Micro-evolutionary response of *Daphnia magna* to changes in biotic stress associated with habitat degradation and restoration of a shallow lake

Helen Michels

Proefschrift ingediend tot het behalen van de graad van Doctor in de Wetenschappen (Biologie),
in het openbaar te verdedigen op 23 maart 2007.

Laboratorium voor Aquatische Ecologie
Ch. De Bériotstraat 32 B-3000 Leuven
België

Promotor: Prof. Dr. L. De Meester
Laboratorium voor Aquatische Ecologie
Katholieke Universiteit

Overige leden van de examencommissie:

Prof. Dr. L. Brendonck
Prof. Dr. O. Honnay
Prof. Dr. E. Jeppesen
Prof. Dr. F. Ollevier
Prof. Dr. R. Stoks

Het onderzoek voorgesteld in dit proefschrift werd gesteund door een beurs verleend door het Vlaams Instituut voor de bevordering van het Wetenschappelijk-Technologisch onderzoek in de Industrie (IWT)

DANKWOORD

Het mag dan misschien wel een cliché zijn, maar een doctoraat maak je niet alleen. Gelukkig maar, anders zou het maar een beetje saai zijn. Bij deze wil ik dan ook al die mensen die hun steen of steentje hebben bijgedragen tot deze thesis, bedanken.

Eerst en vooral wil ik Luc bedanken. Het waren immers zijn lessen populatiebiologie die me inspireerde om iets over *Daphnia,* micro-evolutie en modder uit een Deens meer als thesisonderwerp te kiezen. Zijn wetenschappelijk enthousiasme zorgde er voor dat ik (zij het via een kleine tussenstop in Bolivië) verder in het onderwerp ging en uiteindelijk er een doctoraat over schreef. De maandagochtendvergaderingen, de talloze (soms nogal moeilijk te ontcijferen) verbeteringen en bijsturingen, het leveren van nieuwe invalshoeken als ik weer maar eens vast zat en de efficiënte opvolging van de laatste loodjes waren zeer stimulerend en cruciaal voor deze thesis en mijn 'vorming' onder meer als evolutionair biologe. Merci, daarvoor!

Ik wil ook Professor Ollevier bedanken om me de kans te geven in een goed draaiend labo, omringd door een groep zeer dynamische mensen, aan de slag te kunnen.

Tijdens mijn jaren aan het labo had ik het geluk om een paar stapjes in de wijde wereld te kunnen zetten en zo de verschillende aspecten van wetenschappelijk onderzoek te leren kennen. Luc Brendonck wil ik bedanken voor de kans die ik kreeg om mee op expeditie naar Zimbabwe te gaan en zo de iets avontuurlijkere kant van wetenschappelijk onderzoek te leren kennen.

I would also like to thank Erik Jeppesen for his hospitality during the months that I spent at NERI institute and the fruitful discussions we had. Thanks also to the rest of the søgroup: Sus, Rikke, Liselotte, Torben, Martin, Frank, Kirsten, Lone, Jane, Marianna and all the others for all the help during my experiments and samplings, for the scientific advice, for the crazy lab days and for all the positive vibes. Also thanks to Romi Burks for the work we did together in Denmark on horizontal migration and all the scientific advice.

Hierbij wil ik ook de overige leden van de jury bedanken voor het grondig nalezen en kritisch beoordelen van dit werk.

In een labo als het onze verkeer je als doctoraatstudent in een behoorlijke luxe situatie: voor elk probleem waar je mee zit, is er wel iemand die je kan helpen. Ik wil dan ook iedereen bedanken die hun steentje hebben bijgedragen tot deze thesis. Bedankt Robby, Ellen en Joost voor het extra wetenschappelijk advies, bijsturingen en hulp bij experimenten, Kim en Conny, voor de hulp bij de hele papierwinkel en kleine en grote computerproblemen, An, Ria, Bart, en Sara voor alle labo-technische hulp, Eddy, Ronny, en Stef, voor de hulp bij proefopstellingen, boorkernen, defecte waterpompen en zieke vissen, thesisstudent Karin voor de hulp bij het parasietenexperiment, Cathy voor het statistisch advies (en de paaseitjes), de overige *Daphnia*

mensen: Kevin, Wendy, Anja, Mieke, Dino en Sarah, voor de praktische hulp en voor de ambiance in het *Daphnia*lokaal. Merci voor alle hulp, maar ook gewoon voor de toffe babbels.

Naast al het werk was het gewoon ook aangenaam vertoeven op het labo en dat komt dan vooral door die fantastische bende collega's-vrienden. Merci, Bram en Jef voor de Allee-momenten en de proefexcursies, merci Francis, Bas, Joachim, Raquel, Hendrik, Miko, Frank, Marjan, Stefanie, Melina, Merlijn, Els D., Tom, Jorge, Liesbet, Priscilla, Jonas, Bram, Ann, Yasmine, Eugéne, Els C., Maarten L., Griet, Maarten S., Joost R., Anton, Greg, Tinne, Jeroen, en Steven voor alle koffie-en-taart momenten, staalnames in weer en wind (en door het ijs), doctoraatsfeestjes, zeilregatta's, fietstochtjes, en badminton wedstrijdjes.

Tenslotte wil ik ook alle vrienden bedanken voor hun steun, en alle de leuke momenten in de afgelopen jaren. Bedankt, Oud-Heverlee meiden, 'Leuven'vrienden en Bios chicas! Ook bedankt aan Jaak, Kate en Shaun voor hun gastvrijheid, waardoor de afwerking van mijn doctoraat in alle rust gebeurde.

Ik wil ook Lieve, René, Liesbet, en Kathleen bedanken voor de goede zorgen en steun die ze me gaven tijdens die laatste jaren van mijn doctoraat. Kathleen, bedankt ook voor alle lay-out hulp.

Als laatste wil ik ook het thuisfront bedanken, Erik, Anne-Sophie, An, Bert, Luk, Carlotta en Lula voor de steun en de gezellige drukte waardoor het steeds fijn thuiskomen is. Erik wil ik in het bijzonder bedanken om mij als grote broer wegwijs te maken door Biologie en de eerste jaren van mijn doctoraat. Ma en Pa, het feit dat ik tot hier ben geraakt heb ik aan jullie te danken! De combinatie wetenschap-natuur heb ik immers van thuis meegekregen, maar vooral ook een open kijk op de wereld, en de wil om te ontdekken. Bedankt voor alle steun tijdens mijn studies, doctoraat en voor alle wilde plannen die nog volgen. En allerlaatste Jan! Bedankt om mij als echte coach door die laatste maanden van doctoraat te loodsen. Maar bedankt ook voor alle steun en liefde, de dromen en plannen, het dagelijkse avontuur, en om er gewoon te zijn voor mij. Samen kunnen we Australië aan!!!

Merci,

Helen

CONTENTS

Introduction **1**

Chapter 1

Interclonal variation in diel horizontal migration behavior of the water flea *Daphnia magna* – searching for a signature of adaptive evolution **27**

Chapter 2

Micro-evolution of antipredator traits in a natural *Daphnia magna* population driven by lake restoration **49**

Chapter 3

Dynamics of evolutionary change in a natural *Daphnia magna* population during eutrophication and recovery of a shallow lake **69**

Chapter 4

Micro-evolution in competitive ability and ecological combining ability of a natural *Daphnia magna* population in response to changes in population density and species composition **89**

Chapter 5

An evolutionary perspective of *Daphnia* ectoparasite susceptibility and immunocompetence in relation to predation in Lake Ring. **109**

General discussion **123**

Summary **133**

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

ABSTRACT

This chapter presents an outline of the present thesis. First, we give a brief overview of the genetic consequences of habitat degradation and restoration, focussing on eutrophication of shallow lakes. We pay special attention to the impact of multiple antagonists on zooplankton populations in shallow lakes. Furthermore, we discuss the relative importance of natural selection and neutral events as driving forces of evolutionary processes. Since the study of evolutionary dynamics in natural populations is difficult because of the time aspect involved, we discuss here the potential of layered dormant egg banks as historical archives of lake inhabitants and their environment. At the end of this chapter, we provide some historical and ecological background information on the study system, and present the model organism *Daphnia magna.* To conclude, we present the objectives and outline of this thesis.

1. Habitat degradation and restoration: genetic consequences

The increasing impact of habitat degradation, which can be amongst others caused by acidification, eutrophication, habitat fragmentation and habitat loss, led to active restoration measurements (Meffe and Carroll, 1997). The goal of ecosystem restoration is to emulate a natural and self-regulating system that is integrated in its ecological landscape (Van Adel and Aronson, 2005). Both habitat degradation and restoration affect the biota inhabiting the local habitat, causing changes in species composition and interspecific interactions (Meffe and Carrol, 1997). Although much less intensively studied, habitat degradation and restoration are expected to also affect genetic structure of resident populations (Frankham et al., 2002).

Habitat degradation can be seen as a reduction in habitat quality and its ability to support biota. Since it often implies habitat loss and habitat fragmentation, habitat degradation might also lead to reduction of patch size and increasing spatial isolation of the remnant fragments (Andrén, 1994). As a consequence of habitat degradation and habitat loss, effective population size decreases, resulting in increased levels of inbreeding and loss of genetic diversity due to genetic drift (Frankham et al., 2002; Ellis et al., 2006). Lower levels of genetic variation might limit a species' ability to respond to changing environmental conditions trough adaptation and selection (Young et al, 1996; Stockwell et al., 2003; Ellis et al., 2006). Moreover, increasing spatial isolation of remnant fragments can lead to higher genetic differentiation between the different fragments (Lowe et al., 2005).

To counteract the negative impact of habitat degradation, habitat restoration measures are increasingly being taken. Although these measurements might lead to an increase in biodiversity (Dodson et al., 2001) and population density of the inhabiting species (Frankham et al., 2002), it does not necessarily imply an increase in genetic diversity in the population. Recovered populations that underwent a genetic bottleneck associated with former reduction of population size, might still suffer from genetic erosion, which could make them more vulnerable to environmental changes (Frankham et al., 2002). Because evolution often happens on a short time scale, both habitat degradation and restoration may not only influence the genetic diversity of local population, but may also determine the contemporary evolution of these local populations (Stockwell et al., 2003). Hairston et al. (1999, 2001) demonstrated that changes in resistance of a local *Daphnia* population in Lake Constance to cyanobacteria toxins were correlated with changes in phytoplankton composition and the occurrence of cyanobacterial blooms associated with eutrophication and subsequent oligotrophication of this large lake. Kerfoot et al. (1999) reported changes in genetic structure of a local *Daphnia* population that

were related to changes in exposure to heavy metals during the recent history of the studied lake (see section 5.1).

2. Habitat degradation: Eutrophication of shallow lakes

Eutrophication is a natural aging process in which a steady increase of biological production is due to gradual accumulation of nutrients, and in the case of shallow lakes, a decrease of lake depth (Gulati and Van Donk, 2005). Artificial eutrophication, a consequence of society's urban, industrial and agricultural use of plant nutrients and their subsequent disposal, accelerates this process. It became a widely recognized problem in the 1940s and 1950s (Harper, 1992), and can be seen as one of the biggest and most wide-spread problems for fresh waters (Harper, 1992; Scheffer, 1998). Over the last century several lakes in Europe and North America strongly suffered from an increased nutrient inflow in the form of sewage discharge (point sources) and agricultural run off, reflecting an increasing pressure from demographic growth and the development of economic activity. In many lakes, eutrophication led to ecological problems such as algae blooms, a reduction of biodiversity, and the loss of species that depend on aquatic vegetation and clear-water conditions. Eutrophication causes, however, also economic damage, as turbid lakes are less attractive for recreational use such as swimming, fishing, and water sports. From the 1970s onwards, governments and private organizations started to increasingly invest in lowering the nutrient load and restore lakes (Harper, 1992; Scheffer, 1993).

Depending on the depth of the lake, nutrients will stay or disappear from the nutrient cycle (Moss, 1998; Scheffer, 1998). Nutrients that enter the lake are taken up by primary producers, leading to an increase in biomass. Dead material and faeces sink to the bottom and are decomposed by bacteria. In deep, stratified lakes, nutrients accumulate in the sediment and hypolimnion (i.e. the lower, unmixed water layer, which is unsuitable for primary producers because of too low light intensity) and thus disappear -at least temporally- from the nutrient cycle. Shallow lakes do not stratify for long periods in the summer (polymictic; Scheffer, 1998) and therefore experience much more mixing of the entire water column, keeping nutrients much longer in the nutrient cycle. This makes shallow lakes much more sensitive to eutrophication than deep lakes (Gulati and Van Donk, 2005).

In shallow lakes, eutrophication can cause a shift from a clear-water state with abundant submersed vegetation, to a turbid state that is characterized by a high algal biomass and the absence of submerged vegetation (Scheffer et al., 1993). Both states (clear-water and turbid state) are stabilized by multiple feedback mechanisms (Scheffer, 1998). The clear-water, highly vegetated state is stabilized by high zooplankton grazing rates that keep algal biomass low, and

high abundance of piscivorous fish that controls densities of planktivorous and bentivorous fish. The turbid state is stabilized by the shading effect, caused by algal blooms reducing the capacity of submersed vegetation to develop, low zooplankton grazing rates that fail to control algae growth, and high planktivorous and bentivorous fish densities that keep zooplankton densities low (Scheffer et al., 1993). Because of these stabilizing factors, restoration of non-vegetated turbid shallow lakes to the clear-water, vegetated state is difficult, and most often requires a strong intervention, such as the removal of >90 % of the planktivorous and benthivorous fishes (Hosper and Meijer, 1993; Jeppesen et al., 1999; Gulati and Van Donk, 2002). A mere reduction of nutrient loading alone is in most cases not enough, as during the process of eutrophication a large amount of phosphorus has been absorbed by the sediment. When the loading is reduced and nutrient concentrations in the water drop, phosphorus is often being released by the sediment because of wind-induced mixing (cf. "internal eutrophication"). This delays or even prevents the response of the lake to the reduction of external loading (Scheffer, 1998). In addition to the process of internal loading, the specific community structure of turbid lakes contributes to stabilization of the turbid state and thus makes restoration difficult.

3. Multiple antagonists and their impact on zooplankton population structure

In natural ecosystems, organisms face multiple antagonists at the same time. Defense mechanisms against these different antagonists may interfere with each other, either directly or because each defense mechanism by itself has a fitness cost. It has been shown that zooplankton defense traits reducing mortality by visually hunting predators, such as a reduction in body size and diel vertical migration, may increase vulnerability towards invertebrate predators (Stibor 1992; Tollrian and Harvell, 1999). Simultaneous exposure to several antagonists may thus induce more complex outcomes in the populations than exposure to only one antagonist at a time. Relatively few studies have, however, considered micro-evolutionary responses to different antagonists at the same time. Interference among defense mechanisms may involve trade-offs or feed-back mechanisms, which are shortly outlined in the next paragraphs.

3.1. Trade-offs

In confrontation with different enemies, resource allocation to defense has often to be divided over defenses against the different kind of enemies. This may cause a trade-off between different defense mechanisms. Rigby and Jokela (2000), for instance, reported a trade-off between predator avoidance behavior and immunocompetence against pathogens in a freshwater pond snail *Lymnaea stagnalis*.

Trade-offs between different defense mechanisms are also possible when a defense mechanism against one antagonist leads to a higher vulnerability towards another antagonist. The presence of the second antagonist can then be seen as an additional cost of the defense mechanism against the first antagonist. Habitat selection behavior is an important predator-avoidance mechanism in which *Daphnia* reside deeper in the water column to avoid predation by visually hunting predators. Negatively phototactic clones suffer less by predation of fish. However, the associated higher exposure to parasite spores in the lake sediment has been shown to lead to an increase in parasite infection rates (Decaestecker et al., 2002). The presence of parasite spores in the sediment thus represents an additional cost for predator avoidance behavior (Decaestecker et al., 2002). In addition, the presence of other potential predators such as *Chaoborus,* that reside deeper in the water column during the day, may represent another cost of diel vertical migration (Dawidowicz, 1990). A similar trade-off may be present between the response to predators and to competitors. In response to fish kairomone, i.e. a chemical substance which is excreted by the predators, *Daphnia* exhibit a smaller size at maturity, and in this way avoid size selective fish predation of planktivorous fish (Stibor, 1992). One of the cost of this predator defense mechanism may be, however, that smaller individuals have a higher threshold food concentration, and are thus weaker competitors (Gliwicz, 1990). Trade-offs caused by simultaneous exposure to different antagonists is one of the factors that may lead to the maintenance of genetic polymorphisms for antipredator traits.

3.2. Feedback

One antagonist can also strengthen or weaken the effect that another antagonist has on its victim, irrespective of trade-offs mediated by energy costs or direct interference of defense traits. Duffy et al. (2005) observed that predation indirectly controlled parasite virulence. Predators not only affect their prey population, but also the parasites of this prey, and this may lead to predator control of parasite prevalence (Duffy et al., 2005). Parasitism, on the other hand, may enforce the effect of predation, because infected individuals are often more visible to the predator or debilitated in their escape responses, resulting in an increased risk of predation (Willey, 1990, Lass and Bittner, 2002). By killing their prey, predators also affect the amount of resources available to the remaining prey population, and their competitors (Scott and Werner, 2001).

To summarize, we can conclude that there is ample scope for interference between different antagonists in ecological communities, with both ecological as genetic consequences for the local populations. To unravel these interactions, however, one has to consider patterns of

occurrence of the different types of antagonists and quantify the corresponding variation in defence traits in the study population.

4. Natural selection versus neutral events: finding the driving force behind evolution

The investigation of the relative importance of natural selection and neutral events (genetic drift) as the driving force behind evolutionary processes has been the focus of an increasing amount of studies in the last decade (see review by Merilä and Crnokrak, 2001, and references herein; Storz et al., 2002; Koch et al., 2004; Evano et al., 2006). Micro-evolution is the result of neutral events (gene flow and genetic drift) and natural selection acting on adaptive variation (Hartl and Clark, 1997). To disentangle the relative importance of both factors as driving force behind micro-evolutionary processes, one can compare genetic differentiation based on molecular markers (F_{ST}) and on quantitative traits (Q_{ST}) (see reviews in Reed and Frankham, 2001; Merilä and Crnokrak, 2001; McKay and Latta, 2002). F_{ST} is a standardized measure of the degree of among-population genetic differentiation (Wright, 1951; Weir and Cockerham, 1984; Weir, 1996; Merilä and Crnokrak, 2001), and is primarily determined by a balance between random genetic drift and migration (Hartl and Clark, 1997). The degree of among-population differentiation at neutral markers loci as estimated by an F_{ST} index, which indicates the expected degree of population differentiation as a result of the combined effect of genetic drift and gene flow (Wright, 1951; Lande, 1992). Analogous to the single locus F_{ST} estimate, an estimate for divergence at quantitative traits (termed Q_{ST} by Spitze (1993)), can be obtained by:

$$Q_{st} = \frac{\sigma^2{}_b}{\sigma^2{}_b + 2\sigma^2{}_w} \qquad (1)$$

With σ_b^2 being the among-population variance and σ_w^2 the within-population variance for a given trait. Divergence in quantitative traits should be similar to divergence in allele frequencies at nuclear loci, if they are evolving neutrally and have an additive genetic basis (Wright, 1951).

Higher genetic divergence in quantitative traits than in neutral markers ($Q_{ST} > F_{ST}$) points to directional selection favoring different phenotypic values in different populations. If Q_{ST} equals F_{ST}, drift may explain the observed genetic differentiation among populations. This does not prove that natural selection did not influence the evolutionary trajectory, but rather implies that one can not differentiate the impact of natural selection from the response that might have been observed under neutral evolution. The Q_{ST}/F_{ST} approach thus functions as a test against the

neutral model. $Q_{ST} < F_{ST}$ suggests that natural selection favors the same phenotype in different populations (Merilä and Crnokrak, 2001).

Reviews of Q_{ST} - F_{ST} comparisons indicate that the degree of quantitative trait differentiation typically exceeds that of neutral marker genes (e.g. Merilä and Crnokrak, 2001). This suggests a prominent role for natural selection in determining the population genetic structure for quantitative traits (see reviews in Reed and Frankham, 2001; Merilä and Crnokrak, 2001; McKay and Latta, 2002). Up till now most of this research has focused on patterns of genetic difference among spatially separated populations. Only Cousyn et al. (2001) compared values of Q_{ST} and F_{ST} (see further) to document the evolutionary dynamics within one single population through time, starting from an analysis of variation in neutral markers as well as ecologically relevant traits in a dormant egg bank.

Comparison of the two measures of genetic differentiation has also important applications in conservation genetics. Evolutionary potential is largely determined by the genetic diversity in ecologically relevant traits on which natural selection can act, which can be estimated by Q_{ST}. The majority of information on genetic differentiation among wild populations, however, is limited to patterns obtained by applying DNA markers or allozymes (Reed and Frankham, 2001). Estimating Q_{ST} in wild populations is often difficult to achieve (Frankham et al., 1996). Obtaining F_{ST} estimates only requires DNA samples, which involve less invasive sampling and is easier to accomplish. Therefore a much recurring theme in conservation genetics is whether, and to what extent, there is a relationship between divergence in quantitative traits and divergence in neutral marker loci, reflecting the degree to which F_{ST} estimates can be used for determining evolutionary potential. The answer to this question seems not very clear. Merilä and Crnokrak (2001) stated that there is a strong correlation between Q_{ST} and F_{ST} values, suggesting that the degree of differentiation in neutral marker loci is closely predictive to differentiation in quantitative traits. McKay and Latta (2002) and Frankham and Reed (2001), however, came to the opposite conclusion. McKay and Latta (2002) stipulated that F_{ST} estimates may still reflect allelic variation at QTL (quantitative trait loci), which is an evolutionary potential that is highly relevant for longer term conservation. This statement is, however, based on theoretical arguments and should be empirically tested.

5. Dormant egg banks: historical archives of lake inhabitants and their environment

Stratified lake sediments harbor valuable information on the history of a lake and its surroundings (Jeppesen et al., 2001). Because of its high resolution and its ability to cover a large period of time, sediment layers constitute a good archive (e.g. Verschuren et al., 2000).

Paleolimnology, the study of organisms and chemistry of lake bottom sediments, has recently been used for a wide range of disciplines, ranging from climate change and ecosystem ecology of freshwater bodies to the reconstruction of micro-evolution (e.g. see Jeppesen et al., 2001). In this short overview, we focus on the use of dormant egg banks in paleolimnology (see also De Meester et al., in press) and their importance for the reconstruction of micro-evolution.

5.1. Reconstruction past population abundance

Because of their tolerance to harsh conditions, ephippia are well-preserved in the lake sediment, and can be used for reconstructing the history of a population (Jankowski and Straile, 2003). Ephippia have the advantage over other fossil zooplankton remains, such as mandibles and postabdominal claws, that they are relative large, which makes them easier to handle. They are also easier to identify to species groups. Several studies used ephippia densities to reconstruct past population abundance, species invasions, and recovery after restoration of lakes (Verschuren and Marnell, 1997; Cousyn and De Meester, 1998; Mergeay et al., 2004). This approach assumes a correlation between the number of ephippia produced and the standing stock of the population (Frey, 1964). A potential problem in this assumption is the fact that production of dormant eggs is genotype dependent (Innes, 1997). The tendency to produce dormant stages is, however, largely correlated with habitat characteristics such as predictability and permanence of the habitat (Tessier and Cáceres, 2004). This problem is therefore more pronounced in the interpretation of data on among-population comparisons than for monitoring changes within one habitat. Verschuren and Marnell (1997) found a good correlation between changes in densities of cladoceran ephippia and densities of other subfossil remains, such as abdominal claws, which are generally believed to accurately reflect zooplankton densities (Frey, 1964). Jankowski and Straile (2003) tested the potential of using the egg bank to reconstruct historical data of a *Daphnia* population in Lake Constance, by comparing it to the well documented year-to-year changes of the active *Daphnia* population. They found a close match between the number of ephippia in the sediment cores and the historical data of *Daphnia* population abundance. They also showed, however, that there are problems to assess the relative abundance of the different taxa of the *D. longispina* complex. In general, for both within habitat comparisons, and comparisons between non hybridizing species, it seems relatively safe to assume that there is a good correlation between number of ephippia and historical changes in active population density (Jankowski and Straile, 2003).

5.2. Reconstruction of changes in environmental variables

Daphnia and other pelagic cladocerans fulfill a prominent role in freshwater food webs, being efficient grazers of planktonic algae and being themselves important prey to fish and other predators (Lampert, 1987). Analyzing the dynamics of *Daphnia* through paleolimnological research may offer important information on past lake functioning (Jeppesen et al., 2002). Size selective predation by fish is a potent structuring force determining zooplankton community structure (Lampert, 1987). Furthermore, most cladocerans respond to the presence of fish kairomone by reducing body size (Stibor, 1992), which is translated into smaller ephippium sizes (Verschuren and Marnell, 1997). Therefore the size of ephippia of *Daphnia* can be used as a proxy for changes in predation pressure (Verschuren and Marnell, 1997; Cousyn et al. 2001; Jeppesen et al., 2002).

5.3. Resurrection ecology

Demonstrating an evolutionary response in a natural system is rather difficult since evolution occurs over several generations. Although evolution rates are sometimes very high (Hendry and Kinnison, 1999; Hairston et al., 2005), genetic adaptation to a new environment still takes at least some generations. Only few studies succeeded in tracking genetic changes in populations over an appropriate time scale (Reznick et al., 1997; Grant and Grant, 2002). Many studies circumvented this time aspect by employing a 'space for time' approach, in which genetic differentiation among spatially separated populations is measured, to verify whether the observed pattern of among-population genetic differentiation is in concordance with assumptions of local adaptation. Although this approach has been proven powerful in many cases, there remains a problem of interpretation. It is difficult to prove that the differentiation observed among populations is caused by natural selection leading to adaptive changes, and does not reflect neutral events such as founder effects, genetic drift or gene flow from other populations. Interpretation can be strengthened by comparing genetic differentiation for ecologically relevant traits to that of neutral markers (see section 4). An alternative approach, however, involves the reconstruction of past historical events from the information archived in dormant egg banks of natural populations. Such studies involve a combination of expertise in evolutionary biology and paleolimnology.

Many aquatic organisms produce dormant stages that are resistant to harsh conditions and hatch once environmental conditions improve again (see section 6). Dormancy allows an organism to avoid harsh conditions and contributes to risk spreading over time (Cohen and Levin, 1987). As dormant eggs also offer an efficient mechanism for dispersal (e.g. Green et al., 2002), the production of dormant eggs also contributes to risk spreading in space. Bet-hedging

theory implies that, depending on habitat predictability, a varying fraction of the dormant stages should hatch each cycle of exposure to favorable conditions (Cohen, 1970). Risk-spreading strategies combined with the fact that only a fraction of dormant stages is accessible to hatching stimuli (cf. dormant eggs need light to hatch and many eggs are likely to be covered with sediment), dormant eggs accumulate in the lake sediment forming a dormant propagule (egg) bank (Cáceres, 1998). An important characteristic of lake sediment compared to terrestrial soils is that lake sediments tend to be much less disturbed and enjoy higher sedimentation deposition rates. This leads to a higher reliability and time resolution of the resulting archive. Provided that the lake sediments are relatively undisturbed and well structured, one can thus use the remains of aquatic organisms to reconstruct changes in community composition through time. If dormant eggs still contain enough non-denaturated DNA, they can be analyzed using molecular markers (cf. paleogenetics; Duffy et al., 2000). Provided that the dormant stages are still viable, they can also be hatched and the resulting clonal lineages can be used for quantitative genetic analyses of past populations derived from different periods in the history of a habitat (i.e. resurrection ecology; Kerfoot et al., 1999).

Dormant stages remain viable for decades, even centuries. Hairston et al. (1995) were able to hatch a 330 year old egg of the copepod *Diaptomus sanguineus*, while for *Daphnia* several studies demonstrated that dormant eggs can remain viable for over 80 years (Weider et al. 1997; Cáceres, 1998). The combination of long-term viability of dormant stages, and the high resolution of lake sediment in which they are stored, provides a powerful way to study both the ecological and the evolutionary history of local populations (Brendonck and De Meester, 2003).

Weider et al. (1997) were the first to document the changes in allele frequencies through time from a layered dormant egg bank, using allozyme markers to document genetic changes in association with eutrophication of Lake Constance. They hatched dormant eggs from the sediment to obtain sufficient genetic material to perform electrophoresis. In this study, the capacity for genotyping thus depended on the hatching success of the dormant eggs. Using Polymerase Chain Reaction (PCR)-based DNA markers such as polymorphic microsatellite markers, one can directly genotype the dormant eggs themselves, as the amount of tissue needed is strongly reduced. Genetic analysis can even be performed on unviable eggs. Hairston et al. (1999) were the first to use DNA sequencing to demonstrate that intensive industrial pollution of Onondaga lake preceded a range expansion of *D. exilis* in the lake. Duffy et al. (2000) demonstrated the pollution-facilitated invasion of *D. curvirostris* in the same lake. Limburg and Weider (2002) revealed a genetic shift in a natural *Daphnia* population over a period of 200 years, using microsattelite markers.

Hairston et al. (1999, 2001) documented changes in the resistance of *D. galeata* towards toxic cyanobacteria in Lake Constance. Changes in resistance of the local *Daphnia* population could be correlated with changes in phytoplankton composition, and the occurrence of cyanobacterial blooms associated with eutrophication and subsequent oligotrophication of this large lake. Kerfoot et al. (1999) demonstrated genetic adaptation to changes in exposure to heavy metals in *Daphnia*. Kerfoot and Weider (2004) showed a significant evolutionary response to environmental changes (eutrophication) associated with changing balance in invertebrate and vertebrate predators in a natural *D. retrocurva* population. To obtain insight into the relative importance of selective and neutral processes as driving forces behind evolutionary changes in ecologically relevant traits, it is necessary to combine data on neutral markers and ecologically relevant traits. When changes in ecologically relevant traits are not paralleled by similar changes in neutral markers, this strongly points to natural selection as the evolutionary factor involved (Spitze, 1993). If both neutral markers and ecologically relevant traits show the same pattern, this may reflect that genetic drift or gene flow are important drivers shaping the observed evolutionary change. Cousyn et al. (2001) were the first to combine analyses on both ecologically relevant traits and neutral markers, and demonstrated that the genetic changes in antipredator traits of a natural *Daphnia* population, in response to historical changes in fish stocking, were likely caused by natural selection and were not a side effect of neutral processes.

Analysis of a dormant egg bank yields information on changes in the genetic structure of local populations, and at the same time it also allows to reconstruct changes in environmental conditions which may have acted as selective agents. Whereas most studies up till now focused on reconstruction of micro-evolutionary response to one antagonist (prey-predator, phytoplankton-grazer), there is a potential to look at the micro-evolutionary response toward multiple antagonists. Combining the analysis of ecological relevant traits and neutral markers on hand, and the adaptive response towards multiple antagonists, would lead to a more integrated picture of genetic structure of local populations.

6. *Daphnia magna* as model organism

Because of their short generation time, their cyclical parthenogenetic reproduction and the fact that they are easy to culture, cladocerans and especially *Daphnia* are increasingly recognized as excellent model organisms for evolutionary-ecological research.

Most *Daphnia* species (Crustacea, Branchiopoda, Anomopoda, Daphniidae) have a cyclical parthenogenetic reproduction cycle. Under favorable conditions, they reproduce by amictic parthenogenesis, in which genetically identical offspring are produced. This can last for several generations until environmental conditions deteriorate (high population densities, shortage from food, decrease of temperature, presence of predator-specific kairomone, change in day-length; Zaffagnini, 1987; Hebert, 1978, 1987; Pijanowska and Stolpe, 1996). At this stage haploid eggs and males are produced, and they switch to sexual reproduction. Fertilized sexual eggs are dormant stages of which the development stops in the blastula stage (Zaffagnini, 1987). They are embedded in a special membrane structure which is formed from the brood chamber wall. This structure, called an ephippium, protects the resting egg against frost, drought, and digestive fluids, and ensures survival of the dormant egg until the next growing season. Since not all dormant eggs hatch the following season, they accumulate in the sediment and form a dormant egg bank (Cáceres, 1998).

Dormant eggs of *D. magna* may survive for several decades, are easy to identify up to species level, and easy to hatch, which makes it relatively straightforward to isolate individuals from different time periods of the dormant egg bank and bring them into culture. The possibility to generate a large number of genetically identical individuals allows clear-cut experimental designs to determine genetic variance in quantitative traits. As there are several tens of microsatellite markers available for *D. magna*, extensive analysis of variation in neutral markers is possible (Bastiaan Jansen, pers. comm.).

The water flea *Daphnia* plays an important ecological role in shallow lakes. Its presence or absence can profoundly influence the structure of a freshwater ecosystem (Lampert, 1987). *Daphnia* can exert a strong grazing pressure on the phytoplankton community, but they experience strong selection pressure from different types of antagonists. First of all, *Daphnia* are important and preferred prey for invertebrate predators such as *Chaoborus sp.* and *Notonecta sp.* as well as for vertebrate predators (planktivorous fish). *Daphnia* have developed a wide range of life-history, behavioral, and morphological inducible defense mechanisms to lower the risk of mortality by predation (Boersma et al., 1998; Tollrian and Harvell, 1999). Because of the costs involved in these defense mechanisms, they are only induced when the risk of a confrontation with a predator is high. In aquatic systems the stimuli that are used to induce

defense mechanisms are in most cases chemical substances (kairomone) which are excreted by the predators and can be detected by the prey. Boersma et al. (1998) demonstrated that different *D. magna* clones responded to fish kairomone with a different subset of inducible defense mechanisms. One of the key defense mechanism was however diel vertical migration.

Besides predators, *Daphnia* may experience strong selective pressure from both intra- and interspecific exploitative competition. *D. magna* is a non-selective filter feeder of which the food spectrum overlaps to a large degree with that of other large *Daphnia* species such as *D. pulex* (Kerfoot et al., 1985). The selective impact exerted by competition is expected to change with changing population densities.

Finally, a large number of parasitic and epibiontic infections have been detected in natural *Daphnia* population (Stirnadel and Ebert, 1997; Decaestecker et al., 2005). Because of their ubiquity, their narrow host range, their negative effects on host reproduction and survival, and their host genotype dependent infectivity, parasites are expected to exert a strong selection pressure on their host population (Woolhouse et al., 2002). *Daphnia* populations have been reported to show evolutionary adaptations in response to parasite infections (Little, 2002; Duffy et al., 2007).

7. Study site

Lake Ring (55° 57’ 51.83’’ N, 9° 35’ 46.87’’ E) is a relatively small (22.5 ha) eutrophic and shallow lake (average depth 2.9 m) located in mid-Jutland, Denmark (Fig. 1). The lake suffered from eutrophication during the 20th century due to sewage inflow from a nearby town. In 1970 this pollution source was diverted from the lake (Berg et al., 1994) and the lake gradually recovered. In the mid 1980s, total phosphorus (TP) levels declined (from 1.78 mg/l to 0.629 mg/l) and in between 1979 and 1987 lake water transparency started to improve (Fig. 2). As the catchment area is relatively small, these improvements can probably be ascribed to nitrogen limitation (Erik Jeppesen, pers. comm.), as TP concentrations remained high. Whitefish (*Coregonus lavaretus,* a planktivorous fish) was stocked in the lake in 1989-1990 to investigate its effect on the zooplankton community of the lake (Berg et al., 1994).

Based on the ephippia density profiles and the water and organic material content of the cores, three periods in the history of Lake Ring can be distinguished: a Pre-eutrophication period (1908-1937), a Turbid period (1960-1980) and a Clear-water period (1986-2000).

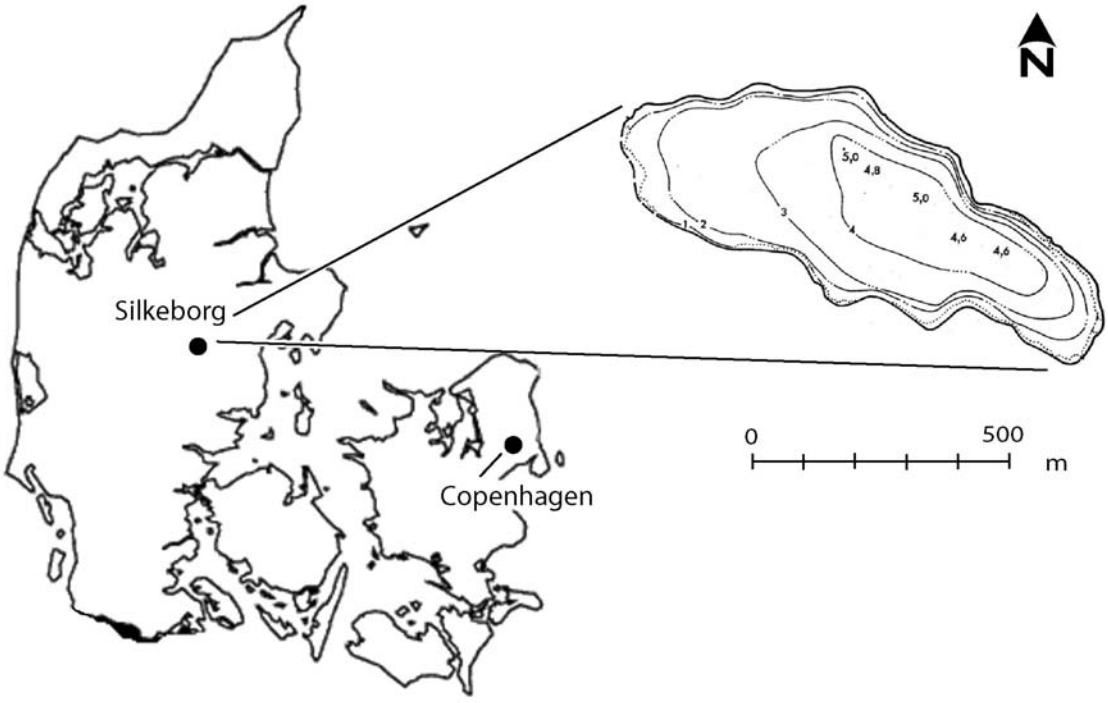

Fig. 1: Map indicating the geographic location of Lake Ring, Denmark, and bathymetric map of the lake; modified from Berg et al. (1994).

In 1989, the only submerged macrophyte in the lake was *Potamogeton pectinatus* L., which appeared along the north-east coast of the lake (Berg et al., 1994). At present, sparse stands of submerged macrophytes are found in other parts of the lake too. Reed belts, consisting of *Phragmites communis* (Lam.), *Limnophila indica (*L.*)* and *Typha* sp., occur along the shore. The fish community of the lake consists of pike (*Esox lucius* L.), perch (*Perca fluviatilis* L.), eel (*Anquilla anquilla* L.), roach (*Rutilus rutilus* L.) and burbot (*Lota lota* L.). The piscivores: planktivores biomass ratio is high (Berg et al., 1994). It is much higher than expected from the TP concentration (Jeppesen et al., 2000), likely as a result of low TN.

In Lake Ring sediment, we retrieved ephippia of *D. magna*, of the *D. pulex* group (most probably *D. pulex)* and of the *D. longispina* group (probably *D. hyalina and/or D. galeata*, and potentially also hybrids between these two species). To identify ephippia of taxa of the *D. pulex* and the *D. longispina* group to species level, genetic analyses are necessary.

Reconstructions based on zooplankton remains (L. Johansson and R. Bjerring Olsen, NERI) and data from local monitoring reports (County of Vejle, unpublished data, 1999) allow us to provide a rough sketch of the ecology of Lake Ring over a time span of the past 150 years. Fig. 2 shows the reconstructed density profiles of ephippia of the different *Daphnia* taxa in the sediment core, the reconstructed values for macrophyte coverage (derived from the analysis of

zooplankton remains) and fish predation pressure (derived from the analysis of *Daphnia* ephippia; see Chapter 1), and changes in Secchi disk depth, total nitrogen, total phosphorus and chlorophyll a in Lake Ring (abiotic factors measured as part of a monitoring program between 1971 and 1999; County of Vejle (unpublished data), 1999). The reconstruction of macrophyte coverage indicated that around 1850 the macrophyte coverage was highest, afterwards slowly decreased, and never really recovered to its former density. Inferred fish predation pressure was also highest in the second half of the 19th century and fluctuated in the 20th century, but overall did not show pronounced variation. The vertical profiles of ephippia abundance indicated that the *D. longispina* species complex was present in very high abundance around 1850, disappeared until the beginning of the 20th century, and from then onwards sporadically reappeared. Around 1960, densities of species of this species group increased a bit, but never reached former densities. *D. magna* and *D. pulex* appear for the first time around 1908 and reach their first peak around 1930. During this period the abundance of ephippia of the *D. longispina* complex stayed low. Around 1970 *D. magna* and *D pulex* peaked for the second time. In 1990 the density of *D. magna* increased again, contrary of *D. pulex* density, which stayed low.

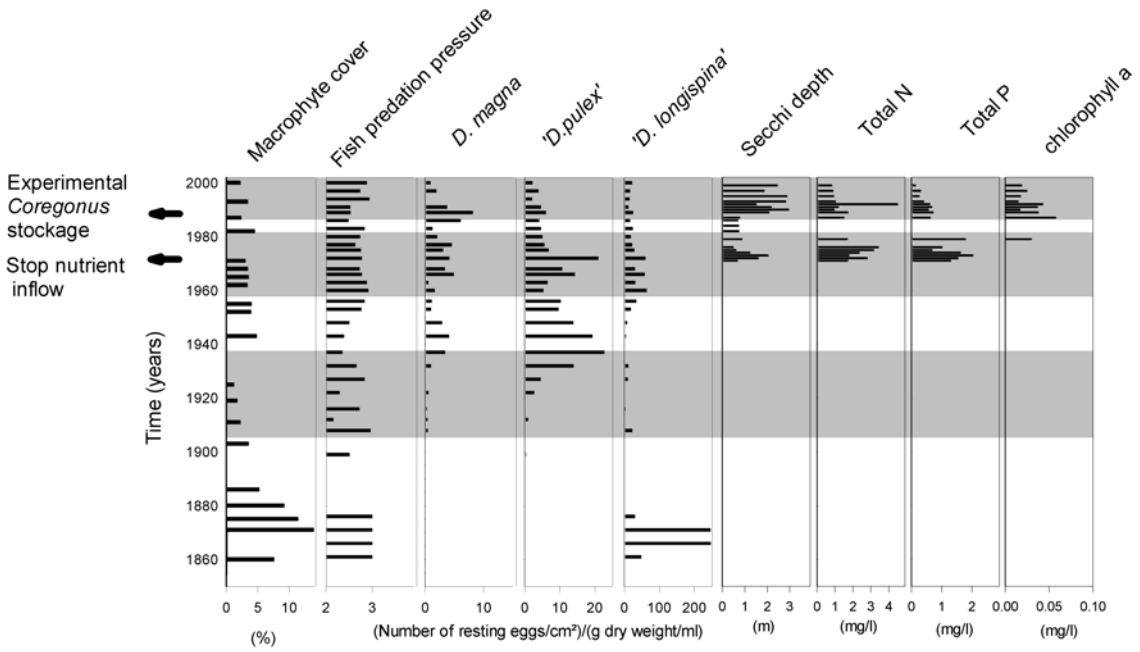

Fig. 2: Cladoceran inferred macrophyte coverage (%), inferred fish predation pressure based on Daphnia *ephippial size, density profiles of ephippia of the different co-occurring* Daphnia *species, Secchi disk depth, total nitrogen, total phosphorus and chlorophyll a in Lake Ring between 1860 and 1999 (data on abiotic factors from County of Vejle, unpublished data, 1999). Note the scale difference on the X-axis for the different* Daphnia *species. Dating of the sediment core was carried out using 210Pb radiodating, and is shown on the Y-axis. The Pre-eutrophication period (1908-1937), the Turbid period (1960-1980) and the Clear-water period (1986-2000) are indicated by grey shading.*

8. Objective and Outline of the thesis

In this dissertation, the main aim was to analyse the evolutionary response of a natural *D. magna* population inhabiting a shallow lake to changes in biotic stress associated with habitat degradation and restoration. More specifically, our approach was to use the dormant egg bank of the lake as an archive of past events, and study patterns of genetic change both for ecologically relevant traits as well as for neutral marker loci. Comparing the obtained patterns for these two categories of traits can provide insight in the driving forces behind the evolutionary process. Lake Ring provides a good study system for an investigation on the evolutionary consequences of environmental change due to eutrophication and subsequent habitat restoration. It has a history of eutrophication and from the 1970s onwards it slowly recovered. Besides some historical information on the recovery of the lake, which was monitored during the past 30 years, we were able to obtain information on changes in macrophyte coverage and fish predation pressure over the past 150 years through an analysis of zooplankton remains. We also reconstructed changes in population abundance of different *Daphnia* species present in Lake Ring during that same 150 year time interval. Finally, Lake Ring meets other key criteria for this kind of research: the sediment of Lake Ring could be dated (by ^{210}Pb radiodating) and contains viable *D. magna* dormant eggs. Our aim was to analyse micro-evolutionary changes with respect to different traits related to selection pressures associated with different types of antagonistic biotic interactions (predation, parasitism, and competition), in an effort to obtain an integrated picture on the potential causes of micro-evolution in the local *D. magna* population.

The framework of our research is illustrated in Fig. 3. We first focused on changes in fish predation pressure, inspired by the clear-cut results that were obtained in earlier work (Cousyn et al., 2001). Diel horizontal migration is a well-known antipredator trait in *Daphnia*, with animals migrating towards the littoral zone during the day to avoid mortality by fish. Both changes in macrophyte cover and fish predation pressure are expected to exert a strong selection pressure onto this behavior. An adaptive response in diel horizontal migration might allow persistence of the local *Daphnia* population as the environment changes, because of eutrophication and habitat restoration, which was associated with shifts from the clear-water to the turbid state and vice versa. Therefore, in Chapter 1 we set out to study the genetic variation in diel horizontal migration of the local *D. magna* population in Lake Ring and its micro-evolutionary response in relation to changes in macrophyte cover and fish predation.

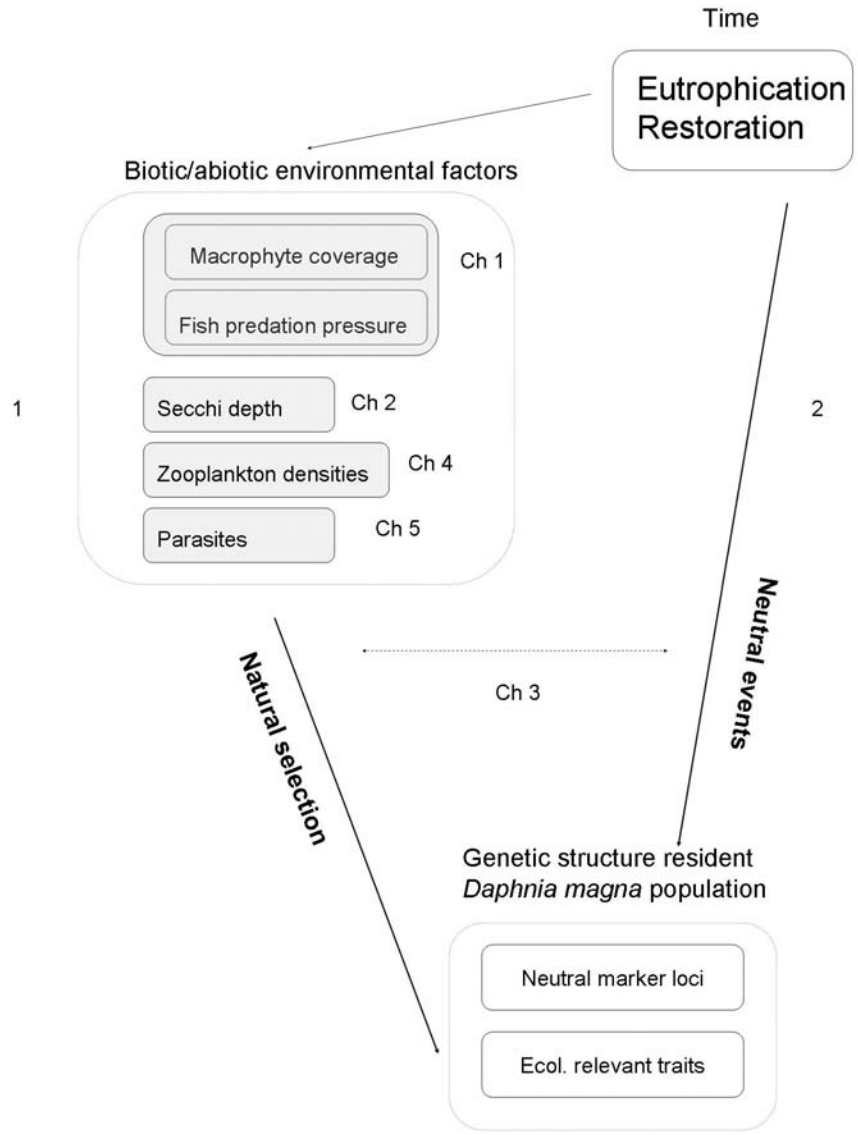

Fig. 3: General framework of research design. The process of habitat degradation and restoration impacts the genetic structure of resident Daphnia magna *population in two ways. (1) Through natural selection exerted by changes in biotic and abiotic environmental factors associated with eutrophication and restoration. (2) Through neutral events either or not associated with habitat changes. The different biotic and abiotic environmental factors are indicated in grey. The chapters in which the different factors of the framework are discussed are indicated next to every item.*

Daphnia are known to respond with several antipredator traits to fish predation (Boersma et al., 1998). Our study of variation in diel horizontal migration behavior showed that clones derived from the Clear-water period exhibit a weaker response to hide among plants even though fish predation pressure did not significantly decrease during this period. This suggests that these clones may use other antipredator traits to protect themselves against fish predation. In Chapter 2 we therefore consider a broader array of traits. We quantified genotypic values in the absence and presence of fish kairomone for six major life history and behavioral antipredator traits in a set of 26 clones isolated from the sediment core of Lake Ring.

The results presented in Chapter 1 and 2 of this work indicate that there is evidence of a micro-evolutionary response in antipredator traits in the local *D. magna* population of Lake Ring. To ensure that this pattern is the result of adaptive evolution in response to natural selection rather than of neutral processes, we compared the amount of genetic differentiation in quantitative traits among populations separated in time (Q_{ST}) with that in neutral marker loci (F_{ST}).

Whereas in the first three chapters the focus was on antipredator traits and their micro-evolution, in the last two chapters of this thesis we focus on defense mechanisms against two other important antagonists. Fig. 2 shows that besides changes in macrophyte cover and fish predation pressure, there are also strong changes in the relative abundance of the two large *Daphnia* species, *D. magna* and *D. pulex*, in Lake Ring. We anticipated that this could lead to changes in competitive ability and ecological combining ability (character displacement). More specifically, Chapter 4 focuses on, whether character displacement and/or changes in the capacity to dominate in competition took place in intra- and interspecific interactions, and to what extent these changes are related to changes in (relative) population density of the two large *Daphnia* species inhabiting Lake Ring.

Decaestecker et al. (2004) observed low levels of parasite and epibiont spores in Lake Ring sediment. Given that this study used rather low amounts of sediment, we hypothesized that actual levels of epibiont and parasite infections may have changed substantially in Lake Ring, and that these may thus potentially also have caused evolutionary changes. In response to these findings, we also focused on micro-evolutionary changes in response to host-parasite interactions. We also considered the interaction between fish predation and resistance against parasites.

In a final discussion chapter, we integrate our findings with respect to the evolutionary responses with respect to the different antagonistic interactions. Although we cannot claim that we considered all possible selection factors (e.g. our study did not involve abiotic stresses), the major contribution of our approach is that we focused on the micro-evolutionary response to

several antagonistic interactions in the same system and time interval. Our results do show substantial micro-evolutionary changes associated with human-induced changes in habitat quality.

We note that all chapters of this thesis are presented as manuscripts and can be read on their own. This may result in some overlap between chapters when referring to literature, data, methods (e.g. on the reconstruction of historical events of ephippium abundance profiles), and to results obtained in other chapters. In case specific results are presented in more than one chapter, they are then part of a broader study (e.g. data on Diel Horizontal Migration (DHM) as presented in Chapter 1 are also used as part of the much larger data-set involving different traits in Chapter 2).

9. References

Andrén, H., 1994. Effects of habitat degradation on birds and mammals in landscapes with different proportions of suitable habitat, a review. Oikos 71: 355-366;

Berg, S., E. Jeppesen, M. Sondergaard, & E. Mortensen, 1994. Environmental-effects of introducing Whitefish, Coregonus- Lavaretus (L), in Lake Ring. Hydrobiologia 276: 71-79.

Boersma, M., P. Spaak, & L. De Meester, 1998. Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: the uncoupling of responses. American Naturalist 152: 925-927.

Brendonck, L., & L. De Meester, 2003. Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. Hydrobiologia 491: 65-84.

Cáceres, C.E., 1998. Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. Ecology 79: 1699-1710.

Cohen, D., 1970. A theoretical model for optimal timing in diapauze. American Naturalist 12: 389-400.

Cohen, D., & S.A. Levin, 1987. The interaction between dispersal and dormancy strategies in varying and hetergenous environments. Lecture Notes in Biomathematics 71: 110-122.

Cousyn, C., & De Meester L., 1998. The vertical profile of resting egg banks in natural populations of the pond-dwelling cladoceran *Daphnia magna* Straus. Archiv für Hydrobiologie 52: 127-139.

Cousyn, C., L. De Meester, J.K. Colbourne, L. Brendonck, D. Verschuren, & F. Volckaert, 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. Proceedings of the National Academy of Sciences of the United States of America 98: 6256-6260.

Dawidowicz, P., 1990. Effectiveness of phytoplankton control by large-bodied and small-bodied zooplankton. Hydrobiologia 200: 43-47.

De Meester, L., J. Mergaey, H. Michels, & E. Decaestecker, Cladocera resting eggs and antropogenic changes. (in press): Alekseev, V. & De Stasio, B. (eds.), Diapause in aquatic invertebrates: role for ecology, physiology and human uses. Springer

Decaestecker, E., L. De Meester, & D. Ebert, 2002. In deep trouble: habitat selection constrained by multiple enemies in zooplankton. Proceedings of the National Academy of Sciences of the United States of America 99: 5481-5485.

Decaestecker E., Lefever C., De Meester L., & Ebert D., 2004. Haunted by he past: Evidence for dormant stage banks of microparasites and epibionts of *Daphnia*. Limnology and Oceanography 49: 1355-1364.

Decaestecker, E., S. Declerck, L. De Meester, & D. Ebert, 2005. Ecological implications of parasites in natural *Daphnia* populations. Oecologia 144: 382-390.

Dodson, S.I., & R.A. Lillie, 2001. Zooplankton communities of restored depressional wetlands in Wisconsin, Usa. Wetlands 21: 292-300.

Duffy, M.A., L.J. Perry, C.M. Kearns, L.J. Weider, & N.G. Hairston, 2000. Paleogenetic evidence for a past invasion of Onondaga Lake, New York, by exotic *Daphnia curvirostris* using mtDNA from dormant eggs. Limnology and Oceanography 45: 1409-1414.

Duffy, M.A., S.R. Hall, A.J. Tessier, & M. Huebner, 2005. Selective predators and their parasitized prey: Are epidemics in zooplankton under top-down control? Limnology and Oceanography 50: 412-420.

Duffy, M.A., & L. Sivars-Becker, 2007. Rapid evolution and ecological host-parasite dynamics. Ecology Letters 10: 44-53.

Ellis, J.S., M.E. Knight, B. Darvill, & D. Goulson, 2006. Extremely low effective population sizes, genetic structuring and reduced genetic diversity in a threatened Bumblebee species, Bombus Sylvarum (Hymenoptera : Apidae). Molecular Ecology 15: 4375-4386.

Evano, G., E. Castella, & J. Goudet, 2006. Evolutionary aspects of population structure for molecular and quatitative traits in the freshwater snail *Radix baltica*. European Society of Evolutionary Biology 19: 1071-1082.

Frankham, R., 1996. Relationship of genetic variation to population size in wildlife. Conservation Biology 10: 1500-1508.

Frankham, R., J. D. Ballou, & D. A. Briscoe, 2002. Introduction to Conservation Genetics, Cambridge University Press, Cambridge.

Frey, D.G., 1964. Remains of animals in Quaternary lake and bog sediments and their interpretation. Archiv für Hydrobiologie Ergebnisse der Limnologie 2: 1-114.

Gliwicz, Z.M., 1990. Food thresholds and body size in cladocerans. Nature 343: 638-640.

Grant, P.R., & B.R. Grant, 2002. Unpredictable evolution in a 30-year study of Darwin's Finches. Science 296: 707-711.

Green, A.J., J. Figuerola, & M.I. Sanchez, 2002. Implications of waterbird ecology for the dispersal of aquatic organisms. Acta Oecologica-International Journal of Ecology 23: 177-189.

Gulati, R.D., & E. Van Donk, 2002. Lakes in the Netherlands, their origin, eutrophication and restoration: state-of-the-art review. Hydrobiologia 478: 73-106.

Gulati, R.D., & E. Van Donk, 2005. Restoration of freshwater lakes. In: Van Adel, J. & Aronson, J. (eds.), Restoration Ecology . Blackwell Publishing, 158-173. Hairston, N.G.,

R.A. Vanbrunt, C.M. Kearns, & D.R. Engstrom, 1995. Age and survivorship of diapausing eggs in a sediment egg bank. Ecology 76: 1706-1711.

Hairston N. G., R.A. Van Brunt, C.M. Kearns, & D.R. Engstrom, 1995. Age and survivorship of diapauzing eggs in a sediment egg bank. Ecology 76: 1706-1711.

Hairston, N.G., W. Lampert, C.E. Caceres, C.L. Holtmeier, L.J. Weider, U. Gaedke, J.M. Fischer, J.A. Fox, & D.M. Post, 1999. Rapid evolution revealed by dormant eggs. Nature 401: 446.

Hairston, N.G., C.L. Holtmeier, W. Lampert, L.J. Weider, D.M. Post, J.M. Fischer, C.E. Caceres, J.A. Fox, & U. Gaedke, 2001. Natural selection for grazer resistance to toxic cyanobacteria: evolution of phenotypic plasticity? Evolution 55: 2203-2214.

Hairston, N.G., S.P. Ellner, M.A. Geber, Yoshida T., & Fox J. A., 2005. Rapid evolution and the convergence of ecological and evolutionary time. Ecology Letters 8: 1114-1127.

Harper, D., 1992. Eutrophication of freshwater, Chapman & Hall, London.

Hartl, D. & A. G. Clark, 1997. Principles of population biology, Sinauer Associates, Inc., Massachusetts, US.

Hebert P.D.N, 1978. The population biology of *Daphnia* (Crustacea, Daphnidae). Biological review 53: 387-426.

Hebert P.D.N., 1987. Genetics of *Daphnia*. R.H. Peters , R. de Bernardi (Eds.) '*Daphnia*' Memorie dell' Instituto Italiano di Idrobiologia 45: 439-460.

Hendry, A.P., & M.T. Kinnison, 1999. Persective: The pace of modern life: Measuring rates of contemporary microevolution. Evolution 53: 1637-1653.

Hosper, H., & M.L. Meijer, 1993. Biomanipulation, will it work for your lake - a simple test for the assessment of chances for clear water, following drastic fish-stock reduction in shallow, eutrophic lakes. Ecological Engineering 2: 63-72.

Innes, D.J., 1997. Sexual reproduction of *Daphnia pulex* in a temporary habitat. *Oecologia* 111: 53-60.

Jankowski, T., & D. Straile, 2003. A comparison of egg-bank and long-term plankton dynamics of two *Daphnia* species, *D-hyalina* and *D-galeata:* Potentials and limits of reconstruction. Limnology and Oceanography 48: 1948-1955.

Jeppesen, E., M. Sondergaard, B. Kronvang, J.P. Jensen, L.M. Svendsen, & T.L. Lauridsen, 1999. Lake and catchment management in Denmark. Hydrobiologia 396: 419-432.

Jeppesen, E., E.P. Jensen, M. Sondergaard, T. Lauridsen, & F. Landkildehus, 2000. Trophic Structure, species Richness and biodiversity in Danish Lakes: change along a nutrient gradient. Freshwater Biology 45: 201-218.

Jeppesen, E., P. Leavitt, L. De Meester, & J.P. Jensen, 2001. Functional ecology and palaeolimnology: Using cladoceran remains to reconstruct anthropogenic impact. Trends in Ecology & Evolution 16: 191-198.

Jeppesen, E., J.P. Jensen, S. Amsinck, F. Landkildehus, T. Lauridsen, & S.F. Mitchell, 2002. Reconstructing the historical changes in *Daphnia* mean size and planktivorous fish abundance in lakes from the size of *Daphnia* ephippia in the sediment. Journal of Paleolimnology 27: 133-143.

Kerfoot, W.C., W.R. Demott, & C. Levitan, 1985. Nonlinearities in competitive Interactions - component variables or system response. Ecology 66: 959-965.

Kerfoot, W.C., J.A. Robbins, & L.J. Weider, 1999. A new approach to historical reconstruction: combining descriptive and experimental paleolimnology. Limnology and Oceanography 44: 1232-1247.

Kerfoot, W.C., & L.J. Weider, 2004. Experimental paleoecology (resurrection ecology): Chasing Van Valen's Red Queen hypothesis. Limnology and Oceanography 49: 1300-1316.

Koch, A.M., G. Kuhn, P. Fontanillas, L. Fumagalli, I. Goudet, & I.R. Sanders, 2004. High genetic variability and low local diversity in a population of arbuscular mycorrhizal Fungi. Proceedings of the National Academy of Sciences of the United States of America 101: 2369-2374.

Lampert W., 1987. Feeding and nutrition in *Daphnia.* R.H. Peters , R. de Bernardi (Eds.) '*Daphnia*' Memorie dell' Instituto Italiano di Idrobiologia 45: 143-192.

Lande, R., 1992. Neutral theory of quantitative genetic variance in an island model with local extinction and colonization. Evolution 46: 381-389.

Lass, S., & K. Bittner, 2002. Facing Multiple Enemies: Parasitised Hosts Respond to Predator Kairomones. Oecologia 132: 344-349.

Limburg, P.A., & L.J. Weider, 2002. 'ancient' DNA in the resting egg bank of a microcrustacean can serve as a palaeolimnological database. Proceedings of the Royal Society of London Series B-Biological Sciences 269: 281-287.

Little, T.J., 2002. The evolutionary significance ŕf parasitism: do parasite-driven genetic dynamics occur ex-silico? Journal of Evolutionary Biology 15: 1-9.

Lowe, A.J., D. Boshier, M. Ward, C.F.E. Bacles, & C. Navarro, 2005. Genetic resource impacts of habitat loss and degradation; reconciling predicted theory for neotropical trees. Heredity 95:255-273.

McKay, J.K., & R.G. Latta, 2002. Adaptive population divergence: markers, qtl and traits. Trends in Ecology & Evolution 17: 285-291.

Meffe, G. & C. R. Carroll, 1997. Principles of conservation biology, Sinauer Associates, Inc., Massachusetts, US.

Mergeay, J., 2005. Paleoecology and paleogenetics in the tropics: the *Daphnia* of Lake Naivasha, Kenya. PhD thesis, KULeuven, Belgium.

Merilä, J., & P. Crnokrak, 2001. Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology 14: 892-903.

Moss, B., 1998. Ecology of fresh waters: man and medium, past to future, Blackwell Publishing, UK

Pijanowska, J., & G. Stolpe, 1996. Summer Diapause in *Daphnia* as a reaction to the presence of fish. Journal of Plankton Research 18: 1407-1412.

Pulido, F., & P. Berthold, 2004. Microevolutionary response to climatic change. Advances in Ecological Research 35: 151-183.

Reed, D.H., & R. Frankham, 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55: 1095-1103.

Reznick, D.N., F.H. Shaw, F.H. Rodd, & R.G. Shaw, 1997. Evaluation of the rate of evolution in natural populations of guppies (Poecilia Reticulata). Science 275: 1934-1937.

Rigby, M.C., & J. Jokela, 2000. Predator avoidance and immune defence: costs and trade-offs in snails. Proceedings of the Royal Society of London Series B-Biological Sciences 267: 171-176.

Scheffer, M., 1998. Ecology of shallow lakes, Chapman & Hall, London, UK.

Scheffer, M., S.H. Hosper, M.L. Meijer, B. Moss, & E. Jeppesen, 1993. Alternative equilibria in shallow lakes. Trends in Ecology & Evolution 8: 275-279.

Schindler, D.W., 1988. Effects of acid-rain on fresh-water ecosystems. Science 239: 149-157.

Scott, D.P., & E.E. Werner, 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. Proceedings of the National Academy of Sciences of the United States of America 98: 3904-3908.

Spitze, K., 1993. Population-Structure in *Daphnia-obtusa* - quantitative genetic and allozymic variation. Genetics 135: 367-374.

Stibor, H., 1992. Predator induced life-history shifts in a fresh-water Cladoceran. Oecologia 92: 162-165.

Stirnadel, H.A., & D. Ebert, 1997. Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. Journal of Animal Ecology 66: 212-222.

Stokwell, C.A., A.P. Hendry, & M.T. Kinnison, 2003. Contemporary evolution meets conservation biology. Trends in Ecology & Evolution 18: 94-101.

Storz, J.F., 2002. Contrasting patterns of divergence in quantitative traits and neutral DNA markers: analysis of clinal variation. Molecular Ecology 11: 2537-2551.

Tessier, A.J., & C.E. Cáceres, 2004. Differentiation in sex investment by clones and populations of *Daphnia*. Ecology Letters 7: 695-703.

Tollrian R., & C.D. Harvell, 1999. The evolution of inducible defences . Princeton University Press, princeton

Van Adel, J., & Aronson, J., 2005. Restoration Ecology, Blackwell Publishing

Verschuren, D., & L.F. Marnell, 1997. Fossil zooplankton and the historical status of Westslope Cutthroat Trout in a headwater lake of Glacier national Park, Montana. Transactions of the American Fisheries Society 126: 21-34.

Verschuren, D., K.R. Laird, & B.F. Cumming, 2000. Rainfall and drought in equatorial East Africa during the past 1,100 years. Nature 403: 410-414.

Weider, L.J., W. Lampert, M. Wessels, J.K. Colbourne, & P. Limburg, 1997. Long-term genetic shifts in a microcrustacean egg bank associated with anthropogenic changes in the Lake Constance ecosystem. Proceedings of the Royal Society of London Series B-Biological Sciences 264: 1613-1618.

Weir, B.S., & C.C. Cockerham, 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Weir, B.S., 1996. Genetic Data Analysis II. Sinauer, Massachusetts.

Woolhouse, M.E.J., J.P. Webster, E. Domingo, B. Charlesworth, & B.R. Levin, 2002. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nature Genetics 32: 569-577.

Wright, S., 1951. The genetical structure of populations. Ann Eugenics 15: 323-354.

Young, A., T. Boyle, & T. Brown, 1996. Estimating population structure in diploids with multilocus dominant DNA markers. Molecular Ecology 8: 907-913.

Zaffagnini F., 1987. Reproduction in *Daphnia*. Memorie dell' instituto Italiano di Hydrobiologia, 245-284.

CHAPTER 1 INTERCLONAL VARIATION IN DIEL HORIZONTAL MIGRATION BEHAVIOR OF THE WATER FLEA *DAPHNIA MAGNA* – SEARCHING FOR A SIGNATURE OF ADAPTIVE EVOLUTION

Helen Michels, Susanne Lildal Amsinck, Erik Jeppesen and Luc De Meester

ABSTRACT

In shallow temperate lakes, zooplankton populations may exhibit diel horizontal migration (DHM) and move towards macrophytes during the day to avoid fish. Using a natural *Daphnia magna* population, we undertook an experimental investigation aimed to describe the genetic variation for DHM and to study whether an adaptive micro-evolutionary response occurred to changes in macrophyte coverage and fish predation pressure through time. Twenty-seven *D. magna* clones were hatched from ephippia in the sediment of shallow Lake Ring, Denmark. This lake was eutrophied during the 20th century and was subject to restoration measures in the 1970s. The DHM behavior of the clones was observed both in the presence and absence of fish kairomone. Significant interclonal variation in DHM behavior occurred in both treatments. To study the micro-evolutionary response of the Lake Ring *D. magna* population, two approaches were used. First, we compared the DHM behavior of clones derived from ephippia collected at different depths. A comparison was conducted between clones resurrected from the period of eutrophication (1960-1980) and from the period of recovery (1986-2000). A significant treatment (presence and absence of fish kairomone) x period interaction effect was identified, suggesting a significant micro-evolutionary response for DHM behavior. The *D. magna* clones exhibited a significantly stronger horizontal migration response during the period of eutrophication than in the recovery phase. Secondly, clonal means, representing the influence of the genotype on the trait, were correlated with environmental conditions (macrophyte cover, fish predation pressure and Secchi depth). The results of this analysis also suggest that a micro-evolutionary response by *Daphnia* has occurred in reaction to changes in fish predation pressure. In periods with high fish predation pressure, *Daphnia* migrated more strongly towards the plants.

Parts of this chapter are in press in Hydrobiologia.

1. Introduction

Recently, there is a rapidly growing interest in the influence of anthropogenic impact on the genetic structure and the micro-evolutionary response of local populations (Schlaepfer et al., 2002; Stockwell et al., 2003; Pulido and Berthold, 2004). Habitat deterioration of shallow lakes through eutrophication is widely spread globally. Similarly, there are an increasing number of successful cases of habitat recovery upon restoration measures. Due to habitat changes during eutrophication and recovery, the selective forces that local zooplankton species experience are altered. This may potentially lead to a micro-evolutionary response in ecological relevant traits, such as antipredator traits.

An important antipredator trait is diel vertical migration (DVM) in zooplankton, which has been studied intensively for more than two centuries (Cushing et al., 1951; Haney, 1988, 1993). Results show that zooplankton tend to reside deeper in the water column during the day than during the night (Ringelberg, 1999; De Meester et al., 1999). DVM is known to occur as a response to changes in light intensity, modulated by factors such as food and not the least the presence of chemical cues from predators (Ringelberg 1991; Van Gool and Ringelberg, 1995). The adaptive significance of DVM in zooplankton is to reduce the risk of detection by visually hunting predators such as fish. However, in shallow lakes, this may not always be a successful strategy, as the possibility of seeking refuge in deeper water layers is diminished due to lack of stratification (Davies, 1985; De Meester, 1993; Lauridsen and Buenk, 1996); the situation may be different is the case of low-lying submerged macrophytes (Cerbin et al., 2003). Although diel horizontal migration (DHM) has been less studied than DVM, recent studies demonstrate that zooplankton in shallow temperate lakes often perform DHM, i.e. migrating towards the vegetated littoral during the day (Davies, 1985; Kvam and Kleiven, 1995; Lauridsen and Buenk, 1996; Burks et al., 2001, 2002). Like DVM, DHM may be induced by chemical cues from fish (Lauridsen and Lodge, 1996) and invertebrate predators (Burks et al., 2001; Van De Meutter et al., 2005), which strongly points to a function as antipredator trait. Macrophytes indeed represent a refuge for zooplankton against visually hunting predators such as fish (Timms and Moss, 1984; Lauridsen and Buenk, 1996), because the foraging efficiency of fish is lower in structurally complex microhabitats. Residing among the macrophytes, however, may have costs. First, the zooplankton may experience an increased exposure to epiphytic and benthic invertebrate predators. Secondly, the macrophytes themselves may release substances that reduce the growth of *Daphnia* (Burks et al., 2000). Lauridsen and Lodge (1996) showed that *Daphnia* tend to avoid macrophytes in the absence of fish kairomone (see also Pennak, 1966), but move towards the plants when exposed to fish kairomone. Abiotic cues, such as light,

temperature, dissolved oxygen and pH seem to be of less importance for DHM than for DVM, because the horizontal gradients tend to be lower than the typically strong vertical gradients in deep lakes (Burks et al., 2001).

Analysis of zooplankton dormant egg banks in stratified lake sediments enables reconstruction of population dynamics and genetic changes in the recent past (Brendonck and De Meester, 2003). The long-term viability of dormant propagules (> 100 years) allows quantitative genetic analyses of 'resurrected' populations (Hairston et al., 1999; Kerfoot et al., 1999; Cousyn et al., 2001). Besides being an archive of genetic information on a local population, stratified lake sediment may also harbor information on changes in environmental conditions which may have acted as selective agents. The changes in key environmental conditions, such as macrophyte coverage and fish predation pressure, can be inferred through paleolimnological analysis of zooplankton remains in the sediment (Jeppesen et al., 1998, 2001; Amsinck et al., 2005). *Daphnia* plays an ecologically important role in shallow lake communities. It exerts a strong grazing pressure on the phytoplankton community and at the same time constitutes an important prey item for visually hunting fish (Lampert, 1987). *D. magna* typically occurs in shallow water bodies, but is very sensitive to fish predation pressure (Cousyn et al., 2001).

Whereas the presence of genetic variation in DVM is well established (Weider, 1984; De Meester, 1993, 1996; De Meester et al., 1995) and rapid adaptive evolution for DVM behavior in response to changes in environmental condition has been reported (Cousyn et al., 2001), no studies have yet been conducted to reveal the ecological genetics of DHM. An adaptive response in DHM to changing environments may allow *D. magna* populations to persist locally, which may contribute to the maintenance of a clear-water state of the lake (Scheffer et al., 1993; Lauridsen et al., 1996). The capacity of a population to show a genetic response in DHM behavior to changing selection pressures is critically dependant on the heritability of the trait. The concept of heritability refers to the proportion of phenotypic variance of the trait in a population, which is due to genotypic variance (Falconer and MacKay, 1996). The cyclic parthenogenetic reproduction of *Daphnia* allows working with clones in an experimental design. Heritability in clonal organisms can be estimated through a clonal repeatability analysis (Lynch, 1984, Falconer and MacKay, 1996).

In this study, 27 *D. magna* clones were obtained by hatching dormant eggs from a sediment core sampled from Lake Ring, a shallow Danish lake exposed to eutrophication and restoration during the 20th century. We analyzed the DHM behavior of these clones both in the presence and absence of fish kairomone. Our aims were to investigate 1) whether genetic variation for DHM is present in this *Daphnia* population; 2) whether a micro-evolutionary

response in horizontal migration behavior in *D. magna* can be observed in relation to changes in habitat characteristics associated with eutrophication and habitat restoration, and 3) whether the presence of fish kairomone induces a stronger DHM behavior towards the plants in this population.

2. Material and methods

2.1. Study site

Lake Ring (55° 57' 51.83'' N, 9° 35' 46.87'' E) is a relatively small (22.5 ha) eutrophic and shallow lake (average depth 2.9 m) located in mid-Jutland, Denmark. The lake suffered from eutrophication during the 20th century due to sewage inflow from a nearby town. In 1970 this pollution source was diverted from the lake (Berg et al., 1994) and the lake gradually recovered. In the mid 1980, total phosphorus (TP) levels had declined (from 1.78 mg/l to 0.629 mg/l) and between 1979 and 1987 lake water transparency began to improve (Fig. 1). As the catchment area is relatively small, the improvements can probably be ascribed to nitrogen limitation (E. Jeppesen; unpubl. data) as TP was still high. Whitefish (*Coregonus lavaretus;* a planktivorous fish) was stocked in the lake during the period 1989-1990 to investigate its effect on the zooplankton community of the lake (Berg et al., 1994).

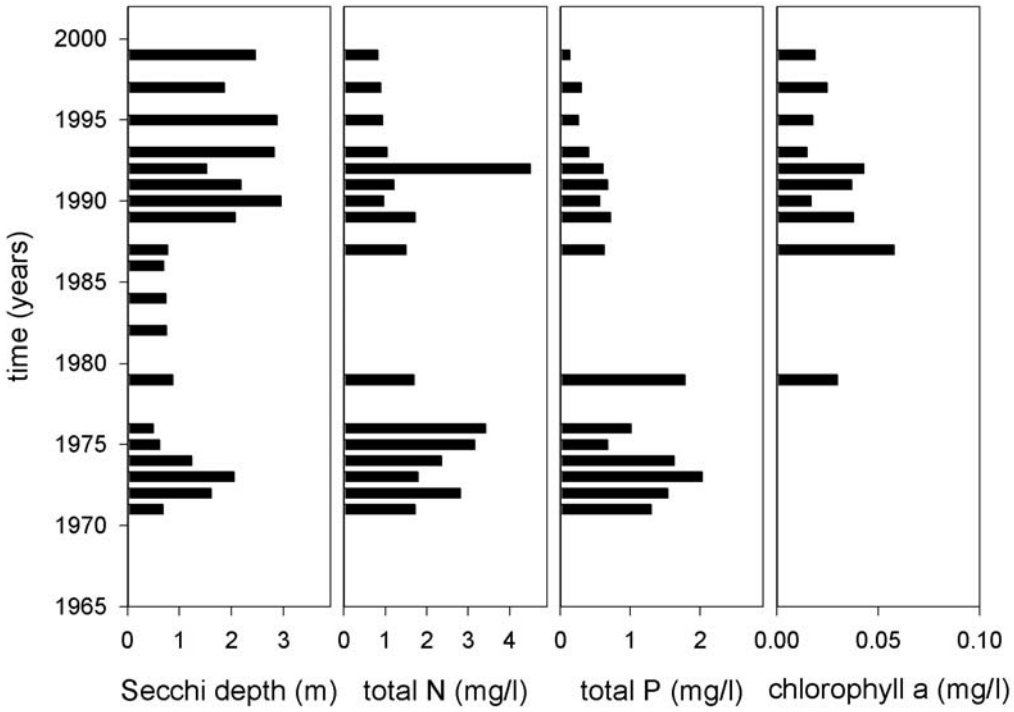

Fig. 1: Secchi disk depth, total nitrogen, total phosphorus and chlorophyll a in Lake Ring between 1971 and 1999 (County of Vejle (unpublished data) (1999)).

In 1989, the only submerged macrophyte in the lake was *Potamogeton pectinatus* L., which appeared along the north-east coast of the lake (Berg et al., 1994). At present, sparse stands of submerged macrophytes are found in other parts of the lake too. Reed belts, consisting of *Phragmites communis* (Lam.), *Limnophila indica (L.)* and *Typha* sp., occur along the shore. The natural fish stock consists of pike (*Esox lucius* L.), perch (*Perca fluviatilis* L.), eel (*Anquilla anquilla* L.), roach (*Rutilus rutilus* L.) and burbot (*Lota lota* L.). The piscivores: planktivores biomass ratio is high (Berg et al., 1994) and much higher than expected from the TP concentration (Jeppesen et al., 2000) likely as a result of low TN.

2.2. Paleolimnological analysis

- **Field methods**

In October 2000, four cores (A, B, C, D; approximately 45 cm each) were recovered from Lake Ring using a Kajak corer (internal diameter 5.2 cm) mounted on a stick. Four cores were taken in order to provide sufficient material for hatching. The cores were sectioned horizontally into 1 cm thick slices, packed in a light-tight recipient and stored at 4 °C until preparation for analysis.

- **Physical analysis and radiometric dating**

For all the core samples, 1 ml of homogenized wet sediment was analyzed for dry weight (at 110 °C) and ash free dry weight (at 550 °C). Radiometric dating of the sediment was conducted by P.G. Appleby, Radioactivity Research Centre, University of Liverpool, UK, on core B only. Homogenized subsamples of core B were analyzed for 210Pb, 226Ra, 137Cs and 241Am by direct gamma assay (Appleby et al., 1986). Radiometric dates were calculated using a standard CRS (Constant Rate of Supply) 210Pb dating model (Appleby and Oldfield, 1978). The independent $^{137}Cs/^{241}Am$ dating method suggests that the CRS method is appropriate for this core (P.G. Appleby, pers. comm.).

- **Profiles of fossil egg abundance**

The sediment of the four cores was filtered manually through a 250 µm mesh sieve. The sediment fraction larger than 250 µm was checked for *D. magna* ephippia in a tray kept on ice to prevent spontaneous egg hatching. We focused on *D. magna*, because due to its size, this species was expected to be the most sensitive to fish predation pressure. Egg-containing ephippia were isolated and stored in the dark at 4 °C. Subsequently, the remaining ephippia of all *Daphnia* species were counted for all 1 cm sample slices using a stereomicroscope (40 x, Leica MZ APO). The identification of ephippia was based on morphological characteristics according to the keys of Brooks (1957), Hebert (1995) and Flößner (2000).

- **Inference of macrophyte coverage and fish predation pressure**

Submerged macrophyte coverage was inferred by use of previously established transfer functions based on macrophyte and macrophyte-sediment associated sedimentary cladoceran remains for Danish lakes (Jeppesen, 1998). The coverage of submerged macrophytes is an estimate of the lake bottom covered by macrophytes and was reconstructed from the sediment core using a weighted-average (WA) model (Johansson et al., 2005). A total of 25 sediment sections of core A were used for inference of submerged macrophyte cover.

The reconstruction of fish predation pressure was not based on a transfer function based on cladoceran remains, because only two of the lakes used to establish the transfer function for fish predation pressure contained *D. magna* and *D. pulex.* As these two species play an important role in Lake Ring, this method is not appropriate in this case. Instead, the inference of fish predation pressure was based on the average size of the ephippia of the different *Daphnia* species present in the sediment. Jeppesen et al. (2002) found a strong correlation between ephippial size and fish abundance (r^2= 0.66, $p<0.001$); size of ephippia decreased, when fish density increased. Information on the average size of ephippia of the different species was collected from literature (Benzie, 2005). This way, there is no interference between the reconstruction of the fish predation pressure and size related traits used in later chapters. We used the following formula for fish predation pressure:

$$e^{-\left[2-\left(\frac{S_{DM}\times A_{Dm}+S_{Dp}\times A_{Dp}+S_{Dl}\times A_{Dl}}{T}\right)\right]} \quad (1)$$

In which S in the average size (µm) of the ephippia (*D.m. = D. magna*; *D.p. = D. pulex*; *D.l. = D .longispina*) and abundance is the abundance of ephippia. T= total number of ephippia.

- **Core alignation**

In order to combine data obtained from the different cores, the four sediment cores were aligned using the density profiles of *D. pulex* ephippia. The core alignment was achieved independently of the pattern observed for *D. magna*, i.e. the study species. The peak in the density profile of *D. pulex,* dated at 1936 using radiometric markers (^{210}Pb, ^{226}Ra, ^{137}Cs and ^{241}Am), was used as reference date. Dating of the peak allowed calculation of the average sedimentation rate and of the corresponding cumulative dry weight. Assumption of constant sedimentation through time (see also Cousyn et al., 2001) allowed us to calculate an alternative time line based on cumulative dry weight. The resulting time line was very similar to that obtained through radiometric dating. The reference date was used for the other cores and a

constant sedimentation rate through time was assumed. The resulting sedimentation rates of the different cores ranged between 0.01-0.03 g dry weight/year per cm². By applying the above procedure, all cores could be dated and aligned to each other.

2.3. Hatching ephippia and culture conditions

Eggs were removed from their protective membranes (decapsulated) and incubated per depth interval in a Petri dish filled with dechlorinated tap water under fluorescence tubes at a long day photoperiod (16 h light - 8 h dark) and at 20 ± 1°C (Schwartz and Hebert, 1987; De Meester and De Jager, 1993). As several ephippia were grouped, when exposed to hatching stimuli, we could not differentiate among hatchlings from the same (full sibs) or different ephippia. Medium was renewed every second day and the hatched individuals were cultured as clonal lineages.

Animals were cultured under standard conditions (30 individuals per litre, 20°C ± 1°C, LD 14:10, high food conditions: 2.5 x 10^5 *Scenedesmus acutus* cells per ml, fed daily). The medium was renewed every other day. One treatment with two levels was applied: presence (F) and absence (NF) of fish kairomone. In the fish treatment, dechlorinated tap water was conditioned by the presence of 3 *Leuciscus idus* (approximately 6 cm) in a 20 litres aquarium for 24 hours. The water was then filtered through a 0.45-µm filter and diluted to a concentration equivalent to 3 fish per 100 litres. In the non-kairomone treatment, dechlorinated tap water was used, which was likewise aged for 24 hours but not conditioned by fish. Each lineage was independently cultured for at least two generations under standard conditions before starting the experiment.

2.4. Horizontal migration experiment

DHM was quantified using a small scale experimental set-up. The set-up consisted of a long PVC gutter (L=1.5 m, W=7 cm, H=4.5 cm) which was closed at both ends (Fig. 2). The gutter was externally divided into 8 compartments, each being 18.7 cm long. In the two outer compartments a littoral zone was simulated. Eleven shoots of natural *Ceratophylum demersum* and 11 shoots of plastic *C. demersum* (plantastics©) were equally divided between both sides of the gutter. Each shoot measured 4 cm, producing a Plant Volume Infested (PVI) of 28 % of the total water volume. The PVI was similar on both sides of the gutter and comparable to the natural situation of mesotrophic-eutrophic shallow lakes (Lauridsen and Lodge, 1996). The gutter was filled with 3 litres of aged tap water or fish conditioned tap water and was thoroughly cleaned after each experiment.

The tests were conducted in an experimental area which was separated from the rest of the walk-in culture room to avoid disturbance and light interference. Light was provided by 2 X 2

fluorescence tubes hanging parallel with the experimental gutters to minimize shadow effects. The light intensity in the experimental area was 1100 lux. After 3-4 hours of acclimatization to the experimental medium, 10 adult females were placed in the middle of the gutter. Preliminary experiments showed that the initial position of the *Daphnia* in the gutter did not affect the final outcome of the experiment. At 15 min. intervals the number of individuals in each compartment was counted. Each experiment lasted 2 hours. The DHM of the test population was quantified by the index HMI=(I-O)/total number animals, averaged over the last hour; with I being the number of animals in the two middle compartments and O the number of animals in the two outer compartments. Values for HMI can range from -1 to 1; value -1 implying that all individuals hide among the plants and +1 that all animals occur in the centre of the gutter. For each of the 2 x 27 treatment x clone combinations, four replicate experimental observations were made. Every replicate consisted of 10 adult females from a different culture or a different generation to avoid confounding maternal effects.

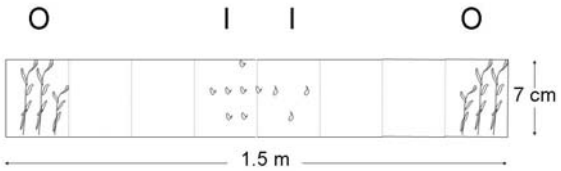

Fig. 2: Experimental set-up for the quantification of horizontal behavior in Daphnia magna *(from above). The set-up consists of a PVC gutter which is divided into 8 compartments, each being 18.7 cm long. In the two outer compartments (O) a littoral zone was simulated. Test animals are place in the centre of the gutter (I).*

2.5. Statistical analysis

The clones used in the experiment were derived from dormant eggs isolated from different time periods. Although they all belonged to the resident population of Lake Ring, they did not belong to the same population in the strict sense of the term (i.e. co-occurring). Therefore, we do not refer to our measures of interclonal variation for DHM behavior as representing heritability estimates. We performed a clonal repeatability analysis to test the interclonal variation of DHM behavior. This analysis quantifies the genetic component to the observed phenotypic variance in the studied trait and the studied set of clones (Falconer and MacKay, 1996). We estimated clonal repeatability in both presence (F) and absence of fish kairomone (NF) as well as for the change in behavior observed upon exposure to fish kairomone (NF-F). The values of the index were transformed $(HMI+1)^3$ to comply with the assumptions of

homoscedasticity (Bartlett's test) and normality (Sokal and Rohlf, 1995). A two-way ANOVA with 'clone' as random factor and treatment as fixed factor was performed to test whether a significant treatment effect could be traced. Clone is set as a random factor, because the experimental clones used, were a random sample of the hatchlings from the dormant eggs isolated from different sediment layers. To calculate clonal repeatabilities, one-way ANOVAs with 'clone' as random factor were carried out for both treatments (absence and presence of fish kairomone) separately and for the change in DHM behavior upon the exposure of the *Daphnia* to fish kairomone.

We used two different approaches to identify whether significant micro-evolution for DHM behavior occurred in the study population. First, we divided the set of clones into subpopulations that were separated in time (further indicate as 'period'), and used a three-way ANOVA to test for an effect of treatment, clone (nested in period), period and treatment x period interaction, with clone as a random factor and treatment and period as fixed factors. We compared the DHM behavior before and after 1970, i.e. the 1906-1968 population versus the 1972-2000 population, to test whether the cut-off of nutrient inflow to Lake Ring in 1970 resulted in a significant genetic change in DHM behavior of the *Daphnia* population. A second logic boundary is the moment on which the lake showed first clear signs of recovery (increased Secchi disk depth, reduced nutrient concentrations). This recovery happened in the mid 1980 (more exactly; between 1979 and 1987). We assume that the decline in phosphate level happened in the middle of this period, thus we tested whether there was a significant difference in DHM behavior between the 1960-1980 and the 1986-2000 populations.

Next, Pearson's correlations were used to check for relationships between clonal averages in HMI and environmental characteristics (macrophyte coverage, fish predation pressure and Secchi disk depth) prevailing during the corresponding time period. All statistical analyses were performed using STATISTICA (Statsoft, 1994).

3. Results

3.1. Interclonal variation

Fig. 3 shows the reconstruction of ephippia density for the different *Daphnia* taxa as well as the reconstructed values for macrophyte coverage and fish predation pressure. Forty one % of the incubated eggs hatched. No relationship was found between the depth and the hatching success. The average time of hatching of the clones that were used in the experiment was 6 days.

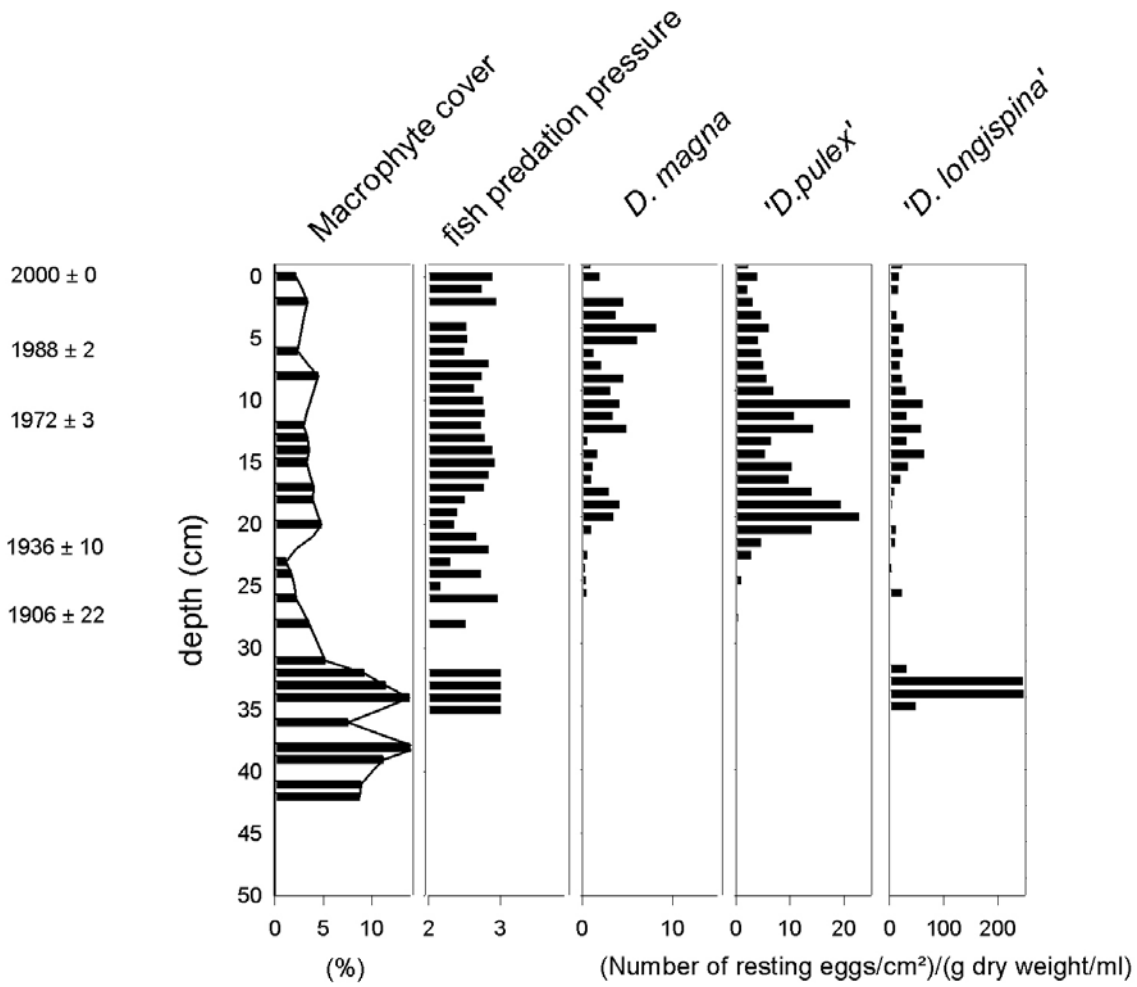

Fig. 3: Cladoceran inferred macrophyte coverage (%) (core A), inferred fish predation pressure based on Daphnia *ephippial size (equation 1, core C) and density profiles of ephippia of* Daphnia *species (core C). Note the scale difference on the X-axis for the* Daphnia *species. 210Pb-based dating is shown to the left (core B).*

Most clones avoided the littoral zone in the absence of fish (HMI ranged from -0.025 to 1). In the presence of fish kairomone, the animals moved towards the plants ($F_{(1, 162)} = 17.12$; $p = 0.0003$). Although the change in DHM behavior in response to fish presence was highly significant, the change in distribution in absolute terms was modest, as the resulting HMI values ranged from -0.42 to 1. We also observed a significant clone effect on horizontal migration behavior ($F_{(26, 162)} = 3.19$; $p = 0.002$). However, no significant clone x treatment interaction effect was observed ($F_{(26, 162)} = 1.12$; $p = 0.31$). Interclonal differences were significant both in the absence and presence of fish kairomone (absence: $F_{(26, 81)} = 1.74$, $p = 0.03$; presence: $F_{(26, 81)} = 2.81$, $p = 0.0002$). The response to fish kairomone did not differ among the clones ($F_{(26, 81)} = 1.04$; $p = 0.41$). Clonal repeatabilities are given in Table 1.

Table 1: Results of one-way ANOVAs testing for interclonal variation in horizontal migration behavior, and the associated clonal repeatability values.

Trait	**df**	**F**	**V_E**	**V_G**	**Clonal repeatability**	**p**
HMI (presence of fish kair.)	26, 81	2.81	3.75	1.70	0.31	<0.001
HMI (presence of fish kair.)	26, 81	1.74	2.69	0.5	0.15	0.031
HMI (response to fish kair.)	26, 81	1.04	6.94	0.082	NS	0.41

3.2. Comparison among populations separated in time

A comparison of DHM behavior of the clones derived from the period before and after 1970 did not reveal a significant treatment x period interaction effect ($F_{(1, 162)} = 0.04$; p=0.84; Fig. 4). In contrast, a comparison between the 1960-1980 period and the 1986-2000 period showed a significant treatment x period effect ($F_{(1, 120)} = 5.50$; p = 0.03; Fig. 5). One-way ANOVAs testing for differences in key environmental characteristics (transparency, inferred fish predation pressure and inferred macrophyte coverage) of Lake Ring between the two time periods (1960-1980 and 1986-2000) revealed a significant difference in Secchi depth between the two periods ($F_{(1, 17)} = 10.15$; p = 0.005). In the period 1960-1980 mean Secchi depth was 1.01 ± 0.17 m and increased to 2.02 ± 0.25 m in the following period (1986-2000). Neither inferred fish predation pressure nor inferred macrophyte coverage showed any significant difference between the two periods.

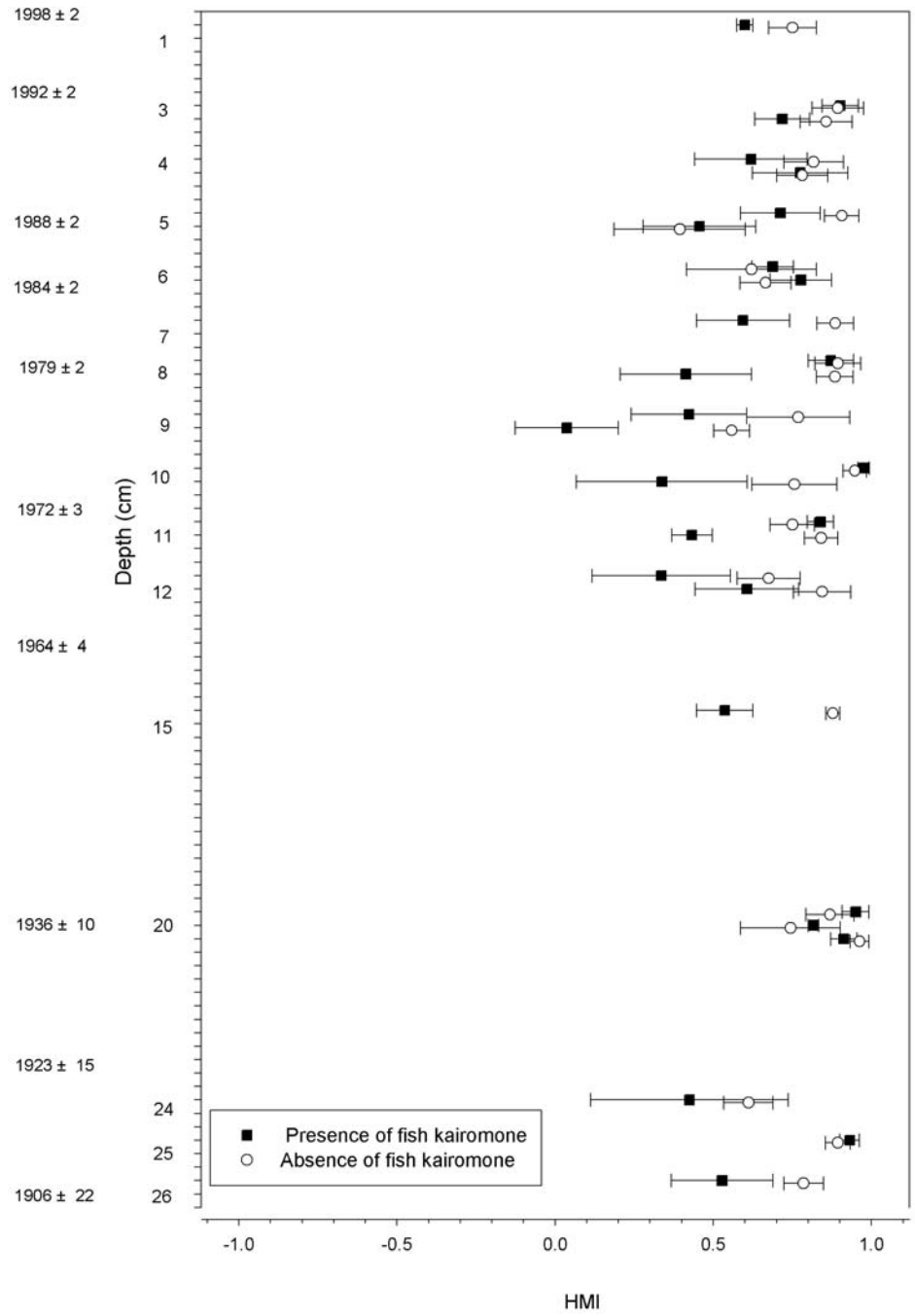

Fig. 4: Horizontal migration index HMI (mean ± SE, n=4) of 27 Daphnia magna *clones isolated from different depth layers of sediment core C from Lake Ring in the presence (solid symbols) and absence (open symbols) of fish kairomone. The data for each clone are plotted at the depth layer from which the clone was hatched. A HMI index of 1 indicates that all individuals occur in the central area of the experimental gutter. Interpretation of depth as age based on ^{210}Pb dating as in Fig. 3.*

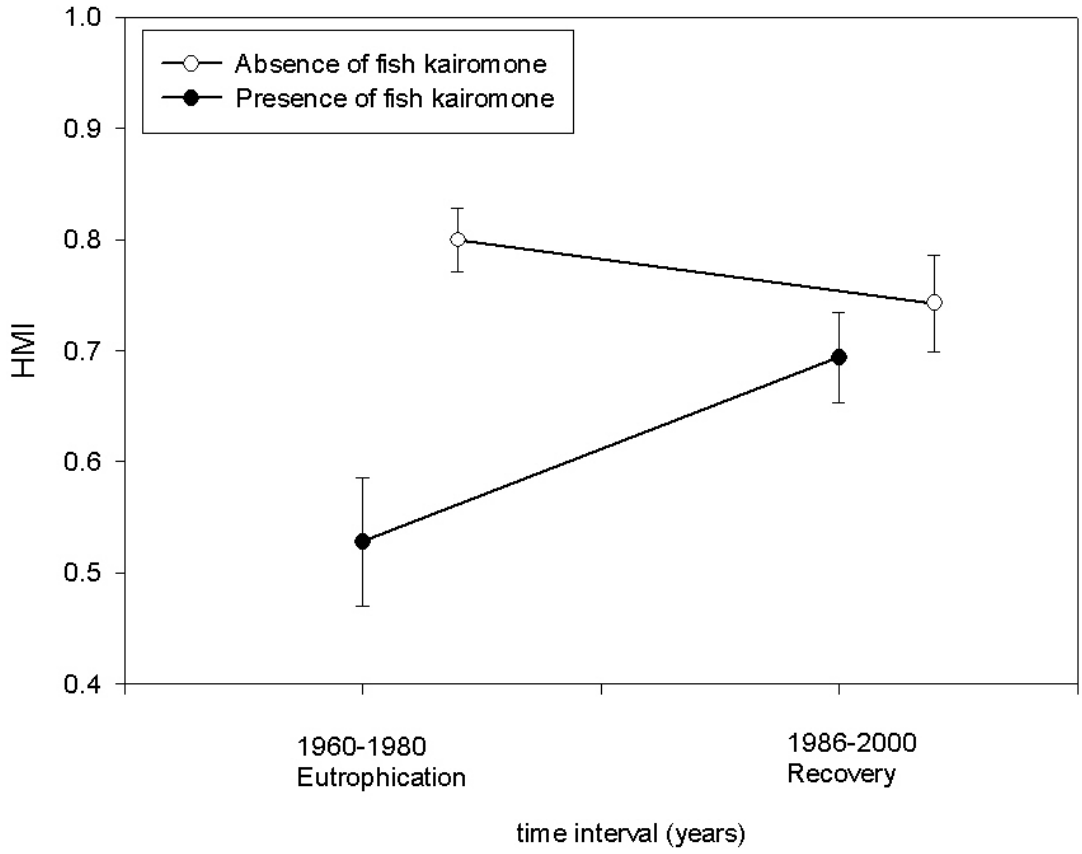

Fig. 5: HMI (mean of clonal averages ± SE (1960-1980): n=11; (1986-2000): n=9) in the presence and absence of fish kairomone of two subpopulations derived from two time periods, characterized by eutrophication (1960-1980) and recovery (1986-2000), respectively.

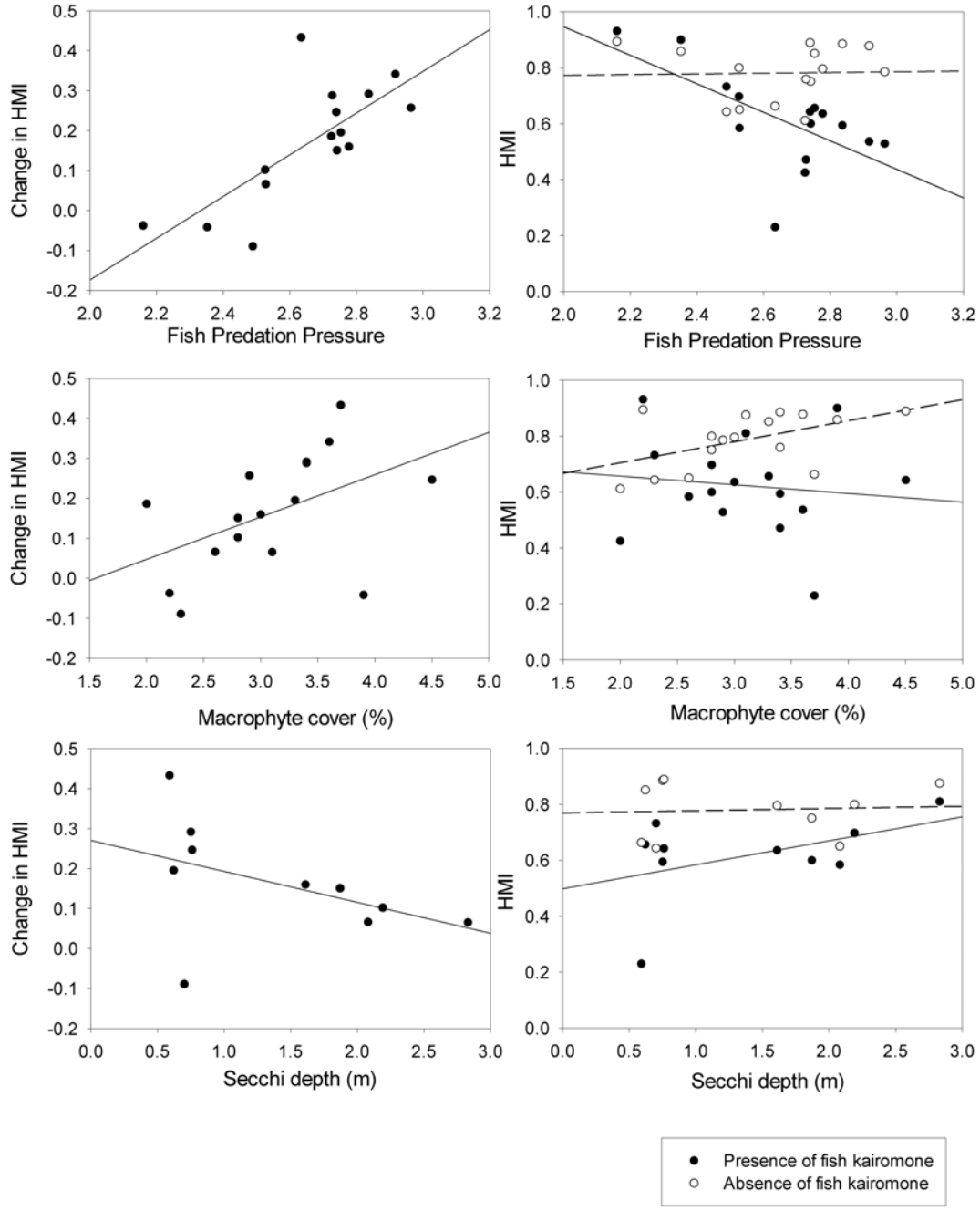

Fig. 6: Scatterplot showing correlations between environmental variables (fish predation pressure, macrophyte cover, and Secchi depth) and HMI in the absence of fish kairomone, HMI in the presence of fish kairomone and change in HMI. Dots represent mean HMI values averaged per depth interval.

As recordings were made of the sediment depths at which dormant eggs were sampled and clones subsequently hatched, correlations could be calculated between the clonal average of HMI, inferred fish predation pressure and inferred macrophyte coverage in the lake at the time corresponding to the depth from which the clones were hatched. Correlations between HMI traits and Secchi depth were also calculated. We averaged the value of HMI of all clones isolated from a particular 1 cm interval to prevent pseudo-replication. As we did not possess data on inferred fish predation pressure and inferred macrophyte coverage for all 1 cm layers, the values for the missing sediment layers were obtained by interpolation. In order to comply with the assumption of normality, 'Non fish HMI' was exponentially transformed. The correlation between HMI in the presence of fish kairomone and inferred fish predation pressure was significantly negative (Pearson's Product Moment correlation $r = -0.62$; $p = 0.012$; Table 2, Fig. 6). The correlation between exp(HMI non fish) and inferred macrophyte coverage was significantly positive (Pearson's Product Moment correlation $r = 0.52$; $p = 0.04$). Moreover, the correlation between the change in horizontal migration behavior upon exposure to fish kairomone of the *Daphnia* clones and inferred fish predation pressure was significant and positive (Pearson's Product Moment correlation $r = 0.75$; $p = 0.001$).

Table 2: Results of Product Moment correlations between HMI Fish, exp(HMI non fish), ΔHMI and the environmental variables: macrophyte coverage, fish predation pressure and Secchi depth.

Trait	**Macrophyte coverage**		**Fish predation pressure**		**Secchi depth**	
	r	p	r	p	r	p
HMI presence fish kairomone	-0.11	0.69	-0.62	0.012	0.26	0.49
Exp (HMI) absence fish kairomone	0.52	0.04	0.02	0.92	0.06	0.86
ΔHMI	0.48	0.07	0.75	0.001	-0.44	0.20

4. Discussion

Our results confirm the results of earlier studies suggesting that horizontal migration behavior of *Daphnia*, a key characteristic of DHM, may be induced by fish kairomone (Lauridsen and Lodge, 1996; Burks et al., 2001). In the absence of fish kairomone, *Daphnia* strongly avoided the littoral zone and most individuals stayed in the centre of the experimental area, which is in agreement with the findings of Pennak (1966), Davies (1985) and Lauridsen et

al., (1996). In the presence of fish kairomone, the animals moved towards the plant-infested area. However, the relatively high values of HMI observed in the presence of fish kairomone reflect that the animals did not move into the littoral itself, but remained near the edge of the plants. This is in agreement with field observations showing that zooplankton often stay immediately outside the macrophyte beds during the day (Lauridsen and Buenk, 1996; Lauridsen et al., 1996).

Quantitative genetic analysis of the past population of *Daphnia magna* in Lake Ring demonstrated interclonal differences in DHM behavior in the study set of *D. magna* clones both in the presence and absence of fish. Given that genetic variation for DHM behavior was observed, a pattern of local adaptation might be expected, in which the population tracks changes in environmental conditions (Endler, 1986; De Meester, 1996; Cousyn et al., 2001). We used a two-stage strategy to reveal whether an adaptive micro-evolutionary pattern could be identified. First, DHM behavior was compared between subpopulations separated in time, involving a comparison of the populations before and after 1970 (1970 being the year when the nutrient input to the lake ceased). No between-period behavioral differences appeared. However, as phosphorus measurements and Secchi depth indicate that lake recovery did not set in until mid 1980 (Fig. 1), an additional comparison was made between the 1960-1980 population and that of 1986-2000. This revealed a significant interaction effect between period and treatment, indicating a micro-evolutionary response. For the period of eutrophication, 1960-1980, higher migration of *Daphnia* towards the plants in the presence of fish kairomone was observed. The response to the fish kairomone of the 1986-2000 population was significantly less pronounced. Not only did the 1960-1980 population exhibit stronger migration towards the plants in the presence of fish kairomone, in the absence of fish kairomone the opposite pattern was observed, probably due to the repellent effect of the macrophytes (Pennak, 1966; Lauridsen and Lodge, 1996). Avoidance of macrophytes seemed to be most pronounced during the earliest period. Although this analysis gave evidence of micro-evolution, it was not subtle enough to distinguish the selective forces behind the pattern. The second approach, the correlation analysis of environmental conditions and the genotypic value (clonal means) of DHM behavior, confirmed our observation of significant micro-evolution and gave a more subtle view on the micro-evolutionary response. In the presence of fish kairomone, a negative correlation was found between HMI and inferred fish predation pressure. Thus, the higher fish predation pressure there was in the studied habitats, the more intense the migration towards the plants was exhibited by the clones in the presence of fish kairomone. The correlation between the inferred fish predation pressure and the change in behavior upon exposure to fish kairomone was also

positive, indicating a stronger reaction to fish kairomone in time periods with higher fish predation pressure.

Although a correlation does not imply causality, it is likely that fish predation pressure forms a selective force for DHM, as it is known that fish predation pressure is generally an important selective force for natural *Daphnia* populations (Gliwicz and Pijanowska, 1989; Lampert, 1987; Cousyn et al., 2001). Furthermore, DHM is a well-established predator avoidance trait (Burks et al. 2001; Lauridsen and Lodge, 1996). DHM can be seen as one of a wide array of predator-induced defence mechanisms (Boersma et al., 1998, 1999), as *Daphnia* seek refuge among the macrophytes in the presence of fish (Lauridsen and Lodge, 1996; Burks et al., 2002). Therefore, the observed result might be interpreted as an adaptive micro-evolutionary response to change in strength of fish predation pressure. In the absence of fish kairomone, however, there was a positive correlation between exp(HMI) and macrophyte coverage, indicating that in the absence of fish kairomone, the avoidance of macrophytes in *Daphnia* increased in periods of higher macrophyte coverage. Whether the decline in DHM in the 1986-2000 period was caused by a subtle decrease in macrophyte coverage cannot be traced by the methods used due to uncertainties involved in the inference of macrophyte coverage. Since the fish predation pressure did not change, the decline of DHM implies that other antipredator traits must have been involved. This suggestion has been tested in Chapter 2, in which we aimed to quantify other antipredator traits (vertical migration, size at maturity, age at first clutch, neonate size, and first clutch size) in the same set of clones to obtain a more integrated picture on how the antipredator strategy of the local *D. magna* population changed in response to lake restoration.

In conclusion, we found interclonal variation in horizontal migration behavior of *D. magna* in the presence and absence of fish kairomone. Furthermore, both a comparison between populations and a correlation analysis demonstrated a micro-evolutionary response in the horizontal migration behavior of the *Daphnia* population of Lake Ring. More specifically, the correlation analysis showed that clones derived from periods characterized by high fish predation pressure reacted stronger to exposure to fish kairomone than clones derived from low fish predation pressure periods. Taken together, these results provide evidence for local adaptation.

5. Acknowledgments

Special thanks go to Liselotte Sander Johansson and Rikke Bjerring Hansen for identification of cladoceran remains and to Anne Mette Poulsen for manuscript editing. We also thank three anonymous reviewers for constructive comments. This study was financially supported by the project OT/00/14 of the K. U. Leuven research fund and by the EU IP project EUROLIMPACS (GOCE-CT-2003-505540). H. Michels is a fellow of the Institute for the Promotion of Innovation through Science and Technology in Flanders. E. J. and S. L. A. were also supported by the Danish lake restoration project CLEAR.

6. References

Amsinck, S., E. Jeppesen, & F. Landkildehus, 2005. Relationships between environmental variables and zooplankton subfossils in the surface sediments of 36 shallow coastal brackish lakes with special emphasis on the role of fish. Journal of Paleolimnology 33: 39-51

Appleby, P.G., P.J. Nolan, D.W. Gifford, M.J. Godfrey, F. Oldfield, N.J. Anderson, & R.W. Battarbee, 1986. ^{210}Pb dating by low background gamma-counting. Hydrobiologia 141: 21-27.

Appleby, P.G., & F. Oldfield, 1978. The calculation of ^{210}Pb dates assuming a constant rate of supply of unsupported ^{210}Pb to the sediment. Catena 5: 1-8.

Berg, S., E. Jeppesen, M. Søndergaard, & E. Mortensen, 1994. Environmental effects of introducing whitefish, *Coregonus lavaretus* (L.) in Lake Ring. Hydrobiologia 275/376: 71-79.

Benzie, J.A.H., 2005. Cladocera: the genus *Daphnia* (including *Daphniopsis*). Backhuys Publishers, Leiden.

Boersma, M., P. Spaak, & L. De Meester, 1998. Predator-mediated plasticity in morphology, life history and behavior of *Daphnia*: The uncoupling of responses. American Naturalist 152: 237-248.

Boersma, M., L. De Meester, & P. Spaak, 1999. Environmental stress and local adaptation in *Daphnia magna*. Limnology and Oceanography 44: 393-402.

Brendonck, L., & L. De Meester, 2003. Egg banks in freshwater zooplankton: Evolutionary and ecological archives in the sediment. Hydrobiologia 491: 65-84.

Brooks, J.L., 1957. The systematics of North American *Daphnia*. Conneticut Academy of Arts and Science. New Haven, USA.

Burks, R.L., E. Jeppesen, & D.M. Lodge, 2000. Chemicals from macrophytes and fishes suppress *Daphnia* growth and alter life history traits. Oikos 88, 139-147.

Burks, R.L., E. Jeppesen, & D.M. Lodge, 2001. Littoral zone structure as *Daphnia* refugia against fish predators. Limnology and Oceanography 46: 230-237.

Burks, R.L., D.M. Lodge, & E. Jeppesen, 2002. Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. Freshwater Biology 47: 343-365.

Cerbin, S., D.J. Baylayla, & W.J. Van de Bund, 2003. Small-scale distribution and diel vertical migration of zooplankton in a shallow lake (Lake Naardermeer, The Netherlands). Hydrobiologia 491: 111-117.

Cousyn, C., L. De Meester, J.K. Colbourne, L. Brendonck, D. Verschuren, & F. Volckaert, 2001. Rapid evolution of predator-induced avoidance behavior in a natural zooplankton population. Proceedings Of The National Academy Of Science 98: 6256-6260.

Cushing, D.H., 1951. The vertical migration of planktonic Crustacea. Biological Reviews of the Cambridge Philosophical Society 26: 158-192.

Davies, J., 1985. Evidence for a diurnal horizontal migration in *Daphnia hyalina lacustris* Sars. Hydrobiologia 120:103-105.

De Meester, L., 1993. Genotype, fish-mediated chemicals and phototaxis in *Daphnia*. Ecology 74: 1467-1474.

De Meester, L., 1996. Evolutionary potential and local genetic differentiation in a phenotypically plastic trait of a cyclical parthenogen, *Daphnia magna*. Evolution 50: 1293-1298.

De Meester, L., & H. De Jager, 1993. Hatching of *Daphnia* sexual eggs. I. Intraspecific differences in the hatching responses of *D. magna* eggs. Freshwater Biology 30: 219-226.

De Meester, L., L.J. Weider, & R. Tollrian, 1995. Alternative antipredator defenses and genetic polymorphism in a pelagic predator-prey system. Nature 378 483-485.

De Meester, L., 1996. Local genetic differentiation and adaptation in freshwater zooplankton populations: Patterns and processes. Ecoscience 3: 385-399.

De Meester, L., P. Dawidowicz, E. Van Gool, & C.J. Loose, 1999. Ecology and evolution of predator-induced behavior in zooplankton: depth selection behavior and diel vertical migration. In: R. Tollrian, & C.D. Harvell (eds.), The ecology and evolution of inducible defenses. Princeton University Press, Princeton: 160-176.

Endler, J.A., 1986. Natural selection in the wild. Princeton University Press, Princeton, New Jersey.

Falconer, D.C., & T.F.C. MacKay, 1996. Introduction to quantitative genetics. Fourth edition. Longman, London.

Flößner, D., 2000. Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas. Backhuys Publishers, Leiden, The Netherlands.

Gliwicz, Z.M., & J. Pijanowska, 1986. The role of predation in zooplankton succession. In Sommer, U. (ed.), Plankton Ecology: Succession in plankton communities. Springer-Verlag, Heidelberg.

Hairston, N.G. Jr., W. Lampert, C.E. Cáceres, C.L. Holtmeier, L.J. Weider, U. Gaedke, J.M. Fisher, J.A. Fox, & D.M. Post, 1999. Rapid evolution revealed by dormant eggs. Nature 15: 446.

Haney, J.F., 1988. Diel patterns of zooplankton behavior. Bulletin of Marine Science 43: 583-603.

Haney, J.F., 1993. Environmental control of diel vertical migration behaviour. Archiv für Hydrobiologie Beiheifte Ergebnisse der Limnologie 39: 1- 17.

Hebert, P.D.N., 1995. The *Daphnia* Of North-America: an illustrated fauna (on CD-rom). CyberNatural Software, Guelphe, Ontario, Canada.

Jeppesen, E., 1998. The Ecology of Shallow Lakes – Trophic interactions of the pelagical. Doctor's dissertation, National Environmental Report No. 247, pp. 37-39.

Jeppesen, E., J.P. Jensen, M. Søndergaard, T. Lauridsen, & F. Landkildehus, 2000. Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient. Freshwater Biology 45: 201-213.

Jeppesen, E., P. Leavitt, L. De Meester, & J.P. Jensen, 2001. Functional ecology and palaeolimnology: using cladoceran remains to reconstruct anthropogenic impact. Trends in Ecology and Evolution 16: 191-198.

Jeppesen, E., J.P. Jensen, S. Amsinck, F. Landkildehus, T. Lauridsen, & S.F. Mitchell, 2002. Reconstructing the historical changes in *Daphnia* mean size and planktivorous fish abundance in lakes from the size of *Daphnia* ephippia in the sediment. Journal of paleolimnology, 27: 133-143.

Johansson, L.S., S.L. Amsinck, R. Bjerring,, & E. Jeppesen, 2005. Mid- to late-Holocene land-use change and development at Lake Dallund, Denmark: trophic structure inferred from cladoceran subfossils. The Holocene, 15:1-9.

Kerfoot, W.C., J.A. Robbins, & L.J. Weider, 1999. A new approach to historical reconstruction: Combining descriptive and experimental paleolimnology. Limnology and Oceanography 44: 1232-1247.

Kvam, O.V., & O.T. Kleiven, 1995. Diel horizontal migration and swarm formation in *Daphnia* in response to *Chaoborus*. Hydrobiologia 307: 177-184.

Lampert, W., 1987. Predictability in lake ecosystems: the role of biotic interactions. In: Schultze, E.D and H. Zwölfer, (eds.), Ecological studies. Springer- Verlag, Berlin Heidelberg. 333-346.

Lauridsen, T.L., & I. Buenk, 1996. Diel changes in the horizontal distribution of zooplankton in the littoral zone of two shallow eutrophic lakes. Archiv für Hydrobiologie 137: 161-176.

Lauridsen, T.L., & D.M. Lodge, 1996. Avoidance by *Daphnia magna* of fish and macrophytes: chemical cues and predator-mediated use of macrophyte habitat. Limnology and Oceanography 41: 794-798.

Lauridsen, T.L., L. Pedersen, E. Jeppesen, & M. Søndergaard, 1996. The importance of macrophyte bed size for cladoceran composition and horizontal migration in a shallow lake. Journal of Plankton Research 18: 2283-2294.

Lynch, M., 1984.The limits to life history in *Daphnia.* Evolution 38:465-482.

Pennak, R.W., 1966. Structure of zooplankton populations in the littoral macrophyte zone some Colorado lakes. Transactions of the American Microscopical Society 85: 329-349.

Pulido, F.,, & P. Berthold, 2004. Micro-evolutionary response to climate change. Birds and Climate change in Ecological Research 35: 151-183.

Ringelberg, J., 1991. Enhancement of the phototactic reaction in *Daphnia hyalina* by a chemical mediated by juvenile perch (*Perca fluviatilis*). Journal of Plankton Research 13: 17-25.

Ringelberg, J., 1999. The photobehaviour of *Daphnia* spp. as a model to explain diel vertical migration in zooplankton. Biological Reviews of the Cambridge Philosophical Society 74: 397-423.

Scheffer, M., S.H. Hosper, M.-L. Meijer, Moss B., & E. Jeppesen, 1993. Alternative equilibria in shallow lakes. Trends in Ecology and Evolution 8:275-279.

Schlaepfer, M., M.C. Runge, & P.W. Sherman, 2002. Ecological and evolutionary traps. Trends in Ecology & Evolution 17: 474-480.

Schwartz, S.S., & P. Hebert, 1987. Methods for activation of the dormant eggs of *Daphnia*. Freshwater Biology 17: 373-379.

Sokal, R., & F.J. Rohlf, 1995. Biometry. Third edition. Freeman, New York.

Statsoft, 1994. STATISTICA for the windows operating system. Inc. Tulsa, UK.

Stockwell C.A., A.P. Hendry, & M.T. Kinnison, 2003. Contemporary evolution meets conservation biology. Trends in Ecology & Evolution 18: 94-101.

Timms, R.M., & B. Moss, 1984. Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing in the presence of zooplanktivorous fish, in a freshwater wetland ecosystem. Limnology and Oceanography 29: 472-486.

Van De Meutter, F., R. Stoks, & L. De Meester, 2005. Spatial avoidance of littoral and pelagic invertebrate predators by *Daphnia.* Oecologia 142: 489-499.

Van Gool, E., & J. Ringelberg, 1995. Swimming of *Daphnia galeata x hyalina* in response to changing light intensities: influence of food availability and predator kairomone. Marine and Freshwater Behaviour and Physiology 26: 259-265.

Willey, R.L., P.A. Cantrell, & S.T. Threlkeld, 1990. Epibiotic euglenoid flagellates increase the susceptibility of some zooplankton to fish predation. Limnology and Oceanography 35: 952-959.

Weider, L.J., 1984. Spatial heterogeneity of *Daphnia* genotypes: Vertical migration and habitat partitioning. Limnology and Oceanography 29: 225-235.

CHAPTER 2 MICRO-EVOLUTION OF ANTIPREDATOR TRAITS IN A NATURAL *DAPHNIA MAGNA* POPULATION DRIVEN BY LAKE DEGRADATION AND LAKE RESTORATION

Helen Michels, Robby Stoks, Liselotte Johansson, Rikke Bjerring, Erik Jeppesen and Luc De Meester

ABSTRACT

We reconstructed the micro-evolutionary response of six antipredator traits of *Daphnia magna* in response to habitat degradation and restoration, which was accompanied with a shift in water transparency. Life history and behavioral traits of a set of clones, hatched from sediments that were deposited in three successive periods in the history of Lake Ring: the Pre-eutrophication period (1908-1937), the Turbid period (1960-1980) and the Clear-water period (1986-2000), were scored in the absence and presence of fish kairomone. In all periods equal proportions of clones reacted with at least one trait to the presence of fish kairomone, but the nature of the antipredator response differed. In the Turbid period, clones responded to fish kairomone with behavioral traits, in the Pre-eutrophication period and the Clear-water period they reacted most strongly with life history traits. The observed micro-evolution of antipredator response to lake restoration, likely reflects a differential efficiency of these different traits in reducing mortality by predation at different levels of water turbidity. This may have been facilitated by their apparent uncoupling.

1. Introduction

Evidence is accumulating that anthropogenic changes can drastically alter selection pressures on species traits (Pulido and Berthold, 2004; Davis et al., 2005). The locally present evolutionary potential of traits, that allows species to cope with these new selection factors, may determine in situ population persistence (e.g. Hairston et al., 1999; Cousyn et al., 2001; Hoffmann et al., 2003; Hairston et al., 2005). Most studies to date have focused on individual traits (but see e.g. Etterson and Shaw, 2001). To deal with selective pressures in the real world, however, changes in a combination of traits may be necessary, and individuals may differ in trait sets used (Boersma et al., 1998). Moreover, the evolutionary potential of a trait may depend critically on genetic correlations with other traits (Lynch and Walsh, 1998). Phenotypic integration may therefore both act as an adaptation and as a constraint (Merilä and Björklund, 2004).

Habitat degradation and subsequent remediating measures like lake restorations cause drastic changes in several abiotic and biotic variables. They likely impose new challenging selective regimes on lake biota (Anderson et al., 2005; Jeppesen et al., 2005). Many restorations efforts on shallow lakes aim at enforcing a switch from a eutrophic turbid state toward a clear-water state (Hosper, 1993; Jeppesen et al., 1999). As water turbidity may strongly affect predator-prey interactions (Scheffer et al., 1993, Abrahams and Kattenfeld, 1997), organisms may have to adjust their antipredator strategies to maintain active populations after lake restoration. Several studies explored how water turbidity affects the expression of specific antipredator traits (Van De Meutter et al., 2005 and references herein). Knowledge on how changes in turbidity affect the evolution of sets of traits is, however, limited. More general, joint studies on the evolutionary potential and micro-evolution of sets of (antipredator) traits are lacking (Relyea, 2004).

In this study, we reconstruct the micro-evolution of six antipredator traits of the water flea *Daphnia magna* Straus, in response to the degradation and restoration of Lake Ring (Denmark), a shallow eutrophied lake. As the lake suffered from severe cultural eutrophication, the point source of sewage effluents from the nearby village was diverted in 1970. With quite some time delay, this resulted in the mid 1980s in a shift from a turbid to a clear-water state. This was not associated, however, with a significant change in fish predation pressure as judged from inferred fish predation pressure using *Daphnia* ephippia in the sediment as a proxy (Jeppesen et al., 2001, Chapter 1). This gives us the unique opportunity to quantify changes in sets of antipredator traits as a function of water turbidity that are not confounded by changes in total predation by fish, the main predator of *Daphnia* (Brooks and Dodson, 1965). Several life history

and behavioral antipredator traits to fish are known for *D. magna* (e.g. Boersma et al., 1998). In Chapter 1, we focused on only one antipredator trait. We could demonstrate that clones isolated from sediment layers dating from before lake restoration showed stronger horizontal migration towards vegetation than clones isolated from more recent sediments, corresponding to the Clear-water period. We here hypothesize that lake restoration is associated with a shift towards other antipredator traits than horizontal migration by the local *Daphnia magna* population. Furthermore, we also take the Pre-eutrophication period (1908-1937) into account, and compare the antipredator strategy of this period with the one of clones isolated from the other two periods. Since this is a period that took place before eutrophication of the lake, it is also characterized with high water transparency. We present an integrated look at six major life history and behavioral antipredator traits, quantify their response to fish kairomone, their evolutionary potential, and their realized micro-evolution in response to lake restoration.

2. Material and methods

2.1. Study site

Lake Ring is a relatively small, shallow lake located in Mid-Jutland, Denmark. The lake was subjected to strong eutrophication during the 20th century due to sewage inflow. To restore the lake, the point source of sewage effluents from the nearby village was diverted in 1970. This initially led to a very gradual recovery, culminating with a drastic increase in water clarity from the mid-1980s and onwards (for more details see Introduction).

To reconstruct associated changes in sets of antipredator traits, we hatched *D. magna* clones from eggs obtained from three successive periods that differed strongly in water turbidity. 6, 11 and 9 clones from the Pre-eutrophication period (1908-1937), the Turbid (1960-1980) and the Clear-water period (1986-2000), respectively, were cultured for at least two generations under standardized culture conditions to minimize maternal effects (30 individuals per liter, 20 ± 1 °C, L:D 14:10, 2.5 10^5 *Scenedesmus obliquus* cells ml^{-1}). Animals were fed daily and the medium was refreshed every second day.

2.2. Life history experiment

To test for changes in sets of antipredator traits between periods associated with lake degradation and recovery, we reared all clones in the presence and absence of fish kairomone. We scored four key life history traits known to play a role in antipredator strategies (Boersma et al., 1998): age at release of the first clutch, size at maturity, neonate size, and size of the first clutch. Each of the treatment x clone combinations had at least three replicates. To avoid

pseudo-replication, every replicate consisted of animals from a different culture vial or from a different generation.

In the fish treatment, dechlorinated water was conditioned by the presence of three *Leuciscus idus* fish (approximately 6 cm) in a 20 l aquarium for 24 h. The water was then filtered through a 0.45-µm filter and diluted to a concentration equivalent to 3 fish per 100 l. In the non-fish treatment, dechlorinated tap water was used which was also aged for 24 hours, but not conditioned by fish.

The experiment started with inoculation of second clutch neonates (< 24 h old). Per replicate a cohort of 30 neonates was inoculated in a 1 l glass jar. The animals were monitored every 24 h until the release of the second clutch in the brood pouch. Average size at maturity was determined for every cohort by averaging the size of six individuals. Twenty adult animals were used to score the behavioral traits (see below). Body size was measured from the top of the eye till the base of the spine. Average age at release of the neonates of the first clutch was determined to the nearest 24 h. Average clutch size per cohort was determined by the total number of juveniles produced, divided by the number of adult individuals present (taking into account accidental losses of adults). Average neonate size per cohort was determined by averaging the body size of ten randomly chosen neonates, measured within 24 hours after birth.

2.3. Behavioral experiments

Two key behavioral antipredator traits were measured: diel vertical migration and horizontal migration. The quantification of the phototactic behavior, a key trait in diel vertical migration (De Meester et al., 1999; Ringelberg, 1999), was based on the method developed by De Meester (1993). For each replicate, the phototactic behavior of ten adult females (carrying their second clutch in an early developmental stage) was quantified. The set-up consisted of a glass column of 10 cm height, which was filled with aged tap water or fish conditioned tap water, depending on the treatment. The column was illuminated from above with a fiber light source (Müster 150 W). The entire set-up was placed in a light-tight PVC box. Prior to the experiment, animals cultured in dechlorinated tap water or fish conditioned water were acclimated during 3-4 h to the corresponding medium in the absence of food (i.e. more transparent water). After a 5 minute dark acclimatization in the experimental column, the experiment started with switching on the light. Each experiment lasted 10 minutes. The animals in the column were filmed with a video-camera, the images were transmitted to a PC, and analyzed using an adapted version of the bbe Toximeter (see http://www.bbe-moldaenke.de) modified to quantify phototactic behavior. The vertical distribution of the test animals was measured every 2 seconds. The index of phototactic behavior (PI) was calculated as (U-L) /

(U+M +L), with U being the number of animals in the upper 2.5 cm, L the number of animals in the lower compartment and M the number of animals in the middle 5 cm of the column. The index was averaged over the last 5 minutes of the experiment to minimize influences of the initial escape reaction. Values of PI can range from -1 (extremely negative phototactic behavior) to +1 (extremely positive phototactic behavior).

Horizontal migration behavior was quantified using the experimental design described in Chapter 1. Briefly, the set-up consisted of a long PVC gutter (L = 1.5 m, W = 7 cm, H = 4.5 cm) which was closed at both ends. The gutter was externally divided into 8 equal-sized compartments. In the two outer compartments a littoral zone was simulated by a combination of real and plastic plants (a total of 11 shoots each, to create a PVI of 28%). The gutter was filled with 3 l aged tap water or fish conditioned water. After 3-4 h of acclimatization to the experimental medium, ten adult females were placed in the middle of the gutter. The number of individuals in each compartment was counted at 15 min intervals. Each experiment lasted 2 h. The horizontal migration index (HMI) of the test population was calculated as (M-O) / (total number animals), averaged over the last hour; with M being the number of animals in the two middle compartments and O the number of animals in the two outer compartments. Values for HMI can range from -1 (all individuals hide among the plants) to 1 (all animals are found in the center of the gutter). The data on horizontal migration behavior are described and discussed in detail in Chapter 1.

2.4. Data analysis

We tested for the effects of clone, period, and kairomone treatment on the combined set of antipredator traits, using a three–way MANOVA. MANOVA was used both to control the Type I error, and to assess effects on the combined set of response variables. Wilk's lambda was used as test statistic. Period and kairomone treatment were considered fixed factors, and clone nested in period was considered a random factor. Response variables in this analysis were age at first clutch, size at maturity, clutch size, neonate size, phototactic index (exponentially transformed), and horizontal migration index (transformed as $[HMI+1]^3$). Transformations were carried out in order to comply with assumptions of normality. The analysis was performed with the GLM procedure in SAS 9.1 (SAS Institute, 2005). Non-significant interactions were removed from the final model.

To directly evaluate which response variables contributed most heavily to significant effects in the MANOVA, we examined standardized canonical coefficients, which quantify the importance of each variable in the dominant eigenvector in MANOVA. They describe the relative contribution of response variables to the effect observed for each factor (Scheiner,

2001). In addition, they describe the covariation among the dependent variables themselves. Given the significant period x kairomone treatment interaction (see results), we also performed separate two-way MANOVAs in the presence and absence of fish kairomone to evaluate which variables contributed mostly to the differentiation between the three periods in both situations. These MANOVAs include clone nested in period as a random factor, and period as a fixed factor. For completeness, we also report significance of univariate tests. Note, however, that in contrast with MANOVA, these assess effects on each response variable independent from effects on other response variables, and may therefore not completely match the pattern given by the standardized canonical coefficients.

To compare the periods characterized by a clear-water state (Pre-eutrophication period and Clear-water period) with each other, and with the Turbid period, a contrast analysis was performed, using separated two-way ANOVAs in the presence and absence of fish kairomone. These ANOVAs included clone nested in period as a random factor, and period as a fixed factor.

We also compared between the different periods the proportion of clones that reacted significantly to fish kairomone and the proportion of reacting clones that reacted with behavioral traits with separate RXC Exact tests. To obtain these proportions, we tested for each combination of clone and trait, separately, whether there was a significant reaction to fish kairomone by determining the contrast between the absence and presence of fish kairomone within the MANOVA model.

To assess the evolutionary potential of the antipredator traits, we estimated clonal repeatabilities following Falconer and MacKay (1996) for the three periods separately. We tested the significance of the broad-sense clonal repeatabilities, quantifying the impact of the clonal effects on phenotypic variation for all the different traits measured in the presence and in the absence of fish kairomone, and for the trait response to fish kairomone, using one-way ANOVAs with clone as a random factor. Finally, to specifically evaluate (genetic) trait integration, we tested the significance and estimated genetic correlations by calculating Pearson product moment correlation coefficients between clonal means of each pair of traits (Via, 1984). Correlations were calculated for the three periods separately for traits measured in the presence of fish kairomone, in the absence of fish kairomone, and for clonal trait differences between both situations.

3. Results

We found significant effects of period, clone, kairomone treatment, and of the two-way interaction between period and kairomone treatment on the combined set of six antipredator

traits (MANOVA, Table 1). Inspection of the standardized canonical coefficients supplemented with the univariate tests showed that the period effect was mainly due to differences in horizontal migration (standardized canonical coefficient: -0,852), and in neonate size (standardized canonical coefficient: 0.737). The clone effect was mainly due to clonal variation in both behavioral antipredator traits (standardized canonical coefficient horizontal migration: 0.628; and vertical migration: 0.782), and to a lesser extend in first clutch size and age at release of first clutch. Differences in how clones reacted to fish kairomone between different periods were mainly due to vertical migration (standardized canonical coefficient = 0.757), horizontal migration (standardized canonical coefficient = 0.745), and to a lesser extend size at maturity (standardized canonical coefficient = -0.515) (Table 1, Fig. 1 and 2). The difference in value and sign of the coefficients for specific traits indicates differences between the periods in the sets of traits used under fish predation risk. Clones derived from the Pre-eutrophication period and the Clear-water period showed a similar response towards fish kairomone: in the presence of fish kairomone their size at maturity decreased more than that of clones from the Turbid water period (Fig. 1 and 2), while clones of the Turbid period did not show this response. In the Turbid period, clones mainly reacted behaviorally to fish kairomone by migrating towards the vegetation (lower HMI) and towards the bottom (lower PI). A contrast analysis indicated that in the presence of fish kairomone, age at first clutch, size at maturity, neonate size, and horizontal migration behavior did not significantly differed between the Pre-eutrophication period and the Clear-water period (Table 2), but clones derived from the Turbid period did significantly differ from the two other periods for these traits (Table 2).

Table 1: Three-way MANOVA testing for effects of period, kairomone treatment, and clone, nested in period, on four life history and two behavioral antipredator traits (HMI = horizontal migration index, PI = Phototactic index). Standardized canonical coefficients from MANOVA are given. An asterisk indicates which effects are significant when tested univariate.

				Standardized Canonical coefficients					
Source of variation	df	F	p	Age at first clutch	Size at maturity	Neonate Size	First clutch size	HMI	PI
Period	12, 226	2.71	0.001	-0.132	0.352	0.737*	-0.351	-0.852*	0.108
Clone(period)	138, 666	2.16	<0.0001	0.418*	-0.334*	0.147	-0.521	0.628*	0.782*
Kairomone	6, 113	7.47	<0.0001	0.329*	0.711*	0.283	-0.326*	0.546*	0.513*
Kairomone x Period	12, 226	2.43	0.005	0.375	-0.515	0.086	-0.001	0.745*	0.757*

Table 2: Contrast analysis testing for the difference between Pre-eutrophication period and the Clear-water period and for the difference between the Turbid period and the Pre-eutrophication period and the Clear-water period. Significant values are indicated in bold.

	Pre-eut/Clear-water		**Turbid /Pre-eutr-Clear water**	
Absence of fish kairomone	F	p	F	p
Age at first clutch	0.36	0.54	0.5	0.48
Size at maturity	3.26	0.07	0.04	0.84
Neonate size	0.85	0.35	14.1	**0.0003**
First clutch size	0.38	0.53	0	0.95
HMI	0.38	0.53	0.02	0.86
FI	0.45	0.5	2.1	0.15
Presence of fish kairomone	Pre-eut/Clear-water		Turbid /Pre-eutr-Clear water	
	F	p	F	p
Age at first clutch	0.63	0.42	5.52	**0.02**
Size at maturity	0.26	0.61	5.12	**0.008**
Neonate size	0.9	0.34	7.71	**0.007**
First clutch size	4.22	**0.04**	2.11	0.15
HMI	3.03	0.08	12.46	**0.0007**
FI	0.45	0.5	2.1	0.15

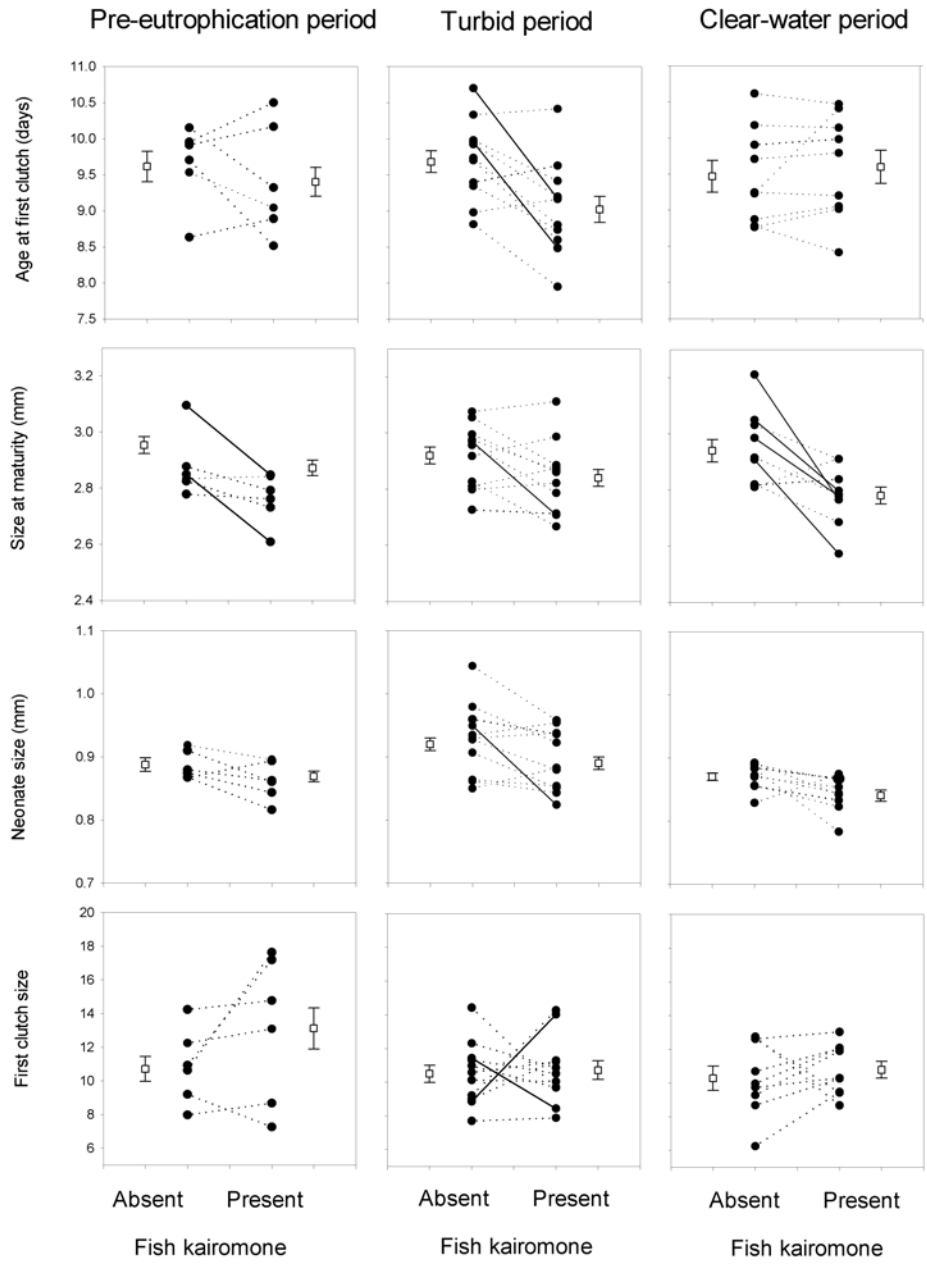

Fig. 1: Clonal reaction norms for four life history antipredator traits (age at first clutch, size at maturity, neonate size, and size of the first clutch) of Daphnia magna *clones isolated from sediments of Lake Ring corresponding to a Pre-eutrophication period (1908-1937), a Turbid period (1960-1980) and a Clear-water period (1986-2000) in response to fish kairomone. Circles represent clonal means. Squares represent means for each combination of kairomone treatment and period and are shown with error bars representing ± SE. Clonal means that differ significantly between the absence and presence of fish kairomone are connected by solid reaction norm lines.*

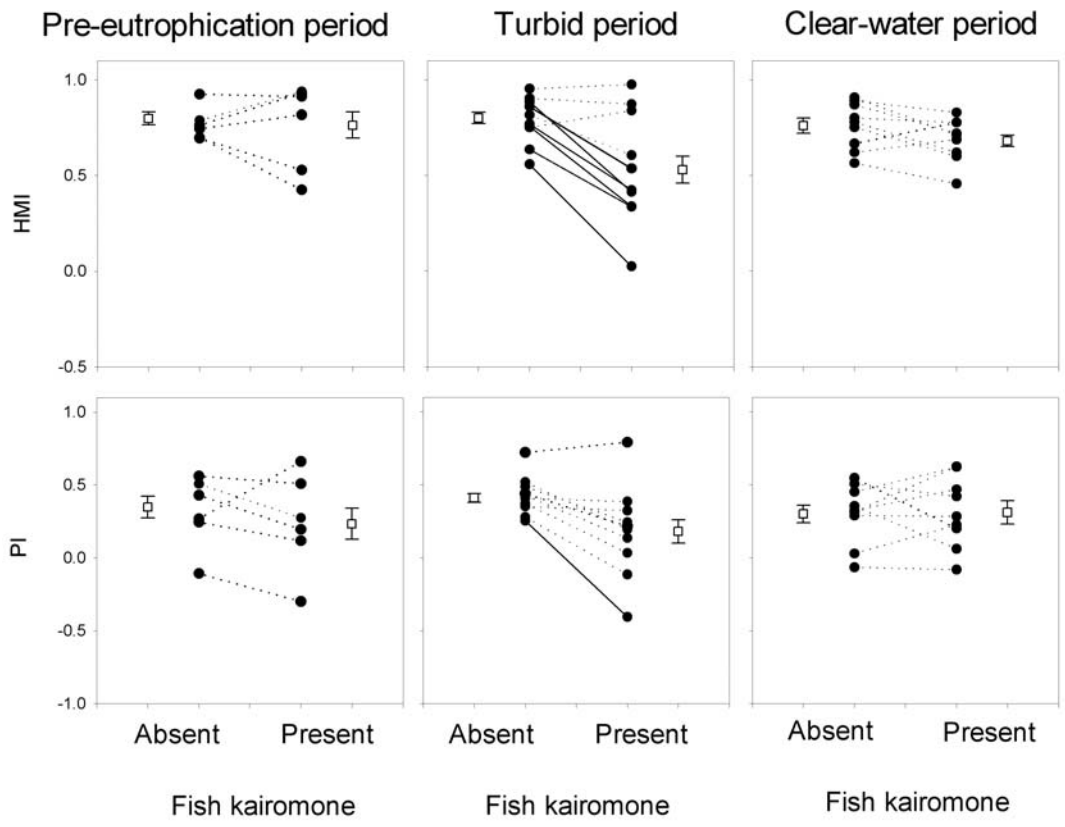

Fig. 2: Clonal reaction norms for two behavioral antipredator traits (HMI = horizontal migration index, PI = phototactic index) of Daphnia magna *clones isolated from sediments of Lake Ring corresponding to a Pre-eutrophication period (1908-1937), a Turbid period (1960-1980) and a Clear-water period (1986-2000) in response to fish kairomone. Circles represent clonal means. Squares represent means for each combination of kairomone treatment and period and are shown with error bars representing ± SE. Clonal means that differ significantly between the absence and presence of fish kairomone are connected by solid reaction norm lines.*

The differential responsiveness to fish kairomone between clones from the different periods was also obvious when analyzing the number of clones that reacted significantly with a certain trait to fish kairomone (Table 3). The proportion of clones that reacted with at least one trait to fish kairomone did not differ between the periods (Pre-eutrophication period: 2 out of 6, Turbid water period: 6 out of 11, Clear-water period: 4 out of 9, Exact test, $p = 0.88$). Of those clones that reacted, however, a larger set reacted with at least one behavioral trait in the Turbid period (6 out of 6) compared to the Clear-water period (0 out of 4) and compared to the Pre-eutrophication period (0 out of 2) (Exact test, $p < 0.05$). Three clones (all of the Turbid period) reacted both with behavioral and life history traits. Of the nine clones that reacted with only one type of trait, both clones from the Pre-eutrophication period and all four from the Clear-water

period used a life history trait, and all three from the Turbid period used a behavioral trait (exact test, $p < 0.05$).

Table 3: Reaction matrix of responses of Daphnia magna *clones to the presence of fish kairomone. Given are p-values of the contrast between two kairomone treatments. Only clones with at least one significant response are given. Total number of clones in Pre-eutrophication period = 6; total number of clones in Clear-water period= 9, total number of clones in Turbid period= 11.*

	Pre-eutr Period		**Turbid period**						**Clear-water period**				
Trait	P 2	P6	T2	T3	T4	T6	T8	T11	C2	C3	C6	C8	Sign
Age at first clutch	...	...	...	...	0.04	...	...	0.05	…	...	...	...	2
Size at maturity	0.02	0.01	...	...	...	...	<0.001	...	0.01	<0.001	<0.001	0.05	7
Neonate size	...	...	...	...	...	...	<0.001	...	...	...	...	...	1
First clutch size	...	...	...	...	...	...	0.04	...	...	...	...	...	1
HMI	...	...	0.01	0.04	0.03	0.05	<0.001	0.02	...	...	...	...	6
PI	...		...	...	...	...	0.02	...	...	...	...	...	1
Total significant	1	1	1	1	2	1	5	2	1	1	1	1	18

Separate MANOVAs per kairomone treatment showed that both in the absence and in the presence of fish kairomone there was interclonal variation for the set of antipredator traits (Table 4). The period effect was significant in the presence of fish kairomone ($p = 0.04$). In the presence of fish kairomone, clones from the different periods differed mainly in neonate size (standardized canonical coefficient =-0.847) and age at first clutch (standardized canonical coefficient = 0.649), with clones from the Pre-eutrophication period and Clear-water period being characterized by a smaller neonate size and a higher age at first clutch.

Table 4: Two-way MANOVAs testing for the effects of period and clone nested in period for four life history and two behavioral antipredator traits (HMI = horizontal migration index, PI = Phototactic index). Standardized canonical coefficients from MANOVA are given. An asterisk indicates which effects are significant when tested univariate.

				Standardized Canonical coefficients					
Source of variation	df	F	p	Age at first clutch	Size at maturity	Neonate size	First clutch size	HMI	PI
Absence of fish kairomone									
Period	12, 36	1.31	0.25	-0.050	0.303	0.976*	0.029	-0.326	0.558*
Clone(Period)	138, 270	1.80	<0.0001	0.269	-0.834*	0.501	-0.821	0.506*	0.339*
Presence of fish kairomone									
Period	12,34	2.12	0.04	0.649*	-0.285	-0.847	0.277	0.546*	0.161
Clone(period)	132, 246	1.57	0.0012	0.005*	-0.712*	0.604	0.2403	0.908*	0.369

For only a minority of traits we could demonstrate clonal repeatabilities that were significantly larger than zero. For most combinations of period and fish kairomone treatments, significant among-clone differences were found for size at maturity (Clonal repeatabilities: Turbid period, absence of fish kairomone: 0.28; presence of fish kairomone: 0.28; Clear-water period, presence of fish kairomone: 0.33, Pre-eutrophication period, absence of fish kairomone: 0.41) and phototactic index (Clear-water period, absence of fish kairomone: 0.32; presence of fish kairomone: 0.02; Turbid period, presence of fish kairomone: 0.12). Additionally, in the Turbid period, heritable variation was detected in the horizontal migration index for both kairomone treatments (presence of fish kairomone: 0.15; absence of fish kairomone: 0.15), in neonate size in the absence of fish kairomone (0.27) and in Pre-eutrophication period in the presence of fish kairomone in age at first clutch (0.44) and in horizontal migration (0.38). The clonal repeatabilities for the response to fish kairomone only were significantly different from zero for vertical migration in the Turbid period (0.35) and in the Clear-water period (0.30). Only a very low percentage of the genetic correlations was significant (6 %, 8 out of 135). Only a trade-off between vertical migration and size at maturity could be detected, and this only in the Turbid period in the absence of fish kairomone (r = -0.64). To test whether traits covaried when taking all other traits into account, we also ran multiple regressions where one of the studied

traits was regressed against all other ones. Results of forward stepwise multiple regressions did, however, not reveal any other negative (partial) correlations between the traits.

4. Discussion

We could demonstrate that the degradation and restoration of Lake Ring, was associated with micro-evolution of antipredator traits in relation to fish predation in the water flea *Daphnia*. In line with our hypothesis, new strategies to cope with fish predation developed after lake restoration in the population, shifting from behavioral avoidance to reduction in size. Moreover, we found that clones derived from the Pre-eutrophication period used the same antipredation strategy as clones from the Clear-water period. Antipredator traits were largely uncoupled, suggesting that they could evolve independently from each other. Most traits, however, showed little clonal variation when the three subpopulations were considered separately, indicating relatively low evolutionary potential.

Whereas during both the Pre-eutrophication period and the Clear-water period, the emphasis of the antipredator strategy was on life history traits, this shifted towards behavioral responses in the Turbid period. During the Turbid period, clones exhibited a behavioral response upon exposure to fish kairomone, both in terms of vertical (towards the bottom) and horizontal (towards the vegetation) migration behavior. These behavioral responses are well-known for zooplankton (De Meester et al., 1999; Burks et al., 2001), and have been shown to be a defense mechanism against fish predation (e.g. De Meester et al., 1995). Adaptive micro-evolution has been shown for both vertical migration (Cousyn et al., 2001), and horizontal migration (Michels et al., Chapter 1). In turbid water, prey do not have to migrate far to get out of sight of the predator (Abrahams and Kattenfeld, 1997), and migration might be the most efficient antipredator trait (e.g. Vos et al., 2002). In the post-recovery period, however, water transparency increased strongly and given the fact that the lake is shallow, this may have resulted in a disappearance of a true refuge at depth. The littoral zone in this lake has earlier been shown to be used as a day-time refuge for various zooplankters, including *Daphnia* (Lauridsen and Buenk, 1996). However, the coverage of submerged vegetation is relatively low (2.7 %), and only marginally increased when shifting from turbid to clear-water condition (see Fig. 2, in Chapter 1). This makes DHM in the Clear-water period not an efficient predator avoiding strategy for the majority of *Daphnia*. This may have shifted the balance in favor of life history response to fish, namely a reduction in size at maturity (see also Vos et al., 2002). A similar, but opposite shift in antipredator traits took place between the Pre-eutrophication period and the Turbid period. This points to turbidity as the responsible factor for the shifts in antipredator traits. Abrahams and Kattenfeld (1997), and Van De Meutter et al. (2005) showed

that prey exhibit a stronger antipredator behavior in clear-water than in turbid water. Our study, however, revealed a shift in antipredator behavior rather than an increase going from a turbid to clear- water condition. These contrasting results probably reflect that different preys were used in the different studies. For both fish (Abrahams and Kattenfeld, 1997), and damselfly larvae (Van De Meutter et al., 2005) vision is important to detect predators. As turbidity decreases the distance at which prey detect their predator, it makes antipredator behavior ineffective (Abrahams and Kattenfeld, 1997). In *Daphnia,* however, detecting predators relies on the detection of fish kairomone, a chemical substance released by the predator. Detection of the predator by *Daphnia* is thus independent of turbidity.

Clones from the different periods did not only differ in their response to fish kairomone, but also in the values of antipredator traits in the absence and presence of fish kairomone. For instance, both in the absence and presence of fish kairomone, clones from the Turbid period showed a larger neonate size than clones from the Clear-water period or from the Pre-eutrophication period. Also, age at release of the first clutch in the presence of fish kairomone was higher in the clones of the Clear-water period and the Pre-eutrophication period compared to those of the Turbid period. When confronted with fish predation risk, *Daphnia* clones typically reduce their age at first clutch to avoid death before reproduction (Stibor, 1992). The reduced age at release of the first clutch may explain why the larger neonate size of the clones of the Turbid period did not translate into a significant overall difference in size at maturity. The head-start of being born large seems to be translated largely into a shorter period to reach maturity. The near absence of genetic correlations between antipredator traits suggests that strong phenotypic integration of antipredator traits was limited in our study species during both periods. Given the relatively small number of clones, we did not perform a correction for multiple testing. Applying such correction would, however, make our conclusion of trait uncoupling stronger. Although we cannot fully exclude the possibility that some traits were strongly coupled to non-studied traits, we included the major life-history and behavioral antipredator traits known for our study species. Moreover, our present findings are in line with a previous study on *D. magna* that showed no associations between a larger set of 12 traits (Boersma et al., 1998). The previously documented trait association between size at maturity and degree of phototactic behavior (De Meester et al., 1995) was only detected in one of the four period x treatment combinations (namely in the absence of fish kairomone in the Turbid period). The fact that this association was not observed in the Clear-water period or the Pre-eutrophication period may reflect that phototactic behavior was not an important antipredator strategy during these periods. Despite the absence of strong genetic correlations, the evolutionary potential of most traits, especially the trait responses to fish kairomone, was low as

indicated by the non-significant among-clone variation of most traits. Yet, micro-evolution of several traits is evident from the significant period x kairomone interaction effect in our analysis. There may be several reasons for this. First, we cannot exclude the possibility that, although our analyses were powerful enough to detect micro-evolution (i.e. a significant period x kairomone interaction effect), the limited number of clones analyzed for each period may have impeded detection of existing genetic variation within the turbid water period. An alternative explanation is that the observed changes are the result of clones with different trait values having invaded Lake Ring after lake restoration. Genetic analyses using microsatellite markers, however, suggest only very low genetic differentiation between the three periods (Chapter 3). This makes the scenario of evolutionary change through immigration unlikely. Our results are in line with a previous study on *D. magna,* where in situ micro-evolution of vertical migration behavior in response to changes in fish predation pressure could be demonstrated in the absence of allele frequency changes in neutral markers (Cousyn et al., 2001).

We here demonstrate that changes in water turbidity, associated with lake degradation and restoration, may cause micro-evolution of antipredator traits in the key zooplankton taxon *Daphnia*. The observed evolutionary changes may be explained by a differential antipredator effect of behavioral, versus life history traits, under clear-water and turbid conditions. So far, only a few other studies have elucidated how recent anthropogenic changes in abiotic conditions have changed predator-prey interactions and the associated set of antipredator traits (e.g. Fox and Morin, 2001). Given the impact of abiotic factors likely to be under anthropogenic influence (e.g. temperature Sanford, 1999) on predator-prey interactions, and the strong selection pressure imposed by predation (e.g. Cousyn et al., 2001), micro-evolutionary responses in antipredator traits in response to anthropogenic changes can expected to be widespread. The observed uncoupling of antipredator traits in *D. magna* (Boersma et al., 1998) has, however, been hypothesized to be the exception (Relyea, 2004). The potential for micro-evolutionary response in other prey species than *Daphnia* may, therefore, be more restrained. There is need for more studies that evaluate genetic integration of antipredator traits. Our study also indicates that attention should be paid to indirect evolutionary responses with respect to biotic interactions to anthropogenic-induced changes in abiotic factors. Finally, our results point to the fact that both anthropogenic-induced habitat deterioration, and the efforts done to restore habitats may cause genetic shifts. The often drastic restoration measures needed to fight habitat deterioration may significantly affect the genetic architecture of the resident populations.

5. Acknowledgements

We thank S. Usé for technical support and A-M Poulsen for valuable editorial comments. H. Michels is a doctoral fellow of the Institute for the Promotion of Innovation through Science and Technology in Flanders. R. Stoks is a postdoctoral fellow of the Fund for Scientific Research Flanders (FWO). Financial support came from the EU IP project EUROLIMPACS (GOCE-CT-2003-505540) and from research grants FWO G.0269.04 and KULeuven Research Fund OT/04/23 to L. De Meester. E. Jeppesen, R. Bjerring and L. Johansson were supported by CONWOY and the lake restoration project CLEAR (Villum Kahns foundation).

6. References

Abrahams, M., M. Kattenfeld, 1997. The role of turbidity as a constraint on predator-prey interactions in aquatic environments. Behavioral Ecology and Sociobiology 40: 169-174.

Anderson, N.J., E. Jeppesen, & M. Sondergaard, 2005. Ecological effects of reduced nutrient loading (oligotrophication) on lakes: an introduction. Freshwater biology 1589-1593 .

Boersma, M., P. Spaak, & L. De Meester, 1998. Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: the uncoupling of responses . American Naturalist 152: 925-927.

Brooks, J.L., & S.I. Dodson, 1965. Predation, bodysize and composition of plankton. The effect of a marine planktivore on lake plankton illustrates theory of size, competition and predation. Science 15: 28-35.

Burks, R.L., E. Jeppesen, & D.M. Lodge, 2001. Littoral zone structures as *Daphnia Refugia* against fish predators. Limnology and Oceanography 46: 230-237.

Cousyn, C., L. De Meester, J.K. Colbourne, L. Brendonck, D. Verschuren, & F. Volckaert, 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. Proceedings of the National Academy of Sciences of the United States of America 98: 6256-6260.

Davis, M.B., R.G. Shaw, & J.R. Etterson, 2005. Evolutionary responses to changing climate. Ecology 86: 1704-1714.

De Meester, L. An analysis of the phototactic behaviour of *Daphnia magna* clones and their sexual descendants. Hydrobiologia 225, 217-227. 91.

De Meester, L. Genotype, fish-mediated chemical, and phototactic behavior in *Daphnia Magna*. Ecology 74(5), 1467-1474. 93.

De Meester, L., P. Dawidowicz, E. Van Gool, & C.J. Loose, 1999. Ecology and evolution of predator-induced behavior of zooplankton: depth selection behavior and diel vertical migration. In: Tollrian R. & Harvell C.D. (eds.), The ecology and evolution of inducible defenses. Princeton University Press, New Jersey: 160-176.

De Meester, L., L.J. Weider, & R. Tollrian, 1995. Alternative antipredator defences and genetic polymorphism in a pelagic predator-prey system. Nature 378: 483-485.

Etterson, J.R., & R.G. Shaw, 2001. Constraint to adaptive evolution in response to global warming. Science 294: 151-154.

Falconer, D.C., & Mackey, T.F.C., 1996. Introduction to quantitative genetics, Longman, London, UK.

Fox, J.W., & P.J. Morin, 2001. Effects of intra- and interspecific interactions on species responses to environmental change. Journal of Animal Ecology 70: 80-90.

Hairston, N.G., W. Lampert, C.E. Caceres, C.L. Holtmeier, L.J. Weider, U. Gaedke, J.M. Fischer, J.A. Fox, & D.M. Post, 1999. Lake ecosystems - rapid evolution revealed by dormant eggs. Nature 401: 446.

Hairston, N.G., S.P. Ellner, M.A. Geber, T. Yoshida, & J.A. Fox, 2005. Rapid evolution and the convergence of ecological and evolutionary time. Ecology Letters 8: 1114-1127

Hoffmann, A.A., R.J. Hallas, J.A. Dean, & M. Schiffer, 2003. Low potential for climatic stress adaptation in a rainforest Drosophila species. Science 301: 100-102.

Hosper, H., & M.L. Meijer, 1993. Biomanipulation, will it work for your Lake - a simple test for the assessment of chances for clear water, following drastic fish-stock reduction in shallow, eutrophic lakes. Ecological Engineering 2: 63-72.

Jeppesen, E., P. Leavitt, L. De Meester, & J.P. Jensen, 2001. Functional ecology and palaeolimnology: Using cladoceran remains to reconstruct anthropogenic impact. Trends in Ecology & Evolution 16: 191-198.

Jeppesen, E., M. Sondergaard, B. Kronvang, J.P. Jensen, L.M. Svendsen, & T.L. Lauridsen, 1999. Lake and catchment management in Denmark. Hydrobiologia 396: 419-432.

Lynch, M., & Walsh, B., 1998. Genetics and analysis of quantitative traits, Sinauer Associates, Sunderland, USA.

Merila, J., & M. Bjorklund, 2004. Phenotypic integration as a constraint and adaptation. In: Pigliucci, M. & Preston K. (eds.), Phenotypic integration Studying the ecology and evolution of complex phenotypes. Oxford University press, New York: 107-129.

Michels, H., S.L. Amsinck, E. Jeppesen, & L. De Meester Interclonal variation in diel horizontal migration behavior of the water flea *Daphnia* magna-searching for a signature of adaptive evolution. Hydrobiologia In Press.

Pulido, F., & P. Berthold, 2004. Microevolutionary response to climatic change. Advances in Ecological Research 35: 151-183.

Relyea, R.A., 2004. Integrating phenotypic plasticity when death is on the line: Insights from predator-prey systems. In: Pigliucci, M. & Preston K. (eds.), Phenotypic integration Studying the ecology and evolution of complex phenotypes. Oxford University Press, New York: 176-190.

Ringelberg, J., 1999. The photobehaviour of *Daphnia spp.* as a model to explain diel vertical migration in zooplankton. Biological Reviews 74: 397-423.

Sanford, E., 1999. Regulation of keystone predation by small changes in ocean temperature. Science 283: 2095-2097.

SAS, 2001. Statisitics, North-Carolina, US.

Scheffer, M., S.H. Hosper, M.L. Meijer, B. Moss, & E. Jeppesen, 1993. Alternative equilibria in shallow lakes. Trends in Ecology & Evolution 8: 275-279 .

Scheiner, S.M., 2001. MANOVA: multiple response variables and multispecies interactions. In: Scheiner, S. M. & Gurevitch, J. (eds.), Design and analysis of ecological experiments. Chapman and Hall, New York, USA:

Stibor, H., 1992. Predator induced life-history shifts in a fresh-water cladoceran. Oecologia 92: 162-165.

Van De Meutter, F., L. De Meester, & R. Stoks, 2005. Water turbidity affects predator-prey interactions in a fish-damselfly system. Oecologia 144: 327-336.

Via, S., 1984. The quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlations in larval performance within and among host plants. Evolution 38: 896-905.

Vos, M., B.J.G. Flik, J. Vijverberg, J. Ringelberg, & W.M. Mooij, 2002. From inducible defences to population dynamics: Modelling refuge use and life historychanges in *Daphnia*. Oikos 99: 386-396.

CHAPTER 3 DYNAMICS OF EVOLUTIONARY CHANGE IN A NATURAL *DAPHNIA MAGNA* POPULATION DURING EUTROPHICATION AND RECOVERY OF A SHALLOW LAKE

Helen Michels and Luc De Meester

ABSTRACT

Lake Ring, a shallow lake in central Denmark, eutrophied in the first half of the twentieth century, but returned to a clear-water state in the 1980s. Using animals, hatched from dormant eggs isolated from dated sediment cores of the lake, we compared genetic variation for ecologically relevant traits (Q_{ST}), and neutral microsatellite markers (F_{ST}) among three subpopulations of *Daphnia magna.* These populations derived from three successive periods in the history of Lake Ring: the Pre-eutrophication period (1908-1937), the Turbid period (1960-1980), and the Clear-water period (1986-2000). We performed a quantitative genetic analysis on six antipredator traits in the presence and absence of fish kairomone, and a molecular genetic analysis on nine microsatellite markers. F_{ST} values were very low (average = 0.009). Q_{ST} values were much higher (average = 0.14), ranging from 0.05 to 0.45 for the six traits in the different treatments, and indicated that natural selection caused micro-evolution in phenotypic plasticity in diel vertical migration and diel horizontal migration, and size at maturity. Moreover, we saw that evolution was reversed over time. Whereas plasticity in response to fish kairomone increased from the 1st to the 2nd period, it decreased again from the 2nd to the 3rd period. Traits values were fairly similar between the subpopulation of 1908-1937 and that of 1986-2000.

1. Introduction

Anthropogenic impact on natural habitats and their biota becomes more pronounced every day (Meffe and Carroll, 1997; Pulido and Berthold, 2004). Habitat deterioration, but also habitat restoration measurements influence the ecology and evolution of species. Many studies considered the effect of human influence on species composition. Studies that consider the impact of anthropogenic stress on the genetic structure and evolution of wild populations are often difficult to conduct because of the time aspect involved. Many studies are therefore restricted to a comparison of impacted and non-impacted populations separated in space (Frankham et al., 2002).

Genetic analysis of stratified dormant egg banks of aquatic organisms provides a way to document micro-evolutionary dynamics in one particular habitat trough time (Jeppesen et al., 2001; De Meester et al., in press). In habitats with a well-documented history of environmental changes, it allows relating changes in genetic structure to changes in environmental conditions. Many aquatic organisms produce dormant stages, which accumulate in the sediment, forming a dormant egg bank (Cáceres, 1998). This forms an archive of past conditions that can be tapped into, provided that the sediment is stratified and can be dated. The long-term viability of dormant propagules (> 100 years) allows quantitative genetic analyses of 'resurrected' populations (Hairston et al., 1999; Kerfoot et al., 1999; Cousyn et al., 2001).

Micro-evolution is the result of neutral events (gene flow and genetic drift) and natural selection acting on adaptive variation (Hartl and Clark, 1997). To disentangle the relative importance of both factors as driving forces behind micro-evolutionary processes, one can compare genetic differentiation based on quantitative traits (Q_{ST}) and molecular markers (F_{ST}) (see reviews in Reed and Frankham, 2001; Merilä and Crnokrak, 2001; McKay and Latta, 2002). Higher genetic divergence in quantitative traits than in neutral markers ($Q_{ST} > F_{ST}$) points to directional selection favoring different phenotypes in different populations. If Q_{ST} equals F_{ST}, drift and gene flow may explain the observed genetic differentiation among populations. $Q_{ST} < F_{ST}$ suggests that natural selection favors the same phenotype in different populations (Merilä and Crnokrak, 2002).

Many shallow lakes in Western Europe have suffered from severe cultural eutrophication in the previous century, which reached a peak in the 1960s (Harper, 1992; Moss et al., 1997; Scheffer, 1998). From the 1970s onwards, restoration efforts were taken for an increasing number of lakes, often resulting in a decrease of nutrient load. Lake Ring, a shallow lake located in central Jutland (Denmark) is a case at hand. As this lake suffered from severe cultural eutrophication, the point source of sewage effluents from the nearby village was diverted in

1970. With quite some time delay, this resulted in the mid 1980s in a shift from a turbid to a clear-water state.

Using animals, hatched from dormant eggs retrieved from different sediment layers of a dated sediment core of Lake Ring, we could demonstrate (Chapter 2) that the mid 1980s shift in water transparency was associated with a micro-evolutionary change in antipredator traits in the *Daphnia magna* population inhabiting this lake. In the present study, we build further on this research. We worked with three *Daphnia magna* subpopulations derived from three successive periods in the history of Lake Ring: the Pre-eutrophication period (1908-1937), the Turbid water period (1960-1980), and the Clear-water period (1986-2000). We scale the observed response in quantitative traits to the corresponding variation in neutral markers, and consider the dynamics of the evolutionary response. We performed a quantitative genetic analysis on six antipredator traits, in the presence and absence of fish kairomone, as well as a molecular genetic analysis on nine microsatellite markers, for the three subpopulations separated in time. We carried out pairwise comparisons among all three populations, calculated population genetic variation for quantitative traits (Q_{ST}), and compared Q_{ST} with the among-population genetic variation for neutral markers (Fst).

Finally, we used also a different approach to reveal the genetic structure of *Daphnia magna* dormant egg bank. Instead of working with a priori defined populations, we also aimed to find the period around which the highest population differentiation was found, by calculating the running Q_{ST} and the running θ (cf. comparable in reasoning to calculating a running average).

Our aim is to document the micro-evolutionary dynamics of the *Daphnia* population, and to test the hypothesis that the evolutionary response was triggered by natural selection, and was reversed when the habitat was restored.

2. Material and Methods

2.1. Study site

Lake Ring is a small, relative shallow lake located in central Jutland (Denmark). The lake was subjected to strong eutrophication during the 20th century due to sewage inflow. To restore the lake, the sewage point source was diverted in 1970. This initially led to a very gradual recovery, culminating in a significant increase in water clarity from the mid-1980s onwards (for more details see Introduction).

2.2. Isolation ephippia and molecular genetic analysis

Sediment cores were taken from the Lake Ring sediment, sectioned horizontally in 1 cm thick slices, and stored in the dark at 4 °C until further analysis. The sediment samples were then filtered through a metal sieve (250 µm mesh); *Daphnia magna* ephippia were isolated, viable eggs were incubated under hatching conditions (20 °C, 14: 10 L D cycle, fresh medium every other day), and hatched individuals were cultured as clonal lineages. Eggs that failed to respond to hatching cues after 20 days or started to show signs of degeneration, were analyzed for microsatellite markers (n = 209). Together with specimens of all established clonal lineages (n = 108), a total of 317 individuals were thus genotyped for nine *D. magna* microsatellite loci (DMA3, DMA3R, DMA35, DMA11, DMA12 DMA15, S6-38, S5-157, S199). Eggs and juveniles were transferred to 105 µl proteinase K-buffer (16mM $(NH_4)_2SO_4$, 67 mM Tris-HCl pH 8, 0.01% Tween-20, 7% DTT and 0.5 mM Proteinase K) in 200µl microtubes, and incubated for 1 h at 56 ° C and 10 min at 95° C, followed by 2 min centrifugation. Because of the small amounts of DNA in the *Daphnia* dormant eggs, PCR preparations were carried out in a no-PCR-laboratory.

PCR amplifications were carried out in 12.5 µl reaction volumes containing 2 x QIAGEN Multiplex PCR Master Mix, 10 X primer mix, 1 µl template DNA, 0.2 µM of each primer, and RNAse free water. PCR amplifications involved a denaturation step of 15 min at 95° C, followed by 30 cycles of 95 °C denaturation for 30 sec, annealing at 56 °C for 90 sec, extension at 72 °C for 90 sec, followed by a final 72 °C extension for 10 min. Microsatellite alleles were separated using an ABI PRISM 3130 capillary DNA sequencer (Applied Biosystems, Foster city CA, USA), and visualized with the program Genemapper 3.7 (Applied Biosystems, Foster city CA, USA).

2.3. Quantitative genetic analysis

Experimental methods for the quantitative genetic analysis were described in detail elsewhere (Chapter 2). Briefly, 6, 11 and 9 clones from the Pre-eutrophication period (1908-1937), the Turbid (1960-1980), and the Clear-water period (1986-2000), respectively, were cultured for at least two generations under standardized culture conditions (30 individuals per liter, 20 ± 1 °C, L:D 14:10, 2.5 10^5 *Scenedesmus obliquus* cells ml^{-1}). Animals were fed daily, and the medium was refreshed every second day. Clonal lineages were reared in the presence and absence of fish kairomone, and four life history traits (age at release of the first clutch, size at maturity, neonate size and size of the first clutch) were scored. Each of the clone-treatment combination had at least three replicates. The experiment started with inoculation of second clutch neonates (< 24 h old). The animals were monitored every 24 h until release of the second clutch in the brood pouch.

Two behavioral traits were quantified each in both absence (NF) and presence (F) of fish kairomone: diel vertical and horizontal migration. Measurement of phototactic behavior, a key trait in diel vertical behavior (De Meester et al., 1999), is described in Chapter 2 and followed existing work (e.g. Cousyn et al., 2001). The experimental set-up to quantify horizontal migration behavior is described in Chapter 1. Briefly, the set-up consisted of a long PVC gutter (L = 1.5 m, W = 7 cm, H = 4.5 cm) which was closed at both ends. The gutter was externally divided into 8 equal-sized compartments. In the two outer compartments a littoral zone was simulated by a combination of real and plastic plants (a total of 11 shoots each, to create a PVI of 28%). The gutter was filled with 3 l aged tap water or fish conditioned water. After 3-4 h of acclimatization to the experimental medium, ten adult females were placed in the middle of the gutter. The number of individuals in each compartment was counted at 15 min intervals. Each experiment lasted 2 h. The horizontal migration index (HMI) of the test population was calculated as (M-O) / (total number animals), averaged over the last hour, with M being the number of animals in the two middle compartments and O the number of animals in the two outer compartments. Values for HMI can range from -1 (all individuals hide among the plants) to 1 (all animals are found in the center of the gutter).

2.4. Data analysis

Within-population allelic genetic diversity was quantified as expected heterozygosity. Exact tests for Hardy-Weinberg equilibrium were performed in TFPGA (Tool for Population Genetic Analysis) (Miller, 1997). Fixation indices for deviation of Hardy-Weinberg equilibrium were calculated in GENETIX (Belkhir et al., 1996). Genetic differentiation among subpopulations separated in time was quantified using Weir and Cockerham's (1984) F_{ST}

estimator θ. 95 % confidence intervals were obtained by bootstrapping over loci. Significance of genetic differentiation was tested using a randomization on allelic frequencies in TFPGA (Miller, 1997).

The degree of population subdivision for each trait (Q_{ST}) was estimated as described in Spitze (1993).

$$Q_{st} = \frac{\sigma^2{}_b}{\sigma^2{}_b + 2\sigma^2{}_w} \quad (1)$$

with σ_b^2 the among-population variance and σ_w^2 the within-population variance for a given trait. The variance components were estimated by comparing observed mean squares to their expectations. The mean squares (MS) were obtained from a variance analysis with population as factor, and clonal means as replicates within population. MS 'within' is an unbiased estimate of the within-estimate population variance (σ_w^2) and MS 'between' yields an unbiased estimate of the among-population variance $\sigma_w^2 + n_0\sigma_b^2$ (2) with n_0 being the average sample size (Storz, 2002):

$$n_0 = \frac{1}{a-1 \times \left(\frac{\sum_{ni}^{a} - \sum_{a} n^2}{\sum_{a} n} \right)} \quad (3)$$

Q_{ST} was calculated for every trait-treatment combination (presence and absence of fish kairomone), and for every trait response to fish kairomone (= clonal mean in absence of fish kairomone - clonal mean in presence of fish kairomone), the latter expresses the phenotypic plasticity of a trait. 95 % confidence intervals for Q_{ST} were calculated by bootstrapping over clonal means. All Q_{ST} analyses were performed in Matlab (MathWorks).

In a first step, pairwise F_{ST} and Q_{ST} values between the three a-priori determinated temporal subpopulations (Pre-eutophication, Turbid, and Clear-water subpopulation) were compared. In a next step, we also calculated Q_{ST} and F_{ST}–values amongst populations without an a priori subdivision among specific periods. To this end, we calculated between two successive populations, in which each population contained an equal number of clones ($F_{ST:}$ n = 30; $Q_{ST:}$ n = 5-10), and the separation between both populations shifted in time (running Q_{ST} and running θ). Given restriction on the total number of clones, a total of 14 pairwise comparisons

were made for the running θ, and 4 pairwise comparisons were made for the running Q_{ST}. Rates of evolution (haldanes) were estimated as described in Hendry and Kinnison (1999).

$$H = \frac{\left(\frac{\overline{X}_2}{S_p}\right) - \left(\frac{\overline{X}_1}{S_p}\right)}{g} \tag{4}$$

$$S_p = \frac{\left[SS_1 + SS_2\right]}{\left(n_1 - 1\right) + \left(n_2 - 1\right)} \tag{5}$$

In equation four, we assumed one sexual generation per year to estimate g (number of generations). Cyclical parthenogenetic organisms like *D. magna* have multiple parthenogenetic generations (10-15), and one sexual generation per year (associated with the presence of dormant egg bank). Although clonal selection also contributes to the micro-evolutionary response in *Daphnia*, part of the response during the parthenogenetic phase is lost upon sexual recombination, because of genetic slippage (Lynch and Deng, 1994). We therefore consider it more logic to interpret the life cycle of *Daphnia* essentially as an annual organism with clonal propagules. n_1 and n_2 indicate the number of clones per population. Haldanes were calculated for every trait-treatment combination, and for every trait in response to fish kairomone, between the Pre-eutrophication period and the Turbid period, the Turbid period and the Clear-water period, and between the Pre-eutrophication period and the Clear-water period. 95 % confidence intervals for haldanes were calculated by bootstrapping over clonal means.

3. Results

Table 1 shows the number of analysed individuals, the expected and observed heterozygosity of the polymorphic microsatellite loci, the fixation indices, and the expected and observed heterozygosity over the different loci. Two out of nine microsatellite markers were monomorphic (DMA 35, and DMA 3), and are not included in the table. After sequential Bonferroni correction only two significant departures of Hardy-Weinberg equilibrium were observed. A positive fixation index points to an excess of homozygotes at locus DMA 11 in the Pre-eutrophication period and at locus S1-157 in the Turbid period. Expected heterozygosity over all loci, which is a good measure for the genetic diversity within a population, does not differ between the different periods.

Table 1: Number of analysed individuals (N), expected (h_e) and observed heterozygosity (h_o), fixation indices (F_{IS}) at seven polymorphic microsatellite loci in three Daphnia magna *populations derived from the Clear-water period, the Turbid period and the Pre-eutrophication period. (H_e) expected heterozygosity and (H_o) observed heterozygosity over the analyzed microsatellite loci for the different populations. Fixation indices significantly deviating from Hardy-Weinberg equilibrium after sequential Bonferroni correction are marked with *.*

Locus		**Clear-water period**	**Turbid period**	**Pre-eutrophication period**
DMA 11	N	97	86	51
	h_e	0.60	0.56	0.51
	h_o	0.57	0.44	0.24
	F_{IS}	0.05	0.22	0.54*
DMA 12	N	100	90	57
	h_e	0.32	0.35	0.35
	h_o	0.30	0.34	0.29
	F_{IS}	0.08	0.03	0.18
DMA 15	N	102	96	57
	h_e	0.43	0.46	0.50
	h_o	0.41	0.39	0.35
	F_{IS}	0.04	0.16	0.30
DMA 3R	N	78	76	44
	h_e	0.53	0.63	0.44
	h_o	0.42	0.51	0.30
	F_{IS}	0.21	0.19	0.34
S5-157	N	78	80	51
	h_e	0.60	0.60	0.51
	h_o	0.53	0.38	0.33
	F_{IS}	0.11	0.37*	0.34
S199	N	79	95	53
	h_e	0.36	0.371	0.26
	h_o	0.25	0.36	0.25
	F_{IS}	0.30	0.04	0.04
S6-38	N	97	104	61
	h_e	0.43	0.42	0.38
	h_o	0.45	0.42	0.31
	F_{IS}	-0.05	-0.01	0.19
All loci	H_e	0.36	0.37	0.33
	H_o	0.31	0.31	0.23

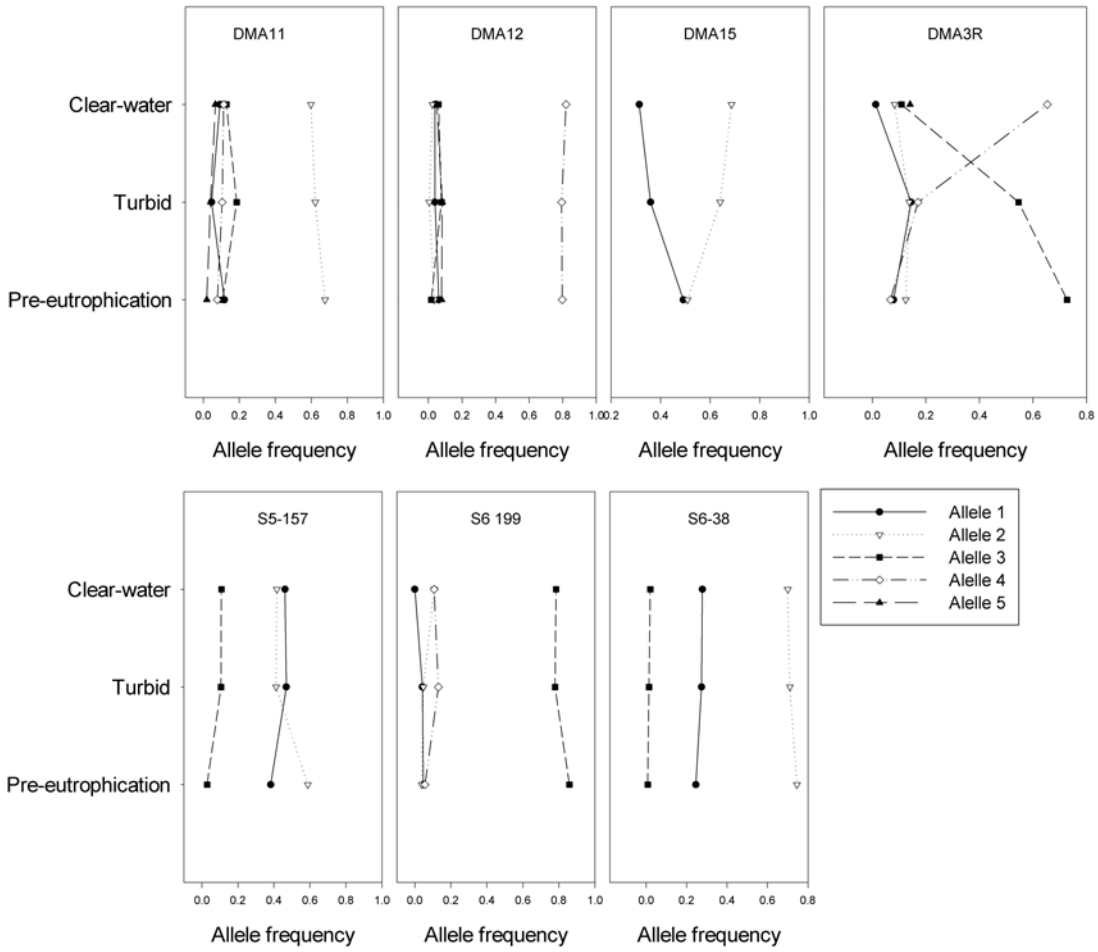

Fig. 1: Allele frequencies of the different allele at seven polymorphic microsatellite loci in Daphnia magna *derived from Pre-eutrophication period, the Turbid period, and Clear-water period.*

Genetic differences for microsatellite markers between subpopulations separated in time were very low (Fig. 1); with all F_{ST} values being lower than 0.05. Pairwise Fisher exact tests indicated that microsatellite allele frequencies in *Daphnia* significantly differed between Clear-water and the Pre-eutrophication period ($p = 0.0003$), and between Turbid and Pre-eutrophication period (<0.0001). The level of differentiation was, however, very low in both cases (Clear-water period/Pre-eutrophication period: $\theta = 0.014$, 95 % CI: -0.0003-0.03; Turbid period/ Pre-eutrophication period: $\theta = 0.015$, 95 % CI: 0.004-0.02; Fig. 2). The running θ varied from -0.009 to 0.05, and was the highest around 1960 ($\theta = 0.05$, 95 % CI: 0.006-0.10; Fig. 3).

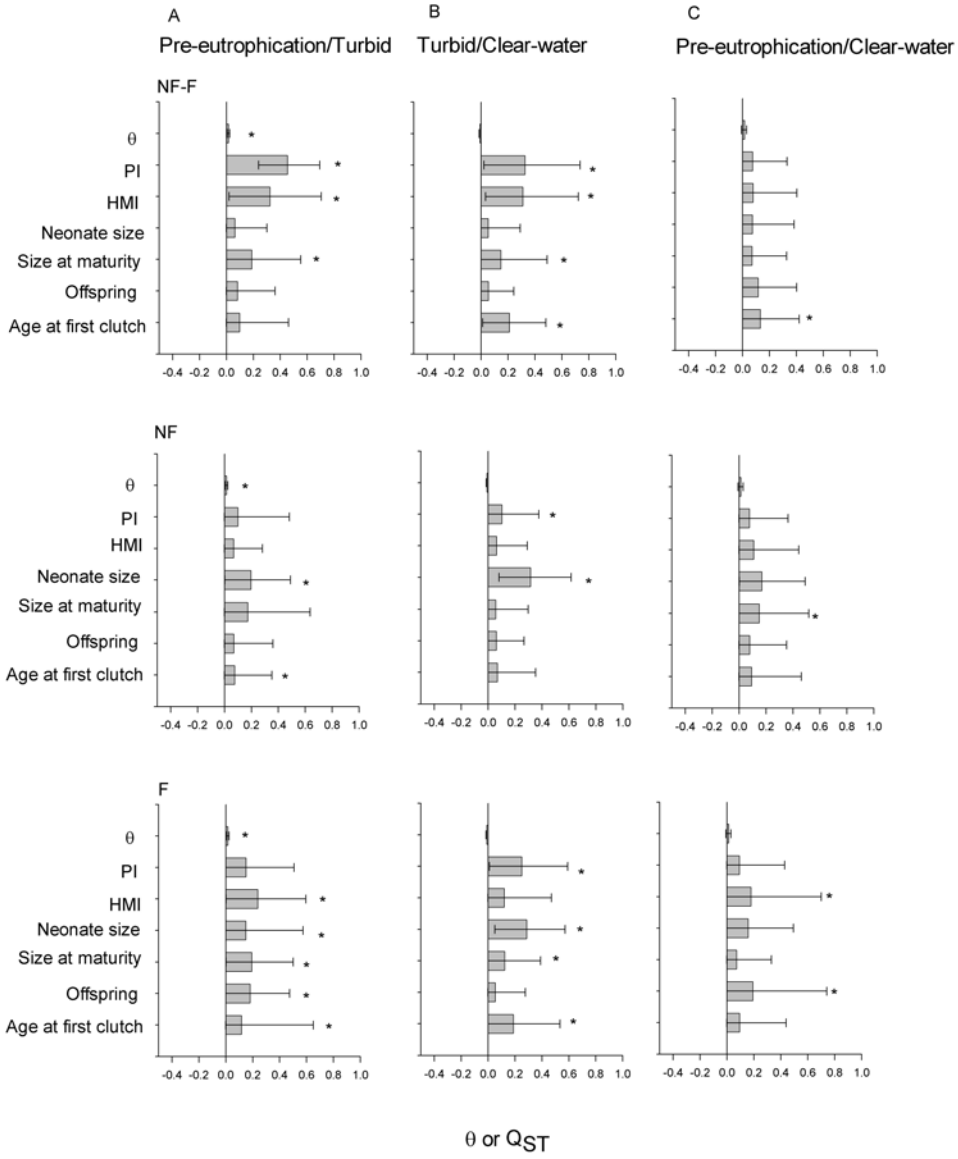

Fig. 2: A) Among-population genetic differentiation based on neutral marker loci (θ) and six antipredator traits (PI = phototactic index; HMI = horizontal migration index, neonate size, size at maturity, number of offspring in first clutch, age at first clutch) (Q_{ST}) between Daphnia magna *populations that are separated in time. A) Comparison of the populations of the Pre-eutrophication period (1908-1937) with that of the Turbid period (1960-1980). B) Comparison of the populations of the Turbid period (1960-1980) with that of the Clear-water period (1986-2000). C) Comparison of the populations of the Pre-eutrophication period (1908-1937) with that of the Clear-water period (1986-2000). NF-F: in response to fish kairomone, NF: in absence of fish kairomone; F: in presence of fish kairomone. Error bars indicate 95% confidence interval; confidence intervals that do not include zero are indicated with an asterisk.*

Genetic differentiation (Q_{ST}) for ecological relevant traits was often substantial. Comparing the population from the Pre-eutrophication and Turbid period revealed pronounced genetic differentiation in the change in vertical migration behavior (PI) in response to fish kairomone (genetic differentiation for phenotypic plasticity) (Q_{ST} = 0.45; 95 % CI 0.23-0.69). To a lesser extent, there was also among-population genetic differentiation in the change in horizontal migration behavior and size at maturity to fish kairomone (Q_{ST} HMI = 0.32; 95% CI 0.02-0.70; Q_{ST} sam = 0.19; 95% CI 0.001-0.55) (Fig. 2). The genetic divergence in the change in vertical (PI) and horizontal migration (HMI) in response to fish kairomone, significantly exceeded genetic differentiation for neutral markers θ (Fig. 2). In the absence of fish kairomone, genetic differentiation in behavioral and life history traits was low and only marginally significant for neonate size (see Fig. 2). In the presence of fish, there was significant differentiation in several traits. Values for Q_{ST} were lower then for changes in traits upon exposure to fish kairomone. Yet, Q_{ST} values are still up to approximate 10 times higher then θ values.

Pairwise Q_{ST} values between the Turbid and the Clear-water period population also showed that genetic differentiation in the changes in vertical migration (PI), horizontal migration (HMI), size at maturity, and age at first clutch in response to fish kairomone are significantly different from zero. The quantitative genetic divergence in the change in vertical migration (PI), horizontal migration (HMI), and age at first clutch as a response to fish kairomone exceeded the genetic differentiation based on neutral marker loci (Fig. 2). In the absence of fish kairomone there was a significant among-population genetic differentiation in neonate size (Q_{ST} = 0.31; 95% CI 0.08-0.61), and PI (Q_{ST} = 0.10; 95% CI 0.0001-0.37). In the presence of fish kairomone, a significant among-population genetic differentiation is observed in vertical migration (PI) (Q_{ST} = 0.25; 95% CI 0.01-0.59), neonate size (Q_{ST} = 0.28; 95% CI 0.05-0.57), size at maturity (Q_{ST} = 0.12; 95% CI 0.0001-0.39), and age at release of first clutch (Q_{ST} = 0.18; 95% CI 0.0009-0.53). A comparison between the Pre-eutrophication and the Clear-water period indicated that differences for the different traits in response to fish kairomone barely differed from zero.

The running Q_{ST} for the different treatment-trait combinations confirms the data based on the a priori subdivision of populations. For size at maturity in the presence of fish kairomone however, this analysis also indicates a significant difference around 1968 (Q_{ST} = 0.25; 95% CI 0.0008-0.59) (Fig. 3).

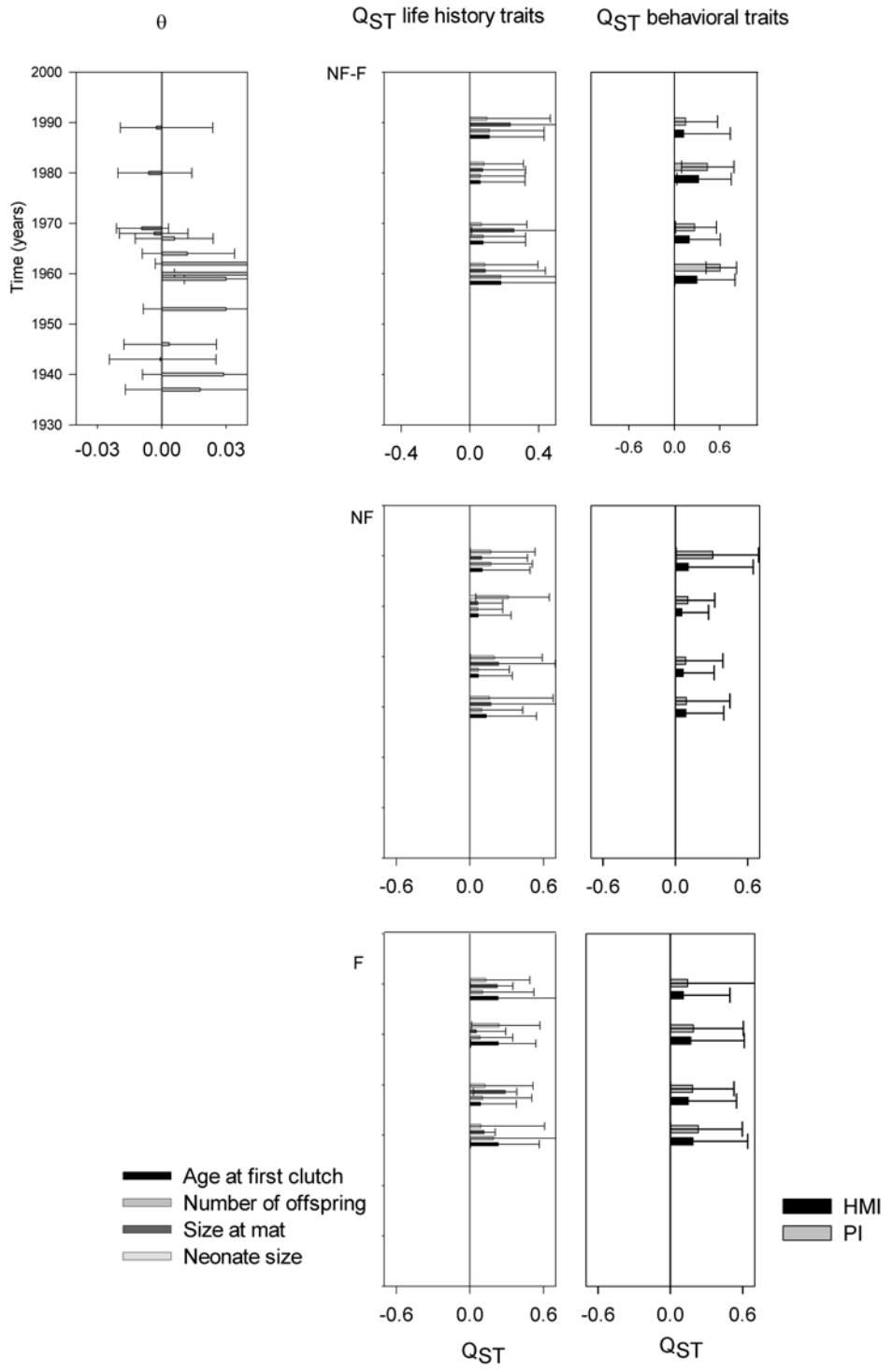

Fig 3: Vertical time profile of among-population genetic differences based on nine microsatellite DNA loci (θ), and vertical time profile of among-population genetic differences based on genetic variances of six antipredator traits (Q_{ST}) in the presence (F), absence (NF), and in response to fish kairomone (NF-F). Values were obtained by pairwise comparisons of successive populations as a running value. Every pair of compared populations contained an equal number of individuals.

Haldanes were significantly different from zero for only few trait-treatment combinations (neonate size in the absence of fish, the change in PI and HMI upon exposure to fish kairomone) (Table 2). Interestingly, when comparing the shift from the Pre-eutrophication period to the Turbid period, with the shift from the Turbid period to the Clear-water period, three out of four significant values (size of neonates in the absence of fish, and the change in two behavioral traits upon exposure to fish kairomone), had opposite signs, reflecting that the direction of evolution from the Pre-eutrophication to the Turbid period was opposite of the direction of evolution from the Turbid period to the Clear-water period. None of these values were significant when we compared the two extreme time periods (Pre-eutrophication period and the Clear-water period).

Table 2: Rate of evolution (haldanes) of six traits in the absence (NF) and presence (F) of fish kairomone and of the change in traits in response to fish kairomone (NF-F) between the Pre-eutrophication and the Turbid period the Turbid period and the Clear-water period, and the Pre-eutrophication period and the Clear-water period. 95 % confidence intervals are indicated between parentheses. Values with confidence intervals that do not include zero are indicated in bold.

	Pre-eutrophication/Turbid period			**Turbid period/Clear-water period**			**Pre-eutrophication/ Clear-water period**		
	NF	F	NF-F	NF	F	NF-F	NF	F	NF-F
Age at first clutch	-0.01	-0.02	0.01	-0.04	0.09	-0.10	-0,01	0,009	-0,02
	(-0.07,0.04)	(-0.06,0.01)	(-0.02,0.06)	(-0.20,0.07)	(-0.01,0.25)	(-0.23,-0.01)	(-0.06,0.01)	(-0.02,0.05)	(-0.05,0.02)
Number of offspring	0.00	-0.01	0.00	-0.01	0.00	0.00	-0,002	-0,006	0,01
	(-0.02,0.01)	(-0.04,0.01)	(-0.01,0.01)	(-0.04,0.03)	(-0.04,0.05)	(-0.01,0.02)	(-0.01,0.01)	(-0.03,0.002)	(-0.01,0.01)
Size at maturity	**0.18**	0.17	-0.15	0.02	-0.36	0.37	0,09	0,04	-0,01
	(-0.08,0.7)	(-0.01,0.44)	(-0.40,0.02)	(-0.68,0.58)	(-0.96,0.14)	(-0.09,1.17)	(-0.06,0.34)	(-0.15,0.27)	(-0.15,0.15)
Neonate size	0.38	0.32	0.01	**-2.04**	-1.97	0.09	-0,60	-0,44	0,08
	(0.01,1.06)	(-0.12,0.92)	(-0.66,0.45)	(-5.26,-0.77)	(-0.96,0.14)	(-1.20,2.49)	(-1.49,0.20)	(-1.35,0.22)	(-0.52,1.10)
HMI	0.00	-0.01	**0.15**	-0.02	0.03	**-0.53**	-0,01	-0,01	0,02
	(-0.02,0.02)	(-0.04,0.01)	(0.025,0.47)	(-0.10,0.04)	(-0.01,0.11)	(-1.93,-0.11)	(-0.04,0.01)	(-0.06,0.01)	(-0.12,0.23)
PI	0.03	-0.05	**0.19**	-0.18	**0.27**	**-0.40**	-0,01	0,016	0,01
	(-0.17,0.30)	(-0.04,0.01)	(0.1,0.40)	(-0.57,0.20)	(0.04,0.68)	(-1.27,-0.05)	(-0.1,0.06)	(-0.04,0.10)	(-0.10,0.05)

Fig. 4 shows the population means of the populations derived from the three periods for vertical migration, horizontal migration and size at maturity. We concentrated on variables of which Q_{ST} value was significantly different from zero in response to fish kairomone in both shifts. Both behavioral traits show the strongest change in trait value during the Turbid period; in the Pre-eutrophication and Clear water period the plasticity response almost disappears. In both traits, phenotypic plasticity is largely related to a change in behavior in the presence of fish kairomone, and the behavior in the absence of fish kairomone does not change much between time periods. Size at maturity, on the other hand, shows the strongest phenotypic plasticity response in the Pre-eutrophication and Clear-water period. This is the opposite pattern of the behavioral traits. Interestingly, the trait value in the Pre-eutrophication and the Clear water period, both characterized by relatively clear-water conditions, are very similar for the three traits (see also Chapter 2).

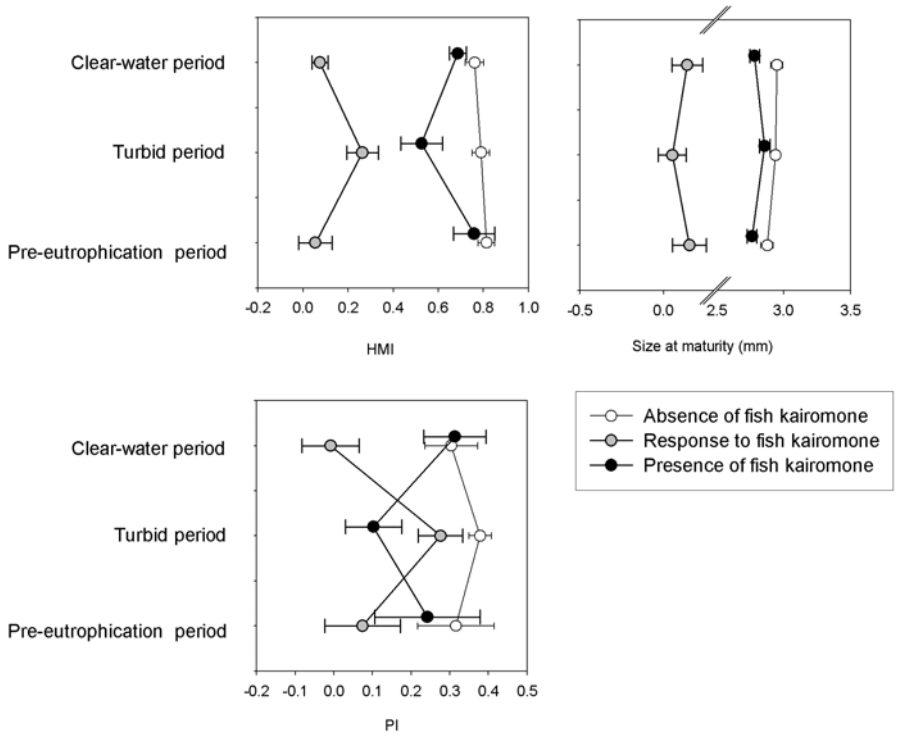

Fig. 4: Population means ± SE for three antipredator traits (PI, HMI, size at maturity) of Daphnia magna *populations derived from the Pre-eutrophication period (1908-1937), the Turbid period (1960-1980) and the Clear-water period (1986-2000). White dots indicate population means ± SE in the absence of fish kairomone, black dots indicate population means ± SE in the presence of fish kairomone, and gray dots indicate population means for the change in antipredator traits in response to fish kairomone in the same three time periods.*

4. Discussion

Our results show only very low or no genetic differentiation for neutral marker loci between the different temporally subdivided subpopulations of *Daphnia magna* in Lake Ring. Comparing three successive and a priori determinated subpopulations of *D. magna* derived from dormant eggs isolated from Lake Ring sediment yielded F_{ST} estimates of 0.02 or lower. Our analyses with the 'running' θ confirmed the genetic continuity of the *D. magna* population in Lake Ring, showing a maximal value of θ 0.05. Our data thus suggest that no, or only a very shallow, molecular genetic subdivision was present in this population over time sequence studied. Furthermore, genetic diversity within the different populations did not substantially differ from each other. This is in line with results of other populations (Cousyn et al., 2001;

Decaestecker et al., 2004) and may reflect a strong buffering effect of large dormant egg bank present in lake sediment.

We did find, however, strong among-population genetic differentiation in antipredator traits, such as traits linked to diel vertical migration, diel horizontal migration, and size at maturity. Pairwise comparisons of the populations derived from the Pre-eutrophication, Turbid and Clear-water period indicate that the population derived from the Turbid period strongly differed from the two other populations, but that the populations derived from the Clear-water and the Pre-eutrophication period did not differ from each other. As Q_{ST} values for some traits exceeded F_{ST} estimates for genetic differentiation in neutral markers, our data provide evidence that natural selection was a driving force the for observed evolutionary changes.

The relative importance of the phenotypic plasticity response of three main antipredator traits appears to shift among periods (see Chapter 2). Clones derived from the Turbid period mostly used behavioral antipredator traits (diel vertical and horizontal migration), whereas clones derived from the clear-water periods (pre- and post eutrophical) responded to fish kairomone with change in life history traits. The running Q_{ST} analysis roughly describes the same pattern for the behavioral traits, i.e. the Q_{ST} for the change in vertical and horizontal migration behavior in response to fish kairomone peaks in 1983 and 1960, i.e. at the transition times.

The fact that Q_{ST} exceeds F_{ST} strongly suggests that natural selection rather than stochastic effects are responsible for the observed genetic differentiation in phenotypic plasticity of behavioral traits and size at maturity (Merilä and Crnokrak, 2002). The values for genetic differentiation for neutral markers are relatively low, suggesting relatively little impact of genetic drift and gene flow. The observed change towards a stronger plasticity response in the behavioral trait during the Turbid period is in agreement with the hypothesis of adaptive evolution, because migration is an efficient antipredator trait in turbid water (e.g. Vos et al., 2002). In clear-water periods vertical migration can be expected to be less efficient in shallow lakes, because of the disappearance of a true refuge at depth. Diel horizontal migration may have lost efficiency in this study system under clear-water conditions, because the littoral zone only marginally increased with a shift from the turbid to the clear-water state (Chapter 1). The plasticity response in size at maturity thus gained importance in the clear-water periods.

Although many studies used Q_{ST} -F_{ST} comparisons to reveal natural selection as driving force behind among-population differentiation (for review see Merilä and Crnokrak, 2002), only one study so far (Cousyn et al., 2001) used this approach on an analysis of a dormant egg bank to document the evolutionary dynamics within one single population through time. Whereas Cousyn et al. (2001) focused on one ecologically relevant trait, we here observe that the resident

Daphnia population in Lake Ring showed an evolutionary response in several antipredator traits in relation to habitat deterioration and restoration. Quantifying the rate of evolution (Haldanes) (Hendry and Kinnison, 1999) shows periods of fast evolution. Our study however, also reveals a reversal in the direction of evolution between the different periods. Comparing the Pre-eutrophication and the Clear-water period thus yields a very low rate of evolution.

The density profile of *Daphnia magna* ephippia in the sediment of Lake Ring, which is an indicator for change in population density of the species in the lake (Verschuren and Marnell, 1997) indicated density decreases during both periods during which the lake shifted from one state to another (around 1960 and 1983). This may indicate that strong selection took place during these periods. Only around 1960 this was associated with a minor shift in allele frequencies at neutral marker loci.

In conclusion, our study indicates that natural selection caused micro-evolutionary shifts in phenotypic plasticity of antipredator traits, more specifically in diel vertical and horizontal migration and size at maturity, in association with habitat deterioration and restoration in a shallow lake. Together with the recovery of the habitat, we observed that the antipredator behavior of a key zooplankton taxon reverted back to its initial condition.

5. Acknowledgments

We thank K. Bollaerts for statistical support. H. Michels is a doctoral fellow of the Institute for the Promotion of Innovation through Science and Technology in Flanders. This research was financially supported by EU IP project EUROLIMPACS (GOCE-CT-2003-505540), FWO G.0269.04 and KULeuven Research Fund project OT/04/23.

6. References

Belkhir K., Borsa P., Goudet J. en Bonhomme F. (1996) - GENETIX 1.3, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome et Population, CNRS UPR 9060, Université de Montpellier II, Montpellier.

Caceres, C.E., 1998. Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. Ecology 79: 1699-1710.

Cousyn, C., L. De Meester, J.K. Colbourne, L. Brendonck, D. Verschuren, & F. Volckaert, 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. Proceedings of the National Academy of Sciences of the United States of America 98: 6256-6260.

De Meester, L., P. Dawidowicz, E. Van Gool, & C.J. Loose, 1999. Ecology and evolution of predator-induced behavior of zooplankton: depth selection behavior and diel vertical migration. In: Tollrian R. & Harvell C.D. (eds.), The ecology and evolution of inducible defenses. Princeton University Press, New Jersey: 160-176.

De Meester, L., J. Mergaey, H. Michels, & E. Decaestecker, (in press) Cladocera resting eggs and antropogenic changes. In: Alekseev, V. & De Stasio, B. (eds.), Diapause in aquatic invertebrates: role for ecology, physiology and human uses. Springer,

Decaestecker, E., C. Lefever, L. De Meester, & D. Ebert, 2004. Haunted by the past: Evidence for dormant stage banks of microparasites and epibionts of *Daphnia*. Limnology and Oceanography 49: 1355-1364.

Frankham, R., J. D. Ballou, & D. A. Briscoe, 2002. Introduction to conservation genetics, Cambridge University Press, Cambridge.

Hairston, N.G., W. Lampert, C.E. Caceres, C.L. Holtmeier, L.J. Weider, U. Gaedke, J.M. Fischer, J.A. Fox, & D.M. Post, 1999. Lake ecosystems - Rapid evolution revealed by dormant eggs. Nature 401: 446.

Harper, D., 1992. Eutrophication of freshwater, Chapman & Hall, London.

Hartl, D. & A. G. Clark, 1997. Principles of population biology, Sinauer Associates, Inc., Massachusetts, US.

Hendry, A.P., & M.T. Kinnison, 1999. Persective: The pace of modern life: Measuring rates of contemporary microevolution. Evolution 53: 1637-1653.

Jeppesen, E., P. Leavitt, L. De Meester, & J.P. Jensen, 2001. Functional ecology and palaeolimnology: Using cladoceran remains to reconstruct anthropogenic impact. Trends in Ecology & Evolution 16: 191-198.

Kerfoot, W.C., J.A. Robbins, & L.J. Weider, 1999. A new approach to historical reconstruction: combining descriptive and experimental paleolimnology. Limnology and Oceanography 44: 1232-1247.

Lynch, M., & H.W. Deng, 1994. Genetic slippage in response to sex. American Naturalist 144: 242-261.

Matlab, 2005. The language of technical computing. The Mathworks, Inc., US.

McKay, J.K., & R.G. Latta, 2002. Adaptive population divergence: markers, Qtl and traits. Trends in Ecology & Evolution 17: 285-291.

Meffe, G. & C. R. Carroll, 1997. Principles of conservation biology, Sinauer Associates, Inc., Massachusetts, US.

Merilä, J., & P. Crnokrak, 2001. Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology 14: 892-903.

Moss, B., M. Beklioglu, L. Carvalho, S. Kilinc, S. Mcgowan, & D. Stephen, 1997. Vertically-challenged limnology; contrasts between deep and shallow lakes. Hydrobiologia 342: 257-267.

Pulido, F., & P. Berthold, 2004. Microevolutionary response to climatic change. Advances in Ecological Research 35: 151-183.

Reed, D.H., & R. Frankham, 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55: 1095-1103.

Scheffer, M., 1998. Ecology of shallow lakes, Chapman & Hall, London, UK.

Spitze, K., 1993. Population-structure in *Daphnia Obtusa* - Quantitative genetic and allozymic variation. Genetics 135: 367-374.

Storz, J.F., 2002. Contrasting patterns of divergence in quantitative traits and neutral DNA markers: Analysis of clinal variation. Molecular Ecology 11: 2537-2551.

Miller P., (1997) TFPGA. Tools for population genetic analysis, US.

Verschuren, D., & L.F. Marnell, 1997. Fossil zooplankton and the historical status of Westslope Cutthroat Trout in a deadwater lake of Glacier National Park, Montana. Transactions of the American Fisheries Society 126: 21-34.

Vos, M., B.J.G. Flik, J. Vijverberg, J. Ringelberg, & W.M. Mooij, 2002. From inducible defences to population dynamics: Modelling refuge use and life history changes in *Daphnia*. Oikos 99: 386-396.

Weir, B.S., & C.C. Cockerham, 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

CHAPTER 4 MICRO-EVOLUTION IN COMPETITIVE ABILITY AND ECOLOGICAL COMBINING ABILITY OF A NATURAL *DAPHNIA MAGNA* POPULATION IN RESPONSE TO CHANGES IN POPULATION DENSITY AND SPECIES COMPOSITION

Helen Michels, Joost Vanoverbeke, Luc De Meester

ABSTRACT

Both intra- and interspecific competition can be strong selection forces, which can lead to evolutionary changes in competitive ability (i.e. the performance in competition) and in combining ability (i.e. the ability to coexist). Given that the selection pressure exerted by competition is density dependent, one may expect that both competitive ability and ecological combining ability may track changes in population densities and the changing relative abundance of competitors. Documenting micro-evolutionary changes in competition is difficult however, because of the time aspect involved, and because character displacement may result in a lowered observed strength of competitive interactions (cf. ghost of competition past). We reconstructed the evolutionary dynamics of competitive interactions of a strong zooplankton competitor, *Daphnia magna*, in relation to changes in densities of conspecifics as well as to changes in population densities of a large bodied congener, *D. pulex.* Here for we used dormant egg banks in layered lake sediments that provide an archive of past population characteristics. We observed interclonal variation in intraspecific competitive ability and in interspecific ecological combining ability, and we found a positive correlation between the interspecific combining ability of the studied *D. magna* clones and the abundance of *D. pulex* as reconstructed from the ephippium density profile in the lake sediment. Furthermore, there was a positive relationship between the ratio of inter- to intraspecific competitive ability of the *D. magna* clones and the relative abundance of *D. magna* and *D. pulex* in the lake as well as between the intraspecific competitive ability of the studied clones and the relative abundance of *D. magna* in the lake. Taken together, these results suggest that the local *D. magna* population of the studied lake was able to genetically track the changes in its competitive environment, both in terms of intraspecific competitive ability as well as interspecific combining ability.

1. Introduction

Competition for limited resources forms the subject of many ecological and evolutionary studies (Gurevitch et al., 1992). Indeed, being relatively fit compared to other genotypes or species is the key to species sorting and natural selection. Even though this entails the capacity to survive the abiotic conditions, and attacks of predators and parasites specific to the local habitat, competition is a central mechanism determining the relative abundance of species and genotypes that can perform reasonably well under the local conditions. Competition has been shown to play a key role in structuring communities (Tilman, 1994; Hu and Tessier, 1995; Càceres, 1998; Chase and Leibold, 2003), and both inter- and intraspecific competition are important drivers of natural selection (see Schluter, 2001). In this respect, there are two strategies that may lead to success, expressed as the ability to coexist or dominate in a community or population: an increase in competitive ability or an increase in ecological combining ability (Aarssen, 1983). Aarssen (1983), introducing evolutionary dynamics in the study of competition, concluded that evolutionary responses to competition may either involve changes in competitive ability or ecological combining ability. Competitive ability is here defined as the performance in competition (e.g. Capaul and Ebert, 2003). Although combining ability has been somewhat neglected in the literature, the recent upsurge in research on character displacement (e.g. Schluter 2000; Pritchard and Schluter, 2001; Steiner et al., 2007) essentially deals with the evolution of an increase in ecological combining ability. The concept of character displacement implies that the intensity of competitive interactions declines over time, and that the descendant's competition strength is only a 'ghost' of its former strength (Connell, 1980; Pritchard and Schluter, 2001). Given that the selecting pressure exerted by competition is density dependent, however, one may even take a more dynamic view, and expect that both competitive ability and ecological combining ability may track changes in population densities and the changing relative abundance of competitors. Both responses can be partly uncoupled, as they involve different mechanisms, and both may contribute to local persistence and performance of genotypes and species.

One can quantify competitive ability and ecological combining ability separately in competition experiments, as the former is reflected in the relative abundance of a genotype or species in a competition trial, whereas the latter translates into differences in the total number of individuals in the experimental unit.

To find evidence for competition as a driver of natural selection leading to divergence, most studies focused on the comparison of sympatric and allopatric populations (see Schluter, 2000). Although such studies can reveal the divergence between populations and species and have provided powerful avenues in the analysis of character displacement, they can not directly

test whether interaction strength changes through time. This temporal aspect can be incorporated by comparing individuals of different time periods, which is, however, difficult in most study systems, as few studies manage to track adaptive changes in populations for sufficiently long time periods.

Many aquatic organisms produce dormant stages that accumulate in the sediment, forming a dormant egg bank (Cáceres, 1998; Brendonck and De Meester, 2003). If this dormant egg bank builds up in stratified sediment that can be dated, it forms an archive of past conditions that can be consulted to reconstruct ecological and evolutionary dynamics. The long-term viability of dormant propagules (> 100 years) allows quantitative genetic analyses of 'resurrected' populations (Hairston et al., 1999; Kerfoot et al., 1999; Cousyn et al., 2001). Besides being an archive on the changes in genetic structure of a local population, stratified lake sediment may also be used to reconstruct changes in population density of species (e.g. Verschuren and Marnell, 1997; Jeppesen et al., 2001). Steiner et al. (2007) used clones collected from different depth from a dormant egg bank (i.e. different time periods) to test whether *D. ambigua* had undergone selection for reduced competition effects and enhance competitive effects.

Here we use the archive in a dormant egg bank of a shallow Danish lake to reconstruct the evolutionary dynamics of competitive interactions of a strong zooplankton competitor, the water flea *Daphnia magna*, in relation to changes in densities of conspecifics as well as to changes in population densities of an important competing species, *D. pulex*. Fig. 1 shows that during the last 90 years strong fluctuations in the absolute and relative abundances of these two *Daphnia* species occurred in our study lake (Lake Ring). Both these *Daphnia* species are large-bodied (*D. magna*: 0.9-4.5 mm; *D. pulex*: 1.1-3.5 mm), non–selective bulk filter feeders with strongly overlapping food spectra (Kerfoot et al., 1985; Lampert, 1987). Hence we anticipated strong competition between these two taxa. Here we test whether there is genetic variation for competitive ability and ecological combining ability in the resident population of *D. magna* of Lake Ring for both intra- and interspecific (with *D. pulex*) interactions, and test the following hypotheses. First, we investigated whether the *D. magna* population evolves stronger competitive ability with time (Hypothesis 1) or in relation to population density peaks of conspecifics (Hypothesis 2); we test the parallel hypotheses for ecological combining ability (Hypothesis 3 and 4), and also test the parallel four hypotheses for interspecific competitive ability (Hypothesis 5 and 6) and ecological combining ability (Hypothesis 7 and 8). Finally, we wanted to explore whether the responses to intra- and interspecific competition are uncoupled, and whether the relative competitive ability for intra- and interspecific interactions is related to the relative abundance of conspecifics and individuals of the competing congener.

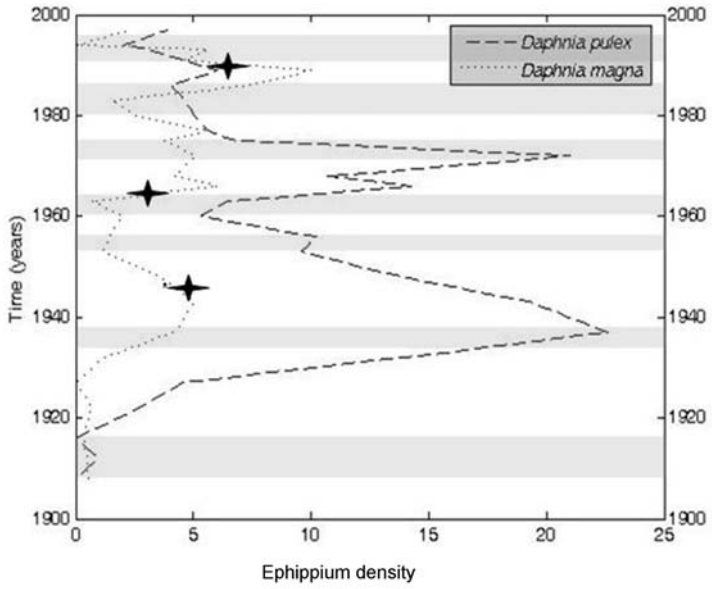

Fig. 1: Vertical profile of ephippium abundance of D. magna *and* D. pulex *in Lake Ring sediment. Grey bars indicate time period from which experimental clones were derived; from every time period three clones were used. Stars indicate the depth from which* D. magna *reference clones were hatched.*

2. Material and methods

2.1. Study site

Lake Ring is a small, relative shallow lake located in central Jutland (Denmark). The lake experienced strong eutrophication during the 20th century due to sewage inflow. To restore the lake, the sewage inflow was cut off in 1970. This initially led to a very gradual recovery, culminating in a drastic increase in water clarity from the mid-1980s onwards (for more details see Introduction).

2.2. Experimental clones

To reconstruct evolutionary changes in intra- and interspecific competitive ability and ecological combining ability, we hatched 21 *D. magna* clones from eggs obtained from 18 depth intervals that covered a 90 years period of lake history (corresponding to the period for which population density data are available; Fig. 1). To increase our power to detect an impact of changes in the density of *D. pulex* and of conspecifics on competition related traits, we carried out a stratified sampling of clones, with three clones being used from each of seven time

periods: before (approx. 1910), at (1940) and after (1955) the first density peak of *D. pulex*, before (1960), at (1975) and after (1985) the second density peak of *D. pulex*, and before (1985) and after (1995) the major density peak of *D. magna* (Fig. 1).

2.3. Experimental design

To estimate intraspecific competitive ability and ecological combining ability, each of the 21 clones (from now on referred to as 'experimental clones') was placed together with *D. magna* reference clones in separate competition trials. To estimate interspecific competitive ability and ecological combining ability, we established competition trials in which each *D. magna* clone was confronted with *D. pulex* reference clones. Testing each of the experimental clones against the same set of reference clones provides us with comparable values of competitive ability for each of the *D. magna* clones (e.g. Capaul and Ebert, 2003). Total density of the competition trial was used as an estimate for combining ability, whereas the frequency of the experimental clone in the competition trial was used as an estimate for competitive ability.

The *D. magna* reference clones were hatched from Lake Ring sediments. To reduce the impact of genotype-genotype interactions with respect to competitive ability and ecological combining ability, we used three replicate *D. magna* reference clones, derived from three time periods of Lake Ring sediment (1951, 1964, 1991). Since we were unable to hatch more than one *D. pulex* clones from Lake Ring sediments, we collected two extra *D. pulex* clones from two different ponds ("Oude Meren" and "Langdorp", both in Belgium).

Each competition trial was started with the inoculation of 10 second clutch neonates (< 24 h old) of the *D. magna* clone to be tested (n = 21) and ten second clutch neonates of one reference clone (either *D. magna* or *D. pulex*) on a 1 l jar. To minimize interference from maternal effects, the clones were cultured for at least two generations prior to the experiment under standardized high food conditions (10 individuals in 500 ml jars, 20 ± 1° C, L:D 14:10, 2.5 10^5 *Scenedesmus obliquus* cells ml^{-1}); the animals were fed daily and the medium was refreshed every second day. For each of the 21 *D. magna* clones, one competition trial was carried out with each of the three *D. magna* reference clones and with each of the three *D. pulex* reference clones. In addition, we established one monoclonal population of each of the 21 clones by inoculating 20 second clutch neonates on 1 l jars. All experimental cultures were fed 1 10^5 cells of the unicellular alga *Scenedesmus obliquus* ml^{-1} daily for the first five day, after which the amount of food was increased to 1.5 10^5 cells ml^{-1} daily. Every second day dead animals and shed carapaces were removed to avoid extensive growth of bacteria and fungi in the cultures. The competition experiment lasted for 35 days. At that moment we determined total density of animals in the cultures as well as the frequency of the experimental clone in each

experimental unit. Total density of the cultures was quantified counting a subsample that contained at least 100 adult individuals. Both adults and juveniles were counted. Remaining individuals of the first generation of inoculated animals, which were easily recognized by their large size (being >30 days old), were not included in the counts. For cultures containing mixtures of *D. pulex* and *D. magna,* we analyzed a representative subsample under a stereomicroscope and determined the total density as well as the frequency of *D. magna* among the adults and subadults. In mixed cultures containing two *D. magna* clones, we randomly took 20 (sub)adults and analyzed them for their genotype at a discriminating locus to quantify the frequencies of the experimental and the reference clone in the culture. Furthermore, the density of the experimental *D. magna* clones was also determined (= total density without density of reference clones). For cultures containing two *D. magna* clones the density of the experimental clone was calculated as frequency multiplied by total density of the mixed population. All *D. magna* clones were, prior to the experiment, characterized for their genotype at four allozyme loci by cellulose-acetate electrophoresis: glucose-6-phosphate-isomerase (*GPI*), mannose-6-phosphate-isomerase (*MPI*), amino-aspartate transferase (*AAT*), and malate dehydrogenase (*MDH*). All 21 experimental clones that we used in the competition trials could be recognized from the reference clones by their genotype at least one (diagnostic) locus.

2.4. Data analysis

We took a dual approach in analyzing the data. First, we analyzed the data in a repeated measure ANOVA with the three different reference clones as repeats, and period as fixed factor, to take the seven time periods into consideration. As the seven time periods are, however, quite evenly spread over time, we also carried out correlation analyses in which we used clonal means as input data.

To test for the effect of time and reference clone on competitive ability and ecological combining ability, we performed repeated measure analyses of variance (RM ANOVA) in STATISTICA (Statsoft, 1994), separately for the two different treatments (competition with conspecifics and with *D. pulex*) and the two different variables (competitive ability and ecological combining ability). Time period was considered a fixed factor. We used clonal means as input data (cf. clones are considered replicates within time periods) and we considered trait variables in the presence of the three different reference clones as repeats for each experimental clone.

To test for interclonal variation, we performed one-way analyses of variance with clone as fixed factor. In this analysis, we used our measurements for competition with the three different

reference clones as replicate observations for a given clone, which is a conservative approach, as it is likely to lead to an underestimation of genetic differences.

Spearman Rank correlations were used to investigate relationships between clonal means for competitive ability and ecological combining ability on one hand, and characteristics of the competitive environment of the corresponding time period (sediment depth intervals, 1 cm slices) the clones were hatched from on the other hand. The latter characteristics included a relative measure of the population density of *D. pulex* and *D. magna*, given by the density of ephippial eggs in the sediment core (Fig. 1), a relative measure of the total biomass of *Daphnia*, to which end the relative measures of the population densities of the different species were weighed by a scaling factor and summed (see Equation 1), and the ratio of the abundances of *D. pulex* to *D. magna*. In these analyses, we used clonal means over the different reference clones, separately for *D. pulex* (interspecific competitive ability and combining ability) and *D. magna* (intraspecific competitive ability and combining ability). We averaged the values of the experimental data of all clones isolated from a particular 1 cm interval to prevent pseudo-replication. This resulted in n = 18 data points.

The "biomass" of *Daphnia* per depth interval was calculated as:

$$Biomass = 1.5 \times E_{Dm} + 1 \times E_{Dp} + 0.5 \times E_{Dl} \quad (1)$$

With

$E_{D.m}$ =abundance *D. magna* ephippia

$E_{D.p}$ =abundance *D. pulex* ephippia

$E_{D.l}$ = abundance *D. longispina* ephippia

Equation 1 is not intended as a measure of true biomass, but the weighing factors are intended to reflect "competitive impact" of the different species, similar to competition coefficients, and are based on the fact that *D. magna* is larger than *D. pulex,* and that animals of the *D. longispina* species complex, of which members also occur in Lake Ring but at relatively low densities during the time period considered, are considerably smaller than the two former species.

If it is difficult to optimize interspecific and intraspecific competitive ability and/or combining ability at the same time, one may expect that there is a change in adjustment (optimization) of the relevant traits depending on the relative abundance of the two species in the habitat. To test for this, we calculated the ratio between the performance of *D. magna* clones in the presence of *D. pulex* reference clones and in the presence of the *D. magna* reference

clones, using both the frequency (competitive ability) and the total density (combining ability), and correlated these ratios with the ratio in abundance of the two species in the field.

3. Results

3.1. Effect of time and reference clone

Table 1: Repeated measure analysis of variance (RM ANOVA) testing for the effect of time, treatment and reference clone, for D. magna *and* D. pulex *reference clones separately, on two different response variables Competitive ability (frequency), Combining ability (total density). Significant p-values are indicated in bold.*

	D. magna						***D. pulex***					
	Competitive ab. (Freq.)			Combining ab. (Tot. dens.)			Competitive ab. (Freq.)			Combining ab. (Tot. dens.)		
Source	df	F	p	df	F	P	df	F	p	df	F	p
Time (Ti)	6,10	1.48	0.29	6,14	0.40	0.86	6,14	0.35	0.89	6,14	2.26	0.09
Reference cl (Re)	2,12	25.6	**<0.0001**	2,28	1.46	0.24	2,28	16.05	**<0.0001**	2,28	0.21	0.80
Re*Ti	12,20	1.83	0.11	12,28	1.11	0.38	12,28	1.35	0.24	12,28	0.51	0.89

In none of the variable-treatment combinations (neither for competitive ability, nor for combining ability, neither for competition with *D. magna,* nor for competition with *D. pulex* reference clones) did we observe a significant effect of time period the experimental clones were isolated from, or a significant time x reference clone interaction effect (Table 1). There was, however, a highly significant effect of reference clones for competitive ability. This implies that the *D. magna* reference clones strongly differed in average competitive ability (frequency: $F_{2,12} = 25.6$; $p < 0.0001$) when they are competing with the 21 experimental clones (Fig. 2A). The 1991 *D. magna* reference had a significant higher competitive ability than the 1964 reference clone ($F_{1,10} = 36.81$; $p = 0.0001$), and than the 1951 reference clone ($F_{1,10} = 14.07$; $p = 0.003$), and the 1951 reference clone had a significantly higher competitive ability than the 1964 reference clone ($F_{1,10} = 17.67$; $p = 0.002$) (Fig. 2A). There was also a significant difference in competitive ability between the *D. pulex* reference clones (frequency: $F_{2,12} = 16.05$; $p < 0.0001$; density $F_{2,12} = 137.3$; $p < 0.0001$). Overall, the Lake Ring reference clone had a higher competitive ability than the 'Oude meren' reference clone ($F_{1,14} = 13.94$; $p = 0.002$) and the 'Langdorp' reference clone ($F_{1,14} = 43.0$; $p < 0.0001$ (Fig. 2B). The fact that the reference clones differ so strongly in their competitive characteristics, lowers the power of our analysis to

detect differences among time periods. Combining ability did not differ between the *D. magna* reference clones, nor between the *D. pulex* reference clones.

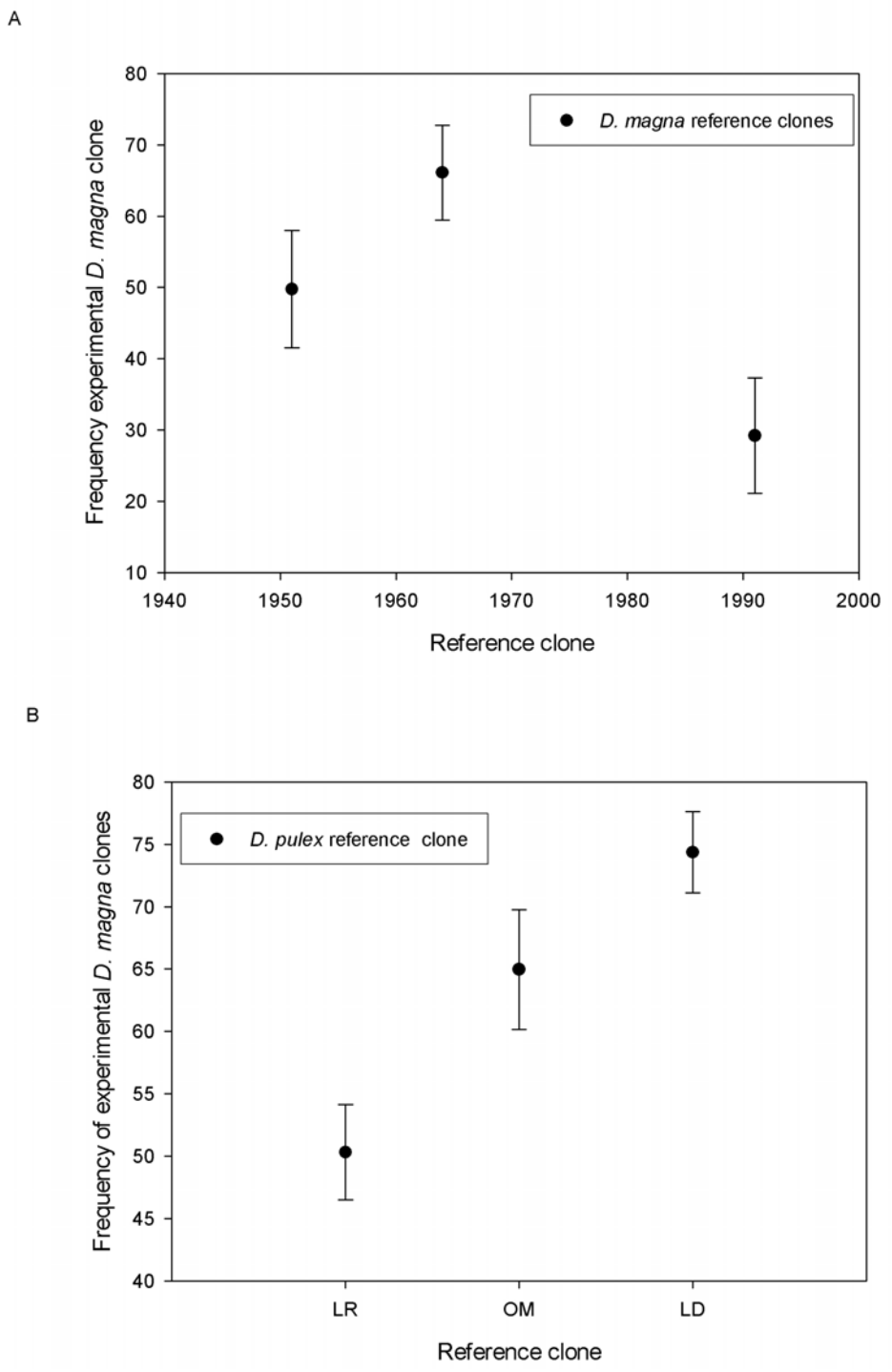

Fig. 2: A: Mean frequency ± SE of 21 experimental clones in the presence of the three D. magna *reference clones. B: Mean frequency of experimental clones in the presence of* D. pulex *reference clones (LR=Lake Ring; OM=Oude Meren, Belgium; LD=Langdorp, Belgium).*

3.2. Interclonal variation

One-way ANOVAs testing for interclonal variation in competition characteristics revealed differences between the 21 experimental clones for intraspecific competitive strength (frequency in presence of *D. magna*: $F_{20,38} = 3.15$; $p = 0.001$, Fig. 3A). No interclonal variation was observed, however, for intraspecific combining ability (total density: $F_{20,42} = 0.69$; $p = 0.80$), nor for competitive ability or combining ability in the presence of *D. pulex* (frequency: $F_{20,42} = 1.68$; $p = 0.07$; total density: $F_{20,42} = 1.59$; $p = 0.10$) (Table 2). A contrast analysis indicated that the average total density of the experimental *D. magna* clones in the *D. pulex* competition trials was significantly higher than average total density of the experimental *D. magna* clones in the *D. magna* competition trials ($F_{(1, 80)} = 5.10$; $p = 0.03$, Fig. 3B). The total densities of the pure cultures did not significantly differ from the clonal means of the total density in the intraspecific competition trails ($F_{(1, 80)} = 2.06$; $p = 0.15$, Fig. 3B).

Table 2: Interclonal variation for the response variables frequency of experimental D. magna *clones, and total density in the presence of* D. pulex *reference clones and in the presence of* D. magna *reference clones. Significant values are indicated in bold.*

***D. pulex* reference clone**	df	F	p-value
Competitive ability (Frequency)	20, 42	1.68	0.07
Combining ability (Total density)	20, 42	1.59	0.10
***D. magna* reference clone**			
Competitive ability (Frequency)	20, 38	3.15	**0.001**
Combining ability (Total density)	20, 42	0.69	0.80

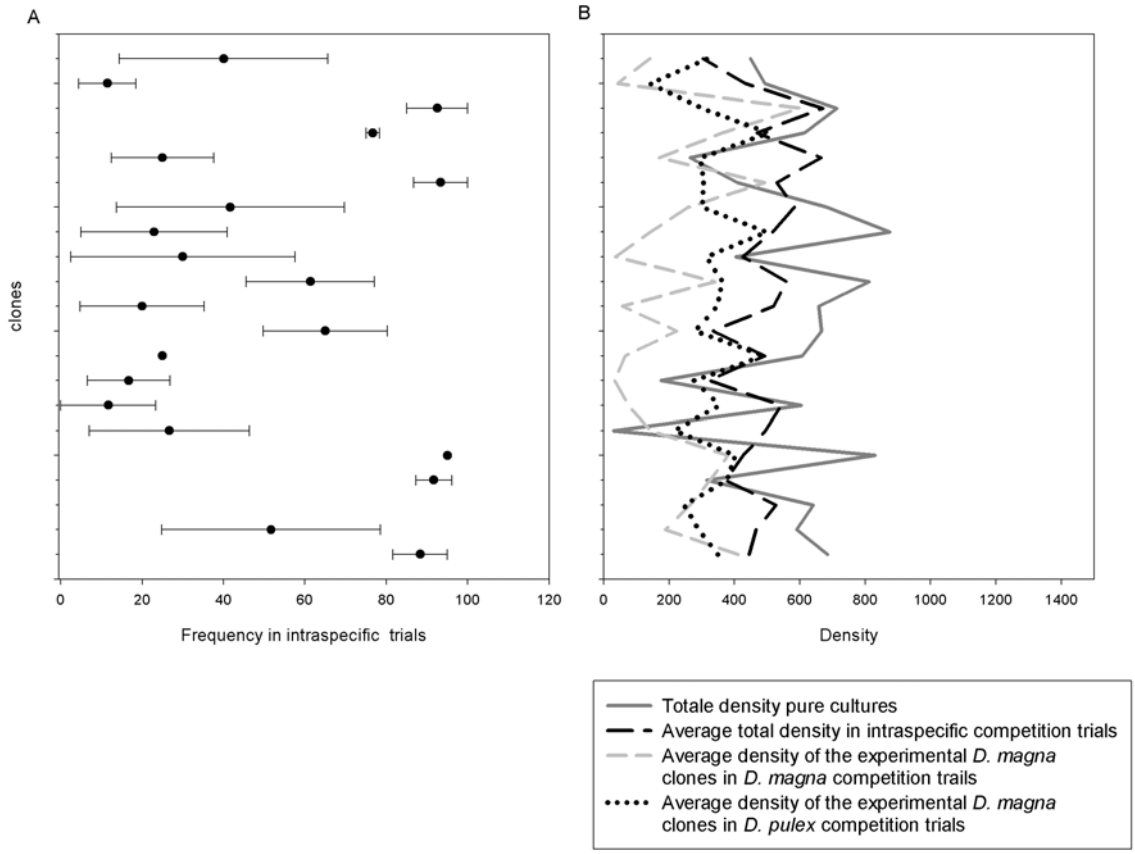

Fig. 3 A: Clonal means of frequency ± SE of 21 experimental D. magna *clones in intraspecific trials. B: Comparison of the total density of the pure cultures, the average total density in intraspecific trials, the average density of the experimental* D. magna *clones in the* D. magna *competition trials, and the average density of the experimental* D. magna *clones in the* D. pulex *competition trials for the 21 experimental clones.*

3.3. Competitive and combining ability in relation to population densities in the field

None of the correlations between competitive ability and combining ability, neither for intraspecific nor for interspecific interactions, with time were significant, indicating that competitive characteristics do not steadily increase nor decrease over time (Table 3). Table 3 also reports the results of the correlation analysis on competitive characteristics and the number of ephippia of *D. magna* and *D. pulex* in the lake sediments. The frequency of the experimental clones (competitive ability) in intra- and interspecific trials was not significantly correlated with the density of ephippia in either of the two species. Interspecific combining ability was, however, strongly correlated with the density of *D. pulex* (r = 0.61) in the field (Fig. 4C).

Ecological combining ability in intraspecific trials was not correlated with ephippium density in the field.

Table 3: Spearman Rank correlation matrix for all combinations between six experimental variables (Intersp. competitive ab.= Interspecific competitive ability; Intersp. combining ab.= Interspecific combining ability; Intrasp. competitive ab.=Intraspecific competitive ability; Intrasp. combining ab.= Intraspecific combining ability; Intersp. comp. ab/Intrasp. comp. ab = Interspecific competitive ability/Intraspecific competitive ability; Intersp. comb. ab./ Intrasp. comb. ab. = Interspecific combining. ability/Intraspecific combining ability) .and five environmental variables (time, D. pulex *density,* D. magna *density, total biomass and ratio* D. pulex/D. magna*). Significant values are indicated in bold.*

	Time	***D. pulex***	***D. magna***	**Biomass**	**Ratio**
Intersp. competitive ab.	-0.11	0.03	0.28	0.19	-0.04
Intersp. combining ab.	-0.009	**0.61**	0.44	0.42	**0.48**
Intrasp. competitive ab.	-0.18	-0.36	0.22	-0.16	**-0.66**
Intrasp. combining ab.	0.17	-0.05	0.19	0.06	-0.06
Intersp. comp ab./ Intrasp. comp ab.	0.007	0.1	-0.22	-0.09	**0.58**
Intersp. comb. ab./ Intrasp comb. ab.	-0.19	0.13	0.03	0.22	0.22

We observed a significant positive correlation between competitive ability with respect to conspecifics and with respect to *D. pulex* in our study set of 21 clones (r = 0.47, p = 0.03, Fig. 4A). There was, however, no significant correlation between combining ability with conspecifics and with *D. pulex* reference clones (Fig. 4B).

We observed that interspecific combining ability was positively related to the ratio in ephippial density of *D. pulex* to that of *D. magna* in the field (Table 3, Fig. 4D). We also observed a significantly positive correlation between the ratio of competitive ability in inter- to intraspecific trials and the ratio in ephippial density of *D. pulex* to that of *D. magna* in the field (Fig. 4E). Finally, we observe that competitive ability of the experimental clones in intraspecific trials was significantly negatively correlated with the ratio of the density of ephippia of *D. pulex* to that of ephippia of *D. magna* (Table 3; Fig. 4F). In other words, competitive strength in intraspecific trials increased as the relative abundance of *D. magna* in the field increased compared to that of *D. pulex*. The data thus suggest that the ability of clones to share resources with the competing species is associated with the relative abundance of conspecifics compared to that of the other species in the natural habitat.

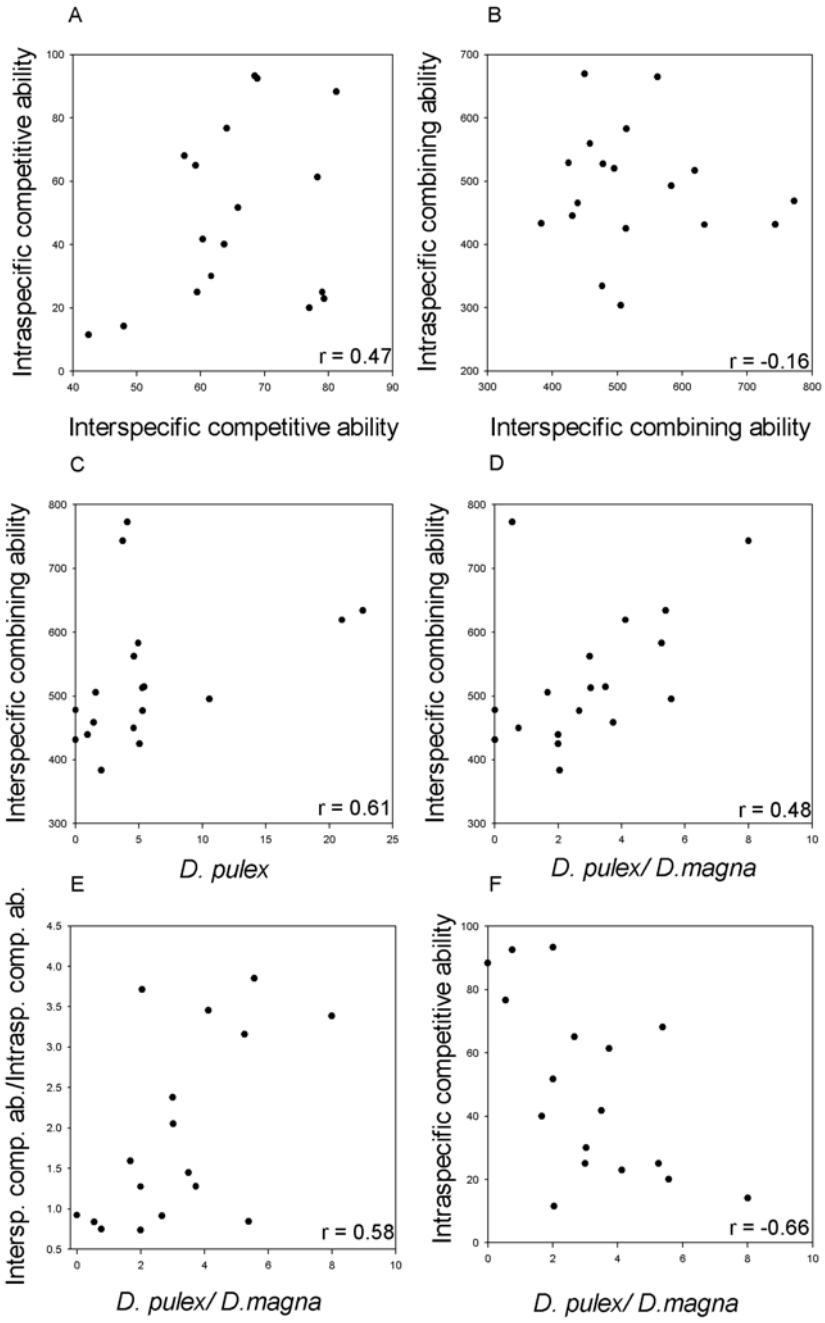

Fig. 4 A) Correlation between intra- and interspecific competitive ability; B) Correlation between inter- and intraspecific combining ability. C) Correlation between interspecific combining ability and the field density D. pulex. *D) Correlation between interspecific combining ability and the ratio* D. pulex/D. magna. *E) Correlation between the ratio of inter- and intraspecific competitive ability and the ratio* D. pulex/D. magna. *F) Correlation between the ratio of inter- and intraspecific combining ability and the ratio* D. pulex/D. magna.

4. Discussion

Our experiments yield evidence for micro-evolutionary changes in both competitive as well as ecological combining ability of a natural population of *D. magna* in relation to competition with conspecifics and with another large filterfeeding cladoceran, *D. pulex*. We observed interclonal variation in competitive ability in relation to conspecifics. The relative abundances of the 21 experimental clones in our competition trials with conspecifics differed significantly, even though our replicate observations involved different reference clones, which introduced variation at the within-clone level, thus reducing the likelihood to detect interclonal differences. We also observed strong differences in the three reference clones we used in our competition trials with *D. magna*. These three reference clones were derived from Lake Ring sediment, and differed strongly in their average competitive ability when confronted with our set of 21 experimental clones. We did not observe significant interclonal variation in competitive ability with respect to competition with *D. pulex*. Our ANOVA failed to detect interclonal variation in combining ability in interaction with conspecifics and with *D. pulex*. This may in part be due to lower power of the test because of the use of different reference clones. As we observed a relationship between combining ability in interspecific interactions and the density of *D. pulex* in the field (see Results), we also screened for the presence of interclonal variation by ranking the clones according to ephippium density of *D. pulex* in the corresponding depth interval, and then checking for differences in combining ability between the 25 percentile clones isolated from depth intervals with lowest ephippium density of *D. pulex,* and the 25 percentile clones isolated from depth intervals with the highest *D. pulex* ephippium density, using clonal means as input data. These two groups of clones indeed significantly differed in combining ability (total density: $p = 0.01$), showing that there is genetic variation for interspecific combining ability in our set of 21 clones. Given that genetic variation for competition traits is observed, a pattern of local adaptation might be expected, in which the local *D. magna* population adapt to a changing competitive environment (Connell, 1980; Schluter, 2001).

We did observe structure in variation in competitive and combining ability. Unlike Steiner et al. (2007), who found a decline in interspecific competitive interaction over time, we do not find a relation with time in competitive ability or combining ability in neither intra- nor interspecific interactions (Hypothesis 1, 3, 5 and 7). We do, however, observe a relationship between competitive characteristics with density profiles in the field as measured by changes in ephippium density in the sediment core. For instance, we observe a strong correlation between interspecific combining ability and the abundance of *D. pulex* in the field (Hypothesis 8; no significant correlations are observed for intraspecific combining ability, Hypothesis 4). In

addition, there is a relation between interspecific combining ability and the ratio of abundance in ephippia of the competing species *D. pulex* to that of conspecifics. Although we do not observe a direct relationship between intra- and interspecific competitive ability and the amount of ephippia of *Daphnia* species in the field (Hypothesis 2 and 6), there is a relationship between the ratio of inter- to intraspecific competitive ability and the ratio of abundance in ephippia of the competing species to that of conspecifics. The higher the relative amount of ephippia of conspecifics in the sediment layer, the stronger intra- compared to interspecific competitive ability of the experimental clones derived from the corresponding time period.

Our data strongly point to a genetic tracking of the competitive environment by the local *D. magna* population of Lake Ring, both in terms of competitive ability (adjusting strength in competitive interactions) as well as in terms of combining ability (character displacement).

This genetic tracking is not perfect, as other factors no doubt interfere with the pattern, but given the multitude of environmental changes, including other biotic interactions such as predation and parasitism (Kerfoot and Sih, 1987; Cousyn et al., 2001; Gandon et al., 2001; Chapter 1) that may interfere, the pattern we observe is strikingly strong. A potentially important point to consider in the interpretation of our results is that the *Daphnia* community of Lake Ring in the past century was largely dominated by *D. pulex*, with the abundance pattern of *D. magna* running in parallel to that of *D. pulex* during a large part of the time. It is only since the shift to clear-water conditions in the 1980s that the relative abundance of *D. magna* and *D. pulex* switched. This may contribute to our observation that there is a much stronger relationship between ecological combining ability with *D. pulex* and the density of *D. pulex* in the field than between ecological combining ability with conspecifics and the density of *D. magna* in the field. Yet, an alternative explanation is that the population did not respond to changes in *D. magna* densities with changes in combining ability with conspecifics. The lack of a relationship between the ratio of inter- to intraspecific combining ability and the relative abundance of *D. pulex* and *D. magna* is in line with the latter interpretation.

For competitive ability, the observed relationships with population densities in the field are very different. Here too, our data provide evidence that the *D. magna* population in Lake Ring does genetically adjust its competitive ability to the competitive environment. More specifically, the competitive ability of the experimental clones in intraspecific trials is negatively correlated with the ratio of abundance in ephippia of the competing species to that of conspecifics. In other words, competitive strength in intraspecific trials increased as the relative abundance of *D. magna* in the field increased compared to that of *D. pulex.* This pattern is corroborated by the results on the competitive ability of the reference clones, which are based on a broader set of data. Indeed, the ranking of competitive ability of the reference clones (1964<1951<1991; the

significance between the 1991 and the 1964 reference clones is significant) corresponds to the ranking in the density of *D. magna* ephippia in the field (see Fig. 1).

Resource competition is frequent cited as major organizational force within zooplankton communities (Tilman, 1994; Hu and Tessier, 1995; Cáceres, 1998). Despite this recognition, only few studies have experimentally examined the evolutionary role of inter- and intraspecific competitive and combining ability (though see Steiner at al., 2007). In this study we found that competitive characteristics of *D. magna* clones hatched from different sediment layers of Lake Ring were related with density profiles in the field as measured by changes in ephippium density in the sediment core, rather than with time. Interspecific combining ability in interactions with *D. pulex* was strongly correlated with the abundance of this species in the field. Furthermore, there was a positive relationship between the ratio of inter- to intraspecific competitive ability and the ratio of abundance in ephippia of the competing species to conspecifics, and a negative relationship between the intraspecific competitive ability and the the ratio of abundance in ephippia of the competing species to conspecifics. We also found evidence for genetic variation in intraspecific competitive ability and interspecific combining ability. Taken together, these results suggest that the local *D. magna* population is able to genetically track the changes in the competitive environment, both in terms of competitive ability and combining ability. More specifically, it seems that competitive interactions with the congener *D. pulex* are avoided by character displacement, resulting in an increase of ecological combining ability. This was not observed with respect to intraspecific competition, probably reflecting the difficulties for reducing the strength of competitive interactions with conspefics. Rather, for intraspecific interactions we observe that the competitive ability is adjusted to the relative abundance of conspecifics compared to the other large-bodied *Daphnia* present in the lake. Overall, our results thus suggest a subtle interplay between intra- and interspecific competition in shaping the evolution of competition traits.

5. Acknowledgments

We thank J. Verbesselt for statistical support, and C. Van Damme and E. Ryken for assistance with the experiment. H. Michels is a doctoral fellow of the Institute for the Promotion of Innovation through Science and Technology in Flanders. This research was financially supported by EU IP project EUROLIMPACS (GOCE-CT-2003-505540), FWO G.0269.04 and KULeuven Research Fund project OT/04/23.

6. References

Aarssen, L.W., 1983. Ecological combining ability and competitive combining ability in plants - Toward a general evolutionary-theory of coexistence in systems of competition. American Naturalist 122: 707-731.

Brendonck, L., & L. De Meester, 2003. Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. Hydrobiologia 491: 65-84.

Cáceres, C.E., 1998. Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. Ecology 79: 1699-1710.

Capaul, M., & D. Ebert, 2003. Parasite-mediated selection in experimental *Daphnia magna* populations. Evolution 57: 249-260.

Chase, J. M. & M. A. Leibold, 2003. Ecological niches: linking classical and contemporary approaches, University of Chicago Press, US.

Connell, J.H., 1980. Diversity and the coevolution of competitors, or the ghost of competition past. Oikos 35: 131-138.

Cousyn, C., L. De Meester, J.K. Colbourne, L. Brendonck, D. Verschuren, & F. Volckaert, 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. Proceedings of the National Academy of Sciences of the United States of America 98: 6256-6260.

Gandon, S., V.A.A. Jansen, & M. Van Baalen, 2001. Host life history and the evolution of parasite virulence. Evolution 55: 1056-1062.

Gurevitch, J., L.L. Morrow, A. Wallace, & J.S. Walsh, 1992. A metaanalysis of competition in field experiments. American Naturalist 140: 539-572.

Hairston, N.G., W. Lampert, C.E. Caceres, C.L. Holtmeier, L.J. Weider, U. Gaedke, J.M. Fischer, J.A. Fox, & D.M. Post, 1999. Rapid evolution revealed by dormant eggs. Nature 401: 446.

Hu, S.X.S., & A.J. Tessier, 1995. Seasonal succession and the strength of intraspecific and interspecific competition in a *Daphnia* assemblage. Ecology 76: 2278-2294.

Jeppesen, E., P. Leavitt, L. De Meester, & J.P. Jensen, 2001. Functional ecology and palaeolimnology: using cladoceran remains to reconstruct anthropogenic impact. Trends in Ecology & Evolution 16: 191-198.

Kerfoot, W.C., W.R. Demott, & C. Levitan, 1985. Nonlinearities in competitive interactions - component variables or system response. Ecology 66: 959-965.

Kerfoot, W. C. & A. Sih, 1987. Predation, direct and indirect impacts on aquatic communities, University press of New England, Hanover, NH.

Kerfoot, W.C., J.A. Robbins, & L.J. Weider, 1999. A new approach to historical reconstruction: combining descriptive and experimental paleolimnology. Limnology and Oceanography 44: 1232-1247.

Lampert, W., 1987. Feeding and nutrition in *Daphnia*. R.H. Peters , R. de Bernardi (Eds.) '*Daphnia*' Memorie dell' Instituto Italiano di Idrobiologia 45: 143-192.

Michels, H., S. L. Amsinck, E. Jeppesen, & L. De Meester, In press. Hydrobiologia.

Pritchard, J.R., & D. Schluter, 2001. Declining interspecific competition during character displacement: summoning the ghost of competition past. Evolutionary Ecology Research 3: 209-220.

Schluter, D., 2000. Ecological character displacement in adaptive radiation. American Naturalist 156: S4-S16.

Schluter, D., 2001. Ecology and the origin of species. Trends in Ecology & Evolution 16: 372-380.

Statsoft, 1994. STATISTICA for the windows operating system. Inc. Tulsa, UK.

Steiner, C.F., C.E. Cáceres, & S.D.P. Smith, 2007. Resurrecting the ghost of competition past with dormant zooplankton eggs. The American Naturalist 169: 10-20.

Tilman, D., 1994. Competition and biodiversity in spatially structured habitats. Ecology 75: 2-16.

Verschuren, D., & L.F. Marnell, 1997. Fossil zooplankton and the historical status of westslope cutthroat trout in a headwater lake of Glacier National Park, Montana. Transactions of the American Fisheries Society 126: 21-34.

CHAPTER 5 AN EVOLUTIONARY PERSPECTIVE OF *DAPHNIA* ECTOPARASITE SUSCEPTIBILITY AND IMMUNOCOMPETENCE IN RELATION TO PREDATION IN LAKE RING.

Michels H., Decaestecker E., Pauwels K., Kuipers K. & De Meester L.

ABSTRACT

In this chapter we reconstructed evolutionary change through time in ectoparasite susceptibility and immunocompetence of a natural *Daphnia magna* population in a shallow lake. We hatched clones from sediments that were deposited in three subsequent periods in the history of Lake Ring (Denmark): the Pre-eutrophication period (1908-1937), a Turbid water period (1960-1980), and a Clear-water period (1986-2000). These clones were exposed to a standardized suspension of sediment of the lake in the presence and absence of fish kairomone. We observed interclonal variation in susceptibility for infestation by *Brachionus* as well as a significant effect of period. Clones derived from the most recent Clear-water period, were less infected by the epibiont *Brachionus rubens* than clones derived from the Turbid period. No significant changes with time were found in Phenoloxidase activity, a factor involved in *Daphnia* immunocompetence, which might indicate that PO does not play a crucial role in the resistance against *Brachionus*.

We observed a significant interaction between phototactic index (PI) of the studied clone and time period on *Brachionus* infectivity. More specifically, our data indicate that positively phototactic clones from the Pre-eutrophication period show higher *Brachionus* infectivity than clones with a less positively phototactic behavior, whereas no such association was observed in the other two time periods.

1. Introduction

The structuring role at the level of both communities and individual populations of predators, is well studied (Kerfoot and Sih, 1987; Gurevitch et al., 1992; Cousyn et al., 2001). Parasites are also known to influence host population dynamics and genetics, because of their negative impact on host reproduction and survival combined with their host genotype dependent infectivity (Gandon et al., 2001; Little, 2002; Woolhouse et al., 2002). The long viability of dormant stages of zooplankton, and the fact that they are deposited in stratified, datable lake sediment, allows comparing traits of hatched individuals derived from different sediment depths, and thus from different time periods ("resurrection ecology", Kerfoot et al., 1999). Since parasites also produce dormant spores that can remain infective for years (Decaestecker et al., 2004), it is possible to confront hatched *Daphnia* clones with standardized suspensions of infective spores, and thus reconstruct evolutionary changes in *Daphnia* parasite susceptibility and immunocompetence. In the present study, we follow this approach to monitor changes in intrinsic susceptibility to infestation with an epibiont, the rotifer *Brachionus rubens*. *Daphnia* are commonly parasitized by endo- as well as ecto-parasites (Stirnadel and Ebert, 1997; Decaestecker et al., 2005). Endo-parasites have strong negative effects on host fecundity and survival, whereas epibionts (ecto-parasites) infect only external parts of the *Daphnia* and have a small impact on the host population. Nevertheless, epibionts can reduce the buoyancy and mobility of the zooplankton, making it more difficult for the zooplankton to obtain food and to escape predators (Green, 1974; Chiavelli et al., 1993).

There is still insufficient knowledge on mechanisms determining immunocompetence in *Daphnia*, but an important mechanism so far recognized is the expression of Phenoloxidase (PO) acitivity (Mucklow et al., 2004). Phenoloxidase is an active enzyme that is part of the prophenoloxidase activation system (Pro-POAS), in which Prophenoloxidase is transformed via an enzymatic cascade into Phenoloxidase (PO). PO leads to the production of quinines that are directly toxic to bacteria, fungi and eukaryotic parasites, and that produce melanotic capsules that surround parasites (Ashida & Brey, 1997). Ebert & Mucklow (2003) suggested adaptive variation in PO activity of *Daphnia* and showed that PO activity reflects parasite resistance. Their results also indicate that this resistance is costly.

Earlier work has shown that there is a trade-off between the behavioral avoidance of mortality by fish predation through diel vertical migration and the probability to take up parasite spores (Decaestecker et al., 2004). *Daphnia* exhibit a broad range of induced behavioral, morphological, and life history defense mechanisms to avoid predation by fish (e.g. Stibor, 1992; Boersma et al., 1998). Phototactic behavior (day-time depth) may interfere with the probability of infection or infestation intensity with epibionts. Expectations are different for

epibionts than for endoparasites, as infections depend on encounter rates with free-swimming ectoparasites rather than on the take-up of spores from the sediments. In the case of *Brachionus rubens*, for instance, the epibiontic species is capable of sustained population growth in the absence of its host (Iyer and Ramakrishna, 2004).

In this study, we investigated whether a natural *Daphnia* population shows a signature of evolutionary changes through time in epibiont (*Brachionus rubens*) susceptibility and immunocompetence. We also explored whether habitat selection behavior of the host clones (depth preference, measured as phototactic behavior) has an impact on infestation rate of the host. We hatched *D. magna* clones from the layered sediments of a shallow lake, Lake Ring (Denmark), in which the *Daphnia* population exhibited an evolutionary response in predator-induced traits in relation to habitat restoration, reflected by a significant period x fish kairomone interaction in phototactic behavior of the *Daphnia* clones (Chapter 2). These *D. magna* clones were exposed to Lake Ring sediment in the presence and absence of fish kairomone. We tested whether there are differences in parasite susceptibility and PO activity in *Daphnia* among time periods, and whether these variables are influenced by predator avoidance behavior and the presence of fish kairomone.

2. Material and Methods

2.1. Study site

Lake Ring is a relatively shallow lake in central Jutland (Denmark), which was subjected to strong eutrophication during the beginning of the 20th century because of sewage inflow. In an effort to restore the ecosystem, the sewage inflow was stopped in 1970. This led to a very gradual recovery, resulting in a drastic increase in water clarity from the mid-1980s and onwards (for more details and a description of Lake Ring, see Introduction). We can therefore distinguish three successive periods in the history of Lake Ring: the Pre-eutrophication period (1908-1937), the Turbid water period (1960-1980) and the Clear-water period (1986-2000).

2.2. Experimental design and origin of clones

Three sediment cores (length: 30 cm, diameter 15 cm) were recovered from Lake Ring. The cores were sectioned horizontally into 0.5 cm slices, packed in light-tight recipients and stored at 4 °C until further processing. We hatched *D. magna* from the dormant egg banks by exposing the sediments in a thin layer to hatching stimuli (20°C, fresh medium, 16L: 8D photoperiod). To reduce the amount of other cladoceran eggs that would hatch, the sediment was first filtered over a sieve with a mesh size of 250 µm.

Six, eleven and nine clones from the Pre-eutrophication period, the Turbid and the Clear-water period, respectively, were successfully hatched from the sediment of Lake Ring. Prior to the experiment, each clone was cultured as three individual maternal lines for at least two generations under standardized conditions (1 individual per 100 ml, 20 ± 1° C, L:D 14:10, 2 x 10^5 *Scenedesmus obliquus* cells ml^{-1}) to exclude maternal effects. Core slices of 60 depths of three different cores were mixed in order to obtain a standardized sediment suspension. We added 400 ml of standardized sediment suspension in each experimental jar, and diluted it until 500 ml with fish medium or dechlorinated tap water. We then allowed the sediment to settle for one day (per experimental jar ± 28 cm^3 of sediment). For the fish medium, dechlorinated tap water was conditioned by the presence of three *Leuciscus idus* fish (approximately 6 cm) in a 20 L aquarium for 24 h (final concentration in experimental jars was equivalent to 3 fish per 100 L). The water was then filtered over 0.45-µm.

At the start of the experiment, each 500 ml jar was inoculated with five newborns (younger than 24 h) of one clone. For 22 days, the *Daphnia* were exposed to sediment suspension in the absence of presence of fish kairomone (156 experimental jars in total). The first three days, cultures were fed daily with a relatively low amount of food (1 x10^5 algal *Scenedesmus obliquus* cells ml^{-1}) to stimulate a benthic grazing behavior in order to increase contact with the sediment and thus increase the likelihood of infection with parasites and epibionts (Ebert et al., 1997). Afterwards, the amount of food was doubled to enhance parasite growth (Pulkkinen and Ebert, 2004). Every two days, 20 % of the medium was refreshed and juveniles were removed. On day 22, all individuals were visually checked for infection, and infection rate (% infected individuals per experimental jar). Two out of five animals per experimental jar were dissected and thoroughly checked for the presence of parasites using phase-contrast microscopy at 400x magnification.

2.3. Immunocompetence

We measured PO activity in *Daphnia*, based on a protocol of Mucklow and Ebert (2003). After 22 days of exposure, 2 µl hemolymph of two animals from each jar was sampled to assess PO activity. The *Daphnia* were placed on a filter paper for drying until all the excess of water trapped between the carapace shields was removed. The hemolymph was then collected by pricking the *Daphnia* with a needle close to the hart, and collecting it in a microcapillary tube (BLAUBRAND, intraEnd cat. no. 709101). To avoid sampling bias across treatments, the hemolymph samples of animals belonging to different treatments were collected in random order.

PO activity was measured following the protocol used by Mucklow & Ebert (2003). We measured dopachrome production, which is the result of oxidation of L-dopa by the enzyme PO, as an estimate for PO activity. Absorbance of dopachrome can be measured with a spectrophotometer at 475 µm (Reeson et al., 1998). Each sample of 2 µl of hemolymph was deposited in an Eppendorf tube with 150 µl of phosphate-buffered saline (PBS: 0.15 M NaCl, 10 mM $Na_2HPO_4.2H_2O$, pH 7.4 with o-phosphoric acid). This sample was placed (*in duplo,* 2 x 75 µl) in a well on a 96 well plate. Then 225 µl of 20mM L-dopa was added in each well and the plate was transferred into the spectrophotometer. We ran a control measurement (using PBS and L-dopa, but without hemolymph) together with the investigated samples on 10 wells in each well plate. PO activity of the samples was calculated as the difference of the absorbance values measured after 4.5h and at start (absorbance after 4.5h - absorbance at time 0). For convenience, we multiplied this difference by a factor of 1.000 to yield activity units (see also Mucklow and Ebert, 2003). The average activity units of the "nonhemolymph" controls were subtracted from all other samples in each well.

2.4. Data analysis

We screened for the presence of genetic variation for the different response variables (infection rate, PO activity) as an indication of evolutionary potential for the traits in the resident *D. magna* population of Lake Ring. Therefore, we performed one way analyses of variance with clone as a random factor in the presence and absence of fish kairomone.

To test for the effect of time period, fish kairomone and vertical migration (phototactic index as a continuous covariable, exponentially transformed) and their interaction effects, we performed a three-way ANCOVA on the parasite infection rates and PO activity. PO was logarithmic transformed to comply with assumptions of homogeneity of variance. Observations on clones were used as replicates within time period; to this end, clonal means of the response variables were used to avoid pseudo-replication. Phototactic behavior was used as a covariate, to verify to what degree phototactic behavior may modulate the relationship between the independent factors and the response variables. Data on phototactic behavior (phototactic index PI) of the different clones were not collected in this study, but were obtained from an earlier study in which the phototactic behavior of all clones used in the present study was determined in the absence and presence of fish kairomone (Chapter 2).

We tested for the effect of *Brachionus* infection on PO activity in the presence and absence of fish kairomone by performing a general linear model with PO activity as response variable and *Brachionus* infection rate as covariate. All statistical analyses were performed using STATISTICA (Statsoft, 1994).

3. Results

Brachionus rubens, an epibiontic rotifer, was the main species that was picked up by the *Daphnia* upon exposure to Lake Ring sediment. Sporadically, an unknown brood parasite infection was noticed as well, but these infections were relatively minor and occurred at low frequency (only 0.05 % of the individuals was infected), it was not analyzed further.

Table 1: Three-way ANCOVA testing for the effect of period, fish kairomone (discrete, fixed factors) and PI (continuous covariable) on Brachionus *infection rate and PO activity*

	Source of variation	df	F	p
PO	Period	2, 40	0.06	0.94
	Fish kairomone	1, 40	0.01	0.93
	PI	1, 40	0.01	0.97
	Period x Fish kairomone	2, 40	0.20	0.82
	Period x PI	2, 40	0.08	0.92
	Fish kairomone x PI	1, 40	0.05	0.82
	Fish kairomone x PI x Period	2, 40	0.15	0.86
Brachionus Infection rate	Period	2, 40	3.11	**0.05**
	Fish kairomone	1, 40	0.60	0.44
	PI	1, 40	0.42	0.52
	Period x Fish kairomone	2, 40	1.81	0.18
	Period x PI	2, 40	3.64	**0.03**
	Fish kairomone x PI	1, 40	0.38	0.53
	Fish kairomone x PI x Period	2, 40	1.47	0.24

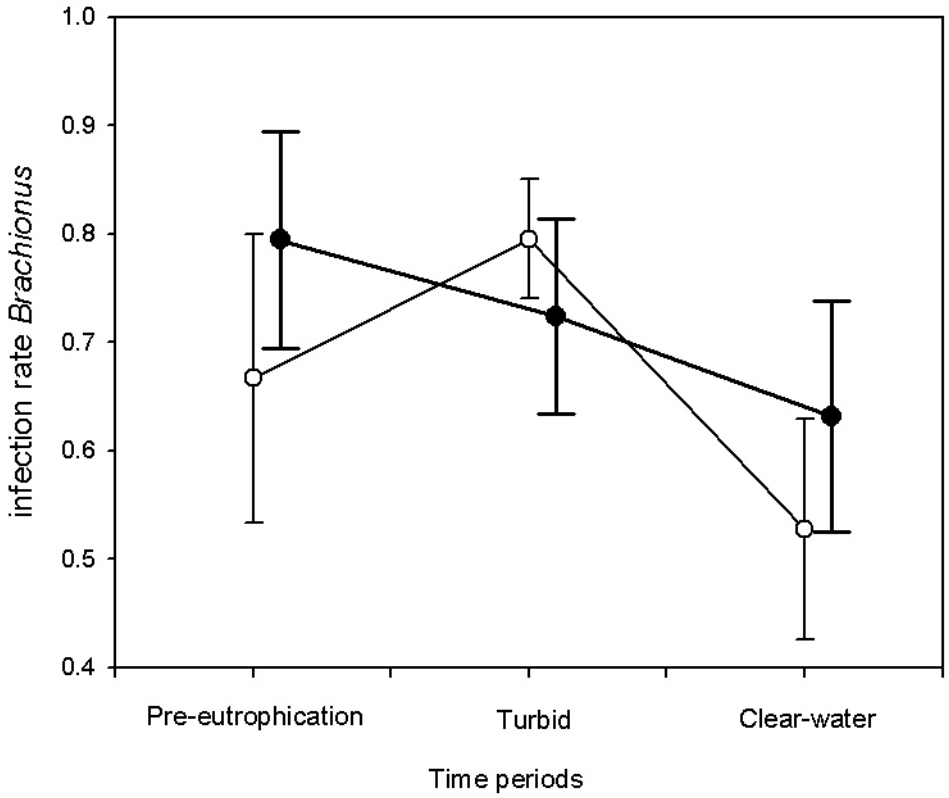

Fig. 1: Population mean ± SE of Brachionus *infection rate in the presence (solid symbols) and absence (open symbols) of fish kairomone for three time periods in the history of Lake Ring. Population means were calculated using clonal means.*

Time period had a marginally significant (p = 0.05) effect on *Brachionus* infection rate (Fig. 1, Table 1). A post-hoc test indicates that *Brachionus* had a significantly lower infection rate in *Daphnia* clones derived from the Clear-water period than in clones derived from the Turbid period (mean *Brachionus* infection rate Clear-water period 0.57 ± 0.07, *Brachionus* infection rate Turbid period 0.76 ± 0.05, p = 0.03). We also observed a significant interaction effect between time period and phototactic behavior on *Brachionus* infection rate. In the Pre-eutrophication period, PI and *Brachionus* infection rate were positively correlated, both in the presence and absence of fish (absence of fish kairomone r = 0.75, p = 0.08; presence of fish kairomone r = 0.67, p = 0.14). This indicates that clones that migrated towards the bottom (low PI) suffer less from *Brachionus* infection (Fig. 2). In the other two periods, this correlation is absent; in the presence of fish in the Clear-water period, there is even a trend for a negative relationship, but the pattern is weak and not significant (r= -0.51, p = 0.15).

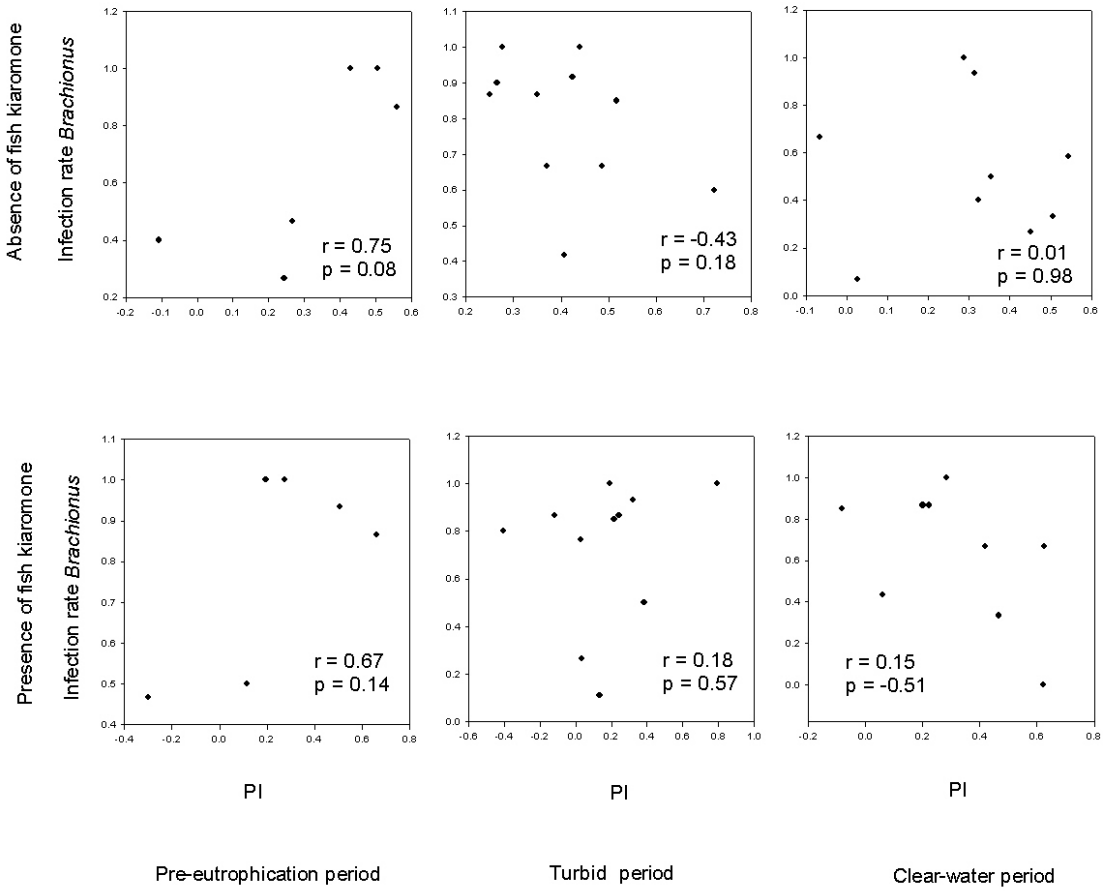

Fig. 2: Scatterplots showing correlations between Brachionus *infection rate and phototactic index (PI) for the three different time periods and in the presence and absence of fish kairomone. For each of the correlations the Pearson correlation coefficient and associated p value is given.*

None of the factors studied (time period, presence of fish kairomone, phototactic behavior) had a significant effect on PO activity in the experimental *Daphnia* as revealed by the three-way ANOVA (Table 1).

Table 2: Interclonal variation for Brachionus *infection rate and for PO activity in the absence and presence of fish kairomone for our study set of clones hatched from Lake Ring sediment*

Absence fish kairomone	**df**	**F**	**p-value**	**Presence fish kairomone**	**df**	**F**	**p-value**
Infection rate *Brachionus*	25, 52	1.50	0.10		25, 48	2.32	0.005
PO activity	25, 38	2.39	0.007		25, 40	0.58	0.92

In the presence of fish kairomone, we observed significant interclonal genetic variation in *Brachionus* infectivity in our set of study clones. In the absence of fish kairomone, interclonal variation was not significant (p=0.10, Table 2). PO activity showed significant interclonal variation in the absence but not in the presence of fish kairomone (Table 2).

4. Discussion

Our results reveal genetic variation in susceptibility to *Brachionus rubens* infection in the resident population of *Daphnia magna* in Lake Ring. Moreover, our data show a change in genetically determined susceptibility with time, as clones derived from the recent Clear-water period (from 1986 onwards) suffered significantly less from *Brachionus* infection than clones derived from the preceding turbid water period. Our data thus clearly indicate micro-evolution with respect to susceptibility to epibiont infection in this *D. magna* population. The driving force of this micro-evolution is at this stage difficult to determine, as Decaestecker et al. (2004) did not report strong differences in *Brachionus* infection rates among different time periods in Lake Ring. The amount of infections obtained by Decaestecker et al. (2004) was, however, much lower than in the present study, reflecting the fact that we exposed our experimental animals to a much higher amount of sediment. It might be that the low amount of infection rates introduced chance effects such that differences in *Brachionus* densities were not revealed in Decaestecker et al. (2004), but this awaits testing. The reduced susceptibility to *Brachionus* of some clones may be the result of a specific response or a more general immunocompetence. Our data do not allow us to make a distinction between these two possibilities. We tested for one trait related to immunocompetence in *Daphnia*, phenoloxidase activity, but did not find a significant relation between PO activity and susceptibility of the clones to *Brachionus* infection. *Brachionus*, and epibionts in general, may exert selection pressure on their host in several ways. They can influence the buoyancy, visibility and mobility of the zooplankton, making it more difficult for the zooplankton to obtain food and to escape predators (Green, 1974; Willey et al., 1990; Chiavelli et al., 1993). In addition, they may also compete with the host for food (Matveeva, 2004)

The presence of fish kairomone did not increase *Brachionus* infection rates, indicating that there was no fish-induced effect that resulted in an increase of the risk of infection by *Brachionus*. We did find, however, a significant interaction effect between PI and period on the *Brachionus* infection rate. In the Pre-eutrophication period there was a positive relationship between phototactic behavior and *Brachionus* infection rate. Staying closer to the water surface (more positively phototactic behavior) increased *Brachionus* infection rates. This observation may be related to the fact that rotifers often tend to stay close to the water surface during the day

(Gilbert and Hampton, 2001). This may result in higher encounter rates of positively phototactic clones with *Brachionus* then for clones that reside deeper in the water column. This pattern is opposite to the observation that negatively phototactic behavior induces higher infection rates of micro-parasites, due to an increased contact with infective spores (Decaestecker et al., 2002). In the case of infection with *Brachionus*, there is a positive association between predation risk (by residing in the upper water layers rather than seeking refuge in the darker water layers close to the bottom of the lake; De Meester et al., 1999) and the risk of infection, rather than a trade-off as reported for predation risk and risk of infection by micro-parasites. No relationship between phototactic behavior and infection rate with *Brachionus* was observed for the Turbid and Clear-water period. One, admittedly speculative, explanation for this observation is that the increased resistance of the *Daphnia* clones derived from the more recent sediments wipes out the difference resulting from variation in encounter rates.

Although we found genetic variation for PO activity in the absence of fish kairomone, our data do not reveal a micro-evolutionary change in time for PO activity in this population.

5. References.

Ashida, M., & P.T. Brey, 1995. Role of the integument in insect defense: pro-phenol oxidase cascade in the cucticular matrix. Proceedings of the National Academy of Sciences of the United States of America 92: 10698-10702.

Boersma, M., P. Spaak, & L. De Meester, 1998. Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: the uncoupling of responses. American Naturalist 152: 925-927.

Chiavelli, D.A., E.L. Mills, & S.T. Threlkeld, 1993. Host Preference, seasonality, and community interactions of zooplankton epibionts. Limnology and Oceanography 38: 574-583.

Cousyn, C., L. De Meester, J.K. Colbourne, L. Brendonck, D. Verschuren, & F. Volckaert, 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. Proceedings of the National Academy of Sciences of the United States of America 98: 6256-6260.

De Meester L., Dawidowicz P., Van Gool E. , & Loose C.J., 1999. Ecology and evolution of predator-induced behavior of zooplankton: depth selection behavior and diel vertical migration. In: Tollrian R. & Harvell C.D. (eds.), The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey:

Decaestecker, E., L. De Meester, & D. Ebert, 2002. In deep trouble: habitat selection constrained by multiple enemies in zooplankton. Proceedings of the National Academy of Sciences of the United States of America 99: 5481-5485.

Decaestecker E., Lefever C., De Meester L., & Ebert D., 2004. Haunted by he past: Evidence for dormant stage banks of microparasites and epibionts of *Daphnia*. Limnology and Oceanography 49: 1355-1364.

Decaestecker, E., S. Declerck, L. De Meester, & D. Ebert, 2005. Ecological implications of parasites in natural *Daphnia* populations. Oecologia 144: 382-390.

Ebert, D., R.J.H. Payne, & W.W. Weisser, 1997. The epidemiology of parasitic diseases in *Daphnia.* Ecological studies. In: Dettner et al. (ed.), Vertical food web interactions. Springer-Verlag, Berlin: 91-111.

Gandon, S., V.A.A. Jansen, & M. Van Baalen, 2001. Host life history and the evolution of parasite virulence. Evolution 55: 1056-1062.

Gilbert, J.J., & S.E. Hampton, 2001. Diel vertical migrations of zooplankton in a shallow, fishless pond: a possible avoidance-response cascade induced by notonectids. Freshwater Biology 46: 611-621.

Green, J., 1974. Parasites and epibionts of Cladocera. Transactions of the zoological society of London 417-515.

Gurevitch, J., L.L. Morrow, A. Wallace, & J.S. Walsh, 1992. A metaanalysis of competition in field experiments. American Naturalist 140: 539-572.

Iyer, N., & R. Ramakrishna, 2004. Effect of the epizoic rotifer *Brachionus rubens* on the population growth of the three cladoceran species . Hydrobiologia 325-332

Kerfoot, W.C., J.A. Robbins, & L.J. Weider, 1999. A new approach to historical reconstruction: combining descriptive and experimental paleolimnology. Limnology and Oceanography 44: 1232-1247.

Kerfoot, W. C. & A. Sih, 1987. Predation, direct and indirect impacts on aquatic communities, University press of New England, Hanover, NH.

Little, T.J., 2002. The evolutionary significance of parasitism: do parasite-driven genetic dynamics occur ex-silico? Journal of Evolutionary Biology 15: 1-9.

Matveeva, L.K., 2004. Interrelations of rotifers with predatory and herbivorous Cladocera: a review of Russian works. Hydrobiologia 186: 69-73.

Mucklow, P.T., & D. Ebert, 2003. Physiology of immunity in the water flea *Daphnia magna*: environmental and genetic aspects of phenoloxidase activity. Physiological and Biochemical Zoology 76: 836-842.

Mucklow, P.T., D.B. Vizoso, K.H. Jensen, D. Refardt, & D. Ebert, 2004. Variation in phenoloxidase activity and its relation to parasite resistance within and between populations of *Daphnia magna*. Proceedings of the Royal Society of London Series B-Biological Sciences 271: 1175-1183.

Pulkkinen, K., & D. Ebert, 2004. Host Starvation decreases parasite load and mean host size in experimental populations. Ecology 85: 823-833.

Reeson, A.F., K. Wilson, A. Gunn, R.S. Hails, & D. Goulson, 1998. Baculovirus resistance in the noctuid spodoptera exempta is phenotypically plastic and responds to population density. Proceedings of the Royal Society of London Series B-Biological Sciences 265: 1787-1791

Schluter, D., 2001. Ecology and the origin of species. Trends in Ecology & Evolution 16: 372-380

Statsoft, 1994. STATISTICA for the windows operating system. Inc. Tulsa, UK.

Stibor, H., 1992. Predator induced life-history shifts in a fresh-water cladoceran. Oecologia 92: 162-165.

Stirnadel, H.A., & D. Ebert, 1997. Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. Journal of Animal Ecology 66: 212-222.

Willey, R.L., P.A. Cantrell, & S.T. Threlkeld, 1990. Epibiotic euglenoid flagellates increase the susceptibility of some zooplankton to fish predation. Limnology and Oceanography 35: 952-959.

Woolhouse, M.E.J., J.P. Webster, E. Domingo, B. Charlesworth, & B.R. Levin, 2002. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nature Genetics 32: 569-577.

GENERAL DISCUSSION

Eutrophication of shallow lakes has a profound impact on the ecology of shallow lakes (Jeppesen et al., 1997; Moss, 1998; Scheffer, 1998). It may lead to changes in fish predation pressure, macrophyte coverage, Secchi depth, parasite abundance, and changes in zooplankton densities, among others. Conversely, lake restoration may have a similarly strong impact on lake ecology, whether or not the lake returns to its original condition. The most intensively monitored recoveries of lakes involve deep lakes (e.g. Lake Washington; Edmondson, 1996; Lake Constance; Sommer et al., 1993), although there are many shorter accounts on changes in community and ecosystem structure upon restoration of shallow lakes (e.g. contributions in Gulati and Van Donk, 2002). Given that there are several studies reporting rapid evolutionary responses to changes in environmental conditions in zooplankton (e.g. Hairston et al., 1999, 2001; Cousyn et al., 2001), and given the importance of conditions such as macrophyte cover and fish predation pressure for zooplankton populations (e.g. Kerfoot and Sih, 1987; Jeppesen et al., 1997; Cottenie and De Meester, 2004), one may expect an impact of lake eutrophication and recovery on the genetic structure of local zooplankton populations. As a matter of fact, the study of Hairston et al. (1999, 2001) actually reports a change in genotypic value for cyanobacteria toxin tolerance in response to the recovery from eutrophication of Lake Constance.

We set out to analyze the genetic response of the resident population of the large water flea, *Daphnia magna*, to eutrophication and subsequent recovery of Lake Ring, Jutland, Denmark. Earlier studies on the reconstruction of micro-evolutionary responses in ecologically relevant traits to environmental changes using zooplankton dormant egg banks mostly focused on one trait at a time (Hairston et al., 1999, 2001; Cousyn et al., 2001; see also De Meester et al., in press).We however, explicitly aimed to look at several different traits that are relevant with respect to different environmental challenges. More specifically, we focused on the three main antagonistic biotic interactions that natural zooplankton populations are faced with: predation, parasitism, and competition. With respect to predation, we focused solely on fish predation pressure, a factor that has been reported before to impact the genetic structure of zooplankton populations (Cousyn et al., 2001). We also looked how abiotic factors, such as Secchi depth could influence the antipredation strategy of *Daphnia magna* towards fish predation.

With respect to parasitism, our approach involved exposing the *Daphnia* to natural lake sediment, thus challenging them with the natural cocktail of infective stages of microparasites and epibionts. Finally, for competition, we quantified both competitive ability (strength in the

competitive interaction) and ecological combining ability (a measure of the ability to share resources with the competitor) for both intraspecific interactions as well as for interspecific competition with the main other large cladoceran in Lake Ring, *D. pulex*. In the following, we shortly outline our main findings for each of the three environmental challenges, and subsequently try to integrate the various results.

1. Micro-evolutionary response in competition related traits in relation to changes in population density and species composition

Changes in population density and species composition in Lake Ring during the course of the past century led to micro-evolution in intraspecific competitive ability as well as interspecific combining ability in the resident *D. magna* population. Instead of a steady increase in competitive or combining ability over time (Connell, 1980), our results point to a much more dynamic pattern, in which trait values are correlated with the abundance pattern of the animals (here counted as ephippia preserved in mud slices) or the relative importance of conspecifics and animals of the congeneric competitor. Also, our data suggest that selection acts on competitive ability for intraspecific interactions and on ecological combining ability (character displacement) in the case of interspecific interactions. We found no indications for changes in combining ability with respect to the interaction with conspecifics, which may reflect the difficulty to engage in niche segregation at the intraspecific level in a generalist filter feeder.

2. Habitat degradation and restoration associated with shifts in antipredator traits

The restoration of Lake Ring resulted, with a delay of approximately a decade, in quite a pronounced increase in water transparency. This change in habitat characteristics was associated with a shift in antipredator traits. In the Turbid period (1960-1980) *Daphnia* exhibited a behavioral response upon exposure to fish kairomone; as they moved more towards the bottom (vertical migration) and into the vegetation (horizontal migration) in the presence of fish kairomone. In the Clear-water period, both these responses disappeared. In turbid water, prey have to migrate less to get out of sight of visually hunting predators than they have to do in more transparent water (Abrahams and Kattenfeld, 1997), and diel vertical migration may be an efficient antipredator behavior in turbid systems (e.g. Vos et al., 2002). The increase in water transparency after recovery may have resulted in a disappearance of the refuge at depth, thus reducing the effectiveness of diel vertical migration as a predator avoidance trait. The littoral zone in this lake has earlier been shown to be used as a day-time refuge for various zooplankters, including *Daphnia* (Lauridsen and Buenk, 1996). However, the coverage of

submerged vegetation is relatively low (2.7 %) and only marginally increased when shifting from the turbid to the clear-water condition (see Fig. 2, Chapter 1). Given the increased visibility of the animals due to the higher water transparency, DHM too may have become a less efficient predator avoidance mechanism in the Clear-water period. Reconstruction from zooplankton remains in the sediments suggests that effective fish predation pressure did not change strongly between the different periods (Chapter 1). As the predator-induced changes in behavioral traits disappeared in the Clear-water period, other antipredator traits became more important. We observed that the *Daphnia* shifted towards a life history response to fish, and reduced their size at maturity. Moreover, they exhibited smaller neonate sizes both in the presence and absence of fish kairomone. The overall antipredator strategy thus differed in the different periods (Pre-eutrophication, Turbid, Clear water).

Similar to the Clear-water period, clones derived from the Pre-eutrophication period showed little response upon fish kairomone in behavioral traits, but showed a strong response in size at maturity. Together with restoration of the habitat, the overall antipredator strategy used by the *Daphnia* thus returned to their initial condition.

3. Parasite susceptibility and fish predation

Besides competition and predation, parasites are also known to influence their host population dynamics and genetics. So far, most studies focused on the effect on micro-evolution of one antagonist at the time (Schluter, 2001, Cousyn et al., 2001, Chapter 1, 2, 3, and 4). However, in natural systems, populations face different antagonists simultaneously, and a defense mechanism against a particular antagonist may be influenced by the presence and defense to other antagonists (Decaestecker et al., 2002). The data presented in Chapter 5 indicate that, at least during one time period of Lake Ring's history, positively phototactic clones exhibited higher *Brachionus* infestation intensity than clones with more negatively phototactic behavior.

4. Patterns of neutral variation and Q_{ST} analysis

Analyses of genetic differentiation between the Pre-eutrophication, Turbid, and the Clear-water period for neutral markers revealed only very low F_{ST} estimates. This leads to the conclusion that the *D. magna* population inhabiting Lake Ring was essentially a continuous population during the past century.

Genetic differentiation based on quantitative traits (Q_{ST}) was only reliably estimated for predator-avoidance traits (Chapter 3) and was much higher than predictions based on the pattern for neutral makers. This suggests that natural selection caused the micro-evolutionary response

leading to a change in phenotypic plasticity of vertical and horizontal migration and size at maturity (Chapter 3) (Merilä and Crnokrak, 2001). We also aimed to find the period around which the highest population differentiation was found, by calculating the running Q_{ST} and the running θ (cf comparable in reasoning to calculating a running average). This approach roughly confirmed the a priori determined subdivisions in populations, which were based on historical events.

5. Synthesis – what is the relative importance of the above factors?

The *D. magna* population in Lake Ring showed micro-evolutionary changes in antipredator traits as well as in inter- and intraspecific competition related traits (competitive ability and combining ability) as a response to anthropogenic induced changes in abiotic factors (Secchi depth) and biotic factors (density of *D. pulex* and *D. magna*, fish predation pressure, macrophyte coverage, parasite abundance). In addition, our data point to a change in resistance to infection by the epibiont parasite *Brachionus*. We thus observed genetic changes in relation to all biotic interactions that we studied, even though, for instance, the micro-evolutionary response to changes in fish predation pressure were less pronounced than those reported by Cousyn et al. (2001). Given that the responses to the different antagonistic interactions are supposed to be costly, one expects trade-offs and conflicts between the different responses. Yet, each of the antagonistic interactions seems to leave a signature in the integrated set of ecologically relevant traits of the study population. The expectation of trade-offs and genetic constraints in the response to multiple environmental challenges leads to the question which of the different factors had the most influence on the genetic structure of the *D. magna* population. In an effort to get some insight into this issue, we tried to integrate the data on the amplitude of changes in the different biotic selection factors in the lake through time and in the amplitude of the micro-evolutionary responses in the corresponding ecologically relevant traits of the resident *D. magna* population. Fig. 1 shows the data points representing the coefficients of variance ($CV = \frac{\sigma}{\bar{X}} \times 100$) based on our reconstruction of lake conditions through time (see Fig. 2 of the Introduction) for the different antagonistic biotic interactions on the one hand, and coefficients of variance of clonal means of the different corresponding ecologically relevant traits that we quantified, on the other. The different antagonistic biotic interactions were estimated by *D. pulex* ephippium density, *D. magna* ephippium density, fish predation pressure, and macrophyte coverage; we additionally included Secchi depth as a variable because water transparency may lead to important changes in effective fish predation pressure. As we do not have data on the changes in parasite abundance over time in Lake Ring, we could not calculate the coefficient of

variance for the selection pressure exerted by parasites. Fig. 1 shows that the variables related to fish predation (reconstructed fish predation, macrophyte coverage and Secchi depth as variables that may strongly impact effective fish predation risk) show the least temporal variation in the studied period (approx. 90 years) in Lake Ring, whereas the resident *D. magna* population experienced strong year-to-year changes in population densities of conspecifics and especially of the competitor *D. pulex*. In agreement with expectations, we see that the traits related to competitive interactions show the strongest interclonal variation. Interestingly, however, genetic variation in traits related to intraspecific competition is higher than in traits related to competition with *D. pulex*, even though the competitive environment showed more fluctuations for inter- than for intraspecific interactions. Overall, Fig. 1 indicates that the stronger environmental variation in the selective force, the more pronounced the genetic signature of that selection factor in our study population. To quantify better the relative power of the different selection factors in shaping the micro-evolutionary responses, we calculated the ratio of the two coefficients of variance. This analysis (Fig. 2) suggests that intraspecific competition has had by far the highest relative impact on evolutionary responses of the resident *D. magna* population in Lake Ring. The relative impact of variation in intraspecific competition (estimated by variation in ephippium density of *D. magna)* on intraspecific competitive ability is more than four time higher than the impact of variation in the density of the competing species *D. pulex* on interspecific combining ability. Our data also suggest that the variables related to fish predation pressure have the lowest impact on evolutionary responses in the resident *D. magna* population in this lake. We conclude that both density of *D. pulex* and *D. magna* represent important driving factors in the micro-evolution in *D. magna* in Lake Ring.

This is a striking result, since most evolutionary ecological research on zooplankton in the past few decades focused more on predation and, more recently, on parasitism than on competition (a notable exception being Steiner et al. 2007).

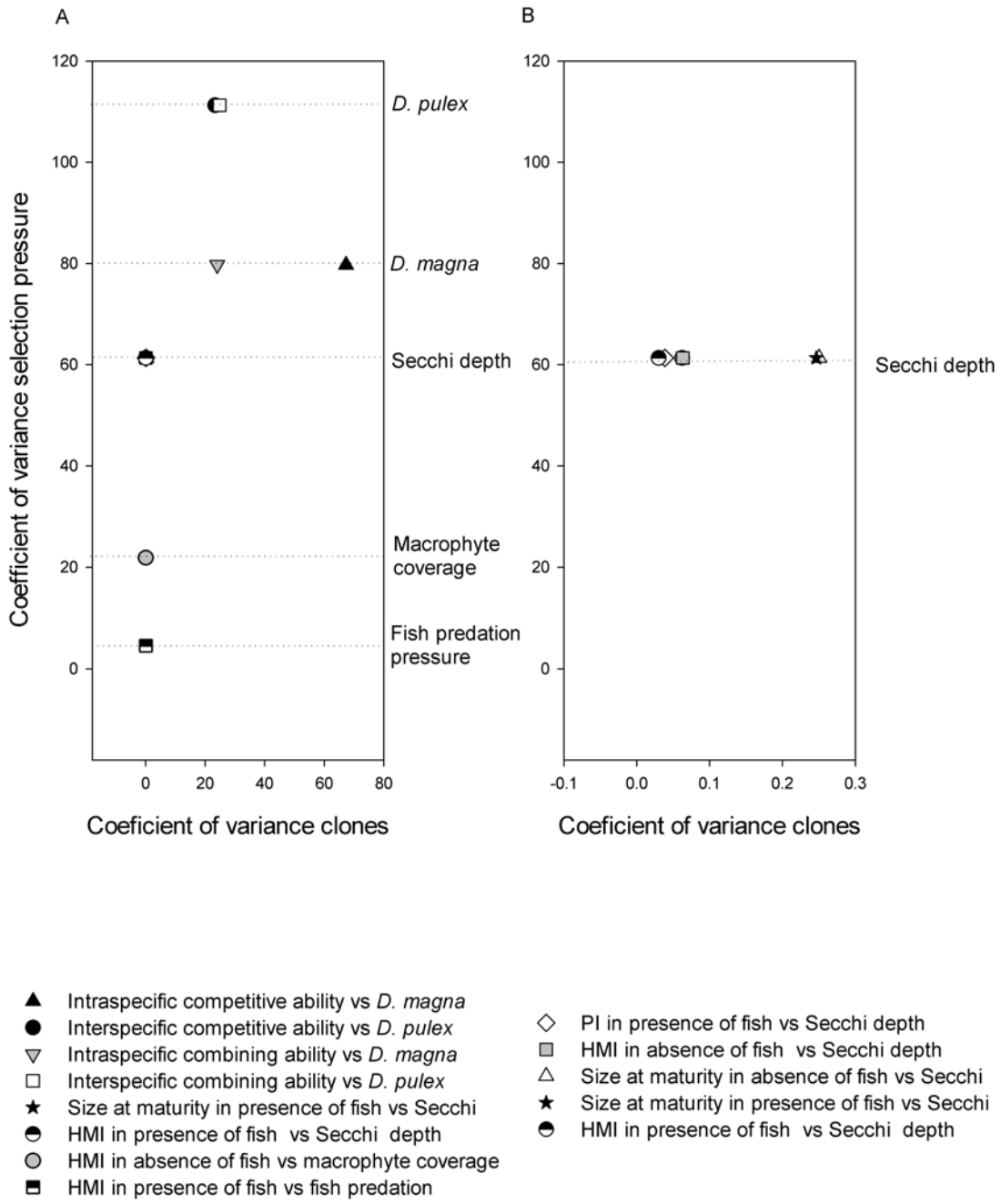

*Fig. 1: A) Relationship between the coeffients of variance of the different selection pressures (*D. pulex *density,* D. magna *density, Secchi depth, and fish predation pressure) and the coefficients of clonal variance of the different traits studied. B) Copy of part of plot A with a higher resolution, so that data points associate with Secchi depth become more visible.*

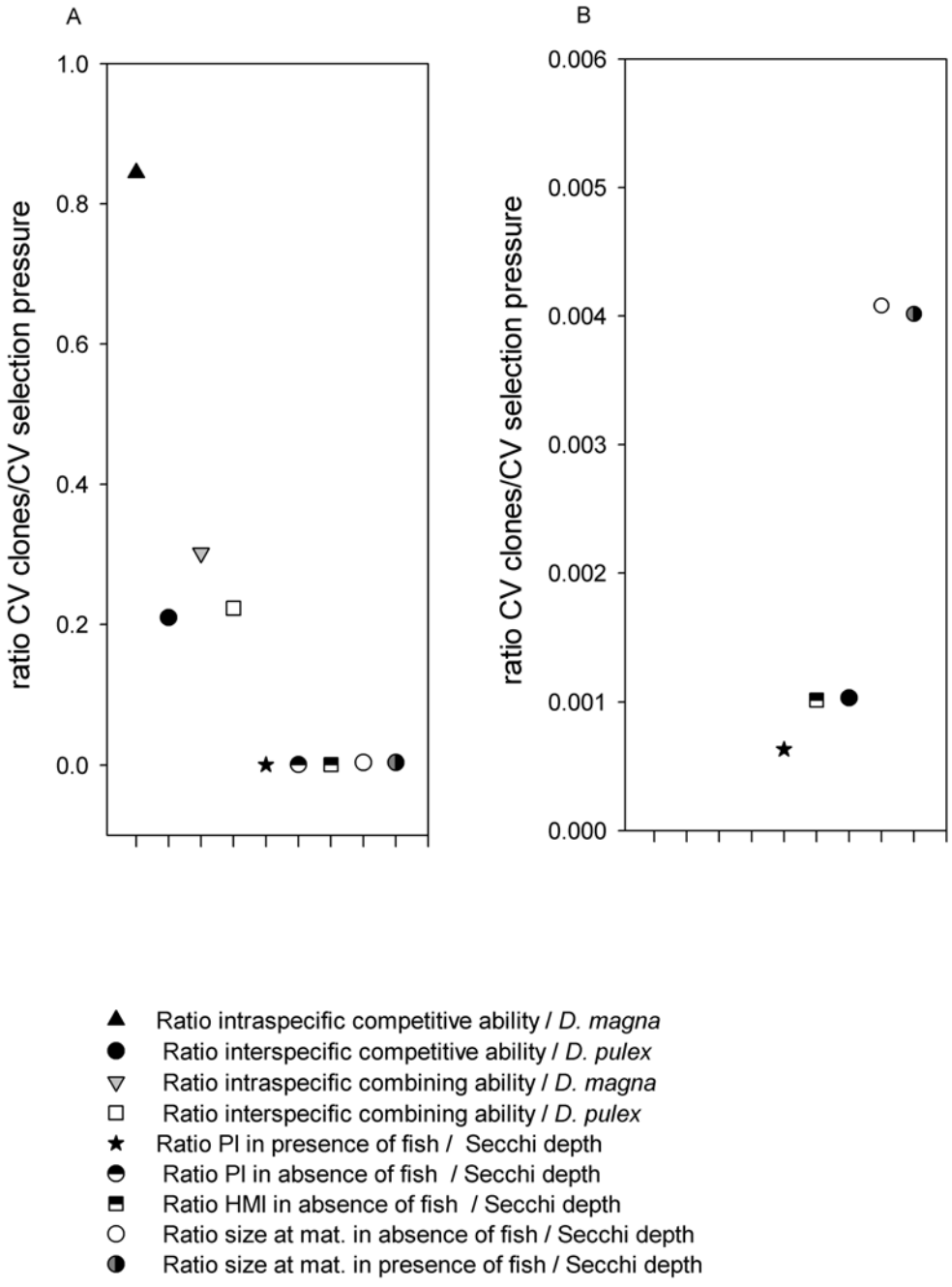

*Fig. 2: A) Ratio of the coefficients of clonal variance of the different traits studied and the coeffients of variance of the different corresponding selection pressures (*D. pulex *density,* D. magna *density, Secchi depth, and fish predation pressure). B) Copy of part plot A with a higher resolution. Please note that even with a higher resolution the different ratios containing clonal variation in HMI can not be distinguished from each other.*

6. Implication for ecological restoration

The primary goal of habitat restoration measures is to counteract habitat deterioration and restore the ecological situation of the habitat in such a way that it becomes a natural and self-regulating system again (Van Adel and Aronson, 2005). An increasing body of evidence suggests however, that both habitat degradation and restoration measurements not only influence the ecology of the habitat, but also directly and indirectly influence the genetic structure of local populations (in plants: Macnair, 1987; in *Daphnia galeata:* Hairston et al., 1999, 2001) and can act as evolutionary forces themself (Palumbi, 2001). In our study we found indications that both habitat deterioration and the subsequent habitat restoration of Lake Ring affected the evolution of the local *Daphnia magna* population through changes in environmental variables. We found micro-evolutionary responses in different ecological relevant traits, such as antipredator traits, and competitive and combining ability. All these micro-evolutionary responses happened on a relative short time scale (within a time interval ranging from a few years to twenty years), and might thus impact the outcome of ecological interactions or interact with anthropogenic perturbations (Stockwell, et al., 2003, Hairston et al., 2005). Although we focused on the evolutionary response of *Daphnia magna*, other studies showed similar contemporary evolution in other zooplankton species (copepods: Hairston and Dillon, 1990; *Daphnia galeata*: Hairston et al., 1999), but also in species with longer generation time (Darwin finches: Grant and Grant, 2002). Since contemporary evolution often happens in the same time frame as many ecological restorations, and other conservation measures, such as conservation of endangered species, and/or control of invasive species take place, both type of processes may interfere with each other. On one hand, conservation measures might influence the genetic structure of local populations, whereas at the other hand contemporary evolution might hamper conservation success. More research is thus necessary to understand how ecological restoration and conservation measures in general interact with the genetic structure of the local populations. Understanding better this interaction might lead to more efficient conservation and restoration efforts.

7. References

Abrahams, M., & M. Kattenfeld, 1997. The Role of turbidity as a constraint on predator-prey interactions in aquatic environments. Behavioral Ecology and Sociobiology 40: 169-174.

Connell, J.H., 1980. Diversity and the coevolution of competitors, or the ghost of competition past. Oikos 35: 131-138.

Cottenie, K., & L. De Meester, 2004. Metacommunity Structure: synergy of biotic interactions as selective agents and dispersal as fuel. Ecology 85: 114-119.

Cousyn, C., L. De Meester, J.K. Colbourne, L. Brendonck, D. Verschuren, & F. Volckaert, 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. Proceedings of the National Academy of Sciences of the United States of America 98: 6256-6260.

De Meester, L., J. Mergaey, H. Michels, & E. Decaestecker, In press. Cladocera resting eggs and antropogenic changes. In: Alekseev, V., & De Stasio, B. (eds.), Diapause in aquatic invertebrates: role for ecology, physiology and human uses. Springer, UK.

Decaestecker, E., L. De Meester, & D. Ebert, 2002. In deep trouble: habitat selection constrained by multiple enemies in zooplankton. Proceedings of the National Academy of Sciences of the United States of America 99: 5481-5485.

Edmondson, W.T., 1996. The uses of ecology: Lake Washington and beyond, University of Washington, US.

Grant, P.R., & B.R. Grant, 2002. Unpredictable evolution in a 30-year study of Darwin's Finches. Science 296: 707-711.

Gulati, R.D., & E. Van Donk, 2002. Lakes in the Netherlands, their origin, eutrophication and restoration: state-of-the-art review. Hydrobiologia 478: 73-106.

Hairston, N.G. & T.A. Dillon, 1990. Fluctuating selection and response in a population of freshwater copepods. Evolution 44: 1796-1805.

Hairston, N.G., W. Lampert, C.E. Caceres, C.L. Holtmeier, L.J. Weider, U. Gaedke, J.M. Fischer, J.A. Fox, & D.M. Post, 1999. Rapid evolution revealed by dormant eggs. Nature 401: 446.

Hairston, N.G., C.L. Holtmeier, W. Lampert, L.J. Weider, D.M. Post, J.M. Fischer, C.E. Caceres, J.A. Fox, & U. Gaedke, 2001. Natural selection for grazer resistance to toxic cyanobacteria: evolution of phenotypic plasticity? Evolution 55: 2203-2214.

Hairston, N.G., S.P. Ellner, M.A. Geber, Yoshida T., & Fox J. A., 2005. Rapid evolution and the convergence of ecological and evolutionary time. Ecology Letters 8: 1114-1127.

Jeppesen, E., M. Sondergaard, M. Sondergaard, & K. Christofferesen, 1997. The structuring role of submerged macrophytes in lakes, Springer, UK.

Kerfoot, W. C. & A. Sih, 1987. Predation, direct and indirect impacts on aquatic communities, University press of New England, Hanover, NH.

Lauridsen, T.L., & I. Buenk, 1996. Diel Changes in the horizontal distribution of zooplankton in the littoral zone of two shallow eutrophic lakes. Archiv Fur Hydrobiologie 137: 161-176.

Macnair, M., 1987. Heavy metal tolerance in plants: a model evolutionary system. Trends in Ecology and Evolution 2: 354-359.

Merilä, J., & P. Crnokrak, 2001. Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology 14: 892-903.

Moss, B., 1998. Ecology of fresh waters: man and medium, past to future, Blackwell Publishing, UK.

Palumbi, S.R., 2001. The evolutionary explosion: How humans cause rapid evolutionary change, Norton.

Scheffer, M., 1998. Ecology of shallow lakes, Chapman, & Hall, London, UK.

Schluter, D., 2001. Ecology and the origin of species. Trends in Ecology & Evolution 16: 372-380

Sommer, U., U. Gaedke, & A. Schweizer, 1993. The 1st decade of oligotrophication of Lake Constance. II.The response of phytoplankton taxonomic composition. Oecologia 93: 276-284.

Steiner, C.F., C.E. Cáceres, & S.D.P. Smith, 2007. Resurrecting the ghost of competition past with dormant zooplankton eggs. The American Naturalist 169: 10-20.

Stokwell, C.A., A.P. Hendry, & M.T. Kinnison, 2003. Contemporary evolution meets conservation biology. Trends in Ecology & Evolution 18: 94-101.

Van Adel, J., & Aronson, J., 2005. Restoration Ecology, Blackwell Publishing

Vos, M., B.J.G. Flik, J. Vijverberg, J. Ringelberg, & W.M. Mooij, 2002. From inducible defences to population dynamics: modelling refuge use and life history changes in *Daphnia*. Oikos 99: 386-396.

SUMMARY

The main goal of this dissertation was to analyse the evolutionary response of a natural *D. magna* population to changes in biotic stress associated with habitat degradation and restoration of a shallow lake. More specifically, the dormant egg bank, present in the sediment of the lake, was used as an archive of past ecological events, to study patterns of genetic changes in a natural *Daphnia magna* population. Genetic changes in both ecologically relevant traits (i.e. life history traits and behavioral traits) as well as in neutral marker loci (i.e. microsatellites markers) were investigated. Comparing the obtained patterns for these two categories of genetic traits, can provide insight in the driving forces behind the evolutionary process. Micro-evolutionary changes with respect to different traits related to selection pressures associated with different types of antagonistic biotic interactions (predation, parasitism, and competition) were analyzed, in an effort to obtain an integrated view on the potential causes of micro-evolution in the studied *D. magna* population.

Lake Ring is a relatively small, shallow lake located in Mid-Jutland, Denmark. The lake was subjected to strong eutrophication during the 20th century due to sewage inflow. To restore the lake, the point source of sewage effluents from the nearby village was diverted in 1970. Initially this led to a very gradual recovery, culminating with a drastic increase in water clarity from the mid-1980s and onwards. To reconstruct the micro-evolutionary responses of the local *Daphnia magna* population, *D. magna* clones were obtained by hatching dormant eggs from a sediment core sampled from the sediment of Lake Ring.

In chapter I, an experimental investigation was undertaken aimed to describe the genetic variation in a natural *D. magna* population for diel horizontal migration (DHM), an important antipredator trait, and to study whether an adaptive micro-evolutionary response occurred to changes in macrophyte coverage and fish predation pressure through time. Furthermore we looked whether horizontal migration behavior among clones differed before and after lake recovery. The results of this study revealed that the *D. magna* clones in Lake Ring exhibited a significantly stronger horizontal migration response during the period of eutrophication than in the recovery phase. Since the fish predation pressure did not change, the decline of DHM implies that other antipredator traits must have been involved. This suggestion was tested in Chapter II. In this chapter we reconstructed the micro-evolutionary response of six antipredator traits (vertical migration, size at maturity, age at first clutch, neonate size, and first clutch size) in response to habitat degradation and restoration, which was accompanied by a shift in water transparency. Life history and behavioral traits of a set of clones, hatched from sediments that were deposited in three successive periods in the history of Lake Ring: the Pre-eutrophication

period (1908-1937), the Turbid period (1960-1980) and the Clear-water period (1986-2000), were scored in the absence and presence of fish kairomone. In all periods equal proportions of clones reacted with at least one trait to the presence of fish kairomone, but the nature of the antipredator response differed. In the Pre-eutrophication period and the Clear-water period, both characterized with high water transparency, clones reacted most strongly with life history traits in presence of fish kairomone, and reduced their size at maturity. In the Turbid period, they responded to fish kairomone with behavioral traits (vertical and horizontal migration).

The observed micro-evolution of antipredator response to lake degradation and restoration likely reflects a differential efficiency of these different traits in reducing mortality by predation at different levels of water turbidity and may have been facilitated by their apparent uncoupling.

To detect whether this micro-evolutionary response was the result of natural selection, a comparison of genetic differentiation based on neutral marker loci and quantitative genetic traits was performed in chapter III. Molecular genetic analysis on nine microsatellite markers revealed only very low genetic differentiation among the different time periods. The observation of low genetic differentiation for neutral traits among these populations indicates that any potential neutral genetic event (genetic drift or gene flow) was buffered by the large dormant egg bank present in Lake Ring sediment. Quantitative genetic analysis of six antipredator traits however, did reveal genetic differentiation between the different time periods. The fact that the quantitative genetic differentiation exceeded the genetic differentiation based on neutral marker loci, indicated that natural selection was the driving force behind the micro-evolutionary response in the antipredator traits.

Besides predation, competition is another important antagonistic biotic interaction. In chapter IV, the evolutionary dynamics of competitive interactions of a strong zooplankton competitor, *Daphnia magna*, was reconstructed in relation to changes in densities of conspecifics as well as to changes in population densities of an important competing species, *D. pulex.* A positive correlation was found between the interspecific combining ability and the abundance of *D. pulex* as measured from the ephippium profile in the sediment. Furthermore, there was a positive relationship between the ratio of inter- to intraspecific competitive ability and the ratio of abundance in ephippia of the competing species to conspecifics, and a positive relationship between the intraspecific competitive ability and the relative abundance of *D. magna.* Taken together, these results suggest that the local *D. magna* population is able to genetically track the changes in the competitive environment, both in terms of competitive ability and combining ability.

Finally, in chapter V the evolutionary change through time in ectoparasite susceptibility and immunocompetence of the natural *Daphnia magna* population in Lake Ring was

reconstructed. Clones derived from the Pre-eutrophication period (1908-1937), the Turbid water period (1960-1980) and the Clear-water period (1986-2000) were exposed to a standardized suspension of sediment of the lake in the presence and absence of fish kairomone. Interclonal variation in susceptibility for infestation by the epibiont *Brachionus rubens* was observed. Furthermore, it was observed that the clones derived from the most recent, clear-water period were less infected by *Brachionus* than clones derived from the Turbid period. No significant changes with time were found in Phenoloxidase activity, a factor involved in *Daphnia* immunocompetence, which might indicate that Phenoloxidase does not play a crucial role in the resistance against *Brachionus*.

The research presented in this thesis demonstrates that *Daphnia magna* showed micro-evolutionary changes in antipredator traits, as well as in inter- and intraspecific competition related traits as a response to anthropogenic induced changes in abiotic factors and biotic factors. All these changes happened on a relative short time scale. These results illustrate that not only anthropogenic-induced habitat deterioration, but also the efforts done to restore habitats may cause genetic shifts and affect the genetic architecture of the resident populations.

NEDERLANDSE SAMENVATTING

De belangrijkste doelstelling van deze thesis is de analyse van de evolutionaire respons van een natuurlijke *D. magna* populatie, in relatie tot veranderingen in biotisch stress, geassocieerd met eutroficatie en het daarop volgende herstel van een ondiep meer, Lake Ring. Meer bepaald werd de rusteibank, die aanwezig is in het sediment van het meer en dienst doet als een archief van ecologische veranderingen tijdens de geschiedenis van het meer, gebruikt om genetische veranderingen in een natuurlijke *Daphnia magna* populatie te onderzoeken. Hiervoor werd gekeken naar het patroon van genetische veranderingen, zowel in ecologisch relevante kenmerken (levensgeschiedenis- en gedragskenmerken) als in neutrale merkers (microsatelliet merkers). De vergelijking van deze patronen in beide soorten kenmerken, kan inzicht verschaffen in de drijvende kracht achter het evolutionaire proces. De micro-evolutionaire veranderingen die aanwezig zijn in verschillende kenmerken en het gevolg zijn van een selectiedruk van een bepaalde antagonistische biotische interactie (predatie, parasitisme, of competitie), werden onderzocht. Hierdoor werd er een meer geïntegreerd beeld verkregen van de mogelijke oorzaken van micro-evolutie in de natuurlijke *D. magna* populatie van Lake Ring.

Lake Ring is een relatief klein, ondiep meer dat gedurende de 20ste eeuw een sterke eutroficatie kende. Om het meer te herstellen naar zijn oorspronkelijke toestand, werd de riolering uit een naburig dorp in 1970 afgeleid. Aanvankelijk leidde dit tot een traag herstel van het meer, maar uiteindelijk werd het meer midden jaren 80 terug helder. Om de micro-evolutionaire veranderingen in *Daphnia* te analyseren, werden er rusteieren uit verschillende diepte van het meersediment ontloken en de zo bekomen clones werden vervolgens in cultuur gebracht.

In hoofdstuk I werd er een experimenteel onderzoek uitgevoerd met als doel de genetische variatie voor Dagelijkse Horizontal Migratie (DHM), een belangrijk verdedigingsmechanisme tegen vispredatie, in de *D. magna* populatie te analyseren. Bovendien werd er nagegaan of de micro-evolutionaire veranderingen gerelateerd zijn met verandering in plantenbedekking en vispredatiedruk. Tenslotte, werd er ook gekeken of het horizontaal migratie gedrag anders is in de periode voor en na het habitatherstel. De resultaten van deze studie tonen aan dat *D. magna* clones in Lake Ring sterker naar de planten migreerden tijdens de eutroficatie dan tijdens de herstelfase. Hoewel de vispredatiedruk tussen de verschillende periodes niet verandert, wordt er toch een daling in horizontaal migratie gedrag waargenomen. Een mogelijke verklaring zou kunnen zijn dat andere verdedigingsmechanismen belangrijk worden in de periode na het herstel. Dit werd getest in hoofdstuk II. In dit hoofdstuk werd de micro-evolutionaire respons op habitatdegradatie en -herstel van zes antipredatiekenmerken (verticale migratie, grootte bij

volwassenheid, leeftijd bij eerste broed, grootte juvenielen) onderzocht. Levensgeschiedeniskenmerken en gedragskenmerken van clones afkomstig van drie opeenvolgende periodes uit de geschiedenis van Lake Ring: de Pre-eutroficatie periode (1908-1937), de Troebele periode (1960-1980) en de Heldere periode (1986-2000), werden beoordeeld in de aan- en afwezigheid van viskairomonen. De resultaten van dit onderzoek tonen aan dat er een verandering is in verdedigingstrategie. In de Pre-eutroficatie periode, een periode die gekenmerkt wordt door helder water, beschermen de *D. magna* clones zich vooral door levensgeschiedeniskenmerken, individuen zijn kleiner bij volwassenheid in de aanwezigheid van vis. In de Troebele periode zijn vooral de gedragkenmerken (verticale en horizontale migratie) belangrijk in de bescherming tegen vispredatie. In de meest recente periode, die ook gekenmerkt wordt door helder water, reageren de clones terug met levensgeschiedeniskenmerken op de aanwezigheid van vis. De algemene defensiestrategie verandert tussen de verschillende periodes. Om na te gaan of deze micro-evolutionaire respons het resultaat was van natuurlijke selectie werd in hoofdstuk III de genetische differentiatie op basis van neutrale merkers en de genetische differentiatie op basis van quantitatieve genetische kenmerken, met elkaar vergeleken. Moleculair-genetische analyse van negen microsatelliet merkers toonde aan dat er slechts zeer weinig genetische differentiatie was tussen de drie periodes. Dit betekent dat de aanwezigheid van een grote rusteibank in het sediment van Lake Ring een buffer is voor potentiële genetisch neutrale gebeurtenissen, zoals genetische drift of genmigratie. Quantitatieve genetische analyse op de zes antipredatiekenmerken wees echter wel op genetische verschillen tussen de verschillende periodes. Het feit dat de quantitatief genetische differentiatie de genetische differentiatie gebaseerd op de neutrale merker loci overschreed, geeft aan dat natuurlijke selectie de drijvende kracht is achter de micro-evolutionaire respons van de antipredatiekenmerken.

Naast predatie, is competitie ook een belangrijke antagonistische biotische interactie. In hoofdstuk IV werd de evolutionaire dynamiek van de competitie tussen twee sterke competitoren gereconstrueerd. Meer bepaald werd het competitieve vermogen en het combinatie vermogen van *D. magna* gereconstrueerd in relatie tot veranderingen in de densiteit van soortgenoten en in relatie tot veranderingen in populatiedenisiteit van een belangrijke concurrent *D. pulex.* We vonden een positieve correlatie tussen het interspecifieke combinatie vermogen en de densiteit van *D. pulex,* zoals die werd weergegeven door het ephippiumprofiel in het sediment. Voorts was er ook een positieve relatie tussen de ratio van inter- en intraspecifiek competitie vermogen en de ratio van de densiteit van ephippia van de concurrerende soorten en de soortgenoten. Er was eveneens een negatieve relatie tussen het intraspecifiek competitie vermogen en de ratio van de densiteit van ephippia van de

concurrerende soorten en de soortgenoten . Alles samen beschouwd, suggereren deze resultaten dat de lokale *D. magna* populatie in staat is om zich genetisch aan te passen aan de veranderingen in de competitieve omgeving, zowel in het competitieve vermogen als in het combinatie vermogen.

Tenslotte, werd in hoofdstuk V de genetische verandering in ectoparasietgevoeligheid en immunocompetentie van een natuurlijke *D. magna* populatie doorheen de tijd gereconstureerd. Hiervoor werden de clones afkomstig uit de drie opeenvolgende periodes in de geschiedenis van Lake Ring, blootgesteld aan een standaard sedimentmengsel in de aan- of afwezigheid van viskairomonen. Er was zowel een interclonale variatie aanwezig in gevoeligheid voor *Brachionus,* als een significant periode effect. Clones die afkomstig waren van de meest recente periode waren minder geïnfecteerd door de epibiont *Brachionus,* dan de clones afkomstig van de Troebele periode. Er werd geen significant tijdseffect werd gevonden in fenoloxidase activiteit, een factor die een belangrijke rol speelt in de immunocompetentie van *Daphnia.* Dit wijst op het feit dat fenoloxidase geen cruciale rol speelt in de resistentie tegen *Brachionus.*

Dit onderzoek toont aan dat *Daphnia magna* een micro-evolutionaire respons vertoonde in antipredatiekenmerken en kenmerken die gerelateerd zijn met inter- en intraspecifieke competitie. Deze veranderingen waren gerelateerd met door de mens geïnduceerde veranderingen in belangrijk biotische interacties. Al deze veranderingen gebeurden op een relatieve korte tijdschaal. Tot slot tonen deze resultaten aan dat niet alleen door de mens-geïnduceerde habitatdegradatie, maar ook de maatregelen die getroffen worden om de habitat te herstellen, genetische veranderingen kunnen teweeg brengen en zo de genetische architectuur van de locale populaties kan beïnvloeden.

© 2006 Faculteit Wetenschappen, Geel Huis, Kasteelpark Arenberg 11, 3001 Heverlee (Leuven).

Alle rechten voorbehouden. Niets uit deze uitgave mag worden vermenigvuldigd en/of openbaar gemaakt worden door middel van druk, fotokopie, microfilm, elektronisch of op welke andere wijze ook zonder voorafgaandelijke schriftelijke toestemming van de uitgever.

All rights reserved. No part of the publication may be reproduced in any form by print, photoprint, microfilm, electronic or any other means without written permission from the publisher.
ISBN [ISBN-nummer] 978-90-8649-088-2
D/ [Wettelijk depotnummer] D/2007/10.705/11