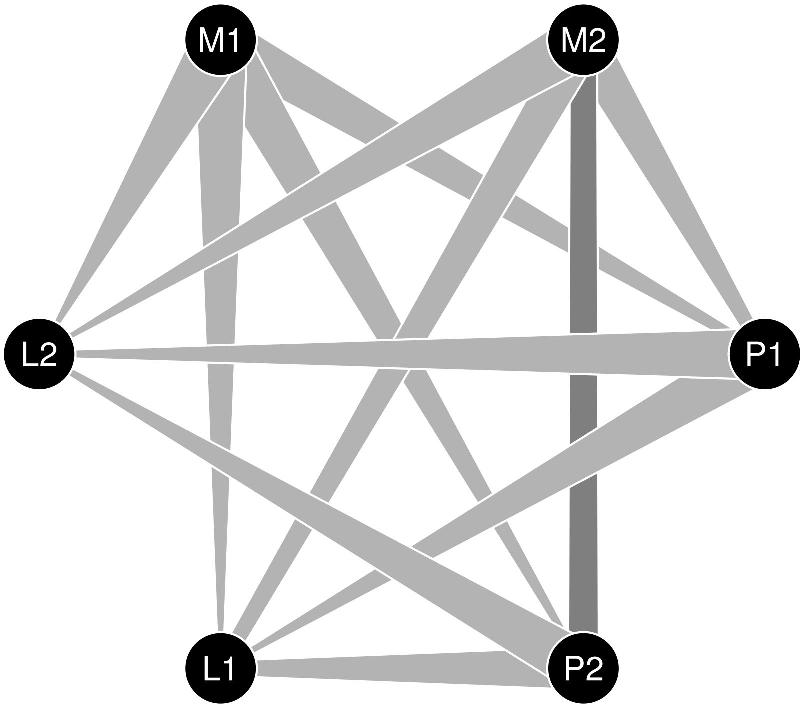

Figure 3.5 Mean proportions of ephippia produced by heterospecific pairs of clones. The end widths of each bar represent the means of the outcomes of the 3 corresponding replicates on a scale of 0 to 1, i.e. opposing end widths add up to one. The combination M2 vs. P2 is the only interspecific combination which does not fit into the general transitive hierarchy and is indicated with a darker hue of gray.

Discussion

Overall, interspecific competitive abilities of *D. magna* clones were found to be organised in a rather strict hierarchy. We demonstrated that if a *D. magna* clone was a better competitor against *D. longispina* it was at the same time with a high probability a better competitor against *D. pulex* and vice versa (Fig. 3.2). Moreover, we could show that the interspecific competitive abilities of the three *Daphnia* species are almost invariably transitive (Fig. 3.5). The results of the correlation experiment per se already suggested the conclusion of transitive competition between the three *Daphnia* species, and the results of the transitivity experiment proved to be congruent.

Interspecific competition in rock pool *Daphnia*

Ignoring the residual variability for the moment, the results comply with the prediction of the size efficiency hypothesis that in the absence of predation larger species are better competitors due to higher feeding efficiency (Brooks & Dodson 1965). *Daphnia magna* has on average a larger body size than *D. pulex* which in turn is on average larger bodied than *D. longispina* (Bengtsson 1986). Larger *Daphnia* species seemed to be more efficient with regard to resource acquisition and subsequent production of ephippia and can thus be regarded as competitively superior to smaller *Daphnia* species. Logically consistent, the intermediately sized *D. pulex* clones were also intermediate with regard to interspecific competitive ability. Although the 40 *D. magna* clones in the correlation experiment differed substantially in their competitive abilities, the majority of clones were competitively superior to both the *D. longispina* and *D. pulex* clones (Fig. 3.1, 3.2). For the intermediately sized *D. pulex* clones, the competitive inferiority to *D. magna* and the competitive superiority to *D. longispina* is indirectly supported by the relatively lower ephippium production of *D. magna* in competition with *D. pulex* compared to the ephippium production in competition with *D. longispina* (Fig. 3.1, 3.2), which is consistent with competitive transitivity.

Hanski & Ranta (1983) hypothesised that in the rock pool metacommunity where we conducted our study, *D. magna*, the largest of the three *Daphnia* rock pool species, should be the best coloniser but worst competitor, *D. longispina*, the smallest species, should conversely be the worst coloniser but best competitor, with the intermediately sized *D. pulex* being intermediate in both respects. With regard to the competition part of their hypothesis, we came to the opposite conclusion in support of the size efficiency hypothesis as did the majority of similar competition studies reviewed by Bengtsson (1987). However, lab experiments with the same three *Daphnia* species suggested *D. pulex* to be the superior competitor with regard to pelagic density, whereas competition between *D. longispina* and *D. magna* depended on the food and temperature regime (Bengtsson 1986). The same study comprised a mesocosm experiment in which no competitive exclusion could be observed (Bengtsson 1986). In more extensive mescosom experiments with experimental communities consisting of all one-, two-, and the single three-species combinations, *D. magna* was found to go less often extinct over the course of several seasons than *D. longispina* and *D. pulex*, but extinction events in the two-species communities occurred only in the smallest mesocosm class of 4 l volume (Bengtsson 1989, 1993). Environmental heterogeneity was brought up as a possible explanation for coexistence in spite of presumably intense competition (Bengtsson 1986, 1989, 1993). In the earlier experiments, pelagic density was used to assess competition (Bengtsson 1986) whereas persistence of mesocosm populations in the later experiments depended on the production of ephippia (Bengtsson 1989, 1993). The methodological differences may thus explain some of the inconsistencies in the results.

Coexistence in rock pools

Observations in the field only partially back the view gained in competition experiments that coexistence is frequent as shown by a long term field monitoring which found that among over 500 rock pools only 22 % of the occupied pools harboured more than one of the three *Daphnia* species concurrently (Pajunen 1986, Pajunen & Pajunen 2003). Niche complementarity of the three species has been suggested to be primarily realised between different rock pools rather than within rock pools (Ranta 1979) and might explain the relatively low percentage of natural two-species *Daphnia* communities. The exclusion of species might therefore not be due to interspecific competition but because of environmental conditions which are beyond species specific physiological tolerance (e.g. Connell 1961). However, coexistence of *Daphnia* species in rock pools might effectively be much more prevalent because species can temporarily be absent in the pelagic zone and persist as sexual eggs in ephippia in the sediment, thus species can remain undetected in the absence of a pelagic population. Instead of intense interspecific competition leading to exclusion, the observed patterns of *Daphnia* occurrence in rock pool metacommunities could rather be caused by avoidance of interspecific competition leading to cryptic coexistence.

In spite of the above proposed mechanism of cryptic coexistence of species, the paradox of the plankton does not appear to be as striking in such rock pool communities as in the originally targeted phytoplankton communities (Hutchinson 1961). Still, coexistence of rock pool *Daphnia* species has been observed over the course of several seasons on a regular basis (Pajunen 1986, Pajunen & Pajunen 2003). The stochasticity of rock pool habitats (Altermatt et al. 2008, 2009) and thus environmental variability may lead to what has been termed lottery competition without the attainment of an equilibrium (Chesson & Warner 1981). Environmental heterogeneity could be expressed as fluctuating local rock pool characteristics that are temporarily at the boundaries of the potentially superior competitor's habitat preferences and thus enable the potentially inferior competitor to persist (Ranta 1979, Pajunen & Pajunen 2007). Persistence might just last over a significantly longer period of time, which occurs as stable persistence to the short-term observer. Moreover, although interspecific competition was generally found to be transitive, the supposedly rare intransitive competitive relationships could be more prevalent in rock pool communities than suggested by our experiments. Intransitive competitive relationships at the species level could thus mediate coexistence like in species assemblages in which interspecific competition adheres to the rock-paper-scissors game (Buss & Jackson 1979, Sinervo & Lively 1996, Frean 2001, Laird & Schamp 2006) even if intransitivity occurs only at modest levels (Laird & Schamp 2006).

The here so far neglected residual variance, the one which is not explained by the positive correlation of interspecific competitive abilities and the putatively exceptional cases of intransitive interspecific competition, might play a role in maintaining diversity at the genetic and species level. Possibly the positive correlation and the transitivity of competitive abilities were found to operate on average, but species coexistence may be fueled by the exceptions to these rules. This idea needs further theoretical work, followed by experiments targeting the variance in competition.

The importance attached to the residual variance is even greater insofar as the clones in our experiments had to compete in a common garden since the mesocosms contained filtered water from the same source rock pool. That all clones were kept in a common garden may be reflected in the pairwise similarity of the competitive profiles of *D. magna* clones originating from the same population (Fig. 3.4). Even though environmental heterogeneity was thus kept at a low level compared to the variability of conditions among natural rock pools, the substantial residual variance in competitive abilities, which was just argued to be important for species coexistence, could arise. Moreover, although the water used in the experiments originated from a rock pool which naturally accommodate a population of *D. longispina*, the smallest sized species tested, the competitive hierarchy was as predicted by the size efficiency hypothesis (Brooks & Dodson 1965).

One part of the residual variance can actually be explained by genetic differences between *Daphnia* clones. *Daphnia magna* clones originating from populations with low and high genetic diversity and young and old populations, respectively, differed in their competitive abilities (Fig. 3.4). Clones from monoclonal populations have a higher chance of being inbred, and since younger populations are on average genetically less diverse than older populations (Haag et al. 2005), the same applies for clones from young populations. Therefore a higher inbreeding coefficient was overall associated with lower competitive abilities of *D. magna* clones (Fig. 3.4), and inbreeding could thus cause deviations from the transitive competition hierarchy.

Another feasible factor explaining the discrepancy between the stringent competitive hierarchy in the experiments and coexistence of *Daphnia* in rock pools could be parasitism (Ebert et al. 2001). The large sized *D. magna* harbour a higher diversity of parasites than the smaller bodied *D. pulex* and *D. longispina* (Stirnadel & Ebert 1997, Ebert et al. 2001) and have been shown to be competitively hampered by parasites (e.g. Haag & Ebert 2004b, Altermatt & Ebert 2008a). Rock pool communities of *Daphnia* species might thus be under a mixture regime of exploitative and apparent competition (Holt et al. 1994). Furthermore, interference competition by means of allelopathy (Whittaker & Feeny 1971) might also mediate coexistence. *Daphnia* are known to produce allelopathic compounds that negatively affect conspecific as well as heterospecific competitors (Seitz 1984, Matveev 1993, Goser & Ratte 1994) and could act as a force opposing exploitative competition.

Ephippium production as a surrogate variable for competitive ability

The proportion of the number of ephippia produced by each competitor is used as a surrogate variable for interspecific competitive ability in our experiments. We argued that this choice is reasonable, though like most surrogate variables ours is not immune to possible impairments either (Goldberg 1996). An obvious limitation ultimately adherent to all measures of competitive ability is the difficulty to predict the return of investment. If all ephippia were equivalent and the number of ephippia improved chances to persist in a linear fashion, the measure would be ideal. But ephippia could either not contain eggs or the eggs could be not viable. The idealised linear relationship could furthermore be hampered by large variation in the offspring's individual fitness if one considers longer-term consequences of competition (Tessier & Consolatti 1991).

Nevertheless, using ephippium production of whole experimental populations as a proxy for competitive ability has several advantages. Although cyclically parthenogenetic *Daphnia* species vary with respect to their strategies to allocate into asexual and sexual reproduction over the course of a season (Innes & Singleton 2000, Yampolsky 1992b), the pelagic rock pool populations in the Baltic Sea perish at the onset of winter and thus the total number of ephippia accumulated until the end of the season provides an integrative measure of population fitness regardless of the clone specific reproductive allocation strategy. In addition, using relatively large experimental populations in natural rock pool water as opposed to small lab populations in artificial medium accommodates to the postulate of Thompson (1988), who stressed the need for a population approach when assessing interspecific interactions. The use of ephippia to directly assess competitive ability of rock pool *Daphnia* clones has to our knowledge not been used so far and certainly explains some of the differences in outcome between our experiments and comparable studies in which increase in pelagic densities or extinction events (which are ultimately mediated by the absence of ephippia) were used as surrogate variables (e.g. Bengtsson 1986, 1989, 1993). Pelagic densities have been used in a vast amount of *Daphnia* competition experiments, which is a self-evident approach in habitats that permanently sustain a pelagic population such as in temperate zone lakes (e.g. Goulden et al. 1982, Tillmann & Lampert 1984, Kreutzer & Lampert 1999). In such habitats, *Daphnia* clones also vary with regard to their investment in sexual reproduction and consequently the production of ephippia (Cáceres 1998), but in rock pool habitats, natural selection has presumably favoured strategies maximising the output of ephippia since their number, and not the number of pelagic individuals, is decisive at the end of the season. A further notable advantage of quantifying ephippium production over the course of a whole season consists in the long-term nature of the measurement as opposed to measurements of changes of pelagic density which are usually performed over much shorter periods of time and represent temporal snapshots, whereas ephippium counts represent a cumulative measure across seasons.

A related problem is the definition of a cutoff point (for a variable serving as a surrogate for competitive ability) above or below which one considers one or the other competitor as superior. This is often implicitly done but does not necessarily reflect the consequences of competition for the involved species. We implicitly assumed cutoff points when trying to order species according to their interspecific competitive abilities to demonstrate transitivity. Nevertheless, the results of the transitivity experiment as well as the findings of the correlation experiment can be considered unequivocal and robust enough to justify our conclusions.

The above considerations could be suggestive of ephippia serving only to ensure local recruitment after winter or droughts, but they are at the same time the dispersal stage of *Daphnia* between localities, i.e. rock pools, in a metacommunity. Because the yearly extinction rate of rock pool populations is approximately 20 % — which is roughly balanced by recolonisations (Pajunen 1986, Pajunen & Pajunen 2003) —, a sufficient production of ephippia in order to be able to colonise new habitats is often the only means by which *Daphnia* can attain longer-term fitness, i.e. fitness beyond the course of a single season (Altermatt & Ebert 2008b, Altermatt et al. 2009). The favouring of immigrant genes imported by dispersed ephippia by strong hybrid vigour of outbred offspring (Ebert et al. 2002) further underlines the contribution of ephippia to fitness.

The alternative types of reproduction in cyclically parthenogenetic *Daphnia* species (Zaffagnini 1987) complicate considerations on fitness and the comparability of intra- and interspecific competitive abilities. If one only assesses the outcome of interspecific competition at the end of the season by quantifying sexual reproduction (i.e. total ephippium production), the fact that rock pool *Daphnia* are known to have different strategies as how to allocate resources to the two alternative paths of reproduction (Innes & Singleton 2000, Yampolsky 1992b) is irrelevant as discussed above. However, the variation in allocation strategies invalidates the use of ephippium production for short-term assessments of interspecific competition. For the assessment of intraspecific competitive ability that last one season, ephippium production could be used albeit one then has to determine the competing clones' genetic contributions for each fertilised, viable sexual egg. Yet for assessments of intraspecific competitive abilities lasting longer than one season, the consequences of inbreeding such as strong hybrid vigour (Ebert et al. 2002, Haag et al. 2002) distort the numerical genetic contributions of clones. Theoretically, if the fitness of hybrids were substantially higher than the fitness of inbreds as shown by Ebert et al. (2002), the fitness contribution of inbred offspring may become insignificant. Asymptotically, i.e. if only outbred offspring contributed significantly to fitness, relative fitness between two competing clones would be shared equally since their genetic contribution to outbred offspring is 50 % each, which would turn the comparison of relative ephippium productions obsolete.

When trying to associate intra- and interspecific competitive abilities over the surrogate variables asexual and sexual reproduction, respectively, two different traits which likely even underlie a trade-off are to be compared (Innes & Singleton 2000, Yampolsky 1992b). In our experiments, besides the assessment of interspecific competition, we separately estimated the strength of intraspecific competition by measuring pelagic densities of asexually produced individuals. Although we cannot provide direct evidence for the relation of intra- and interspecific competitive abilities, the indirect evidence for a positive correlation between the two is convincing (Fig. 3.3). Even though an increased allocation of resources into asexual reproduction entails a decrease of investments into sexual reproduction and vice versa (Yampolsky 1992a, Innes & Singleton 2000), interspecific competitive ability of *D. magna* expressed as ephippium proportion was positively correlated with intraspecific competitive ability of *D. magna* expressed as pelagic density proportion (Fig. 3.3). This indicates that *D. magna* clones which are able to produce more ephippia in competition with another species tend at the same time to be able to clonally expand more efficiently in intraspecific competition.

Conclusion

Recapitulatory, our results suggest that interspecific competition between *Daphnia* species in rock pool metacommunities of the Baltic Sea generally follows a stringent hierarchy as predicted by the size efficiency hypothesis. In spite of the strong correlation of interspecific competitive abilities and predominantly transitive competitive interactions, there is enough variation with the potential to mediate coexistence within and across metacommunity localities.

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Chapter 4

Direct quantification of dispersal in a *Daphnia* rock pool metacommunity

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Abstract

Quantifying spatial and temporal patterns of dispersal is challenging, even more so if direct rather than indirect methodology should be applied. Some organisms depend on propagules, such as seeds in most plants or analogously resting eggs in certain zooplankton species, which are passively transported. It has long been demonstrated that migration occurs in *Daphnia* rock pool metacommunities, however estimates were so far based on indirect genetic or demographic measures and did not attempt to directly quantify dispersal by resting eggs. We present the use of astroturf mats, otherwise used by plant ecologists to quantify seed rain, to trap dispersing *Daphnia* resting eggs in the field and the application of the technique in a well studied rock pool metacommunity. Although the yielded number of resting eggs was modest, we provide direct quantitative evidence for passive dispersal and the possible existence of *Daphnia* resting egg banks outside of rock pools. Whereas colonisation events into natural rock pools are concealed by unsuitable physicochemical conditions or priority effects, the absolute dispersal effort can be estimated more reliably by regarding the astroturf traps as colonisable substitutes for natural rock pools. We suggest that *Daphnia magna* resting eggs are dispersed frequently by wind, and on the other hand we speculate that *D. longispina* resting eggs may be predominantly transported by insects. Thus the presumably species specific dispersal strategies may have direct consequences for extinction-colonisation dynamics in the metacommunity.

Key words ephippia, resting egg bank, migration, colonisation, astroturf, anemochory, zoochory, *Daphnia magna*, *Daphnia longispina*

Introduction

The original metapopulation model by Levins (1970) only considered colonisation of empty habitat patches. Yet dispersal also connects occupied habitat patches (Clobert et al. 2004), which can lead to a reduction of the extinction risk of local populations (Brown & Kodric-Brown 1977). The study of dispersal in metacommunities unites both aspects since the colonisation of a habitat patch by a species is often equivalent to the invasion of a habitat patch already occupied by other species (reviewed in Holyoak et al. 2005).

In the last decades, advances in methods to measure dispersal indirectly surpassed advances in methods to measure dispersal directly (for an overview see Clobert et al. 2001). A decisive disadvantage of indirect measurements however consists in the immanent ignorance of the individuals which dispersed but did not manage to reach a new habitat or to successfully establish themselves in a new habitat due to interactions with the local resident community (e.g. Shurin 2000, De Meester et al. 2002, Calcagno et al. 2006). Therefore dispersal rates based on measures of (realised) gene flow (Neigel 1997, Bossart & Prowell 1998) are bound to underestimate the real extent of dispersal. This often unknown quantity contains information about the absolute and not merely successful dispersal effort. If the spatial expansion of unsuccessful dispersers were taken into account, one could deduce a population's colonisation potential, an important aspect for the conservatory management of endangered species which are organised as metapopulations (Ovaskainen 2002).

The quantity of unsuccessful dispersers may be negligible in species where dispersal is actively aimed towards suitable habitat patches, but it is of importance in species which disperse passively, as is particularly the case in plants (Howe & Smallwood 1982) and freshwater zooplankton inhabiting isolated bodies of water (Maguire Jr. 1963). Similarly to plant seeds, the propagules of zooplankton depend on transport by animals — such as waterfowl (Figuerola & Green 2002, Figuerola et al. 2005), amphibians and reptiles (Bohonak & Whiteman 1999, Lopez et al. 1999), or invertebrates (Bohonak & Jenkins 2003, van de Meutter et al. 2008) — or by wind (Brendonck & Riddoch 1999, Cáceres & Soluk 2002, Vanschoenwinkel et al. 2008a). A further analogy between plant and zooplankton propagules consists in the dual function of serving as the dispersal and the resting stage, the later forming egg banks, the equivalent of seed banks, from which propagules either disperse or contribute to recruitment by hatching of pelagic individuals (De Stasio Jr. 1989, Brendonck & De Meester 2003).

Despite its merits, measuring dispersal directly is a difficult task (Hanski et al. 2000, Bilton et al. 2001), which is reflected in the popularity of indirect methods (Neigel 1997, Bossart & Prowell 1998). Many experimenters installed empty mesocosms in the field and followed their colonisation by zooplankton species from neighbouring water bodies (Shurin 2000, Cáceres & Soluk 2002, Louette & De Meester 2005, Allen 2007). Selectively preventing access to animals even allows for the distinction of dispersal vectors, specifically animals or wind (Cáceres & Soluk 2002). However, an undesirable property of this approach are priority effects favouring early arrivals and impeding successful establishment of latecomers (De Meester et al. 2002, Calcagno et al. 2006), which leads to the inevitable oversight of viable, though unsuccessful invaders. Methods that trap propagules and do not discriminate between establishment success lead to more complete measures of dispersal as exemplified by sticky traps (Brendonck & Riddoch 1999) or wind socks (Jenkins & Underwood 1998, Vanschoenwinkel et al. 2008a), provided an assessment of the viability of propagule eggs is conducted afterwards. In short, all possible vectors have to be monitored but only viable propagule eggs must be included in order to obtain comprehensive measures of dispersal.

These methodological considerations equally apply to attempts to quantify dispersal in *Daphnia* metacommunities on the skerry island coast of the Baltic Sea (Bengtsson 1986, Pajunen 1986). The freshwater filled rock pool habitats are connected by dispersal of ephippia (Haag et al. 2005, 2006), which in turn also build ephippium banks (Pajunen 1986, Pajunen & Pajunen 2003b). Suitable growing conditions combined with environmental stimuli break the diapause of *Daphnia* resting eggs (Banta & Brown 1929, Stross & Hill 1965, Carvalho & Hughes 1983, Kleiven et al. 1992) and cause the sexual (female) offspring to hatch and found new pelagic populations after having survived winter cold or summer desiccation (Cáceres & Tessier 2003, Altermatt & Ebert 2008). Ephippium banks are probably predominantly formed in rock pool sediments from where the following year populations are recruited (Pajunen 1986, Pajunen & Pajunen 2003b). However, these ephippium banks are likely only extant over short periods because dormancy breaking stimuli are numerous and supposedly lead to an almost complete depletion of dormant eggs at the beginning of a new growing season or after droughts (Pajunen & Pajunen 2003b). Alternatively, longer lasting ephippium banks might be built up outside rock pools in vegetation or rock crevices (Pajunen 1986), albeit their dimension and importance as stepping stones for dispersing ephippia remain unclear.

Because only little is known about the absolute dispersal effort of *Daphnia* in rock pool metacommunities, an estimate of the amount of passively dispersed ephippia regardless of the total ephippium production in rock pool communities (Altermatt & Ebert 2008) or actual colonisation success (Haag et al. 2005, 2006) would help to judge the relative importance of either type of ephippium bank, within or outside of rock pools. To obtain an estimate of temporal and spatial fluxes of ephippia, integrative measurements are a practical choice (Vandekerkhove et al. 2005) since dispersal of ephippia is potentially difficult to track. The use of mats fabricated of astroturf — a material usually processed to door mats or artificial lawn for sports — for the study of seed rain is an established method in plant ecology (Henry & Molau 1997, Molau & Larsson 2000). Because *Daphnia* ephippium sizes are in the range of diameters which astroturf mats manage to retain when swept onto and a pilot study had proven the mats' efficacy to retain *Daphnia* ephippia and resting stages of other crustaceans, we used astroturf mats in a 3 years' trial to estimate the amount of dispersing ephippia in a *Daphnia* rock pool metacommunity on the skerry island coast of southwest Finland (Hanski 1983, Pajunen 1986).

Astroturf mats and rock pools are both traps in the sense that they are capable of retaining ephippia, but unlike in rock pools, the detection of colonisation events from external ephippium banks is greatly facilitated in astroturf mats as no local ephippium banks are initially present. We contrast our results of found ephippia with experimental and observational data on ephippium production and metacommunity dynamics conducted in the same area to check the feasibility of our findings. Moreover, the temporal and spatial occurrences of ephippia allow us to draw tentative quantitative conclusions on dispersal rates and metacommunity colonisation events.

Material and methods

The study site is located in southwest Finland at the peninsula of Hanko south of Tvärminne Zoological Station (59° 50′ N, 23° 15′ E, `http://www.helsinki.fi/tvarminne/`). The area harbours a rock pool metacommunity of the freshwater crustaceans *D. longispina* O. F. Müller, *D. magna* Straus, and *D. pulex* Leydig (Crustacea: Cladocera). Since 1982, Pajunen has been sampling over 500 rock pools in the metacommunity twice a year to monitor the presence of the three autochtonous *Daphnia* species (Pajunen 1986, Pajunen & Pajunen 2003b). The samplings took usually place in summer (June) and autumn (August) and allow to estimate colonisation and extinction dynamics of individual rock pool populations.

On two skerry islands, grey polyethylene astroturf mats (FinnTurf, Sandudd Oy, Finland) of rectangular shape and 45 cm side length were fixed to the rock with rust-proof screws at each corner (Fig. 4.1). The mats were placed at the intersection points of a regular rectangular 10 m grid that is congruent with the Finland Uniform Coordinate System KKJ (Kartastokoordinaattijärjestelmä), which corresponds to a random positioning of the grid (Fig. 4.2). Mats were installed at locations that were at maximum 10 m apart from the next neighbouring rock pool and out of reach of maximal water level changes of the Baltic Sea. At some positions, mats were repeatedly ripped off by winter storms and were thus not included in the analysis. The resulting coverage resulted in two coherent areas of astroturf mats and rock pools (Fig. 4.2). The two equipped skerry islands Storgrundet (labelled N) and Granbusken (labelled G) are situated approximately one kilometre apart, the later lying further in the southwestern part of the study area. In the southern quadrant, G is directly exposed to the Baltic Sea while N is more protected by being situated north of a large island of relatively high altitude stretching from west to east.

39 and 63 mats were placed on N and G respectively, corresponding to total areas of 7.9 and 12.76 m^2, respectively (Fig. 4.2). Within 10 m of any astroturf mat, there were 72 rock pools on N and 90 rock pools on G with total surface areas of 285.71 and 399.52 m^2. The area of an astroturf mat was 0.45×0.45 m, the mean surface area of a rock pool was, expressed as a rectangle, 2.06×2.06 m, i.e. approximately 21 times larger.

The surface of astroturf consists of small funnels that are highly efficient in collecting and trapping small particles, thus its common use for door mats (Fig. 4.1). Astroturf and in particular the specific product FinnTurf has been successfully applied in plant seed rain assessments and has been found to be effective for seeds between 0.3 to 10 mm diameter (e.g. Henry & Molau 1997, Molau & Larsson 2000), which includes the size range of ephippia of rock pool *Daphnia* species. Furthermore, traps consisting of funnels are deemed more efficient in retaining plant seeds than sticky traps since the later turn unusable in dry and dusty environments (Johnson & West 1988), which could become especially a problem in long-term exposure on skerry islands. A pilot experiment comprising eight mats arranged in increasing distances around an isolated rock pool confirmed that the material is also suitable for trapping *Daphnia* ephippia, which underlines their functional equivalence to plant propagules (De Stasio Jr. 1989).

The mats were installed in mid May 2006 prior to the production of the first ephippia. In mid September 2006, mid May 2007, mid September 2007, mid May 2008, and mid September 2008, i.e. at the beginnings and ends of the *Daphnia* growing seasons 2006, 2007, and 2008, the mats were removed in dry condition from the ground and emptied by rolling them out and hitting them to the walls of a large vessel, followed by re-installation to the rock. In previous tests, this simple procedure proved to effectively release all ephippia unless the mat surface was moist. The sediments were collected, transferred to plastic zip bags and stored at room temperature.

Following the harvest, each mat sediment sample was suspended in a 1 litre jar filled with a 1:1 mixture of 48 μm filtered rock pool water and artificial *Daphnia* culture medium (Klüttgen et al. 1994). The suspensions were exposed to approximately room temperature and permanent light conditions (sunlight during daytime plus light from fluorescent lamps mimicking the wavelength composition of sunlight: Lumilux 865,

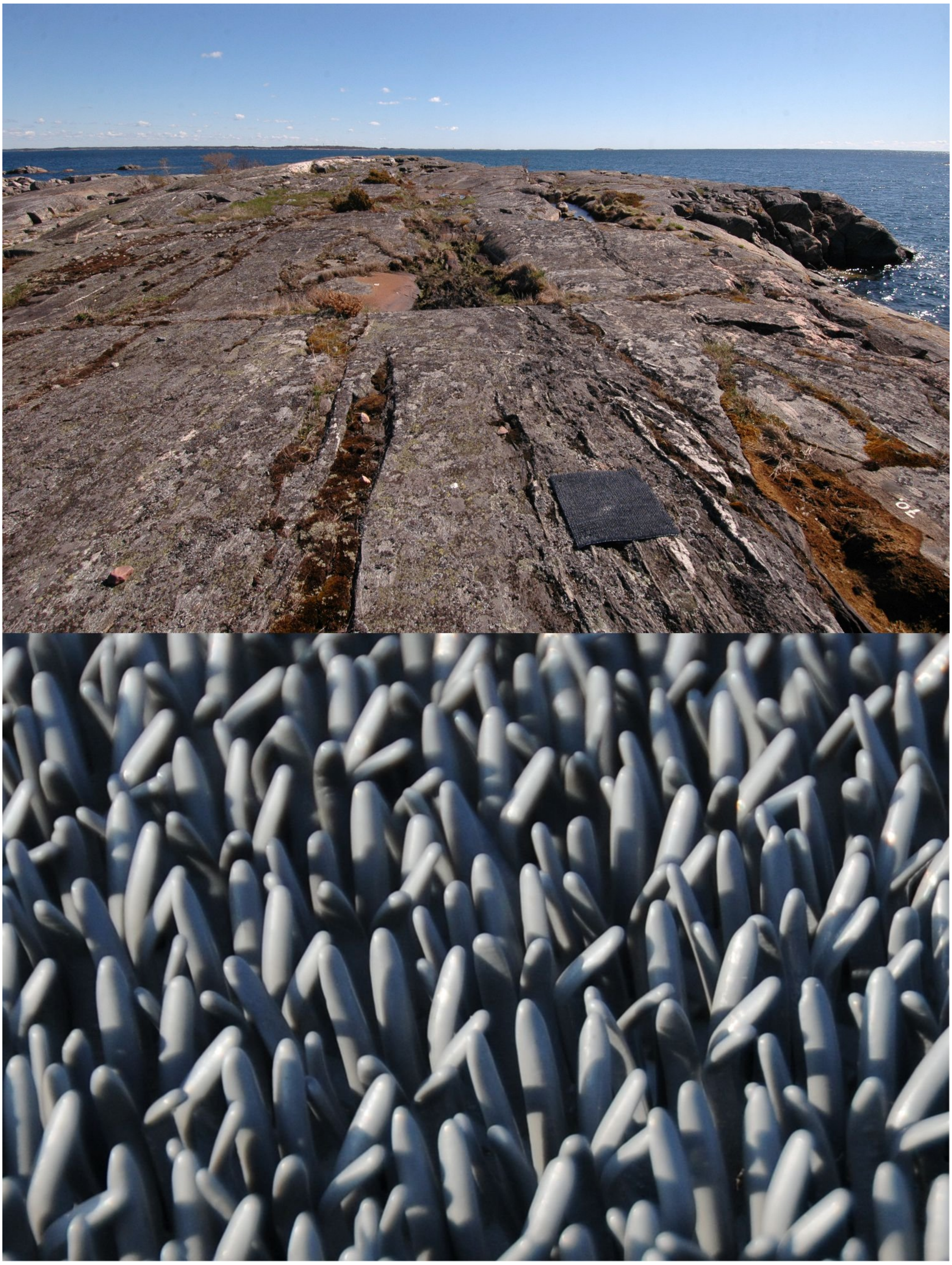
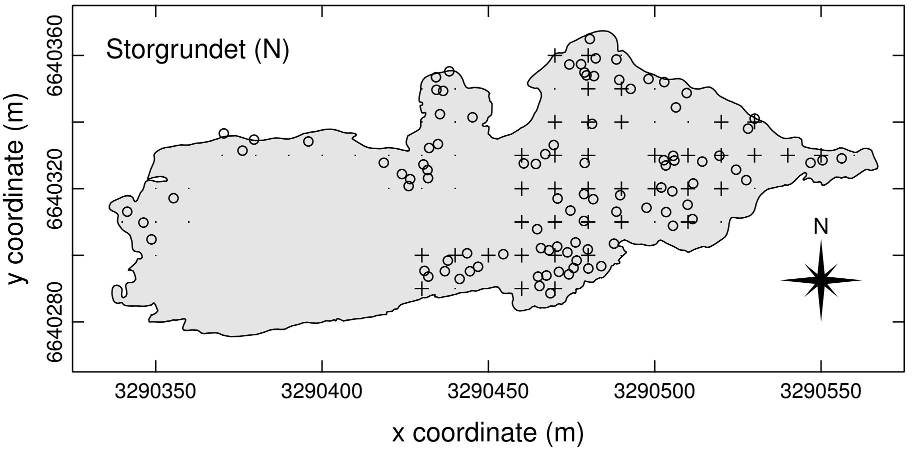


Figure 4.1 Astroturf mat positioned in the field (in the foreground, on island N; top) and close-up of the surface of an astroturf mat (FinnTurf, Sandudd Oy, Finland; bottom). The small funnels in which small particles get trapped are formed by stretched out plastic fingers.



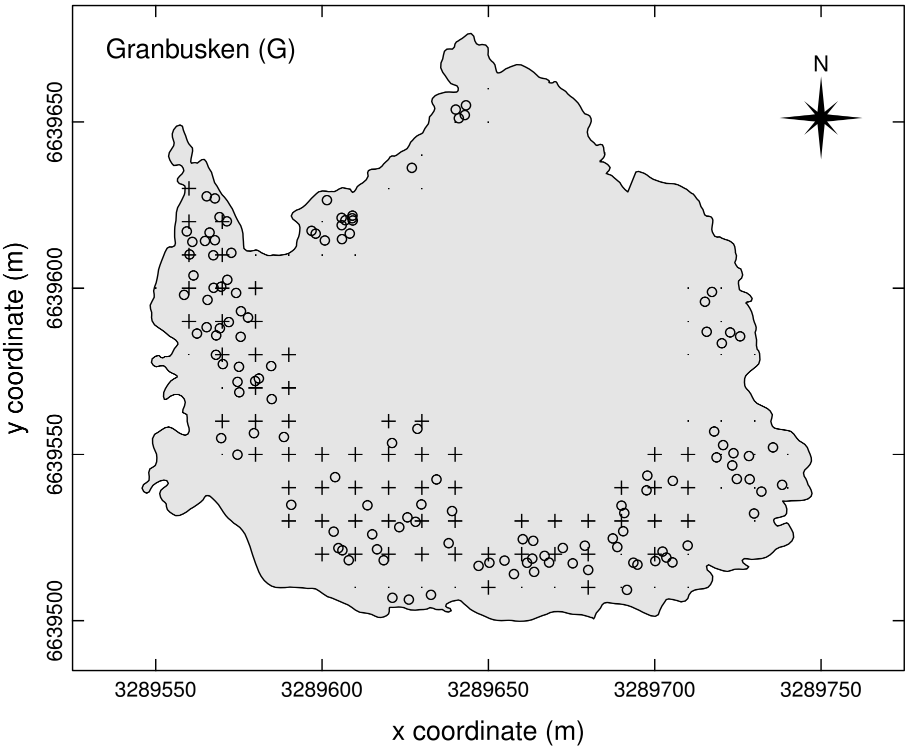


Figure 4.2 Astroturf mat locations on the islands Storgrundet (labelled N, top) and Granbusken (labelled G, bottom). Dots indicate suitable grid points (i.e. those being situated on land lying within 10 m of any rock pool, black crosses show the actual positions of the astroturf mats, and circles represent rock pool positions. The grid points correspond to the 10 m grid positions of the Finland Uniform Coordinate System KKJ (Kartastokoordinaattijärjestelmä).

Osram, Munich, Germany). Over a period of two weeks, the jars were daily visually checked for any emerged meso- and macroplanktonic animal. Besides hatchlings of *D. longispina* and *D. magna*, we notably found copepods and ostracods. An alternative assay would have consisted in visual checks of the sediments for resting stages, but this procedure was not pursued because we were only interested in resting stages with viable eggs since only these were regarded as truly dispersing (Cáceres & Soluk 2002).

Of all *D. magna* hatchlings, isofemale lines were produced by letting the animals reproduce asexually. Then, the hatchlings were checked for endoparasites following the protocol of Ebert et al. (2001). We were particularly interested in the presence of the *D. magna* specific microsporidian endoparasite *Hamiltosporidium tvaerminnensis* HAAG (formerly misidentified as *Octosporea bayeri* JIROVEC, Haag et al. 2011), which can be transmitted vertically from mothers to the sexual eggs of ephippia (Vizoso & Ebert 2004, Vizoso et al. 2005).

As the number of found *Daphnia* ephippia or rather emerged hatchlings was very low, we did not apply inferential statistics but restricted ourselves to descriptive techniques. In order to have a spatial reference to potentially meaningful predictors, we compiled maps of rock pool occupancy by *Daphnia* based on long-term monitoring data (Pajunen 1986, Pajunen & Pajunen 2003a). Further, we contrasted the findings with the estimated production of ephippia calculated from estimates of rock pool volumes by Pajunen & Pajunen (2007) and a linear regression equation by Altermatt & Ebert (2008). Plots were produced with R (R Development Core Team 2011, version 2.13.0) and the packges grid (Murrell 2006, version 2.13.0), lattice (Sarkar 2008, version 0.19-26), gstat and sp (Bivand et al. 2008, versions 0.9-81 and 0.9-82).

Results and Discussion

Origin and number of all emerged crustacean hatchlings are given in Table 4.1 and Fig. 4.3. Throughout the whole study period, only 1 *D. longispina* and 18 *D. magna* hatchlings were found at 7 different locations. One *D. magna* hatchling from the northern finding of 9 hatchlings on island N was infected with *H. tvaerminnensis*, the other *D. magna* were free of endoparasites. *Daphnia pulex* is quite rare on the studied islands (Pajunen 1986, Pajunen & Pajunen 2003b), which might explain its absence in the astroturf harvests.

On the northern island N, only the harvest in spring 2007 revealed any hatchlings but at the same time the largest numbers overall (Fig. 4.3). Conspicuously, all hatchlings on N originated from astroturf mats which were aligned exactly on a straight line in north-south direction. Furthermore, this harvest contained the only non-*Daphnia* resting stages, i.e. hatchlings from copepods and ostracods were found although their

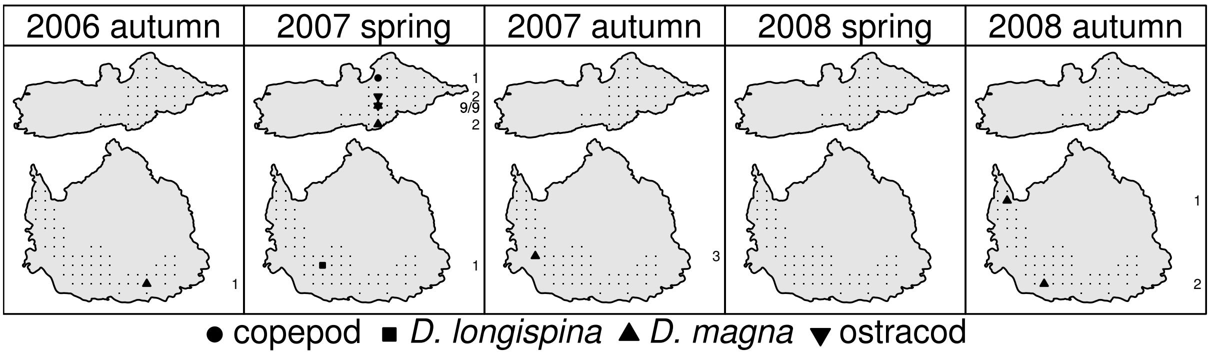


Figure 4.3 Origin of hatchlings in the 5 harvests. The astroturf mat positions are indicated with dots (compare Fig. 4.2). Note that the drawings of the two islands have been artificially placed next to each other to achieve a denser representation of the results. The dates refer to the times the astroturf mats were harvested and the numbers to the right of the symbols indicate how many hatchlings of each taxon were found. In spring 2007, there was one grid location where both *D. magna* and ostracods were found (overlapping symbols forming a star).

resting stages are supposed to be too small to be kept back efficiently by astroturf mats (Molau & Larsson 2000). Whereas from N no harvest was positive apart from spring 2007, *Daphnia* hatchlings were found in four out of five harvests from the southern island G (Fig. 4.3). Interestingly, the only *D. longispina* hatchling stemmed from G from the spring 2007 harvest, which was the only harvest containing non-*Daphnia* hatchlings emerged from relatively small propagules.

We concentrate our discussion on the origin and number of the hatchlings of the two *Daphnia* species *D. longispina* and *D. magna* since we possess long-term demographic data for these species.

Rock pool characteristics and occupancy

For each rock pool, a rich set of ecological variables is available (Ebert et al. 2001, Pajunen & Pajunen 2007) which we tried to associate with the location of the found *D. magna* hatchlings. However, neither rock pool dimension measures such as area or volume, nor hydrological variables such as catchment area, desiccation risk, or the presence of higher plants accelerating evopotranspiration, nor physicochemical properties such as Ca^{2+} concentration, conductivity, humic acid concentration, or pH showed any clear spatial accordance with the sites of *D. magna* hatchling discovery.

A more promising approach consisted in contrasting the findings with the demographic long-term *Daphnia* presence/absence data set by Pajunen (Pajunen 1986,

Table 4.1 Origins and numbers of crustacean hatchlings emerged from the resting stages found on the astroturf mats.

year	sample	island	mat	taxon	individuals	minimal dispersal distance (m)
2006	autumn	G	87	*D. magna*	1	4.8
2007	spring	G	56	*D. longispina*	1	6.5
		N	12	copepod	1	–
			43	ostracod	2	–
			54	*D. magna*	9	5.7
			54	ostracod	9	–
			68	*D. magna*	2	5.1
	autumn	G	42	*D. magna*	3	18.6
2008	autumn	G	10	*D. magna*	1	2.6
		G	80	*D. magna*	2	4.0

Pajunen & Pajunen 2003b) which allows to extract the number of biannual samplings between 1982 and 2008 which were positive for *D. longispina* and *D. magna*. The more samplings for a certain species were positive, the more stable the corresponding rock pool population is likely to be. Breaking down the number of positive samplings to none and one or more positive samplings since 1982, one obtains the number of rock pools that have ever been inhabited by either of the two species in the vicinity of every astroturf mat. The number of rock pools ever inhabited by *D. magna* per 100 m^2 square is given in Fig. 4.4. If a rock pool was never inhabited, it is likely to be not inhabitable at all or at least not over longer periods so that no pelagic population could be detected by the biannual samplings. The *D. magna* hatchlings found in the spring 2007 harvest on N came from within or directly north of the islands' region with the highest density of inhabitable rock pools (Fig. 4.4). On G, the *D. magna* hatchlings of most astroturf mats stemmed also mainly from regions with a high density of inhabitable rock pools, whereas the 3 hatchlings from the autumn 2007 harvest hatched from ephippia found on a mat with no inhabitable pools in the closer vicinity and only a low density of inhabitable pools in the further neighbourhood. These contrasting relations of origin and distance to highly populated regions indicate that the majority of ephippia may not disperse over long distances but a minority does, suggesting a leptokurtic distribution of dispersal distances (Allen 2007).

Ephippium production

Altermatt & Ebert (2008) give a simple linear regression model to estimate the number of ephippia produced by a *Daphnia* rock pool population over the course of a whole

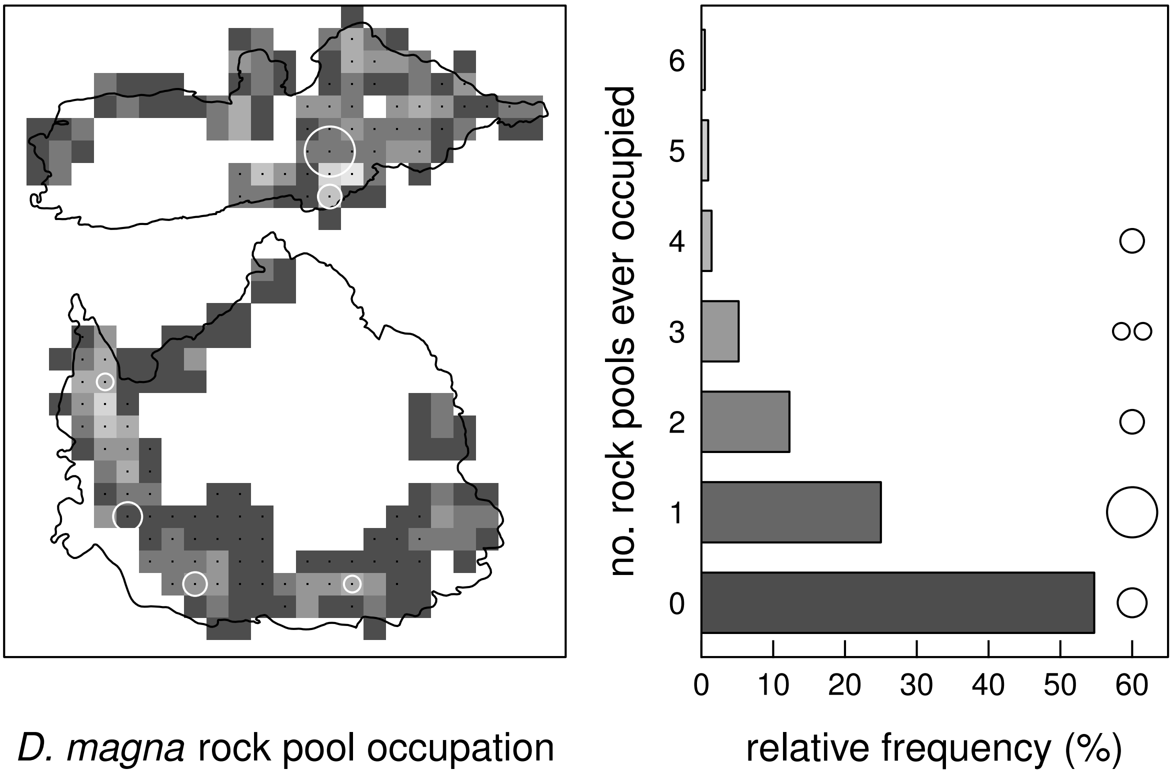


Figure 4.4 Number of rock pools ever occupied by *D. magna* between 1982 and 2008 per 100 m^2 square (left) and the relative frequencies thereof (right). The six finding sites of *D. magna* hatchlings are indicated with circles whose areas are proportional to the number of hatchlings (range 1–9) and are drawn both on the map and in the bar chart. Note that the gray levels in both graphs refer to the same number of occupied rock pools per 100 m^2 square. Astroturf mat positions are indicated with dots, squares represent the 10×10 m area around potential astroturf positions, i.e. positions not further away than 10 m from any rock pool.

season depending on the rock pool volume as the single predictor (provided by Pajunen & Pajunen 2007). We applied the regression equation to the sampling data of 2006–2008 (Pajunen 1986, Pajunen & Pajunen 2003b) and made the simplifying assumption that a population produces the full amount regardless of whether it was present in the first (summer) and/or second (autumn) sampling. The single *D. longispina* hatchling of the spring 2007 harvest came from an astroturf mat just north of the square with the highest estimated ephippium production on G in 2006. On the contrary, the *D. magna* hatchlings came from squares with rather low estimated ephippium production even in the surrounding squares (Fig. 4.5). In general, the positions where viable ephippia were found did not coincide with the estimated hot spots of ephippium production. This finding is in line with the study of Altermatt & Ebert (2008) who postulated that smaller rock pools contribute more migrants to the metacommunity since they are more prone to desiccation in summer. The areas with high estimated ephippium production are at the same time the areas with rock pools with the largest volumes and are thus much less affected by desiccation, which results in a smaller proportion of ephippia exposed to dispersal vectors than in smaller rock pools. Consequently, if we assumed that the found hatchlings originated primarily from rock pools in the immediate neighbourhood, then they would have dispersed from relatively small, desiccation-prone rock pools. However, exposure of ephippia does not only occur by desiccation, it may also happen by through strong storms moving parts of the rock pool water body in liquid or frozen state. Moreover, estimates of ephippium exposure as suggested by Altermatt et al. (2009) nearly coincide with the spatial density of ephippium production because even though larger, less desiccation-prone pools contribute relatively fewer ephippia, they still contribute more ephippia in absolute terms than smaller pools.

Ephippium banks

According to Pajunen & Pajunen (2003b), resting egg banks in rock pool populations of *Daphnia* are supposedly rare because of a wealth of dormancy breaking stimuli and washing out of sediments in shallow pools. The existence of longer lasting accumulations of ephippia in deeper rock pools or outside of rock pools in the surrounding vegetation or rock crevices can however not be excluded (Pajunen 1986, Pajunen & Pajunen 2003b). Egg banks outside of water bodies inhabited by zooplankton are often neglected, possibly because in many systems the bulk of resting eggs accumulates in the sediment as is for instance the case in lake *Daphnia* (e.g. Cáceres 1998, Tessier & Cáceres 2004, Mergeay et al. 2007). Consequently, resting egg banks are commonly viewed as long-term storage facilities through which persistence of species or temporally sequential coexistence of different species can be mediated (reviewed in

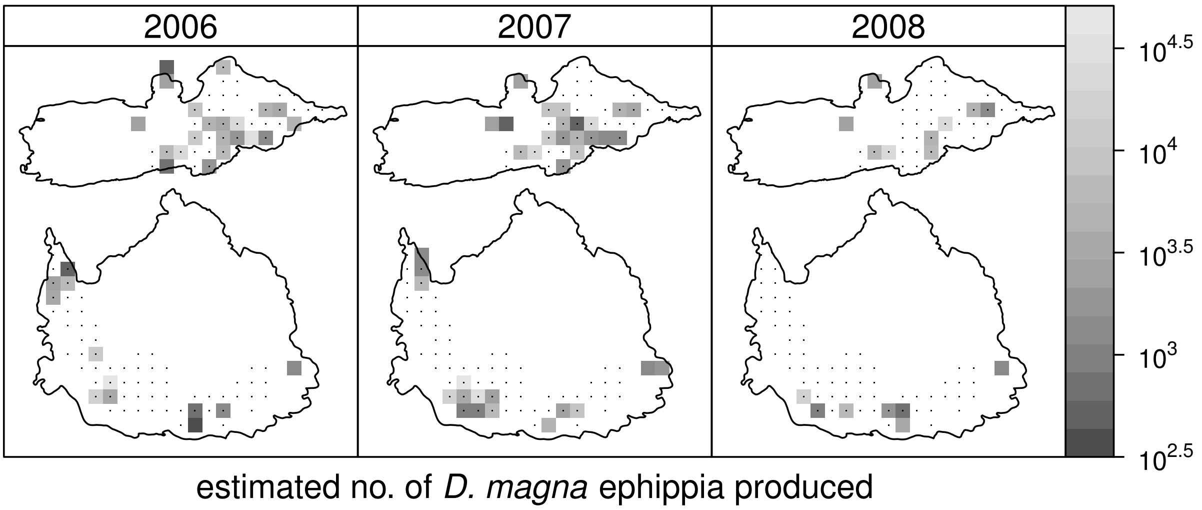


Figure 4.5 Estimated number of *D. magna* ephippia produced during a season according to a linear regression model by Altermatt & Ebert (2008). Areas with no extant *D. magna* population are not shaded. Astroturf mat positions are indicated with dots, squares represent the 10 × 10 m area around each potential astroturf position, i.e. positions not further away than 10 m from any rock pool. Note the logarithmic scale ($\log_{10}$).

Brendonck & De Meester 2003). Although short-lived resting egg banks do probably not serve as reservoirs from where animals adapted to historical regimes of nutrient availability (Hairston Jr. et al. 1999) or parasite infectivity (Decaestecker et al. 2007) can be recruited, they can be crucial for the persistence of metacommunities consisting of small, ephemeral ponds (Vanschoenwinkel et al. 2008a). In permanent aquatic systems, there is a constant flow of individuals from ephippium banks in the sediment back to the pelagic population (Cáceres 1998), whereas in more stochastic systems such as our *Daphnia* rock pool metacommunity, the back flow from rock pool ephippium banks is probably very limited (Pajunen & Pajunen 2003b). But since the dimension and longevity of ephippium banks outside of rock pools is largely unknown, their importance as constant reservoirs and providers of migrants is potentially underrated. The astroturf mats can be regarded as surrogate vegetation or rock structures holding back dispersing ephippia, and since the total area covered by the mats was negligible in relation to the total area surrounding the studied rock pool complexes, ephippium banks outside of rock pools might be larger than previously assumed. It remains to be clarified how well ephippia of rock pool *Daphnia* are adapted to sustain environmental stress outside of water.

Dispersal distances

More specifically, the demographic data set (Pajunen 1986, Pajunen & Pajunen 2003b) can be used to estimate the minimal dispersal distances of the found ephippia or hatchlings respectively. Under the assumption that ephippia found on astroturf mats must originate from rock pools — and not ephippium banks outside of rock pools — which were occupied at least according to one sampling by Pajunen & Pajunen in the current or preceding season, the minimal dispersal distances are as listed in Table 4.1. Giving each hatchling equal weight, the median dispersal distance for *D. magna* would then be 5.7 m, i.e. smaller than the estimate of 8 m calculated by Pajunen & Pajunen (2003b). But since the estimate of Pajunen & Pajunen (2003b) is based on nearest neighbour distances of rock pool colonisation events and thus represents indirect evidence, our estimate of directly observed dispersal seems feasible. The maximal value of 18.6 m, out of a very small sample size of only six locations and 18 hatchlings, indicates that longer dispersal distances are possible. Furthermore, since the calculations are based on the assumption that the ephippia must have come from the closest previously occupied rock pool, our estimates tend to be very conservative, and thus the median values rapidly increase when taking the second (5.8 m), third (7 m), fourth (8.8 m), etc. closest previously occupied rock pool. Conversely, in the light of ephippium banks outside of rock pools, our estimates might not be conservative at all but rather reflect the relatively short distances that ephippia realistically travel within half a year, i.e. approximately the time between subsequent astroturf harvests.

Dispersal vectors

No astroturf mat location where viable ephippia were found was situated in the overflow region of other rock pools, thus dispersal of ephippia via this course seems unlikely. On island N, all findings — of *Daphnia* and other crustacean propagules — were aligned in north-south direction (Fig. 4.3). These spatial and species richness patterns might be an indication of an extreme meteorological event such as strong winds or even waves from the Baltic Sea in longitudinal direction. A correlation with available meteorological data is however difficult to realise since such events probably occur on a very local scale. On island G, *Daphnia* ephippia were found in 4 out of 5 harvests (Fig. 4.3). One reason for this might be that G is more exposed to the Baltic Sea and therefore also less protected from winds blowing from southwestern, southern, or southeastern directions. However, although these explanations indicate that wind is favoured as the major dispersal agent, dispersal by animals cannot be excluded (Bilton et al. 2001) since the design of our propagule trap is not vector specific as opposed to e.g. in the study of Cáceres & Soluk (2002).

Hanski & Ranta (1983) speculated that *D. magna* might be better dispersers since their ephippia, unlike the ephippia of *D. longispina*, have appendages which may facilitate uplift by wind or adherence on birds or insects. The fact that we only found a single *D. longispina* hatchling is supportive, although *D. longispina* ephippia are substantially smaller than *D. magna* ephippia and may have been retained less efficiently by the astroturf mats. Furthermore, *D. longispina* tends to occur in larger rock pools (Pajunen & Pajunen 2007) with a lower desiccation risk (Altermatt et al. 2009), which consequently would lead to a lower probability of mass exposure of ephippia to wind (Altermatt et al. 2009). A screening by van de Meutter et al. (2008) showed that *Daphnia* ephippia are dispersed by backswimmers (Notonectidae) but that dispersal was size-selective towards smaller ephippia. Furthermore, ephippia had to be buoyant to be dispersed with the consequence that *D. magna* ephippia were never observed to be transported (van de Meutter et al. 2008). This is because they do not float on the water surface as opposed to ephippia of *D. longispina* which are actively deposited at the water surface by the females (Slusarczyk & Pietrzak 2008). In the studied rock pool system, corixids are very abundant (Pajunen & Pajunen 2003a), and we speculate that they might play the role of backswimmers as vectors of *D. longispina* ephippia. Taken together, we suggest that *D. magna* ephippia are sporadically exposed when rock pools dry up and *D. longispina* ephippia are continuously exposed at the rock pool water surface. A possible consequence could be the dependence on different dispersal vectors with *D. magna* adapted to wind and *D. longispina* adapted to insect transport.

The relative importance of wind and animals as vectors in the *Daphnia* rock pool metacommunity might currently remain unresolved, however, wind certainly contributes decisively to the dispersal of ephippia which are exposed in masses, which is the case for *D. magna* in desiccated rock pools. In a similar zooplankton rock pool metacommunity of African anostracans, initial trials to estimate wind dispersal with sticky propagule traps resulted in only 8 viable resting stages within 3 years, 7 of which were found at the same of three study sites (Brendonck & Riddoch 1999). Not only the total trap area and rock pool area were similar to the ones in our trial, also the study period and the number of found viable propagules was in the same order of magnitude (Brendonck & Riddoch 1999). The conclusion that the importance of wind dispersal was overestimated was later refuted by Vanschoenwinkel et al. (2008a,b). By using wind socks, they found about 850 propagules of 17 invertebrate taxa during a single month (Vanschoenwinkel et al. 2008a). Wind direction, but not wind speed, was found to be the best predictor for the trap yield (Vanschoenwinkel et al. 2008a). These results indicated that wind dispersal, besides water overflow and amphibians (Vanschoenwinkel et al. 2008b), is frequent at least over short distances and leads to fast recolonisations of emptied habitats, but also to the erosion of resting egg banks in rock pools (Vanschoenwinkel et al. 2008a). In direct comparison, ephippia of *D. longispina* and *D. magna* are larger than anostracan resting eggs and might thus not be as readily dispersed by wind, which is why trials with wind socks in the *Daphnia* rock pool metacommunity would probably not be more successful in detecting viable, dispersed ephippia.

The possibility of dispersal of *D. magna* ephippia by waterfowl and other birds may not be excluded because ephippia remain viable after a gut passage (Proctor 1964). On the one hand, at least the propagule findings on island N arranged in a straight line suggest that wind or waves are the more probable dispersal agents (Fig. 4.3). On the other hand, whereas wind is a potent short-distance disperser, animals such as waterfowl are certainly more efficient in long-distance dispersal of relatively large, heavy propagules as illustrated by Figuerola et al. (2005) who found that waterfowl migration routes coincide with gene flow of *Daphnia*.

Colonisation events

Arrivals of ephippia on astroturf mats may alternatively be regarded as pseudo-colonisation events and can thus be contrasted with real colonisation events in the surrounding metacommunity based on the long-term monitoring data set (Pajunen 1986, Pajunen & Pajunen 2003b). As many regions did either not contain inhabitable rock pools or all rock pools were already occupied, information on colonisation events is sparse. Colonisation events can be expressed as the fraction of colonisable rock pools which were actually colonised in the current sampling, or more specifically rock pools that did not contain a specific *Daphnia* species for at least a full year are now colonised (Pajunen 1986, Pajunen & Pajunen 2003b). Not unexpectedly, a spatial correspondence between the sparsely definable fraction of colonisation events and the origins of the *Daphnia* hatchlings is not easily established. Even though no clear temporal accord is visible, the hatchling origin sites lie within or very close to regions where colonisations in the metacommunity happen on a regular basis (data not shown). These regions might be more exposed to dispersing agents such as wind or incoming waves of the Baltic Sea, which would explain the good overall correspondence with the origin sites of the found hatchlings. On the other hand, regions with lots of colonisation events supposedly harbour smaller, more desiccation-prone rock pools and therefore more extinction-prone *Daphnia* populations, and at the same time these populations probably also have a higher exposure of ephippia, which in turn would also explain the correspondence.

As outlined in the introduction, estimates of colonisation events into natural rock pools are difficult to obtain since rock pools are either not inhabitable because *Daphnia* habitat preferences do not match the pool's physicochemical conditions or are already harbouring a community, which renders the disentanglement of local recruitment and immigration practically very difficult even if destructive measurements would be taken. 2 and 4 *D. magna* pseudo-colonisation events took place on N and G during the whole study period, whereas *D. longispina* only pseudo-colonised 1 astroturf mat on G. If one relates these events to the total number of colonisable astroturf mats, the pseudo-colonisation fractions for *D. magna* are 5.1 % on N and 6.3 % on G. For comparison, 8.2 % and 13.6 % of the colonisable rock pools on N and G were colonised by *D. magna* according to the long-term data set (Pajunen 1986, Pajunen & Pajunen 2003b). Compared to the long-term data, the pseudo-colonisation fractions for astroturf mats might seem small but they are still in the same order of magnitude. However, if one adjusts for the available surface area and divides these numbers by the total astroturf surface area and rock pool surface area per island respectively, the pseudo-colonisation fraction into astroturf mats was 0.65 % m^{-2} on N and 0.5 % m^{-2} on G for *D. magna*. These numbers are considerably larger than the corresponding numbers for the long-term data set where the colonisation fraction into rock pools was 0.02 % m^{-2} on N and 0.03 % m^{-2} on G for *D. magna*.The higher numbers for pseudo-colonisation events might be an indication that only a fraction of the hatchlings manages to establish itself (e.g. due to priority effects, compare De Meester et al. 2002, Calcagno et al. 2006), which is expressed in the much lower numbers for the colonisation events extracted from the long-term data set. More likely however, lots of colonisation events are not detectable because they occurred in already occupied rock pools. Furthermore, astroturf mats and rock pools act as filters of passing ephippia, and the efficiency of such a filter probably depends strongly on its spatial extension.

Haag et al. (2006) and Louette et al. (2007) suggested that the founding of new populations is accomplished by very few or single individuals in the *Daphnia* rock pool metacommunity and a system of artificially created ponds, respectively. The numbers of found *Daphnia* hatchlings per astroturf mat (Table 4.1) do not fully back this explanation because in one case, 9 hatchlings were found. The 9 hatchlings probably stemmed from a cluster of ephippia which was dispersed as a whole. If dispersal events of such ephippium conglomerates occur frequently, habitat monopolisation by residents (De Meester et al. 2002) and consequent preemption of invaders (Calcagno et al. 2006) may explain the low number of migrants inferred from genetic differentiation of local populations in the rock pool metacommunity (Haag et al. 2005). Even though inbreeding depression in the *Daphnia* metacommunity is high and immigrant genes have a selective advantage due to hybrid vigour of outbred offspring (Ebert et al. 2002), priority effects might still prevent the successful establishment of the majority of immigrants.

Eventually, *Daphnia* were not always dispersing on their own: one out of 18 *D. magna* hatchlings was found to be infected by the microsporidian *H. tvaerminnensis*. Although previously assumed, this is direct proof that the horizontally and vertically transmitted endoparasite (Vizoso & Ebert 2004, Vizoso et al. 2005) is dispersed by phoresis in ephippial *D. magna* eggs. It remains however unclear whether phoresis or direct transport of spores is more important in short and long distance dispersal of this parasite.

Conclusions

Indirect quantifications of dispersal in the *Daphnia* rock pool metacommunity tend to underestimate dispersal since priority effects can prevent the establishments of immigrants (De Meester et al. 2002, Calcagno et al. 2006). Furthermore, using long-term demographic data to estimate dispersal conceals colonisations of already occupied rock pools (Pajunen 1986, Pajunen & Pajunen 2003b) and is thus also not appropriate. In contrast, astroturf mats, by simulating colonisable habitat patches, have the advantage that all viable ephippia can be detected, and so they proved to trap *D. magna* ephippia to a degree that is comprehensible. Although the found quantities of viable *Daphnia* ephippia are small, one has to bear in mind that only about 0.2 % of the rock pool areas of the two skerry islands were covered with astroturf mats. Extrapolating the number of *D. magna* hatchlings to the total area would result in almost ten thousand potential migrants in the study area (Fig. 4.2). Thus temporally irregular dispersal, likely predominantly by wind, of *D. magna* might be much more frequent than previously thought. In combination with the speculated temporally more regular insect-mediated dispersal of *D. longispina*, species sorting processes (Leibold et al. 2004) could be driven similarly as in the African anostracan rock pool metacommunity studied by Vanschoenwinkel et al. (2007, 2008a). The differential occurrence of *D. longispina* and *D. magna* in larger, more stable and smaller, more desiccation-prone rock pools, respectively (Pajunen & Pajunen 2007) might thus be a direct consequence of the presumably species-specific dispersal strategies. That we mainly found *D. magna* ephippia underlines the finding of Altermatt et al. (2008) who demonstrated that *D. magna* is the most dynamic species in the rock pool metacommunity, which further nourishes the speculation that *D. longispina* and *D. magna* may disperse generally by animals and wind, respectively. Dispersal, although often difficult to measure (Hanski et al. 2000, Bilton et al. 2001), is the subject of a vast amount of studies, but the question why specific dispersal strategies evolved is often not raised (Dieckmann et al. 1999). *Daphnia* rock pool metacommunities provide an opportunity to catch up on this omission and the results at hand may indicate where to place the emphasis in future investigations.

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Chapter 5

Parasite adaptation and similarity selection in a host-parasite system

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(manuscript revision no. 2393)

Abstract

Sexual recombination is common among higher organisms. The costs associated with male production, mate finding and the break-up of co-adapted gene complexes makes the widespread occurrence of sex still a puzzle for evolutionary biologists. A recent model suggested that under conditions of even a small degree of mother-offspring parasite transmission, coevolving parasites may account for the evolutionary maintenance of sexual reproduction under a wide range of parameters (Agrawal 2006). Under these conditions the genetic similarity between mother and offspring becomes important because vertical transmission of host-genotype specific parasites would be facilitated if offspring resembled the genotype of the mother. We used an outdoor mesocosm experiment to test if the observation that outbred offspring suffer less from parasitism in a *Daphnia magna* - microsporidian system can be explained by the similarity selection hypothesis. We compared parasite transmission to sexual host offspring from mothers infected with either adapted or novel isolates of the parasite *Hamiltosporidium tvaerminnensis* and found that the adapted parasites had the highest transmission success. This result supports the similarity selection hypothesis and suggests a direct benefit for sexual reproduction, mediated by the genotypic dissimilarity between mothers and their sexual offspring. We further found that the advantage of sexual reproduction is reduced when the host population was kept in competition with *D. longispina*, suggesting that resistance to the parasite is costly.

Introduction

Parasites and hosts are engaged in antagonistic coevolution where selection favours hosts which minimise the fitness costs imposed by the parasite and parasites which maximise survival and transmission while exploiting the hosts (Thompson 1989, Woolhouse et al. 2002). During such arms races, the parasite population is believed to track common host genotypes, which results in negative frequency dependent selection with the allele frequencies of the coevolving hosts and parasites cycling over time (Clarke 1976, Lively & Dybdahl 2000). Under conditions of such coevolutionary dynamics, genetic recombination may be beneficial because it creates novel host genotypes by breaking up formerly successful gene complexes which are now disadvantageous since the parasite population adapted to them (Hamilton 1980, Jaenike 2007, Salathé et al. 2008). The idea that sexual reproduction may be maintained by coevolving parasites is known as the Red Queen hypothesis (Bell 1982) and has become one of the leading models explaining maintenance of sexual reproduction (de Visser & Elena 2007, Salathé et al. 2008). However, this idea has been criticised for working only under a very restrictive set of parameters (Parker 1994, Otto & Nuismer 2004). Recently, the introduction of a further assumption showed that the parameter space under which Red Queen dynamics select for sexual reproduction (Agrawal 2006). The assumption is that the encounter between a host and a parasite is not entirely random within the population but instead a notable portion of the offspring might encounter parasites which had previously adapted to their mother (Rice 1983, Agrawal 2006). Asexual offspring of an infected mother will be equally susceptible to the parasite as its mother (because of having the same genotype), while sexual offspring may gain an advantage by being dissimilar and therefore are expected to be on average less susceptible (Fig. 5.1a). Thus, parasite success and the consequent selection exerted on the host are not only a function of the current host genotype, but also of the genetic similarity to its mother and the degree to which the parasite is adapted to the mother's genotype. The main reason for similarity selection to shift the balance in favour of genetic recombination is that in the presence of vertically transmitted parasites, sexually reproducing mothers have an advantage by producing dissimilar offspring because the mean fitness of their offspring is higher than the mean fitness of offspring of mothers reproducing asexually. This is a direct benefit of sex. Without similarity selection, the benefits of sex under conditions of host-parasite arms races are considered to be indirect (Salathé et al. 2008).

Similarity selection in the context of Red Queen coevolution may play a role whenever at least some proportion of transmission is between mother and offspring. This may be the case in typical horizontally transmitted parasites with some degree of vertical transmission due to little or late offspring dispersal, parental care, or viscous population structure. It is also found in systems with parasites with both vertical

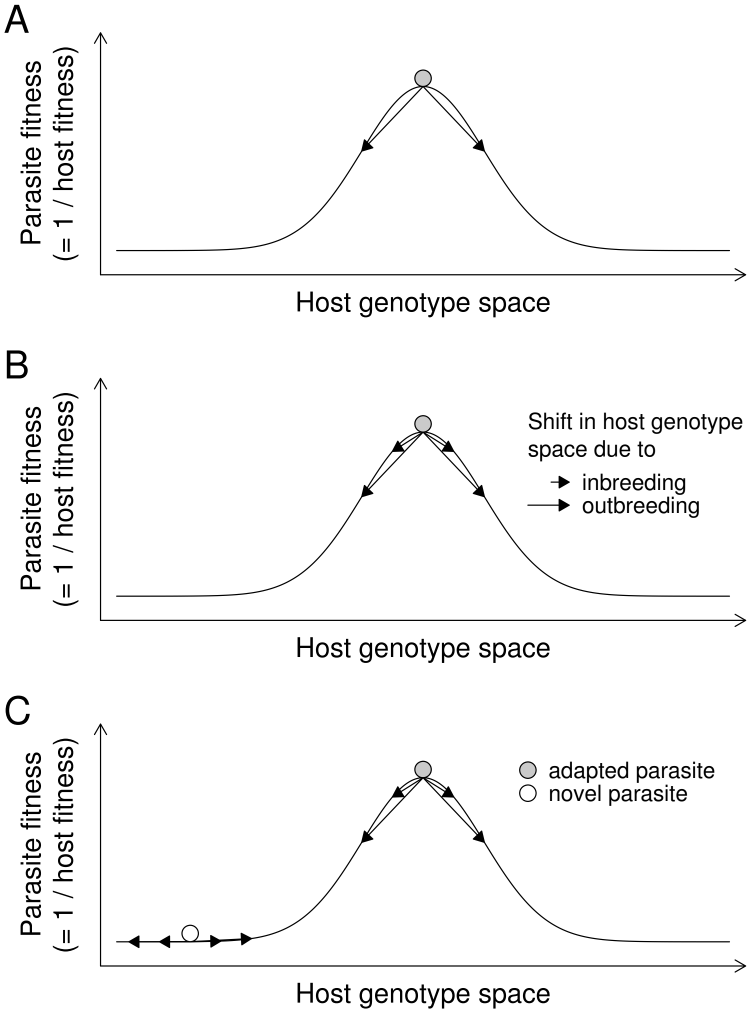

Figure 5.1 A graphical version of the similarity selection hypothesis. The graphs show a fitness landscape for the parasite, which is an inverse landscape for the host. Adapted parasites sit at or near the top of the hill (grey dot). With asexual host reproduction the parasite will stay were it is, because the host genotype does not change. The three graphs show three ways to test the similarity selection model. a) Sexual reproduction would shift the host genotype away from the peak. The parasite would loose fitness (in the case of our study, reduced vertical transmission). This test is not possible with the *Daphnia* system, as sexual reproduction is obligatorily linked to resting. b) The effect of inbreeding versus outbreeding hosts. Inbreeding shifts the genotype space only a little bit (short arrows), while outbreeding shifts it stronger. c) The effect of the parasite being adapted to a host genotype or being novel. Host sexual reproduction reduces parasite fitness if the parasite is adapted, but not if the parasite is novel. In the later case, a shift in host genotype space does not affect host or parasite fitness.

and horizontal transmission as part of their normal transmission strategy (Schuster & Coyne 1974, Agnew & Koella 1997, Kaltz & Koella 2003). Finally it is the case in mostly vertically transmitted parasites which maintain virulence by some degree of horizontal transmission (Mangin et al. 1995, Lipsitch et al. 1996, Vizoso et al. 2005). The later is the case for the here investigated microsporidian parasite *Hamiltosporidium tvaerminnensis* infecting the planktonic crustacean *Daphnia magna* which is well suited to test the similarity selection hypothesis experimentally.

Background and study system

The microsporidium *Hamiltosporidium tvaerminnensis* Haag (formerly misidentified as *Octosporea bayeri* Jirovec, Haag et al. 2011) is a host specific endoparasite of the cyclic parthenogen *Daphnia magna* 1820 Straus, common in the rock pool metapopulation of the Baltic Sea (Ebert et al. 2001). The parasite reduces fecundity, survival and competitive ability of its host (Vizoso & Ebert 2005, Zbinden et al. 2008). In the seasonal environment of the *Daphnia* metapopulation, the parasite is found in intermediate prevalences in spring, while it regularly reaches 100 % prevalence by mid summer (Lass & Ebert 2006, Ebert et al. 2007). The parasite produces environmental spores which allow for horizontal transmission after release from the decaying cadaver of the host. At the same time, *H. tvaerminnensis* is transmitted transovarially from mothers to asexual offspring with 100 % efficiency (Vizoso et al. 2005, Ebert et al. 2007). Vertical transmission is also observed to sexually produced offspring, but up to 30 % of the sexual offspring may escape infection (Vizoso et al. 2005), which is of direct benefit for these offspring as they will not suffer from the costs of infection. This alone, however, does not allow the conclusion that this benefit is due to genetic recombination because in *Daphnia magna*, the process of sexual reproduction is obligatorily linked to a resting phase (all sexual eggs must undergo diapause) while asexual reproduction is not (asexual eggs develop directly). Thus, reduction in parasite transmission to sexual offspring may be a consequence of the diapause of the sexual egg, instead of or in addition to genetic recombination and/or of the diapause of the sexual egg. Therefore experiments are necessary to disentangle mode of reproduction from diapausing.

The similarity selection hypothesis can be tested in different ways. A direct comparison between asexual and sexual offspring (Fig. 5.1a) is, however in most systems not possible because it is confounded with other factors (in the case of *Daphnia* with the resting phase). A different way to test the similarity selection hypothesis is to breed offspring with different degrees of genetic similarity to their mother (Fig. 5.1b). Such an experiment, in which all treatment groups passed through diapause, revealed that outbred *D. magna* offspring escaped infection more often (85.2 % infected) than selfed offspring which were the product of matings of mothers with their asexually produced sons (98.9 % infected; $P < 0.001$) (Ebert et al. 2007). This outcome is consistent with predictions from the similarity selection hypothesis (Fig. 5.1b), as the parasite encounters in the selfed offspring, genotypes relatively more similar to the mother's genotype, while the outbred offspring's genotype is less similar to the mother's genotype. However, the result could also be explained with by the different levels of inbreeding among the treatments. Selfed offspring might suffer from inbreeding depression and are therefore less capable of defending against the infection.

A further way to test predictions of the similarity selection hypothesis is by manipulating the degree to which the parasites are adapted to their hosts. A novel parasite will have no adaptation to the offspring while a parasite adapted to the mother should also have an advantage in the offspring because even sexual offspring resemble their mother to some degree, whereas novel parasites will lack this advantage (Fig. 5.1c). Sexual offspring from mothers infected with adapted parasites should thus suffer more from parasitism than offspring from mothers with novel parasites. Furthermore, when the parasite is novel, the similarity selection hypothesis predicts no difference in parasite success between inbred and outbred host offspring since the performance of the parasite is a consequence of a random association between the host and the parasite genotype (Fig. 5.1c). In contrast, the inbreeding hypothesis predicts that outbred offspring should do better than the presumably immunocompromised inbred offspring, even if infected with a novel parasite.

Previous studies have highlighted the potential of *H. tvaerminnensis* to adapt to its *Daphnia* host. *Hamiltosporidium* exhibits local adaptation to *D. magna* in rock pool populations (Altermatt et al. 2007). This gives immigrant hosts an advantage because the locally adapted parasites are more harmful for the local hosts than for the immigrants. Furthermore, infected field collected host clones were, after curing and cloning, more susceptible to their own parasite isolate than host genotypes which were uninfected at the time of collection from the same population (Altermatt & Ebert 2007). These findings allowed us to assume that parasites which had been kept in monoclonal lines of their hosts for many generations are specifically adapted to these host clones.

Results

To test if the similarity selection or the inbreeding hypothesis is a better explanation for the reduction in vertical transmission of *H. tvaerminnensis* to sexual offspring of *D. magna,* we set up an experiment in which populations of *D. magna* with 100 % prevalence of *H. tvaerminnensis* were allowed to pass through one cycle of sexual reproduction under natural outdoor conditions. Inbreeding and parasite adaptation were manipulated in these populations in a 2-way full-factorial design, producing four treatment groups with 18 replicate populations each (total $N = 4 \times 18 = 72$). Inbreeding was manipulated by forming either monoclonal (1 *D. magna* clone) or polyclonal (4 clones) populations. All hosts were infected under standardised conditions with *H. tvaerminnensis*. As adapted parasites we used *H. tvaerminnensis* isolates which had been cultured with the host clones for at least 25 asexual generations of the host, whereas non-adapted parasites were isolates horizontally transferred to the hosts shortly before the start of the experiment (novel combinations).

One to four days before the release of the animals into the outdoor mesocosms, we tested, whenever possible, 5 asexual offspring from all *Daphnia* clones (with novel and evolved infections), for the presence of the parasite. For the novel infections, these were the second generation offspring of the animals which were exposed to the parasite and which were tested positively in the first generation. Without a single exception, all tested offspring were infected, confirming the high fidelity of *H. tvaerminnensis* transmission during asexual host reproduction, even in case when the parasite was not adapted (in total 492 offspring). Lines which did not become horizontally infected in the first generation were not included in the experiment. Our experiment was thus biased in that only combinations were used which the parasite was able to infect. This makes our test of the similarity selection hypothesis more conservative because incompatible combinations were excluded.

After 96 days in the mesocosms under natural conditions, we collected the sexual eggs from all populations, overwintered them outdoors, and allowed them to hatch in the field in the following spring. Parasite transmission success was determined from the proportion of infected hatchlings from the sexual eggs. The similarity selection hypothesis and the inbreeding hypothesis make contrasting predictions for our experiment. While the predictions for the similarity selection hypothesis are based on the idea that parasite adaptation will facilitate the parasite's transmission from the mother to the sexual eggs, the inbreeding hypothesis is based on the idea that inbred hosts have a poorly functioning immune defence. Consistent with the similarity selection hypothesis, the evolved, presumably adapted parasites were transmitted with higher fidelity than the novel parasites in both monoclonal and polyclonal host populations (Fig. 5.2, Table 5.1). Novel parasites performed equally poorly (reduced vertical transmission) in mono- and polyclonal host populations, which is inconsistent with the inbreeding hypothesis but consistent with the similarity selection hypothesis (Fig. 5.2). Consistent with our earlier study (Ebert et al. 2007), transmission of adapted parasites tended to be higher in monoclonal than in polyclonal host populations (subset of the evolved parasite treatment only: $z = 1.85, P = 0.065$).

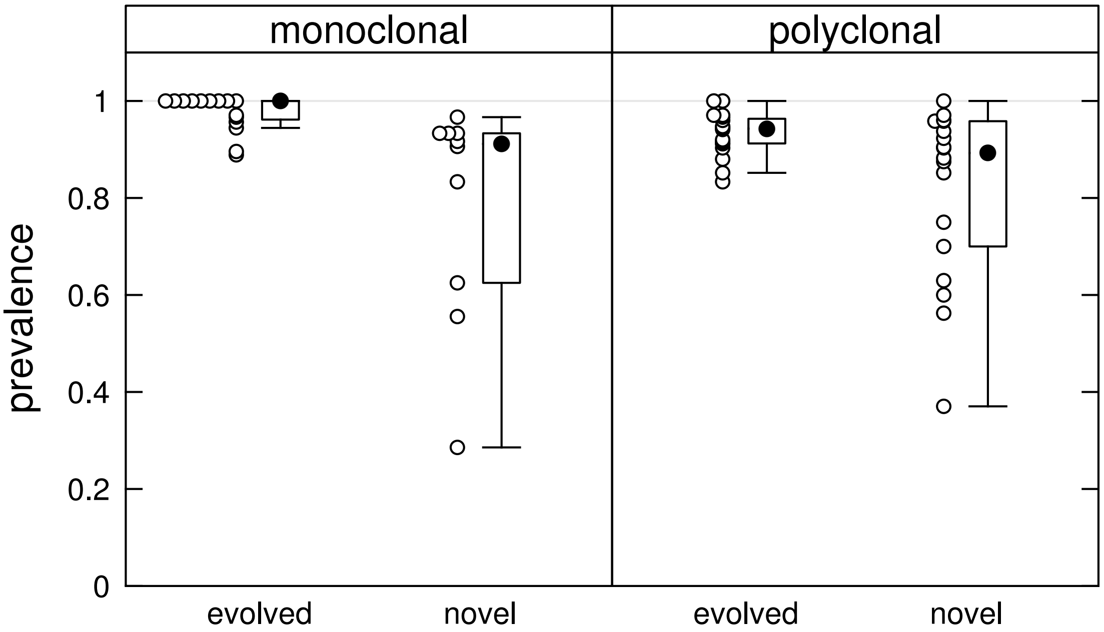

Figure 5.2 Average proportion of infected offspring per mesocosm in the four treatment groups of the first experiment. Data points are displayed individually and summarised as box-and-whiskers plots. Populations were either started from 1 clone (monoclonal) or from 4 clones (polyclonal) and were infected either with evolved parasite lines (evolved) or with new parasite lines (novel).

Table 5.1 Coefficient estimates with associated 95 % confidence intervals for the full model for experiment 1. The estimate for the dispersion parameter for the binomial error was 3.51 and used to correct the standard errors for the partial Wald tests.

Variable	Estimate		95 % CI	*z* value	*p* value
intercept	odds	25.9	9.87 - 68.2	6.60	< 0.0001
host (polyclonal – monoclonal)	odds ratio	0.561	0.177 - 1.77	−0.986	0.32
parasite (novel – evolved)	odds ratio	0.191	0.0608 - 0.600	−2.83	0.0046
host × parasite	ratio of odds ratios	1.52	0.387 - 5.94	0.596	0.55

Table 5.2 Coefficient estimates with associated 95 % confidence intervals for the full model for experiment 2. *D. magna* clone and grid (a blocking treatment) were modelled as random effects (not shown).

Variable	Estimate		95 % CI	*z* value	*p* value
intercept	odds	10.3	3.80 - 27.8	4.59	< 0.0001
inbreeding (inbred – outbred)	odds ratio	3.93	0.884 - 17.5	1.80	0.072
competition (competition by *D. longispina* – no competition)	odds ratio	2.18	1.41 - 3.37	3.52	0.00043
inbreeding × competition	ratio of odds ratios	1.39	0.590 - 3.28	0.753	0.45

Daphnia magna from small rock pools in the metapopulation are often highly inbred, due to strong single clone founder effects (Ebert et al. 2002, Haag et al. 2005). To mimic this bottleneck, we set up a second experiment in the same manner as the previous experiment but with all mesocosm populations being mono-clonal. Half of the clones were previously inbred (inbreeding coefficient $F = 0.5$), the other half outbred ($F = 0$). We crossed these two treatments with a competition treatment factor. Half of the populations were kept without competitor while for the other half we introduced the *D. magna* clones into mesocosms which contained populations of *D. longispina*. *D. longispina* is often co-occurring in rock pools with *D. magna*. Although it shares largely its niche with *D. magna*, it neither crossbreeds with it nor has it ever been found to be infected with the parasite *H. tvaerminnensis*.

The second experiment revealed a significant competition effect, with competition reducing the likelihood of escaping from parasitism (Fig. 5.3, Table 5.2). This may indicate that resistance is costly and less affordable under conditions of intense competition. The effect of the breeding status was in the predicted direction, with outbred hosts producing more offspring escaping the parasite. However, this effect only approached significance (Fig. 5.3, Table 5.2).

Discussion

Consistent with earlier studies (Vizoso et al. 2005, Ebert et al. 2007) *H. tvaerminnensis* showed 100 % success in vertical transmission during asexual *Daphnia* reproduction, regardless of whether it was well adapted to the host genotype or novel. Failure to transmit vertically became only apparent during vertical transmission to sexual host offspring (Figs. 5.2 and 5.3), with the rate of vertical transmission being dependent on the genetic constitution of the host population, the degree of parasite adaptation to the mothers and the presence of a competitor of the host. These findings clearly show that

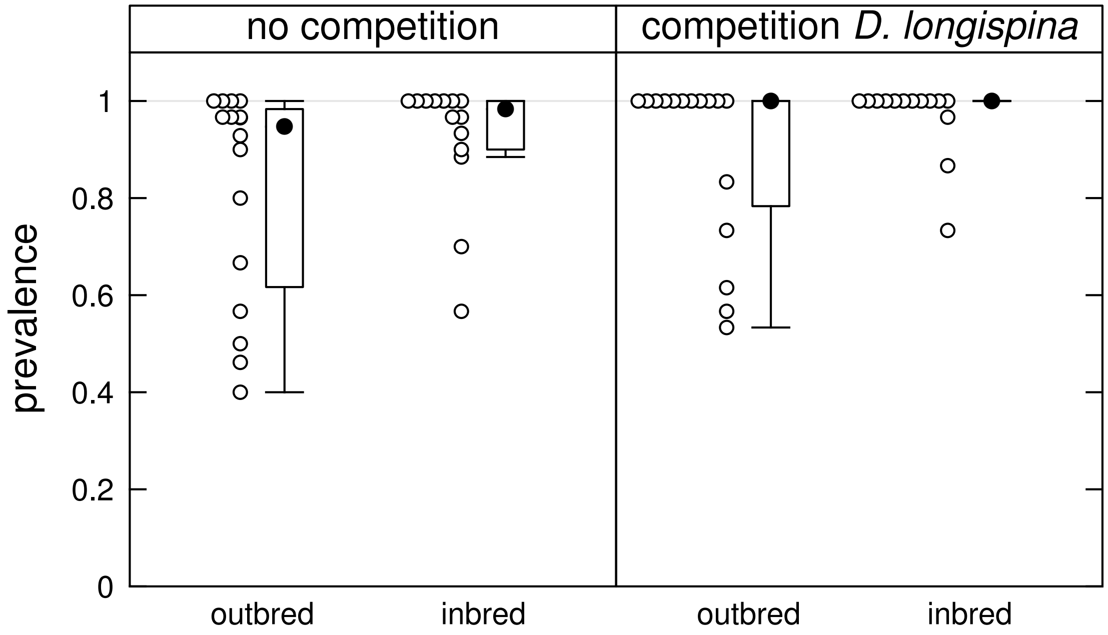

Figure 5.3 Average proportion of infected offspring per mesocosm in the four treatment groups of the second experiment. Data points are displayed individually and summarised as box-and-whiskers plots. *Daphnia magna* populations started with 1 clone which was either outbred or inbred and either faced no competition or faced competition by *D. longispina*.

the reduction in vertical transmission success to sexual host offspring is not a simple consequence of resting stage production and resting which are obligatorily connected to sexual reproduction in *D. magna*.

Parasite adaptation and similarity selection

Parasites which had been living with and presumably adapting to a given host genotype suffered less from the failure of vertical transmission to sexual host eggs than novel parasites. Thus, parasite adaptation to an asexually reproducing host clone comes along with a benefit in vertical transmission during sexual reproduction of the host. This finding is consistent with the predictions of the similarity selection hypothesis (compare Fig. 5.1c). Parasite fitness after host sexual reproduction is thus lower for novel than for evolved parasites. Furthermore, for novel parasites it did not make a difference whether the host offspring were selfed or outcrossed, which is also consistent with the similarity hypothesis.

Most studies on the evolutionary maintenance of sexual reproduction focus on indirect benefits of sex because direct benefits have hardly been reported and are generally considered to be an exception (de Visser & Elena 2007). In contrast to indirect benefits, direct benefits would allow to explain the paradox of sex with ease (Maynard Smith 1978, de Visser & Elena 2007). Our study suggests a direct benefit for genetic recombination because the mean fitness of the (partially uninfected) offspring is higher than the fitness of the infected mother, or her asexual offspring. To our knowledge this is the first demonstration of a direct benefit of sexual reproduction.

Our study suggests that host recombination may be beneficial because parasites adapt to their hosts (Fig. 5.2). Without parasite adaptation, sexual reproduction would not make a difference for the parasite (Fig. 5.1c). This finding is at the core of the coevolutionary hypothesis for the maintenance of sex, the Red Queen hypothesis (Hamilton 1980). Our study stresses that sex is particularly beneficial if there is a temporal autocorrelation in the specific factors which cause selection, i.e. when selection by host-genotype specific parasites acts across generations. If every host generation would encounter a random set of parasite genotypes, sexual reproduction would not be beneficial for the host. In the typical Red queen models (Hamilton 1980, Hamilton et al. 1990, Otto & Nuismer 2004), temporal autocorrelation is produced by negative frequency dependent selection. The parasite genotypes common at the time the mother lived are still common when the offspring are present. In the similarity selection hypothesis, the temporal autocorrelation of selection is strengthened by vertical transmission, because the offspring have a higher likelihood to suffer from the parasites adapted to their mother than without vertical transmission.

The reduction in parasite transmission observed in the here presented experiments and in the previous study seem rather small. In our previous study, we used an epidemiological model streamlined for the *D. magna* - *H. tvaerminnensis* system, to show that even small reductions in transmission rate can have profound fitness consequences (Ebert et al. 2007), with the benefit depending on the background prevalence of the parasite. When nearly all animals in the population are infected, uninfected individuals enjoy a large fitness benefit. Under conditions of low background prevalence, these benefits are low. However, prevalence of *H. tvaerminnensis* is typically high, frequently reaching prevalences of up to 100 % in natural populations (Lass & Ebert 2006, Ebert et al. 2007). Thus, the small differences in transmission observed in our study indicate a strong fitness effect under natural conditions.

Higher vertical transmission rates to sexual eggs cannot be a consequence of selection on improved vertical transmission during evolution in hosts reproducing asexually, because novel and adapted parasites had already 100 % vertical transmission success during asexual host reproduction. Rather, increased vertical transmission through the sexual eggs may be a consequence of selection for improved multiplication or survival of the parasite during the asexual phase of the host. This form of adaptation may lead to higher numbers of parasites being passed into the resting eggs and thus lead to an increased chance for successful vertical transmission to the sexual host offspring. Too low numbers of parasites transferred into the egg may lead to a failure of vertical transmission. This mechanistic explanation can be tested experimentally, using quantitative PCR to assess parasite representation during different phases of resting egg production and resting.

Inbreeding versus similarity selection hypothesis

We have previously shown that outbreeding reduces vertical transmission of *H. tvaerminnensis* as opposed to selfing (Ebert et al. 2007). This is consistent with the similarity selection hypothesis (compare Fig. 5.1b), but this earlier study was not able to exclude a general inbreeding effect (i.e. inbred offspring are poor defenders against parasites). Using a treatment with adapted and non-adapted parasite lines, we provide evidence in favour of the similarity selection hypothesis. The lack of a difference between mono- and polyclonal populations when exposed to a novel parasite is inconsistent with the inbreeding hypothesis, whereas it is in accordance with the similarity selection hypothesis (Fig. 5.2, Table 5.1).

Both hypotheses predicted a difference between the transmission rates among treatments with different degrees of inbreeding, as was also found in the previous study (Ebert et al. 2007). The reason for the a weaker support of the monoclonal versus polyclonal effect in the present study (Fig. 5.2) may be that the parasites in the current study were adapted for much longer times to their host clones (1–7 years in the laboratory with exclusive asexual host reproduction), while in the earlier study females were caught from natural populations after hatching from resting eggs and used directly for the experiment (Ebert et al. 2007). The experiment with the competition treatment also revealed a marginal significant effect of the clone treatment. In this case all clones selfed, but the inbreeding coefficient of the sexual offspring of the outbred mothers was 0.5, while for the inbred mothers it was 0.75. The change from mother to offspring genotype was somewhat larger in the outbred mother treatment, presumably making it harder for the parasite to establish itself in the offspring. The small difference in the change of the genotype from mothers to offspring may have reduced the effect size in this experiment.

Competition

Our second experiment revealed an effect of interspecific host competition on the vertical transmission rate of the parasite to sexual eggs. Resting eggs do not play a direct role in competition within a season. We speculate that the higher success of the parasite in the competition treatment is a consequence of a lower quality of the mothers or of the resting eggs. Mothers being under conditions of interspecific competition may have less means to control parasite multiplication or survival than those experiencing only intraspecific competition and therefore parasites may have better chances to establish themselves in the resting eggs. Moreover, if the eggs or embryos themselves can influence parasite success, the resting eggs of stressed mothers may be less capable of doing so. Maternal effects on offspring resistance to parasites had been reported before for *D. magna* (Mitchell & Read 2005).

Competition between *D. magna* and *D. longispina* is common in the rock pool metacommunity (Ranta 1979, Pajunen & Pajunen 2007). Typically, parasitised *D. magna* are worse competitors than healthy *D. magna* (T. Zumbrunn & D. Ebert, in preparation), giving *D. longispina* an advantage in interspecific competition. Our finding adds another benefit of parasitism of *D. magna* for *D. longispina*, one that is only realised across seasons. In spring parasite prevalence was higher in *D. magna* populations which were in competition with *D. longispina* in the previous season (Fig. 5.3). This reduces the population growth of *D. magna*, and gives a further advantage to *D. longispina*. Thus, competition between the two *Daphnia* species is not only directly influenced by the effect of the parasite on the performance of its hosts but also indirectly by the increased parasite transmission across seasons in *D. magna* due to competition. Comparisons of prevalences in natural *D. magna* population with and without *D. longispina* revealed indeed lower prevalence in the absence of *D. longispina* (T. Zumbrunn, unpublished data).

The results of the competition experiment indicate that mothers under supposedly stressful conditions have a disadvantage with regard to *H. tvaerminnensis* infection. This finding is consistent with a cost of resistance. If resistance against *H. tvaerminnensis* requires resources, stressed mothers may be less able to defend their offspring against parasites. An experimental evolution study with natural *D. magna* populations evolving with and without *H. tvaerminnensis* for 2 growth seasons has previously shown that costs are involved in the defence against *H. tvaerminnensis* (Zbinden et al. 2008). Populations that evolved with the parasite showed higher levels of tolerance towards *H. tvaerminnensis* but paid a cost in terms of reduced growth rates in the absence of the parasite. The costs observed in the current study and those in the earlier study are however based on different mechanisms. The costs revealed by the competition experiment shown here may be based on resource limitation while the costs revealed by the experimental evolution study indicated a genetic trade-off (Zbinden et al. 2008).

Conclusions

The suggestion that parasites being transmitted from mothers to offspring may play a role for the evolution of sex is more than 25 years old (Rice 1983). The sometimes oversimplified statement that vertical transmission will lead to avirulence may initially have reduced the acceptance of this hypothesis, until a formal genetic analysis recently showed that some degree of vertical transmission is particularly favourable for the evolution of sex, and in particular when the parasite is not highly virulent (Agrawal 2006). Our results are clearly in support of the similarity selection hypothesis, suggesting that host recombination is a mean to break down host-genotype-specific parasite adaptation, which happens during the asexual growth phase of the *Daphnia* host. While the *Daphnia-Hamiltosporidium* system does in many aspects not reflect typical host-parasite systems, its somewhat unusual features allowed us to test this hypothesis with simple experiments. There are many other systems where, in an ecological setting, some degree of mother-offspring transmission is likely (Rice 1983, Agrawal 2006), suggesting that similarity selection may be common. The predictions of the similarity selection hypothesis may be tested in some of these systems as well.

Material and Methods

All *Daphnia* and *Hamiltosporidium tvaerminnensis* were isolated from rock pools of the South-Western Finnish skerry archipelago. *Daphnia* clones were kept in the laboratory in artificial medium (Klüttgen et al. 1994) and fed with the unicellular alga *Scenedesmus obliquus*. Lines were kept in triplicate populations in 400 ml jars. The population size in these jars was usually around 40 to 60 individuals. Outdoor experiments were set up on the island Furuskär of the Tvärminne archipelago, close to natural rock pools. Populations were set up in 10-L plastic buckets, filled with 6 L natural rock pool water filtered through a 20 μm nylon net. No food was added during the experiments and the populations were allowed to produce resting eggs over the summer. In September of the same year, we collected the resting eggs (= ephippia) from all buckets by pouring the entire content of the buckets through a sieve. The ephippia from each replicate were then placed separately in 10 ml medium and stored over winter outside in a weatherproof box. In late April of the following spring, the ephippia were placed outdoors in buckets filled with 6 L rock pool water. During the following 4 weeks, all hatchlings were collected and after a minimum of 10 days inspected for the presence of

H. tvaerminnensis infections using phase-contrast microscopy at 400 × magnification. If less than 30 *Daphnia* had hatched in a population, all hatchlings were inspected for the parasite. Otherwise we used a plankton divider to obtain random subsamples of at least 24 hatchlings per population.

First experiment

In the summers of 2001, 2005 and 2006, we collected female *D. magna* from different rock pools, cloned them in the laboratory (isofemale lines) and tested them for infections by *H. tvaerminnensis*. The offspring of infected clones were kept in the laboratory until spring 2007. Uninfected clones were infected with *H. tvaerminnensis* (taken from other infected clones) in the summers of 2001 and 2005 and kept in the laboratory until spring 2007. In total, about half of all clones used were naturally infected, while the other half was infected in the laboratory. *Hamiltosporidium tvaerminnensis* had been evolving within these *D. magna* clones for 1 to 7 years (about 25 to 175 asexual *Daphnia* generations) of exclusively asexual host reproduction "evolved" lines). To produce clones with novel infections ("novel" lines), we used either naturally uninfected clones or clones cured from their natural infection with an antibiotic (Zbinden et al. 2005). Using *H. tvaerminnensis* from host populations, which had been kept in the laboratory for one to several years, we infected the uninfected *D. magna* clones by adding spores of *H. tvaerminnensis* to females (10'000 spores per female in 20 ml medium). Host and parasites originated from different populations in all cases. The females were then multiplied by asexual reproduction and tested for infections. Uninfected lines were discarded. These novel infections were produced in April 2007.

The infected clones from the laboratory cultures were released in late May 2007 into the mesocosms in the field. For the monoclonal treatments, we used 18 novel and 18 evolved unique combinations. For the polyclonal populations, we produced 18 novel and 18 evolved infected sets of 4 clones each. These polyclonal sets included the clones used in the monoclonal treatment. 14 clones had to be used in two polyclonal sets, but such that in no replicate two clones were the same. 16 females were released in case of monoclonal populations and 4 females per clone in case of the polyclonal populations. In September, we collected the resting eggs, kept them over winter outdoors and placed them in fresh rock pool water in the following spring to allow hatching under outdoor conditions. In total, we obtained hatchlings from 61 of the 72 populations.

Second experiment

Uninfected *D. magna* clones were obtained from crossings of clones originating from the metapopulation and from an ongoing long term outdoor experiment (Altermatt

& Ebert 2008) whose clones originated from the same metapopulation. These clones were exposed to a mixture of *H. tvaerminnensis* spores from randomly sampled *D. magna* from the metapopulation. Their offspring was tested for success of infection. In mid June 2006, half of 120 mesocosms were charged with 40 *D. longispina* individuals from 10 different rock pools each. These *D. longispina* populations were given one month to establish in the mesocosms. In mid July 2006, each of 30 outbred and 30 inbred *D. magna* clones were allowed to invade 2 mesocosms each: an empty mescosom and a *D. longispina* mesocosm population. Ten individuals per *D. magna* clone were released in each case. In mid September, all ephippia were collected and stored over winter, and were induced to for hatching in the following spring as described before. From the 60 mesocosms that produced at least 4 *D. magna* hatchlings, 30 randomly sampled individuals were checked for infection by *H. tvaerminnensis*. If less than 30 individuals were available, all were checked.

Statistical analysis

For the first experiment, prevalence was modelled with the factors host (mono- vs. polyclonal), parasite (evolved vs. novel), and their interaction. We used a generalised linear model with binomial error distribution and (canonical) logit link. Significance of parameter estimates was assessed with partial Wald tests using standard errors that were corrected for possible overdispersion. For the second experiment, prevalence was modelled with the factors inbreeding (outbred vs. inbred), competition (no competition vs. competition by *D. longispina*), and their interaction. We used a generalised linear mixed effects model with binomial error distribution and (canonical) logit link. *D. magna* clone and grid (a blocking treatment) were modelled as random effects (separate intercepts each). Again, significance of parameter estimates was assessed with partial Wald tests. Overdispersion was negligible; therefore uncorrected standard errors were used. Model fitting and the production of figures were accomplished with R (R Development Core Team 2011, version 2.13.0) and package lme4 (Bates et al. 2008, version 0.999375-28).

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Chapter 6

Synthesis and directions for future research

Synthesis

The overarching objective of the presented studies was to identify universal determinants of coexistence of three *Daphnia* species in a rock pool metacommunity. More specifically, I studied how the factors parasitism, inbreeding, and dispersal are associated with the outcome of interspecific competition and how they interact in the process. In the studied *Daphnia* metacommunity, the focus of research was previously on intraspecific and interclonal competition. They key insights can be summarised as follows: First, parasites are known to be a selective force in interclonal host competition (Haag & Ebert 2004) and to exhibit local adaptation to their hosts (Altermatt et al. 2007). Further, inbreeding leads to strong inbreeding depression (Haag et al. 2002), favours immigrant genes through hybrid vigour (Ebert et al. 2002), and decreases the competitive ability in intraspecific competition (Haag et al. 2003). Moreover, dispersal provides only one to very few individuals per colonisation event leading to founder events (Haag et al. 2006) and determines local and global stability of the *Daphnia* metacommunity (Altermatt et al. 2008). I tried to translate these insights, gained mainly at the level of single species populations, to interspecific competition in order to ascertain interactions between the populations that enable the coexistence of species as a community at the local scale and as a metacommunity at the regional scale.

Ebert et al. (2001) reported for *D. magna* that parasite species richness is primarily associated with host population age. In chapter 1, I showed that this finding is not generally valid because in *D. longispina*, parasite species richness is mainly explained by the presence of the interspecific competitor *D. magna*. I put forward that *D. magna* might serve as a reservoir of parasites from which *D. longispina* — which according to my screenings generally harbours a much lower endoparasite and epibiont species diversity — is constantly inflicted.

In chapter 2, I demonstrated in experimental communities that the persistence of resident *D. longispina* populations is enhanced if invading *D. magna* clones are infected with the microsporidian endoparasite *H. tvaerminnensis*. Parasites may thus not only act as a selective force in intraspecific (Haag & Ebert 2004) but also in interspecific host competition. I further showed that the persistence of the resident *D. longispina* populations is enhanced if invading *D. magna* clones are inbred, once again confirming a finding of intraspecific competition (Haag et al. 2003) for interspecific competition. In the associated chapter 5, I could exhibit, by following the development of the experimental communities, that the descendants of the invading *D. magna* clones suffer from higher prevalences of *H. tvaerminnensis* due to interspecific competition with the resident *D. longispina* populations. This indicates that the parasites of the invading *D. magna* clones may reinforce the persistence of the resident *D. longispina* populations in the longer term.

In chapter 3, I showed that the interspecific competitive abilities between *D. longispina*, *D. magna*, and *D. pulex* follow a surprisingly strict hierarchy. Again, as in intraspecific competition (Haag et al. 2003), the prime reason for decreased interspecific competitive ability is thought to be inbreeding. Even though I demonstrated that the interspecific competitive abilities were almost invariably transitive, I propose that inbreeding may enable intransitive interspecific competition and thus potentially stabilise local coexistence of *Daphnia* species.

The direct measurement of dispersal is a difficult and laborious task, which is reflected in the popularity of indirect methods (Neigel 1997, Bossart & Prowell 1998). In chapter 4, I describe the novel application of a direct measurement method borrowed from plant ecology to the quantification of viable dispersing *Daphnia* resting stages. Despite the modest yield, I could show that dispersal may provide sufficient migrants to fuel coexistence of *Daphnia* species.

In addition to the extensively presented material, I conducted a series of smaller, topically related observational and experimental studies which were not recessed. In the following, three selected examples shall be described in more detail for archival reasons. It is hoped that they might provide directions for future research.

Second host of *Larssonia obtusa*

In chapter 1, I concluded that the only endoparasite species found to be common in *D. longispina* was the microsporidian *Larssonia obtusa*. In a similar manner as *Hamiltosporidium tvaerminnensis* is the predominant endoparasite in *Daphnia magna*, *L. obtusa* might

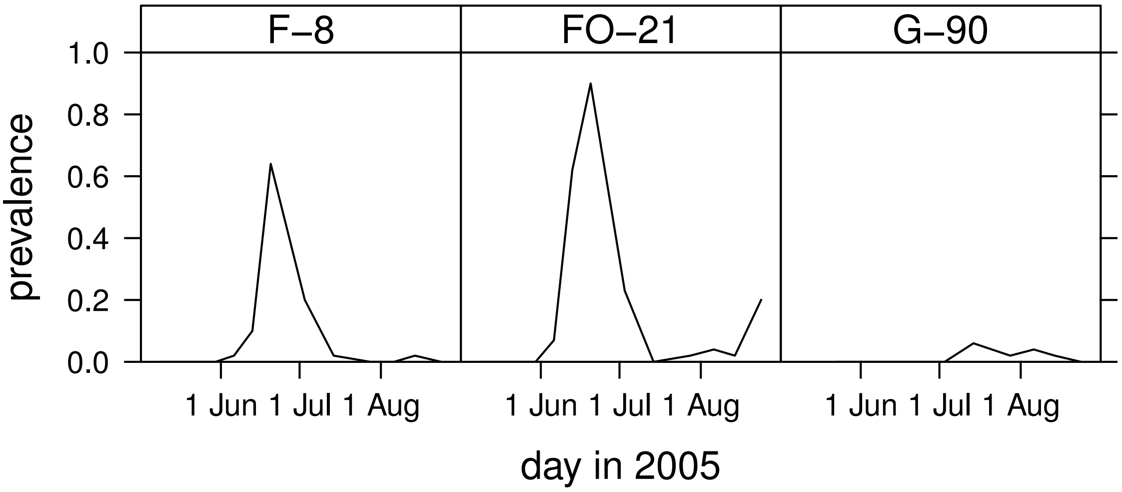

Figure 6.1 *L. obtusa* prevalence in *D. longispina* in three rock pools in summer 2005. Note that rock pools F-8 and FO-21 are less than 200 m apart from each other, while G-90 lies in more than 1.5 km distance. The *L. obtusa* outburst in G-90 is very small compared to the other two pools and might have arisen from spores from the previous years, i.e. there was maybe no potential second host around in which the parasite could develop new spores.

be the predominant endoparasite in *D. longispina*. Unlike *H. tvaerminnensis*, which is specific to *D. magna*, *L. obtusa* is able to infect all three *Daphnia* species occurring in the Tvärminne metacommunity. However, the life cycle of *L. obtusa* is only poorly understood. Actual prevalences might be underestimated since *L. obtusa* is a fast killer and/or the infection only becomes visible shortly before host death. Furthermore, two reasons speak in favour of the existence of a second obligate host — may it be the intermediate or the final host. First, spores that developed in *Daphnia* hosts are not infective for *Daphnia*. Second, microsporidian taxa phylogenetically close to *L. obtusa* have an intermediate host (Vossbrinck et al. 2004).

Five rock pools known to have hosted *L. obtusa* in previous years were followed over an extensive period in 2005. In intervals of 7-10 days, the complete rock pool fauna was sampled, categorised into broad taxonomic groups, and preserved in ethanol for later use. At the same time, parasite prevalence was estimated by checking approximately 50 *Daphnia* individuals for infection (Fig. 6.1):

Two circumstances indicate that mosquito larvae of the Culicidae family might be good candidates for second hosts. First, tn the rock pools that harboured *L. obtusa* (3 out of 5), the peak prevalence occurred roughly two weeks after Culicidae mosquito larvae had been sampled (Fig. 6.2). Second, studies show that closely related microsporidia

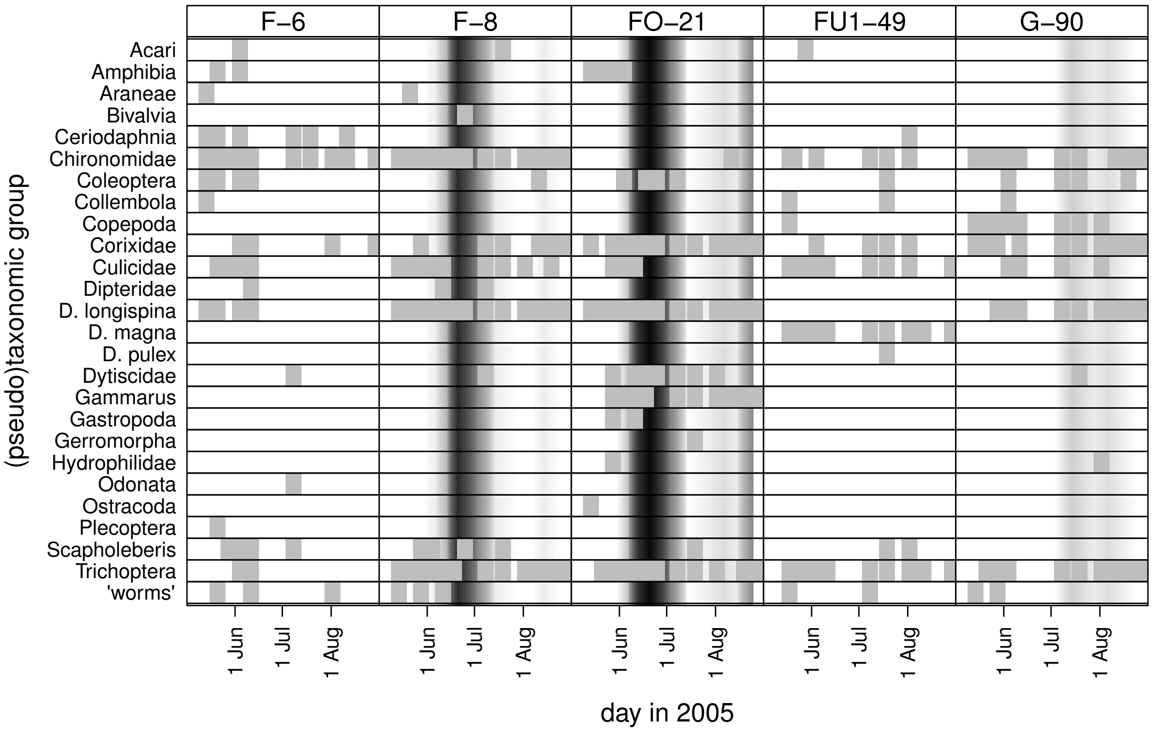

Figure 6.2 Temporal occurrence of (pseudo)taxonomic groups (horizontal bars) in the 5 rock pools on four different islands under investigation. *L. obtusa* prevalence is indicated by the background colouring (black: 100 % prevalence). Among the candidates for second hosts are beetles (other than Dytiscidae and Hydrophilidae), Chironomidae, Corixidae, Culicidae, Dytiscidae, Hydrophilidae and Trichoptera. No beetles were found in F-8, no Chironomidae were found in FO-21 and no Hydrophilidae were found in F-8. Corixidae and Trichoptera occur in all pools and Dytiscidae occur in all but one pool, but no co-occurrence with *L. obtusa* outbreaks could be seen. Culicidae were present in all pools and disappeared before *L. obtusa* outbreaks in *Daphnia*. However, they did not occur before every outbreak. Note that the gap of (pseudo)taxonomic group occurrence at the end of July in F-6, FU1-49, and G-90 is due to missing data.

taxa have an intermediate mosquito host (Andreadis 1985, Andreadis & Vossbrinck 2002, Becnel & Andreadis 1998).

Since primers for the small subunit of ribosomal DNA of *L. obtusa* are available (Refardt et al. 2002), a potential second host should be detectable with PCR. Priority should given to the screening of Culicidae larvae for which further material is available from a second potential host sampling campaign in 2006 focusing on Culicidae.

If a second host were wound, the next step would be to try to cultivate it in order to be able to establish a new study system. Such a one-parasite-two-hosts system raises many questions, one of which is briefly laid out in the following. If *L. obtusa* turned out to have a primary and a secondary host, this would have implications for the selection regime imposed on *Daphnia* hosts. It has been suggested that the time periods when *L. obtusa* primarily parasitises either its primary or its secondary host do not overlap totally (Bengtsson 2005). Consequently, *Daphnia* hosts might not be under constant selection for resistance but rather experience a relaxation period during the time *L. obtusa* is present in the non-*Daphnia* host. If there was a trade-off between resistance and interspecific competitive ability, genotypes that increased in frequency during a first phase of parasitism by *L. obtusa* due to superior resistance might decrease again in frequency as soon as *L. obtusa* switches hosts. At the time *L. obtusa* switches back to *Daphnia* hosts, the predominant genotypes present might be much more susceptible than if *L. obtusa* did not perform host switching and constantly selected for resistant genotypes. I put forward host switching to be a way of counteracting evolution of host resistance and therefore to enhance the parasite's overall fitness.

Predation by Dytiscidae larvae

In chapter 1, the parasite communities associated with *Daphnia* were discussed extensively, and in chapter 2, parasitism was considered as a factor inhibiting the invasion capability of *D. magna*. So far, however, predation and its potential role to mediate coexistence in the *Daphnia* rock pool metacommunity was not even mentioned. Corixids are abundant in the Tvärminne rock pool system (e.g. Pajunen & Pajunen 1993) but although they often co-occur with *Daphnia*, the later are not a preferred prey (VI Pajunen, personal communication). Occasional observations of mass aggregations of Dytiscidae water beetles in rock pools with previously dense *Daphnia* communities and references to Dytiscidae larvae as potential efficient predators of *Daphnia* (VI Pajunen, personal communication) led to the design of a pilot experiment testing the influence of predation on pelagic density and ephippium production. In 16 outdoor mesocosms filled with filtered rock pool water, pairs of *D. longispina* and *D. magna* clones competed in the absence or presence of four Dytiscidae larvae each.

There was a clear tendency that *D. magna* produced fewer ephippia under predation and that *D. longispina* could in turn increase their ephippium production (Fig. 6.3). This indicates that the visually oriented predators might have had a preference for larger prey items, which would have exerted a higher predation pressure on *D. magna* than on *D. longispina*. Predation could thus mediate coexistence of the two differently sized *Daphnia* species by apparent competition. Such stabilisation mechanisms were mostly studied in lake *Daphnia* systems where fish are the predominant predators (e.g. Gliwicz & Wrzosek 2008). As fish do only accidentally get flushed into rock pools by sea waves, they play no role in the studied *Daphnia* metacommunity. The role of beetles and their larvae however might have been underestimated so far, not as predators which are

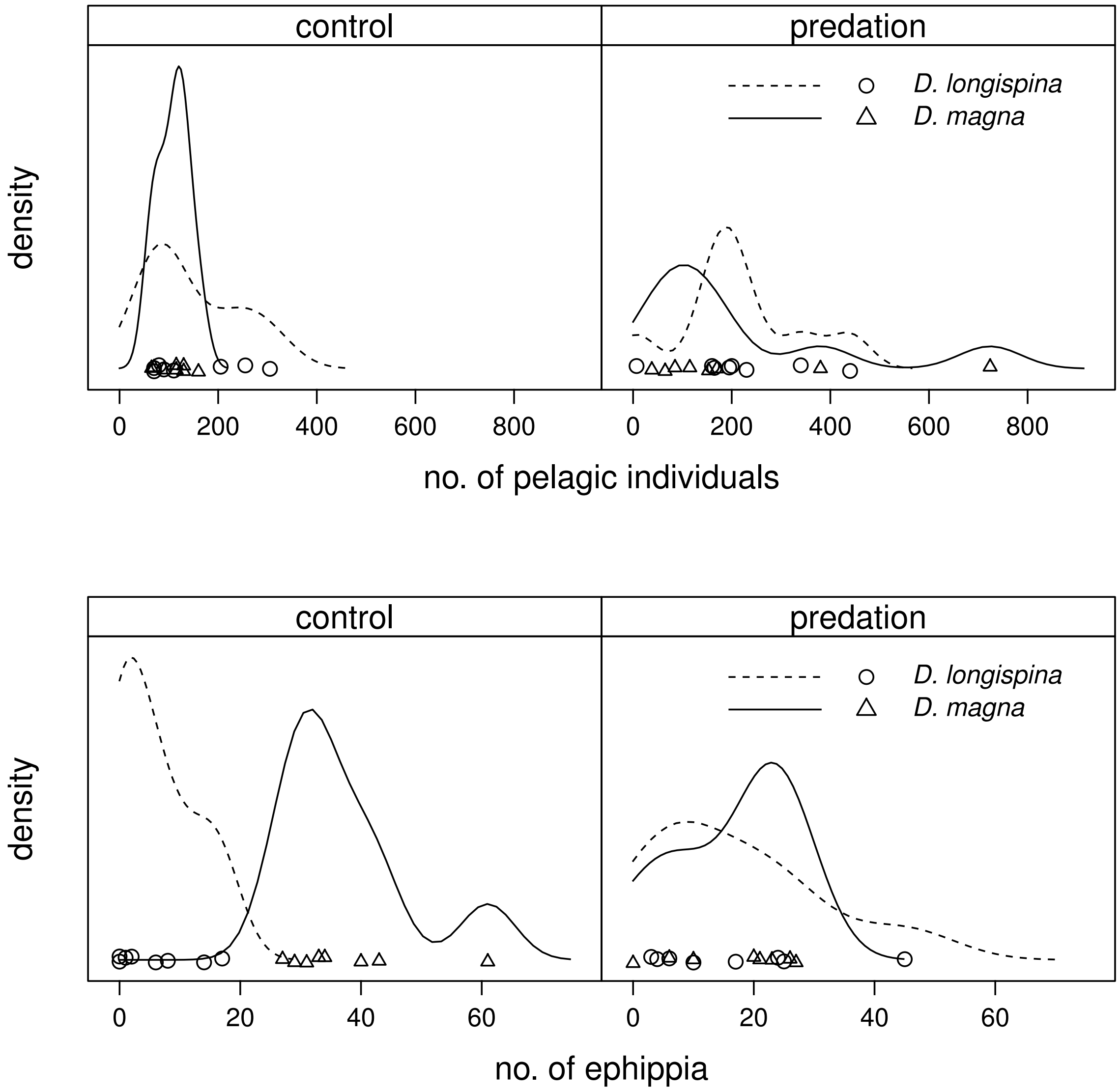

Figure 6.3 Kernel density estimates of the number of pelagic individuals (top) and ephippia (bottom) produced by the *D. longispina* and *D. magna* clones under control conditions (left) and under a predation regime of Dytiscidae larvae (right) in 16 outdoor mesocosms.

able to significantly decimate *Daphnia* populations but as predators which selectively prey on large-sized individuals and thus discriminate against *D. magna*.

Parasite mediated interspecific competition and optimal virulence

If two species compete for shared resources but only one of the species hosts a parasite, the parasite should not be too virulent. If it were, the probability that its host is outcompeted would increase and overall fitness of the parasite would decrease. I therefore hypothesise that virulence should be selected to be lower in host populations where an interspecific non-host competitor is present regularly.

According to chapter 2, *D. magna* fitness was reduced by its interspecific competitor *D. longispina*. If its parasite *H. tvaerminnensis* were too virulent, *D. longispina*, which seemed to be the worse competitor, would have higher chances to outcompete *D. magna* and thus reduce the parasite's fitness dramatically. I speculate that natural selection might exert pressure towards a lower optimal virulence of *H. tvaerminnensis* when its host *D. magna* does regularly compete with *D. longispina*.

In 2007, there were 30 pools in the studied metacommunity that had constantly been harbouring both *D. longispina* and *D. magna* over at least 5 years. If natural selection changed optimal virulence in the predicted manner, enough time may have passed to be able to detect whether the virulence of *H. tvaerminnensis* in these communities was lower compared to the one in pure *D. magna* populations which did not face any competition by *D. longispina*. To test whether the interspecific competitional history of *D. magna* was correlated with *H. tvaerminnensis* virulence, I performed a pilot study where 24 newborn *D. magna* were sampled from each of 16 rock pools harbouring *H. tvaerminnensis*, half of them having experienced interspecific competition by *D. longispina* in the past. The animals were singly raised in wellplates with food ad libitum. After two weeks' time, the animals were checked for *H. tvaerminnensis* infection. The number of eggs in the first clutch was determined and used as a surrogate variable for parasite virulence.

Whereas higher *H. tvaerminnensis* prevalences were found in *D. magna* originating from communities rather than pure *D. magna* rock pool populations, neither the clutch size nor the developmental status of the eggs seemed to have been associated with the origin of the mothers. The hypothesised effect could not be detected either because the suggested selection regime simply does not exist or because there are strong, unknown confounders that superpose any possibly very small effect. Since the evidence at hand is only correlational, no conclusive answer can be given. Experimental evolution with manipulated outdoor mesocosm communities might prove to be a more suitable approach to address the question.

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