GOETHE
UNIVERSITÄT
FRANKFURT AM MAIN

Nora Brede

The Reconstruction of Evolutionary Patterns from *Daphnia* Resting Egg Banks

2008

The Reconstruction of Evolutionary Patterns from *Daphnia* Resting Egg Banks

Nora Brede

Doctoral thesis

July 2008

The Reconstruction of Evolutionary Patterns from *Daphnia* Resting Egg Banks

Dissertation
zur Erlangung des Doktorgrades
der Naturwissenschaften

vorgelegt im Fachbereich Biologie
der Goethe-Universität
in Frankfurt am Main

von Nora Brede
aus Frankfurt am Main

Frankfurt am Main 2008
(D 30)

“Hybridization is an ecologically dependent behavioral phenomenon, with genetic consequences.” (Grant & Grant, 2008)

Table of Contents

General Introduction

Many lakes in Europe are known to have gone through severe ecological changes induced by human influence. Anthropogenic ecological change has become a major subject in biological sciences – especially in environmental studies, biodiversity research and protection of endangered species. Pollution caused by toxicants, medicaments and other chemicals, invasion of exotic plant and animal species and change of natural habitats facilitated by infrastructure, massive cutbacks and herewith associated erosion, draining of wetlands or fragmentation of landscapes in general have a strong impact on many organism groups. These factors imply new selective pressures in addition to natural ones (such as parasites, predators, competition etc.) and fitness challenges for the inhabitants of freshwater systems.

This thesis presents the reconstruction of a population over time exposed to environmental change induced by anthropogenic influences. In the center of this study stand species of the *D. longispina* group, i.e. *D. galeata*, *D. hyalina*, *D. cucullata* and their interspecific hybrids, inhabiting two pre-alpine lakes, Lake Constance and Greifensee. Both lakes went through a phase of eutrophication (in the case of Greifensee hypereutrophication) beginning around mid of the 20th century. By exploring their biological archives burried in sediment layers I studied the effects of eutrophication on species composition, interspecific hybridization, the genetic population structure and the fate of introgression over time.

The Genus *Daphnia*

Species of the genus *Daphnia* belong to the most prominent planktonic organisms in freshwater ecosystems (Manca & Ruggiu, 1998). As one of the most dominant grazers in freshwater plankton, it has been in the center of interest for many decades. *Daphnia* became a model organism in several fields of biology: ecology, limnology, evolutionary biology and genetics (Lynch & Spitze, 1994; Stark & Banks, 2003). The genus itself consists of three subgenera: *Daphnia*, *Ctenodaphnia* and the *D. longispina* group (Colbourne & Hebert, 1996). Although the taxonomic status of some species is not clear, current studies recognize 32 species in Europe (Schwenk et al., 2000; Benzie, 2005). For this study, the *D. longispina* group is of special interest: Of the known eight or nine species five are known to hybridize interspecifically (Salinger, unpubl.; Schwenk et al., 2000). Species of the *D. longispina* group

inhabit different habitats but overlapping of habitats is possible and occurs frequently (e.g. Petrusek, 2008). Single species show unique adaptations to fish predation like formation of helmets (Laforsch & Tollrian, 2004), vertical migration (Lampert et al., 1994), to UV radiation by melanization of the carapace (Rautio & Korhola, 2002) or to food limitation (Lampert, 1988). Where intermediate habitats occur or different 'niches' are available different *Daphnia* species may occur in sympatry (Schwenk, 1997). These characteristics in combination with their reproduction strategy provide a large potential to inhabit small temporary ponds as well as large lakes.

In general, all species of the *D. longispina* group are cyclical parthenogens. Asexual reproduction via parthenogenesis (resulting in clonal lineages) can be replaced by bisexual reproduction under stressful conditions via fertilization of haploid eggs (Hobæk & Larsson, 1990; Kleiven et al., 1992). These eggs are encapsuled in a carapace structure called ephippium and are shed off by the sexual female during moulting or with decomposition of the dead organism. Those resting eggs are not only produced during crowding or to circumvent starvation due to limited food, they also remain viable for long periods of time surviving conditions unsuitable for the adult waterflea like drought, frost and even gut passage of water fowl (Proctor, 1964; Proctor & Malone, 1965). Dormant propagules are also capable to promote the dispersal of *Daphnia*, although this is only one of several possibilities known to facilitate migration (reviewed in Brendonck & De Meester, 2003). Further mechanisms of dispersal, e.g. via wind and attachment to other objects through hook-like structures or spines on the ephippial shell are described (Havel & Shurin, 2004). To ensure that hatching does not start with the first cues when conditions are still unsuitable, resting eggs remain in stasis (depending on the species from a few weeks to several months) and are not capable of developing until the next season to prevent premature hatching in unfavourable conditions (Hairston et al., 2000; Caceres & Tessier, 2003). Subsequently, dormancy can be interrupted by triggers like light and increasing temperatures (reviewed in Brendonck & De Meester, 2003; Vandekerkhove et al., 2005a).

The aim of this study was to exploit the unique opportunity to analyse population dynamics over time, i.e. almost one half of a century, to analyse the effect of anthropogenically induced ecological change on three interspecifically hybridizing species of the *D. longispina* group: Three parental taxa, *Daphnia galeata*, *D. hyalina* and *D. cucullata* as well as their interspecific hybrids (with an emphasis of *D. galeata x hyalina*). *D. hyalina* (which is D.

longispina according to new findings: Petrusek et al., 2008) was described from the Lake Constance region and is known to inhabit mainly large oligotrophic permanent habitats. *D. galeata* is well adapted to eutrophic lakes and seems to rely more on sexual reproduction than the former species. *D. cucullata* is relatively small and well adapted to high fish predation (Flößner, 1972). Besides their small body size this species is known to respond to fish kairomones and even microturbulences with the generation of a relatively large helmet and formation of a long spine on the abdominal end of the carapace (Tollrian, 1990; Laforsch & ollrian, 2004).

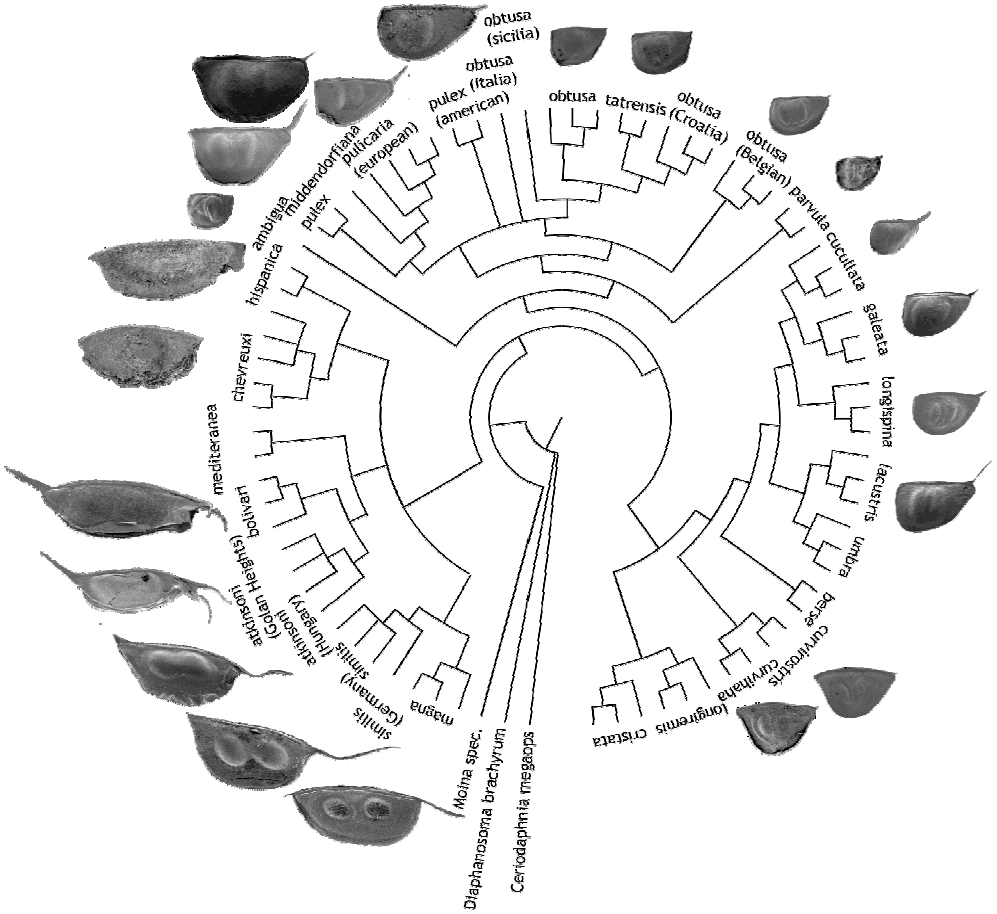

Figure A: Rooted phylogenetic tree based on 12S rDNA sequences of all European *Daphnia* species comprising the subgenera *Daphnia* (branch on lower left), *Ctenodaphnia* (upper branch) and *Hyalodaphnia* (branch on lower right). Added are SEM pictures of ephippia assigned to the according species (Salinger, 2007).

Daphnia Resting Egg Banks

Many freshwater organisms produce resting stages because they are often confronted with unsuitable living conditions. Unsuitable settings for small planktonic organisms might be of abiotic nature like drought, frost, or low oxygen as well as of biological nature like dietary

restrictions, competition (crowding) or predation (reviewed in: Brendonck & De Meester, 2003). Because of their morphological traits resting eggs can stay viable (depending on the species) up to 330 years in certain zooplankters (an extreme case though: Hairston et al., 1995). For *Daphnia*, an ephippium extracted from 125 year old sediments turned out to be still viable (Cáceres, 1998). In this and another study the oldest hatched resting eggs were extracted from sediment layers deposited between 1960 and 1963 (Keller & Spaak, 2004). According to Brendock & De Meester (2003) dormant stages of zooplankton in resting egg banks are found in densities of 10^3-10^5 eggs per m². Natural hatching cues only affect those resting eggs in the upper most 2-10 cm of the sediment since the chance of being whirled up and getting exposed to appropriate stimuli are highest only in this upper region (Herzig, 1985; Cáceres & Hairston, 1998).

It seems that the shape and coloration of dormant stages is species-specific. *Daphnia* ephippia are of filigree ultrastructure which can only be seen using scanning elektron microscopy. Especially the appendages that facilitate the dispersal of the resting eggs and the surface structure which allows the duration in mal-conditioned surroundings are an object of interest (Green & Figuerola, 2005). The feasability to assign ephippia (e.g. extracted from sediments or drawn from habitats that lack living animals) to a certain species could be an important indicator for past ecological conditions since most *Daphnia* species are adapted to specific habitats. In another so far unpublished study, we collected ephippia from all known European *Daphnia* species including a sample from the type locality of every species and verified them by sequencing and blast searching a 12S rDNA fragment (Fig. A). By this we were able to analyse the possibilities of species specific assignment of resting eggs on a computational basis and with this provide a potent tool for ecologist, palaeolimnologists and others allowing a more precise characterization of ancient habitat conditions.

In most habitats the majority of ephippia sinks to the ground and becomes buried by seasonal debris and sediment before hatching cues initiate the development of some eggs (though in permanent lakes probably hatching does not occur because of their depth and thereby inaccessibility of developmental triggers). Since this process is repeated as long as sexual reproduction takes place an archive of dormant life stages accumulates and is then called a resting egg bank. The genetic composition of those *Daphnia* reproducing sexually is thus stored in those layers of the sediment of the season during which they were produced. Especially if the sediment is stratified which can remains well preserved over decades,

Daphnia resting egg banks can become a precious object of study to scientists, since they can be used to reconstruct ecological and evolutionary processes over a certain timescale (i.e. resurrection ecology: Kerfoot et al., 1999). On the one hand, resting eggs up to an age of 45 years can usually be stimulated to hatch (Jankowski & Straile, 2003; Keller & Spaak, 2004) offering the opportunity to study individuals 'produced' under different ecological conditions than the recent ones and on the other hand eggs (independently of their capability to develop) can be conducted to genetic analysis directly to avoid biases due to differential hatching (Reid et al., 2000; Brede, 2004).

So far the genetic archives of *Daphnia* were used to answer questions on the recruitment of populations from the resting egg bank (Cáceres, 1998; Hairston et al., 2000), to understand patterns of dispersal (Havel & Shurin, 2004) or to reconstruct ecological changes in habitats (e.g. Vandekerkhove et al., 2005b). But until now the potential of resting egg banks have not been utilized to understand population dynamics over time. Since resting eggs deposited in a specific sediment layer are produced by the planktonic population inhabiting a habitat at that time and since these dormant stages represent the genetic composition of the that time, they may serve to study the effects of microevolutionary patterns and the impact of interspecific hybridization on parental species.

Interspecific Hybridization

Traditionally, the biological species concept of Mayr (1963) is referred to as the definition best explaining a species in many educational institutions: A species is a group of interbreeding individuals that does not interbreed with other such groups. Over the last decades many revisions of Mayrs definition have been proposed and new concepts have been published. Templeton (1989) defined with the cohesion species concept "… the most inclusive group of organisms having the potential for genetic and/or demographic exchangeability." A major problem of Ernst Mayrs view on the evolution of species can be seen in its restriction of speciation following a tree-like scheme. In order to understand the natural pattern of speciation in my opinion it is necessary to dissociate from the concept of a tree-like order of relationships among organisms which provides a very unflexible understanding of how species interact. In *Evolution Through Genetic Exchange* Arnold (2006) supports Templeton's definition as the most accurate description of what can be

observed in nature itself. He also supports the findings of Doolittle (1999) who states that "… the history of life cannot properly be represented as a tree". Arnold concludes: "This (…) reflects the reality of the web-of-life metaphor as the best representation of long-term evolutionary pattern and process." Especially the proportion of hybridization and introgression (the gene flow between species as a result of ongoing hybridization and backcrossing) has been underestimated until recently (Arnold, 1997). The amount of ongoing natural interspecific crossings and their effect on taxonomic units is best described by the concept of reticulate evolution: "Web-like phylogenetic relationships reflecting genetic exchange (through lateral transfer, viral recombination, introgressive hybridization, etc.) between diverging lineages." (Arnold, 2006).

The above mentioned frequent hybridization among *Daphnia* species contradicts traditional species concepts involving those premises defined by Mayr (1963) and are therefore not applicable. *Daphnia* species are able to respond microevolutionarily to rapid ecological changes (Hairston et al., 1999b; Decaestecker et al., 2007). Based upon these findings, I hypothesize that the combination of resting eggs production as an adaptation to harsh conditions and missing reproductive barriers among sympatrically occurring, related species usually preventing interspecific hybridization allows a quite immediate adaptation to ecological changes due to a subsequently higher genetic variability in these taxa via geneflow between species and may therefore be advantagous.

Preview to This Study

My general aim was to study the biological archive of *Daphnia* (Cladocera; Anomopoda) resting eggs isolated from lake sediments and to unravel population genetic processes covering several decades. Specifically, I addressed the following six questions:

(1) How did the genetic architecture of the species complex change after a hybrid sweep?
(2) Are levels of interspecific hybridization and introgression associated with environmental changes?
(3) What is the level of postzygotic isolation among species, and does the genetic composition in the resting egg bank correspond with the genetic spectrum of hatched individuals?
(4) What is the level of genetic differentiation in neutral genetic loci?

(5) What is the origin of current *Daphnia* populations of Lake Constance?
(6) What effect did introgression have on the current *Daphnia* populations of Lake Constance?

In chapter one I describe the establishment of 32 microsatellite markers for European *Hyalodaphnia* species which are subsequently used to classify the examined taxa, hybrid classes and population structures. Some markers have been established for other *Daphnia* species before, others were tailormade for *D. galeata*. The markers were tested not only for *D. galeata*, *D. hyalina*, *D. cucullata* and *D. curvirostris* but also for the recombinants *D. galeata x hyalina* and *D. galeata* x *cucullata*. In a final step I have identified diagnostic alleles for the tested species.

In chapter two I present a population genetic analysis of the *Daphnia* resting egg banks of Lake Constance and Greifensee that have undergone a phase of anthropogenically induced ecological change. I reconstructed its effect on the species composition and the resulting interspecific hybridization between the inhabiting species *D. galeata* and *D. hyalina* (to some extent also *D. cucullata* formerly present in Greifensee). Chapter three focusses on Lake Constance *Daphnia* and the association of genetic processes and ecological change. I put an emphasis on the fate of interspecific hybrids and the rate of genetic exchange and therefore applied nuclear and mitochondrial DNA restriction fragment analyses as well as the microsatellite markers developed as described in chapter one. Moreover, I studied hatched parental and hybrid individuals from different time periods and analysed their ability to cope with high and low quality and quantity of algael diet.

To better understand the contribution of the resting egg bank to current populations, I compared the genetic composition of eggs, non developing eggs and hatchlings (chapter four) genetically. The processes of developing from an egg to an adult individual that is capable of establishing a clonal lineage is physiologically complex and directly connected to their genetic constitution. Interspecific hybridization may lead to fitness deficiencies due to genetic incompatibilities or – to the other extreme – may result in hybrid vigor. In chapter four the extent of hybrid contribution to the planktonic population was the main focuss of research.

Chapter five finally deals with stabilized introgressants of *D. galeata* and *D. hyalina* occuring in the planktonic population of 2005 and 2006 and occupying adaptive niches formerly

unique to one of the parental species. Of major influence seems to be the cytoplasmic information rather than the nuclear condition when selection acts on organisms via temperature.

Chapter 1: Microsatellite Markers for European *Daphnia*

Published in Molecular Ecology Notes: N. Brede, together with A. Thielsch, C. Sandrock, P. Spaak, B. Keller, B. Streit and K. Schwenk (2006)

1.1. Introduction

Cladoceran species of the genus *Daphnia* have become an important model organism in ecotoxicology, limnology, ecological genetics and recently in genomics (Lynch & Spitze, 1994; Stark & Banks, 2003). This prominence of *Daphnia* is based on several characteristics of this genus: Species are widely distributed, representatives are key species in trophic cascades and occur across all kinds of freshwater habitats, they are easy to rear in the laboratory and because of their reproductive mode (cyclic parthenogenesis) they represent ideal experimental animals. In addition, *Daphnia* diapausing eggs were successfully used to reconstruct changes of taxon composition over evolutionarily relevant time periods (e.g. Hairston et al., 1999a).

The frequent occurrence of interspecific hybridization among species of the *D. longispina* complex resulted in several taxonomic problems, but motivated many studies with regard to the origin, maintenance and fate of hybrid lineages (e.g. Schwenk & Spaak, 1995; Jankowski & Straile, 2004). Previous studies were mainly limited by the small number of fixed loci among species (allozymes: Schwenk & Spaak, 1997) and the limited discriminatory power of other molecular markers (e.g. Billiones et al., 2004).

Although several microsatellite markers have been developed for American species (Colbourne et al., 2004; Fox, 2004), only a small number of markers has been published for European taxa (Ender et al., 1996; Fox, 2004).

1.2. Material and Methods

Here we present 32 microsatellite markers for the European *D. longispina* group, which were partly optimized using the *D. pulicaria* markers by Colbourne et al. (2004; Dp). Furthermore, we tested previously published primer sequences developed either for European *Hyalodaphnia* or for North American *D. galeata mendotae* (DaB: Ender et al., 1996; Dgm: Fox, 2004). An additional set of microsatellite markers (SwiD) was developed using an

Table 1 Characteristics of the 32 microsatellites: Locus name, repeat motif, primer sequences, annealing temperature (T_a), fragment size range, cross-species test with number of alleles.

Locus	Repeats	Primer sequences (5´-3´)	T_a (°C)	Size range (bp)	GenBank accession no.	Cross- species test*				
						D. galeata	*D. hyalina*	*D. rosea*	*D. cucullata*	*D. curvirostris*
DaB10/15[1]	TC_6	F: AGA GAA GTG TTT GCG TTT C R: TGT TTC CTA TAT CCC TCG G	55	75-89	U41402	5	2	NT	4	2
DaB17/17[1]	T_9	F: GAG AAC CTT TTA TCA GCT TCG R: ACT CAT CTG GTG AGA TGG ATC	55	100-109	U41403	4	4	NT	4	1
DaB17/16[×]	GA_{10}	F: AGG GAA CGA GCG GCG ATA AG R: TCT TTG GCA GGC CAC TGC CAA GG	55	189-195	U41403	3	2	NT	2	NO
DaB10/14[1]	CAA_8	F: CTC TTA TAA CCA GCA CCT CG R: CTA TTA TTC CAT CGT CCG TC	55	222-234	U41402	4	4	NT	1	NO
p512[2]	$(TG)_4...(GT)_8$	F: TTT CGT TCT ACC CAG GGA AG R: TTT GCT CGT CTG TGA TAG GC	56	125-141	AY057864	5	4	NT	3	NT
Dp519[2]	$(TG)_6(GA)_7$	F: AGT CGC GAC GAC ATA AAG C R: GTG GTA GTT GTG GAA TCC G	50	144-160	AY057865	5	5	NT	6	NO
Dgm101[3]	$(GA)_{10}AGA$	F: TCT TGC TCG AAT TCT CTC C R: CCT GTC TCA CAC GGA GC	54	162-177	AY542275	3	2	3	3	4
Dgm102[3]	$(TTG)_{10}...(TTG)_4$	F: AGC GTA TTC GAT TTC AGG R: ACG GAT TCG ATG ATG AAC CC	57	118-131	AY542276	1	1	3	1	2
Dgm105[3]	$(CAG)_8AG$	F: ATG TGA GCG CGC GAG CAT TT R: GTC CAG CCG GCC CAT TTC AGT T	55	172-197	AY542269	2	4	3	2	1
Dgm106[3]	$(CAA)_8CCAA$	F: ACC ACC ACC TCC TCC GCC ACA T R: TTC GTC GAT TTC CTC ACC CAT TTC	50	124-155	AY542270	2	2	2	NT	NT
Dgm107[3]	$(TGC)_7$	F: CCT TTG GCA TCG TTT CTT ATT CTT R: CCT GCC AAC CTC CCA GTC CT	51	117-132	AY542271	2	3	NO	1	NO
Dgm109[3]	$(ACC)_7AC$	F: CCA GCT GTT GAC CAC CTG R: TGC GCG AGG ATT TCC AAC AC	61	247-266	AY542272	2	4	3	5	NO
Dgm112[3]	$(TGC)_6TGG$	F: GGA AAT AGG CCT AGA TGC TGT GT R: TTA TTG ATC TTC CGG CTG ACT TTA	55	109-130	AY542274	3	2	1	NO	3
Dgm113[3]	$(GTC)_7$	F: TGC CAC GAA TCG TCT ATA ATG GTG R: AAG CCC ACA TGT AGG CAC AAG TCA	55	133-155	AY542279	3	3	4	NO	2
Dp196NB[+]	AC_5	F: ATT TTC CGC CCT TAT TCT GC R: TCT TGG TCG CGT TCC AGC	50	115-130	WFms0000201	3	3	2	NT	1
Dp238NB[+]	AG_8	F: ACA AGC AAC TCA CCA AAA GG R: CTA GAT GTA CAC TGG GC	52	61-77	WFms0000246	4	1	3	1	2

Dp281NB^{+}	T_{10}	F: AAT AAC ACT CGT AGC ACG R: AGC GGA CCG GAA GTG GTA GG	55	69-78	WFms0000290	2	2	2	1	1
SwiD1	$(TG)_{18}$	F: GCC GTG TTC GAA AGC TAG TC R: AGC CGA ACG AAA AAC ATG C	61	116-142		3	3	2	2	NO
SwiD2	$(TG)_{18}$	F: GTC AAG TTG TTC TTG TTA TTG TGC R: TTT TGT AGG TCC GCG TAA ATG	55	164-194		3	2	2	1	NO
SwiD4	$(CA)_{17}$	F: GAC CCA AAG TCT CTC TCT CCA TC R: TGG AGA TGT ATC ACA TCC ATA CG	56	159-204		2	3	2	1	3
SwiD5	$(GA)_{13}$	F: ACT ATG CAT AAC ACA GAC ACA CG R: GAA GTA CGG CAA GGA GCA AC	61	145-165?		3	1	2	NO	NO
SwiD6	$(CA)_{13}$	F: GAT CAG CAA GAT GAA ATA CAC R: ATT TGA AGG CAT TTC CTG TAC G	55	123-142		4	2	3	1	NO
SwiD7	$(CTG)_{6}$	F: TCA GAC TGG TGA TTA CGA CTG C R: TCT GAT AAA GCG GAT GAG AGA AC	58	159-171		2	2	2	1	2
SwiD8	$(TG)_{4}(TG)_{13}$	F: GAT ATT CTC TTG GAC TGC GTT TG R: GAT ATG ACA AGC CGA CGT CAT	56	117-159		3	1	1	NO	NO
SwiD10	$(TG)_{19}$	F: TGT AGA TAT CAG CCA GCA GCT C R: AAG GTT ATT CTC TCC GCT CGT C	60	182-204		2	2	1	2	2
SwiD11	$(GT)_{20}$	F: ACT CGA CAA ACT TGG AGA GGT C R: GGG GTG GCT ATA GAT AGA CTG G	57	155-177		3	NO	2	1	3
SwiD12	$(TC)_{14}TTA(TG)_{12}$	F: ATT CTT ATT GCC CCA AAT AAC C R: GCC GCT TTT TCT ATC TGC ATA C	55	105-127		4	4	3	NO	1
SwiD14	$(GT)_{12}...(GT)_{7}$	F: AGA CGA TCG TTG GTT CAT CC R: CCG GAT AGT TGC TGG AAA AG	59	173-191		2	1	3	1	1
SwiD15	$(GT)_{14}$	F: TCT TCT TTT TCC CAT ACA GAC TCT C R: CTC CCT CTG ATT TGG CGT AAC T	54	79-99		2	3	1	NO	NO
SwiD16	$(CA)_{15}$	F: CAT CGA CAA TGT ACG GTG GGA G R: GGC TGG TGG TGG TCC AGT GGT T	57	168-187		2	1	2	1	3
SwiD17	$(GA)_{9}$	F: CGT ATG GAT GTG ATA CAT CTC CA R: GGA ATG AGT TGG AAA GAG GGA	53	76-98		1	1	1	2	3
SwiD18	$(CA)_{9}$	F: GGA TGC CAA CTC TCT CCC CCT A R: CGT GTG TCT GTG TTA TGC ATA GT	55	85-97		3	2	3	2	NO

* Cross-species testing results: > 1: variable microsatellite - number of alleles, 1: amplicon obtained but tested invariable, NO: no amplicon obtained, NT: not tested for this species; [1] Ender et al. (1996), [×] primer sequence differs from Ender et al. (1996), [2] Colbourne et al. (2004) , [+] primer sequence differs from Colbourne et al. (2004), [3] Fox (2004).

Table 2 Selection of 16 potentially species specific microsatellite markers: Locus name, detected alleles for tested species and interspecific hybrids.

	Cross-species and interspecific hybrid test*							
Locus	*D. galeata*	*D. hyalina*	*D. rosea*	*D. cucullata*	*D. curvirostris*	*D. galeata x hyalina*	*D. galeata x rosea*	*D. galeata x cucullata*
Dgm102[1]	131	118	118 / 124 / 131	118	**140** / **144**	131	128 / 131	115
Dgm105[1]	186 / 193	184 / 187 / 190 / 195	183 / 185 / 187	**177** / **179**	177	193	186 / 193	193
Dp196NB[+]	115 / 117 / 121	121 / 127	121 / **135**	NT	130	119 / 121	119 / 121	NT
Dp238NB[+]	61 / 65 / 71 / **77**	65	61 / 65 / 69	59	59 / 61	61 / 65	**77**	59
SwiD1	125 / 130 / 134	**116** / 121 / 134	**116** / 125	125 / 130	NO	134	130	125
SwiD2	164 / **182** / **184**	168 / 172	168 / 172	164	NO	172	172	172
SwiD5	158 / 162 / 164	**127**	153 / 158	NO	NO	160	162	NO
SwiD7	158 / 161	161 / 164	161 / 164	158	**144** / **152**	158 / 161	155 / 161	158
SwiD8	122 / **141** / **155**	122	122	127	NO	122	117	**155**
SwiD10	186 / **202**	186 / 192	186	184 / 196	192 / 196	186	184	180
SwiD11	158 / 163 / 175	NT	166 / 175	150	**139** / **141** / **145**	NO	163	NT
SwiD12	111 / 119 / 124 / **127**	111 / 113 / 115 / 119	113 / 115 / 117	NO	**98**	115 / 122	105 / 113	124
SwiD15	**91** / **97**	79 / 84 / 86	79	NO	NO	79 / **97**	**95**	**97**
SwiD16	168 / 172	172	170 / 172	**182**	**180** / **185** / **187**	168 / 172	168 / 172	168
SwiD17	80	80	80	80 / 83	76 / 78 / **98**	80	80	80
SwiD18	85 / 87 / 93	93 / 97	91 / 93 / 97	81 / 91	NO	NO	85	**77**

* Cross-species testing results: bold numbers: alleles potentially species specific, NO: no amplicon obtained, NT: not tested for this species or hybrid; [+] primer sequence differs from Colbourne et al. (2004), [1] Fox (2004).

enriched library from size selected genomic *D. galeata* DNA ligated into SAULA/SAULB-linker (Armour et al., 1994) and enriched by magnetic bead selection with biotin-labelled $(CA)_{14}$ and $(ACAG)_7$ oligonucleotide repeats (Gautschi et al., 2000). Out of 570 recombinant colonies screened, 98 gave a positive signal after hybridization. Plasmids from 72 positive clones were sequenced and primers were designed for 15 microsatellite inserts (Ecogenics GmbH). Of these, 13 were tested for polymorphism.

In order to cover a representative array of species of the *D. longispina* group, we selected laboratory clones originating from a broad geographic range. *D. galeata* (one from each country: Germany, Switzerland, The Netherlands, North Ireland), *D. hyalina* (one from Northern, Middle and Southern Germany, and one from Switzerland), *D. rosea* (one from Northern and two from central Germany), *D. cucullata* (one from each country: Switzerland and The Netherlands) and *D. curvirostris* (two from Northern Germany, one from each of the following countries: Central Germany, Czech Republic) were tested as well as one interspecific hybrid of the following species: *D. galeata x hyalina* (The Netherlands), *D. galeata x rosea* (Israel) and *D. galeata x cucullata* (The Netherlands). Six of the 32 loci (DaB10/15, DaB17/17, DaB17/16, DaB10/14, Dp512, and Dp519) have been tested on 23 populations of *D. galeata* across Europe. The allelic richness (alleles/*N*) ranged from 0.34 to 0.72 (Ø = 0.484) and an average observed heterozygosity of 0.236 (range: 0.114-0.405; Dove et al., in preparation). Since species of the *Daphnia longispina* group form clonal assemblages, generations overlap due to diapause and introgressive hybridization occurs frequently, thus heterozygosity deficiencies and linkage disequilibria are often observed (Schwenk & Spaak, 1995).

DNA preparation of *Daphnia* individuals was carried out in 70 µl H3 buffer (1x: 10 mM Tris-HCl (pH 8.3 at 25°C), 0.05 M potassium chloride, 0.005% Tween-20 and 0.005% NP-40). After adding 2 µl of Proteinase K (Sigma, 10 mg/ml) samples were vortexed and centrifuge*D.* Incubation varied between 4 and 16 hours at 56°C. Samples were then boiled at 96°C for 12 min, centrifuged shortly, and stored at 4°C. PCR reactions were performed in 0.2 ml tubes using either a Biometra T3 or a DYAD thermal cycler. All reactions were first performed with a 10 µl reaction volume containing 2.4 mM $MgCl_2$, 1x PCR buffer, 0.25 mM dNTPs, 0.2 µM of each primer and 0.5 Units Taq DNA polymerase (chemicals and primers by Invitrogen). Cycling conditions started with a 3 min denaturing step at 95°C followed by 35 cycles of 1 min steps at 95°C, 55°C and 72°C. A final 7 min synthesis step at 72°C completed the program.

Depending on the specifity of each primer set, PCR conditions varied mainly in annealing temperature (see Table 1). When pure PCR products were obtained, the PCR was repeated with labeled forward primers (Invitrogen, MWG). Amplicons were diluted and electrophoresed on a CEQ 2000 (Beckman Coulter) or on an A.L.F. sequencer (Amersham) with self-designed size standards based on Lambda virus DNA (Symonds & Lloyd, 2004).

From 32 loci 25 were variable for at least three species. 16 of the markers showed a large shift of fragment lengths, indicating potentially diagnostic insertions or deletions among species (see Table 2). Several primer combinations were tested in multiplex PCR reactions to allow faster and efficient screening of populations. Primer concentrations were adjusted in order to amplify similar amounts of PCR products. For example, primers DaB17/17 (0.1 µM), DaB10/14 (0.075 µM), Dp512 (0.3 µM) and Dgm109 (0.075 µM) were subjected to a multiplex PCR with 3 mM $MgCl_2$, 1x PCR buffer, 2% BSA (NEB), 2% DMSO (Sigma), 1 U Taq (Invitrogen) in a reaction volume of 10 µl. Cycling conditions are identical to those presented above. For the primer set SwiD1, Swid10 and Swid14 (each 0.1 µM) an annealing temperature of 60°C was appropriate. Generally, many primer combinations were successfully tested in multiplex PCR, however, sufficient amplification was only achieved if all primers were labeled with the same dye.

1.3. Results and Discussion

110 primer pairs that positively amplified microsatellite markers in American *D. dentifera* (Colbourne et al., 2004) were tested and resulted in eight polymorphic loci. Only 65 primer pairs successfully amplified a fragment and only 34 amplicons exhibited the expected fragment size (± 50bp; after testing amplification conditions of Colbourne et al. 2004 and alternative conditions using a *D. pulicaria*-clone from The Netherlands as a positive control). DNA Sequencing revealed that only 18 fragments corresponded with the reference sequence, of which eight loci (7.3% of the tested microsatellites) contained repetitive units found in *D. pulicaria* (Colbourne et al., 2004). Due to inefficient amplification yield for the *D. longispina* group, three primer pairs (Dp196NB, Dp238NB, and Dp281NB) containing a variable microsatellite were newly designed. Our study shows that the high sequence divergence of microsatellite flanking regions hampers the application of primer sets originating from sister species. However, considering the large genetic differentiation among

D. pulicaria and members of other subgenera (i.e. *Ctenodaphnia* and *D. longispina* group), we expect that the application of the described markers will be most efficient for closer related species.

These markers together with the newly developed markers provide a powerful “toolbox” of 32 microsatellite loci for *Daphnia* taxonomy, ecology and evolutionary biology. These markers were already applied to determine natural population structure in *D. galeata* (Dove et al., in preparation) and for clonal identification of experimental animals (Seidendorf et al., 2007). In addition, due to the discriminatory power of microsatellite loci among species and hybrid classes, we were able to reconstruct evolutionary changes from *Daphnia* resting egg banks (Brede et al., in preparation).

Chapter 2: The Impact of Man-made Ecological Changes on the Genetic Architecture of *Daphnia* Species

Submitted to PNAS: N. Brede, together with C. Sandrock, D. Straile, P. Spaak, T. Jankowski, B. Streit and K. Schwenk

2.1. Introduction

During the last century most European lakes went through a phase of eutrophication, i.e. an overenrichment with nutrients, and many recovered their original trophic state due to pollution control (Correll, 1998). This process is accompanied by a shift in species composition and diversity of both pelagic and litoral communities, reduction of water quality, and even occasional fish kills (Schindler, 2006). In northern temperate lakes, total phosphorus (P) concentration is regarded as the key factor of eutrophication (Schindler, 1978). Man made increased levels of phosphorus (urban and industrial sewage, erosional runoff and leaching from agricultural areas) causes algal blooms which subsequently affect species of higher trophic levels such as zooplankton and fish. Among the most important planktonic grazers in pelagic foodwebs are species of the genus *Daphnia* (Crustacea: Anomopoda; water fleas). *Daphnia* species serve as food for fish and invertebrates, and feed on algae and bacteria. *Daphnia* produce diapausing stages, which are deposited in the sediments of lakes (Figure 1B). Since subfossil resting eggs are often still viable (up to 100 years: Marcus et al., 1994)) and provide sufficient quality and quantities of DNA for genetic analyses (Schwenk, 1993; Limburg & Weider, 2002), resting egg banks represent an unique biological archive to unravel ecological and evolutionary changes (Hairston et al., 1999b; Brendonck & De Meester, 2003). Although sediment remains have been used to assess the species compositional changes in association with eutrophication (Jeppesen et al., 2005), hardly anything is known about intraspecific processes, such as extinction or origin of lineages, natural selection and adaptation. However, a number of studies suggested an indirect impact of variation in phosphorus on *Daphnia* clonal and species composition through variation in planktivorous fish (Boersma et al., 1998), parasites (Wolinska et al., 2006), and toxic cyanobacteria (Hairston et al., 1999b). Life history surveys of *Daphnia* species indicated that predation levels as well as food quantity and quality (e.g. C:P ratio of algae) determine fitness (Seidendorf et al., 2007). In order to unravel a potential association of species composition and variation in P levels, we reconstructed the taxon composition and patterns of genetic

variation using *Daphnia* diapausing (ephippial) eggs from two peri-alpine lakes, Lake Constance (Austria, Germany, Switzerland) and Greifensee (Switzerland). Both lakes, as the majority of European lakes, were subjected to increased levels of phosphorus mostly due to the intensive application of fertilizers and phosphorus containing detergents (Correll, 1998). However, after the reduction of P inflow, due to the installation of sewage treatment plants, P-levels decreased again to lower levels comparable to those prior to eutrophication. The two main goals of our study were (1) to test for a potential association of inter- and intraspecific genetic variation in *Daphnia* with changes in total P over time. Furthermore, (2) we aimed to assess the evolutionary consequences of rapid ecological changes on the genetic make-up of natural populations.

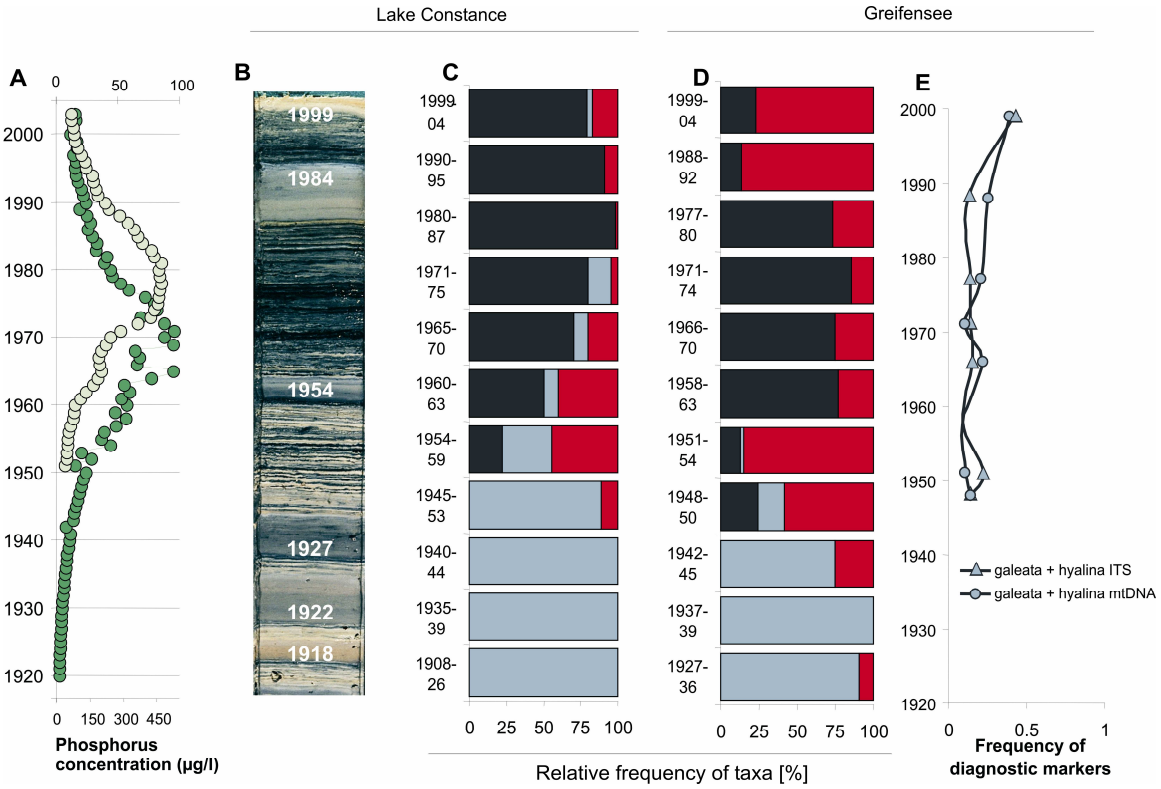

Figure 1. Reconstruction of the *Daphnia* taxon composition and ecological changes in Lake Constance and Greifensee over time. (**A**) Phosphorus concentration in Lake Constance (grey and upper x-axis) and Greifensee (green and lower x-axis) over time . (**B**) Sediment core covering years between 1900 and 2004 used for the isolation of resting eggs. (**C** and **D**) Temporal variation in relative abundances of *Daphnia* taxa in Lake Constance and Greifensee, respectively (light blue = *D. hyalina*, red = *D. galeata x hyalina* and grey = *D. galeata*). Species and hybrid identification is based on ITS-RFLP, mtDNA, and microsatellite analyses . (**E**) Temporal pattern of nuclear and mitochondrial DNA introgression of *D. galeata* in Greifensee. Relative abundance of all those *D. galeata* individuals classified by microsatellite analyses, which exhibit either nuclear (ITS) or mitochondrial (16S) DNA of *D. hyalina*. Blue triangles represent ITS alleles and blue dots mitochondrial haplotypes of *D. hyalina* found in *D. galeata* resting eggs.

2.2. Materials and Methods

Sediment cores of Lake Constance (Germany) were collected in September 2004 from 180 m depth close to the Langenargener Bucht. Greifensee (Switzerland) cores were collected in November 2004 from the middle of the lake at 30 m depth. Largest possible lake depth for sediment sampling was chosen to prevent sampling of biological archives which have been subjected to multiple hatching stimuli. Sediments from Lake Constance were dated by lamination counting (Wessels et al., 1995; Weider et al., 1997). In addition, cores of both lakes were dated using reference cores which have been subjected to 137Cs-dating (Lake Constance (Wessels et al., 1995) and Greifensee (Lotter et al., 1997). Sediments from Greifensee were portioned into 1 cm slices. In order to obtain a sufficient number of resting eggs for both lakes we pooled sediment layers covering on average 5.59 years (range = 3 to 18 years; Table 3). In order to prevent contamination of cores by the movement of the Perspex tube through the sediment, we removed the outer sediment ring (ca. 1 cm) from subsequent treatments. Ephippia were isolated by washing the sediments through a metal sieve (220 µm mesh size). Phosphorus concentrations, either based on direct measurements or reconstructions using diatoms in sediments, were provided by the Landesanstalt für Umweltschutz Baden-Württemberg, Institut für Seenforschung, Langenargen, Germany (Lake Constance) and the Canton of Zürich (Greifensee; Elber et al., 2004).

Resting eggs were isolated from their ephippial shells and DNA was prepared separately in 35 µl H3 buffer (10 mM Tris-HCl, pH 8.3 at 25°C; 0.05 M potassium chloride, 0.005% Tween-20, and 0.005% NP-40) and 1.2 µl Proteinase K (10 µg/ml; Sigma). After an incubation time of 12 hours Proteinase K was deactivated by heating the sample 12 min to 95°C. An ITS fragment (a short piece of the ITS1 region, 5.8S rDNA, and a large part of ITS2 region) was amplified using a total reaction volume of 14 µl. 2 µl of template, 3 mM MgCl2, 1x PCR buffer, 0.2 mM dNTP, 0.3 µM of each primer (ITS2-5.8S: 5´-GGA AGT AAA AGT CGT AAC AAG G-3´ and ITS1-18S: 5´-CGG TGG TCG ACG ACA CTT CGA CAC GC-3´) and one U/reaction Taq polymerase (Invitrogen) were amplified in 94 °C for 3 min, 5 cycles at 94°C for 1 min; 52°C for 1 min; 72°C for 1.5 min; 35 cycles: 94°C for 1 min; 50°C for 30 sec; 72°C for 1 min; final synthesis step at 72°C for 5 min. A restriction fragment length polymorphism analysis (RFLP) was used for taxon identification (Billiones et al., 2004). Amplicons of the ITS region were digested with the restriction enzyme Mwo I for 2.5 hours at

60°C in a total reaction volume of 9.6 µl containing 8 µl PCR product and 10x NEBuffer for Mwo I, 0.8 U of the restriction enzyme and autoclaved dH_2O.

Table 3. Number of *Daphnia* resting eggs subjected to DNA analyses. ITS = Restriction fragment length polymorphism analysis (RFLP) of the internal transcribed spacer region (ITS), 16S = 16S rDNA mitochondrial DNA RFLP and µsat = microsatellite analysis of six (Lake Constance) and eight (Greifensee) polymorphic loci.

Lake	Time	ITS	16S	µsat
Lake Constance	1908-26	2	0	2
	1935-39	3	0	1
	1940-44	1	0	0
	1945-53	9	0	0
	1954-59	27	27	27
	1960-63	30	0	38
	1965-70	71	0	0
	1971-75	64	37	38
	1980-87	81	0	0
	1990-95	56	57	56
	1999-04	100	0	0
	Total	**444**	**121**	**156**
Greifensee	1927-36	30	30	30
	1937-39	15	14	14
	1942-45	15	15	15
	1948-50	38	34	34
	1951-54	66	61	61
	1958-63	44	25	25
	1966-70	97	76	76
	1971-74	29	18	18
	1977-80	100	49	49
	1988-92	66	39	39
	1999-04	56	52	52
	Total	**556**	**413**	**413**

To distinguish between *D. galeata* and *D. hyalina* mitochondrial haplotypes, a digestion of a 16S rDNA fragment with restriction enzymes was conducted (Schwenk et al., 1998). PCR amplifications were performed with 2 µl template in 14 µl reaction volume containing 3 mM MgCl2, 1 x PCR buffer, 0.2 mM dNTP, 0.3 µM of each primer (S1: 5'-CGG CCG CCT GTT TAT CAA AAA CAT-3'; S2: 5'-GGA GCT CCG GTT TGA ACT CAG ATC-3') and 1 U Taq DNA polymerase (all chemicals and primers by Invitrogen). Cycling conditions started with two cycles of 93°C for 2.5 min, 55°C for 1 min, and 72°C for 2 min followed by 41 cycles of 1 min steps at 93°C, 55°C, and 72°C. A final 5 min synthesis step at 72°C completed the

amplification. Four µl of the amplicon were separately digested for 3 hours with three different enzymes: Mnl I, Dde I, and Rsa I (all NEB). The combination of digestion banding patterns identified the mitochondrial haplotypes (Table 4).

Microsatellite analyses are based on either six (Lake Constance) or eight (Greifensee) loci: DaB10/15, DaB17/17, DaB17/16, DaB10/14 (Ender et al., 1996), Dp512, Dp519 (Colbourne et al., 2004), Dgm101, and Dgm109 (Fox, 2004). All amplifications were performed in a 10 µl reaction volume containing 2.4 mM MgCl2, 1x PCR buffer, 0.25 mM dNTPs, 0.2 µM of each primer, and 0.5 U Taq DNA polymerase (chemicals and primers by Invitrogen). Cycling conditions started with a 3 min denaturing step at 95°C followed by 35 cycles of 1 min steps at 95°C, primer specific annealing temperature (see below) and 72°C. A final 7 min synthesis step at 72°C completed the program. Annealing temperatures varied depending on primer pairs: DaB10/15, DaB17/17, DaB17/16, DaB10/14 at 55°C; Dp512 at 56°C, Dp519 at 50°C, Dgm101 at 54°C, and Dgm109 at 60°C. Amplicons were diluted and electrophoresed on an A.L.F. sequencer (Amersham) with self-designed size standards based on Lambda virus DNA.

Table 4. Species specific mitochondrial DNA markers in *Daphnia*. Restriction fragment length polymorphism analysis (RFLP) of 16S rDNA fragments using restriction enzymes *Rsa* I, *Dde* I, and *Mnl* I. Haplotypes *c1*, *g1* and *h1* were reported in previous studies; *x7* and *c3* exhibit identical RFLP patterns, but DNA sequencing of 12S rDNA revealed large sequence differences (11.1% sequence divergence). Each resting egg of *D. cucullata* and a subsample of *D. hyalina* resting eggs were subjected to PCR and sequencing of mtDNA (Taylor et al., 1996). Haplotype *c3* was found only in twelve *D. cucullata* resting eggs during the time period of 1958-1963 indicating a short but unsuccessful invasion of Greifensee.

Species	**haplotype**	***Rsa* I**	***Dde* I**	***Mnl* I**
D. cucullata	*c1*	560	290-180-100	250-230-100
	c3	560	450-100	250-260-100
D. galeata	*g1*	510-50	290-180-100	250-210-100
D. hyalina	*h1*	560	390-100-80	230-180-100-90
	x7	560	450-100	250-260-100

Species and hybrid identification was based on two different approaches, first we pooled all available information (ITS, microsatellites and mtDNA; “total evidence”) and secondly, we identified species based on microsatellite analysis and inferred the relative frequency of a nuclear (ITS) and a mitochondrial (16S rDNA) species specific marker over time (“congruence approach”). Microsatellite data were subjected to two different model-based Bayesian statistical techniques, STRUCTURE version 2.1 and NEWHYBRIDS version 1.1, which utilize the information of highly polymorphic molecular markers (Pritchard et al.,

2000; Anderson & Thompson, 2002). The following options were used for each STRUCTURE run: assignment without any prior information of population membership, two population model (K = 2), 106 replicates after a burn-in of 105, admixture model, α inferred with an initial value of 1, a maximum value of 10, a uniform prior, and the same value for all populations; different values of FST for different subpopulations; prior mean FST of 0.01; a prior SD of 0.0; and constant λ with a value of 1. NEWHYBRIDS analyses are based on more than 106 Markov Chain Monte Carlo (MCMC) simulation sweeps following a burn-in period of 104 sweeps, six genotype frequency classes, and no prior information. Data sets were analysed several times with different starting values, lengths of burn-in period and numbers of sweeps, as recommended by the authors (Anderson & Thompson, 2002).

Table 5. Hardy-Weinberg expectations of *Daphnia* taxa. We used the relative abundances of species, based on ITS and microsatellite DNA markers to estimate expected taxa proportions under random mating in both lakes. We found three of eight (Lake Constance) and three of ten (Greifensee) deviations from Hardy-Weinberg expectations [chi-square test (Peakall & Smouse, 2006)].

Lake	Time	χ^2	P
Lake Constance	1945-53	0.031	0.860
	1954-59	0.270	0.603
	1960-63	0.068	0.794
	1965-70	10.101	**0.001**
	1971-75	45.265	> **0.001**
	1980-87	0.003	0.955
	1990-95	0.122	0.727
	1999-04	4.967	**0.026**
Greifensee	1927-36	0.050	0.823
	1942-45	0.082	0.775
	1948-50	0.919	0.338
	1951-54	33.199	**0.000**
	1958-63	0.502	0.479
	1966-70	1.823	0.177
	1971-74	0.166	0.684
	1977-80	2.323	0.127
	1988-92	38.122	> **0.001**
	1999-04	21.748	> **0.001**

One of the methods used for species and hybrid identification, the ITS-RFLP analysis (Billiones et al., 2004), has been verified using additional restriction sites, since recent findings indicate a polymorphic recognition site of Mwo I for *D. galeata* and *D. cucullata* (Skage et al., 2007). However, we did not detect any ambiguous genotypes in the populations of Lake Constance and Greifensee. We only found a small proportion of *D. cucullata* (and

their hybrids) resting eggs during a short time period (1958-1963) in Greifensee. Identification of *D. cucullata* based on ITS patterns was consistent with mtDNA and microsatellite data. In Lake Constance no *D. cucullata*, *D. cucullata x galeata*, or *D. hyalina x cucullata* were detected.

Gene flow among species was detected by comparisons of ITS, microsatellite, and mitochondrial DNA markers. Each comparison (microsatellites versus ITS; microsatellites versus mtDNA; ITS versus mtDNA; or selected microsatellite loci versus microsatellites) revealed evidence for recombinant genotypes and introgression. In addition, we obtained very similar patterns of introgression irrespectively of the applied statistical techniques, e.g. STRUCTURE (Pritchard et al., 2000) or NEWHYBRIDS (Anderson & Thompson, 2002). Our results, together with previous multiple findings of introgression among *Daphnia* species, provide strong evidence for gene flow between species opposed to alternative explanations such as ancestral polymorphism or homogenization of rDNA intergenic spacers after hybridization (Taylor & Hebert, 1993; Schwenk & Spaak, 1995; Spaak, 1996; Gießler et al., 1999; Taylor et al., 2005).

2.3. Results and Discussion

Lake Constance and Greifensee are currently inhabited by two genetically divergent species of the *Daphnia longispina* complex (*D. galeata* and *D. hyalina*) as well as their interspecific hybrids (Jankowski & Straile, 2003; Keller & Spaak, 2004). These *Daphnia* taxa reproduce via cyclic parthenogenesis, altering clonal propagation with sexually produced resting eggs. The induction of sexual females and males is determined by environmental factors such as the change in food level, crowding (high densities of conspecifics), and photoperiod (Hobæk & Larsson, 1990). Therefore, abundances of resting eggs over time do not necessarily represent the short term success or fitness of taxa or clonal lineages at a given time period (Jankowski & Straile, 2003; Keller & Spaak, 2004). Instead, relative abundances of taxa or genotypes derived from resting egg banks reveal information about lineages which successfully contributed genes to the next generation. Thus, analyses of resting egg banks allows one to trace the long-term evolutionary fate of lineages (Weider et al., 1997).

The changes in species and hybrid composition are associated with rapid environmental shifts in Lake Constance and Greifensee (Figure. 1A, 1C and 1D). In the first half of the last century both lakes were inhabited by *D. hyalina*, which has been shown to be indigenous for Lake Constance (Obersee; Elster & Schwoerbel, 1970). In addition, our data confirm previous observations that *D. galeata* invaded Lake Constance in the mid-1950s (Muckle & Dillmann-Vogel, 1976). Similarly, Greifensee was successfully invaded by the same species in the mid-1940s. Species invasions in both lakes were followed by interspecific hybridization and a subsequent decrease of *D. hyalina* ephippia in the resting egg bank. This reduction and the virtual absence of *D. hyalina* resting eggs during recent decades (Figure 1C and 1D) is probably primarily due to the reduction of sexual activity since planktonic *D. hyalina* individuals have been reported in recent studies (Jankowski & Straile, 2003; Keller & Spaak, 2004). Furthermore, in the resting egg bank of Greifensee we detected *D. cucullata* and the interspecific hybrids *D. hyalina* x *cucullata* and *D. cucullata* x *galeata* in low frequencies.

The occurrence and sexual reproduction of parental taxa seem to be explained by ecological parameters. Both resting egg banks show that an increase of *D. galeata* and a decrease of *D. hyalina* over time are associated with the rise of phosphorus concentrations. Although both lakes went through a very similar history of eutrophication, they differ in the magnitude of phosphorus pollution. Lake Constance phosphorus levels rose from less than 10 µg/l (winter mixis) in the 1940s to a maximum of 87 µg/l in 1979, Greifensee peaked in 1971 with 525 µg/l after an average value of about 40 µg/l in the 1930s (Elber et al., 2004).
These different levels, but very similar shapes of the P curves suggest that the relative change in trophic levels might be more important for species shifts than absolute P values. Alternative explanations for the association of taxon composition and relative changes in P are 1) a general invasion of *D. galeata* in this region during the 1960s, 2) reduced sexual reproduction of *D. galeata* in Lake Greifensee during the first part of the last century or 3) other factors related to P, such as fish abundances, parasites or temperature determine the establishment of *D. galeata*.

Since a number of lakes which have not been subjected to a major phase of eutrophication (and are found in the neighbourhood of eutrophic systems), have never been invaded by *D. galeata* but exhibit only populations of *D. hyalina* or *D. longispina* (a synonym of D. hyalina: Petrusek et al., 2008). Furthermore, many studies have showen that *D. hyalina* is mainly found in oligotrophic lakes, wheras *D. galeata* represents a generalist which also occurs in

strongly polluted habitats (Keller et al., 2008 and references therein). In addition, studies using zooplankton remains of several lakes across Europe covering a large part of the last century revealed that species composition over time was associated with trophic levels (Manca & Armiraglio, 2002; Guilizzoni et al., 2006). In addition, field and laboratory studies showed that *Daphnia* species vary in their habitat preference and fitness and respond to variation in the P content of their food (Hessen et al., 1995; Tessier et al., 2000; Seidendorf et al., 2007). Therefore, or results – the association of P changes and *Daphnia* taxon composition in Lake Constance and Greifensee – are in concordance with previous field observations and current experimental work.

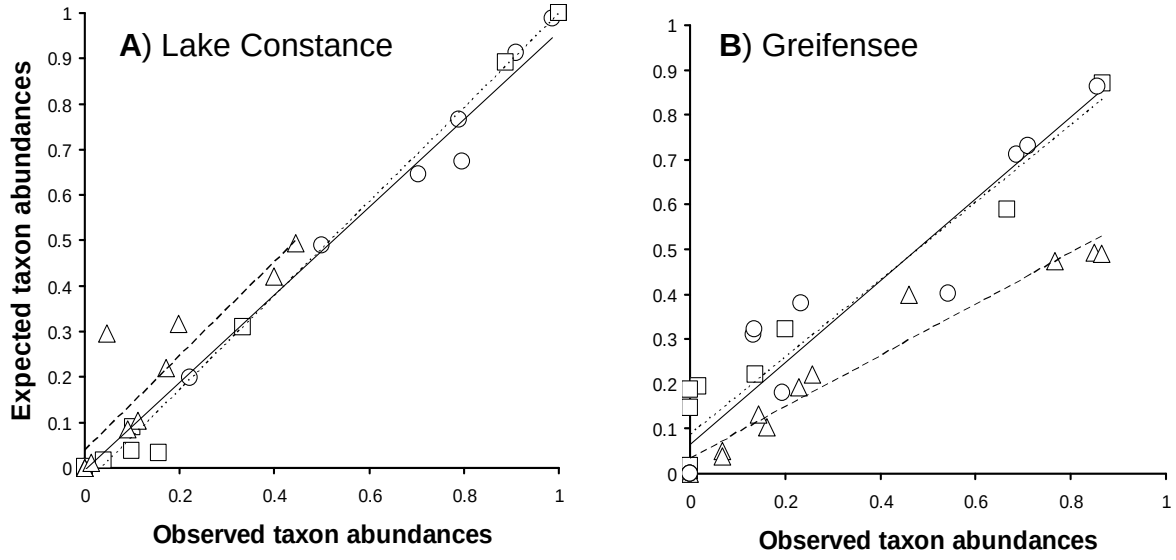

Figure 2. Comparison of observed and expected parental and hybrid genotypes assuming Hardy-Weinberg equilibrium in *Daphnia* populations of Lake Constance (A) and Greifensee (B). Observed species (*D. galeata* = circles; *D. hyalina* = squares) and hybrid (triangles) identification is based on nuclear DNA markers (ITS and microsatellites) and expected taxon abundances are calculated according to Hardy-Weinberg expectations (Table 5). Lines represent linear correlations of observed and expected taxa abundances (stippled = *D. galeata x hyalina*, dotted = *D. hyalina* and solid = *D. galeata*)

Although Greifensee reached relatively high levels of P already during the 1940s, *D. galeata* invaded the lake much later when P concentrations levelled around 100 µg/l. However, the presence of a few interspecific hybrids already during the 1920s suggests that *D. galeata* was present in Greifensee, but did not become established (despite P levels above 20 µg/l). This suggests that other (environmental) factors limited the establishment of *D. galeata*. One of the most important factors, besides P, which alter *Daphnia* communities is fish predation (Vakkilainen et al., 2004). However, predation has different impacts in shallow and deep lakes, e.g. predator control is higher in shallow than deep lakes (Jeppesen et al., 2003). Thus,

the interaction of P and fish predation (and potentially other factors) determines local community structures (Jeppesen et al., 2000; Hobaek et al., 2002; Moss et al., 2004; Gyllenström et al., 2005). Thus, it is not surprising that instead of absolute values of P rather the relative changes in trophic levels correspond with community structure.

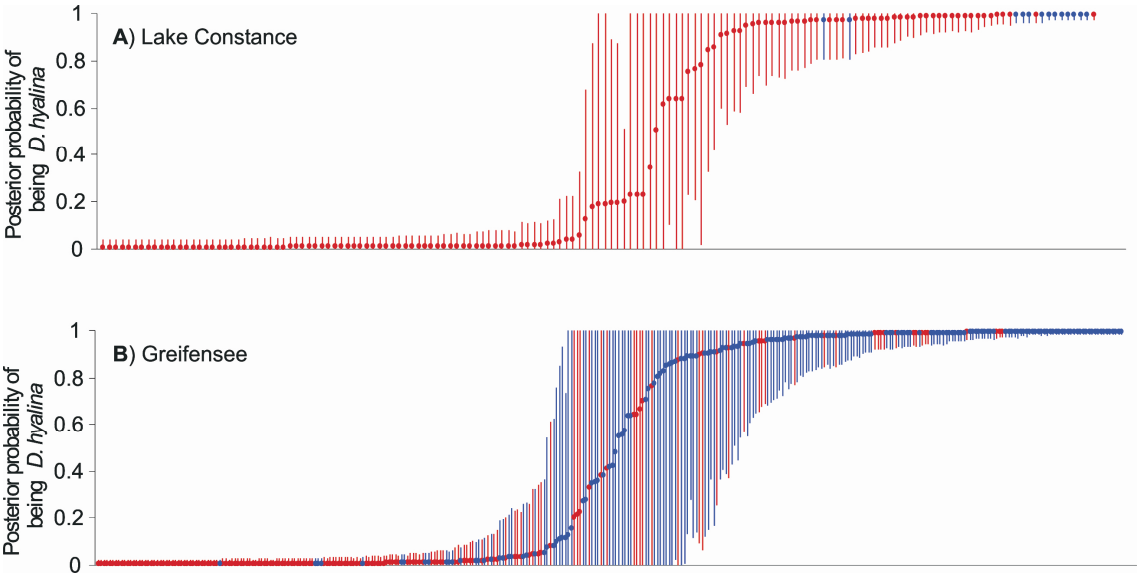

Figure 3. Level of mitochondrial DNA introgression in *Daphnia* species. Genotypic structure of *Daphnia* populations in (**A**) Lake Constance and (**B**) Greifensee based on the posterior probability of belonging to *D. hyalina* [as implemented in STRUCTURE (Pritchard et al., 2000)]. Species specific mitochondrial DNA haplotypes (16S rDNA RFLP analyses) are labeled with different colors (blue = *D. hyalina* and red = *D. galeata*). Error bars represent 95% probability intervals (blue = *D. hyalina* and red lines *D. galeata*).

Recent life history experiments of *D. galeata* and *D. galeata x hyalina* hybrids hatched from Lake Constance sediments provided further support for the association of variation in P-content of food items (algae) and *Daphnia* fitness. Daphnids from three time periods (1960s, 1970s and 2000s) showed a larger differentiation in somatic growth rate (Q_{st}) over time than genetic differentiation (F_{st}; Schwenk unpublished data). This pattern was consistent for both taxa and also prevalent in other traits, such as days to first reproduction and number of juveniles. Since genetic differentiation through time was an order of magnitude higher for the studied life history traits than for neutral markers (DNA microsatellites), natural selection is the most likely driving force behind the observed evolutionary changes (Spitze, 1993; Merila & Crnokrak, 2001). In summary, our data are consistent with field and laboratory experiments which suggest a severe impact of total P on community structure and differential fitness in *Daphnia*. Relative changes in trophic levels seem to facilitating interspecific hybridization and determine the fate of evolutionary lineages in large-lake *Daphnia*.

In addition, we found in both lakes that the occurrence of interspecific hybrids is determined by the relative abundance of parental species suggesting that species form a panmictic population with apparently low levels of pre- or postzygotic isolation (only a few samples deviated from Hardy-Weinberg expectations; see Table 5 and Figure 2; Schwenk et al., 2001). This pattern suggests that the ecological shift facilitated the establishment of *D. galeata* and subsequent interspecific hybridization with *D. hyalina*. *D. galeata x hyalina* resting eggs were detected during times of intermediate phosphorus concentrations (e.g. Greifensee: 185.9 µg/l; range = 18 - 525 µg/l), indicating either hybrid superiority under changing habitat conditions or a different phenology of sexual reproduction of parental taxa which causes an increasing level of interspecific crosses (Lewontin & Birch, 1966). Although we have no direct evidence for increased hybrid fitness under intermediate phosphorus conditions, the existence of backcrosses and mitochondrial DNA introgression suggests that *D. galeata x hyalina* individuals successfully reproduced and contributed to future generations (Figure 1E). Although we can not differentiate between the two potential szenarios, either increased hybrid fitness at intermediate trophic conditions or random mating among parentals, however both scenarios imply that the changes in trophic levels are responsible for the origin of interspecific hybrids.

The overenrichment and later re-oligotrophication of lakes allowed us to reconstruct the biotic consequences of biomanipulation at the species and individual level. Previous studies either observed community changes over short time periods (several years) using observational data at high taxonomic resolution or long time periods (100 to 200 years) using sediment remains and low taxonomic resolution. Here we present data covering 100 years of intraspecific changes based on a high taxonomic resolution (genotype level). This data set allowed us to asses the genetic consequences of the rapid ecological shift in Lake Constance and Greifensee. Comparative analyses of nuclear and mitochondrial DNA markers over time indicate that population genetic changes are mediated by introgression since the onset of interspecific hybridization (Figure 1E). The directionality and level of mitochondrial introgression varied among lakes (Figure 3).

However, in both lakes we found a significant number of *D. hyalina* (defined by microsatellite analysis) exhibiting mitochondrial DNA of *D. galeata*. In addition, we observed a general shift in the genotypic architecture of *D. galeata* and *D. hyalina* in both lakes over time (Figure 4). Introgression, i. e. the spread of genetic material of one species into the gene

pool of the other, facilitates the origin of new recombinant genotypes which may establish new evolutionary lineages.

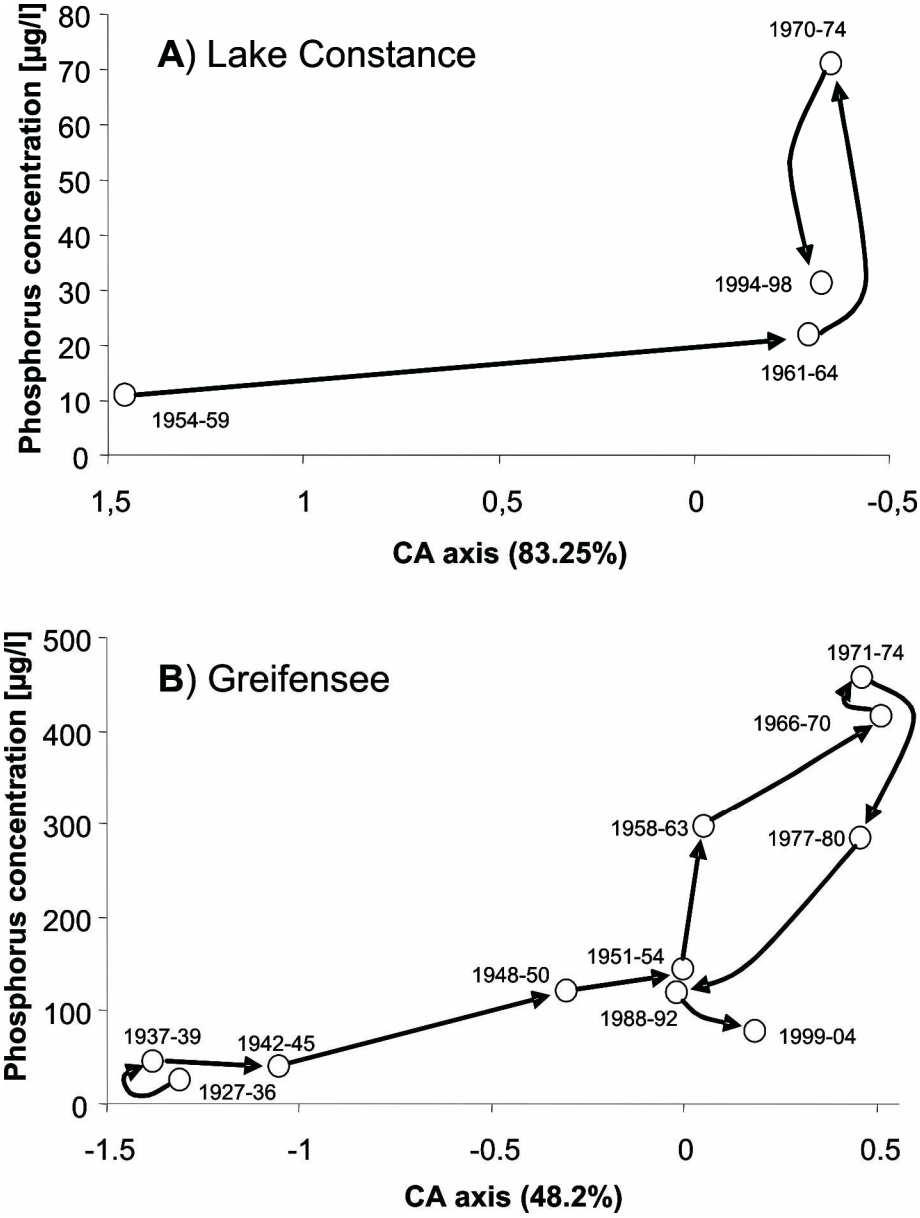

Figure 4. Association of genotypic shifts in the *Daphnia* species complex and environmental change over time. Genetic architecture of the *Daphnia hyalina-galeata* species complex in (**A**) Lake Constance and (**B**) Greifensee based on a canonical correspondence analysis (CA) of microsatellite data (Belkhir et al., 1996-2004). Each dot represents the genetic composition of a sample of *Daphnia* resting eggs (including species and hybrids) isolated from sediment cores of a given time perio*D.* The y-axis represents the average phosphorus concentration (µg/l) for each sample and arrows indicate the sequence of temporal changes.

This process is expected to be irreversible, since it is highly unlikely that assortative mating, genetic drift, or selection may re-establish the original genotypic architecture. Thus, interspecific hybridization of different *Daphnia* species prohibited the recovery of the taxon

composition prior to the eutrophication period despite the fact that both lakes nearly returned to their initial trophic states. Recent global environmental changes, such as the rise of surface temperature and the invasion of alien species, offer ample opportunities for interspecific hybridization because previously geographically isolated organisms come into contact. Since our data show that species introductions not only alter local communities, but also genetic structures of indigenous species, human-mediated introductions pose direct and indirect effects on the diversity of local populations. The environmental problem of eutrophication as well as of alien species invasions might be more serious than previously thought since genetic consequences can be cryptic and persistent.

Chapter 3: Ecological and Genetic Consequences of Interspecific Hybridization in *Daphnia*

3.1. Introduction

During the last two decades more and more cases of recombinant taxa across all animal classes are reported (Schwenk et al., 2008). In *Daphnia* species hybridization is well known and explored thoroughly (Schwenk & Spaak, 1997; Spaak, 1997; Schwenk et al., 2000). Researchers have focused on the extent of hybrid genotypes within populations (Schwenk & Spaak, 1997) and their ecological preferences or fitness traits in life history experiments (Spaak & Hoekstra, 1995; Spaak & Boersma, 2006). Unclear remains the fate of interspecific hybrid lineages which may persist via parthenogenesis.

Hybrids may adapt much faster to ecological changes since recombination of differentiated parental genomes generates a more diverse array of genotypes than mutation. Above this, most novel mutations are unique and will be subjected to selection. With genetic characters 'adopted' from another species evolutionary verification may be overridden (Arnold, 2006). The enrichment of genomes with new but 'validated' genetic traits descending from the other parental taxon may serve as a potential source for genetic novelties in hybrids. Although gene flow between species may be anticipated via enzymatic processes like gene conversion in parts of the genome (Aylon et al., 2004), it can also lead to the merging of sympatrically occurring species.

Daphnia species have become a model organism in ecological and evolutionary studies since sexually produced diapausing eggs can be stimulated to hatch even after 40 years if dormancy (Jankowski & Straile, 2003; Keller & Spaak, 2004). Genotypes produced by *Daphnia* that have inhabited bygone time periods involving other ecological conditions and selective effects can be used to study evolutionary processes over time (Hairston et al., 1999b; Jankowski & Straile, 2003). Moreover, the fate of interspecific hybridization and its consequences can be reconstructed and tested to explore the fitness traits of recombinant genotypes. In this study we focussed on their competitive potential compared to parental taxa and the effect of gene flow between species (i.e. introgression: Grant et al., 2005; Mallet, 2005; Ishida & Taylor, 2007) under the light of altering ecological conditions.

In this study we investigated the taxon composition and fate of interspecific hybrids as well as parental taxa of *Daphnia galeata* and *D. galeata x hyalina* from Lake Constance. We answer the following questions: 1) Did interspecific hybridization result in gene flow between species, i.e. introgression? 2) If so, did introgression and altering ecological characteristics of the habitat lead to the merging or diversification of taxa over time? We attempt to answer these questions by analysing the population genetic structure of the resting egg bank of the last 48 years. Subsequently, we hatched parental and hybrid individuals from different time periods and tested these on the extent of adaptation to different food levels based on the trophic history of Lake Constance.

3.2. Material and Methods

Sampling. Sediment cores of Lake Constance, which is located at the boarder of Germany, Switzerland and Austria, were collected in September 2004 from 180 m depth close to the Langenargener Bucht. Largest possible lake depth for sediment sampling was chosen to prevent sampling of biological archives which have been subjected to multiple hatching stimuli (below the thermocline, anoxic conditions in summer and in the dark). Sediments were dated by lamination counting. In addition, cores were dated using reference cores which have been subjected to ^{137}Cs-dating (Wessels et al., 1995). In order to prevent contamination of cores by the movement of the Perspex tube through the sediment, we removed the outer sediment ring (ca. 1 cm) from subsequent treatments. Ephippia were isolated by washing the sediments through a metal sieve (220 µm mesh size). Phosphorus concentrations, either based on direct measurements or reconstructions using diatoms in sediments, were provided by the Landesanstalt für Umweltschutz Baden-Württemberg and Institut für Seenforschung, Langenargen, Germany (Lake Constance).

Genetic analysis. Out of 214 isolated resting eggs, 48 extracted from the years 1960-1970, 48 from 1971-75, 31 from 1980-1987, 45 from 1990-1995 and 42 eggs from 1999-2004 were analyzed with ITS RFLP and 12 microsatellite loci to identify the taxa present in Lake Constance. Resting eggs were isolated from their ephippial shells and DNA was prepared separately in 35 µl H3 buffer (10 mM Tris-HCl, pH 8.3 at 25°C; 0.05 M potassium chloride, 0.005% Tween-20, and 0.005% NP-40) and 1.2 µl Proteinase K (10 µg/ml; Sigma) at 40°C. After an incubation time of 12 hours Proteinase K was deactivated by heating the sample 12

min to 95°C. An ITS fragment was amplified according to the protocols given in (Billiones et al., 2004; Skage et al., 2007).

Microsatellite analyses are based on twelve loci: Loci SwiD6, SwiD12, SwiD18 and SwiD14, Dp281, DaB10/14 as well as Dgm105, Dgm109, Dgm112 and Dp196, DaB17/17 were amplified separately multiplexed in a 10 µl reaction volume containing 2 µl DNA and 3 mM $MgCl_2$, 1x PCR buffer, 0.2 mM dNTP, 0.2 µM of every primer (only Dp281 with 0.1 µM), and 1 unit of Taq polymerase (primers SwiD6, SwiD12 and SwiD18 by MWG, all chemicals as well as all other primers by Invitrogen). Cycling conditions started with a 3 min denaturing step at 95°C followed by 35 cycles of 1 min steps at 95°C, 55°C annealing (Dp196 and DaB17/17 at 53°C) and 72°C. A final 7 min synthesis step at 72°C completed the program.

Amplicons were diluted and electrophoresed on an CEQ sequencer (Beckman Coulter) with self-designed size standards based on Lambda virus DNA (Brede et al., 2006).

Data analysis. We identified the taxonomic affiliation of each egg based on a factorial correspence analysis (GENETIX v. 4.01, Belkhir et al., 1996-2004) of microsatellite data and partially inferred ITS-RFLP data available as an additional species specific marker for hybrid class detection (see also chapter two: "congruence approach"). Microsatellite data were subjected to two different model-based Bayesian statistical techniques, STRUCTURE version 2.1 and NEWHYBRIDS version 1.1, which utilize the information of highly polymorphic molecular markers (Pritchard et al., 2000; Anderson & Thompson, 2002). The following options were used for each STRUCTURE run: assignment without any prior information of population membership, two population model (K = 2), 10^6 replicates after a burn-in of 10^6, admixture model, α inferred with an initial value of 1, a maximum value of 10, a uniform prior, and the same value for all populations; different values of F_{ST} for different subpopulations; prior mean F_{ST} of 0.01; a prior SD of 0.0; and constant λ with a value of 1. NEWHYBRIDS analyses are based on more than 10^6 Markov Chain Monte Carlo (MCMC) simulation sweeps following a burn-in period of 10^6 sweeps, six genotype frequency classes, and no prior information. Data sets were analysed three times with different starting values, lengths of burn-in period and numbers of sweeps, as recommended by the authors (Anderson & Thompson, 2002).

Life History experiments. *Daphnia* individuals were hatched from ephippia isolated from sediment layers of selected time periods. After genotyping clonal lineages we selected for the taxon *D. galeata* five clonal lineages from different time periods: 1965-1970, 1970-1980 and

1999-2004. For the second life history experiment with *D. galeata x hyalina* we chose five clonal lineages from the time periods: 1970-1975, 1975-1980 and 1999-2004.

We conducted an experiment with two food conditions and five clones of *D. galeata* from five time periods. In up to seven replicates we tested two to four 24h juveniles over four days in 20°C (±1°C) in a light-dark-cycle (16:8 h). In 100 ml jars all juveniles were reared in ADaM medium (Klüttgen et al., 1994) – P+ conditions were equivalent to a C:P ratio of 120 in the fed algae Scenedesmus obliquus, P limited food (-) corresponded to a C:P ratio of 1000. Medium and food were renewed every day during the experiment which lasted four days. Individuals were measured before the experiment under a binocular and after the experiment under a light microscope from the top of the head to the basis of the spine. The somatic growth rate was calculated with: $SGR = [\ln(S_t) - \ln(S_0)] / t$

with S_0 being the mean of body size at the beginning of the experiment and S_t the mean of body size after four days.

In a second experiment we added 'quantity' as a factor to the experimental design. In a flow through system as described in Lampert et al. (1988) Q+ conditions were 1.0 mg C/L, Q- corresponded to 0.1 mg C/L. At a temperature of 18.8°C 20 *Daphnia* individuals were tested in one tube with five replicates per clone. All algae media were stored in 20 l bottles wrapped with aluminium foil and checked daily with a photometer. After four days, *Daphnia* were extracted from the tubes and their body size measured (base of spine to top of head without the potential helmet).

F_{st} and Q_{st} values. A comparison of genetic and ecological differentiation between individuals was used to detect evolutionary processes like diversifying or homogenizing selection (Merila & Crnokrak, 2001). For the analysis of genetic distance between species within one time period and between those individuals used in the life history experiments F_{st} values were calculated with GenAlEx (Peakall & Smouse, 2001). Q_{st} values were calculated with: $Q_{st} = V_z/(V_z+V_i+2V_e)$

With V_z being the variance between populations (mean squares of time), V_i being the variance within populations (mean squares of clones) and V_e the error (mean squares). Mean squares were calculated on the basis of general linear models with STATISTICA (v. 6, StatSoft, Inc. 2003).

3.3. Results

Taxon composition over time. Our results show that the taxonomic composition of *D. galeata*, *D. hyalina* and recombinant genotypes has changed over time and these findings correspond with those of chapter two. We found five taxonomic classes ranging from *D. hyalina* parental genotypes over hybrids to *D. galeata* as the second parental taxon present in the lake. The determination between F1 and F2 *D. galeata x hyalina* was not possible and therefore all ambivalent genotypes were pooled in a general hybrid group.

Between 1960 and 1970 all taxonomic classes, i.e. parental taxa, first generation hybrids, second generation hybrids and backcrosses in both directions, were be found present in the resting egg bank. Between 1971 and 1975 *D. hyalina* produced 20% of the dormant propagules whereas hybrid genotypes are hardly found anymore. All other resting eggs belong to the taxon *D. galeata*. From 1980 until 1995 all resting stages analyzed belong to the previous group. In the sediments of the years 1999 to 2004 both parentals and both backcross classes were detected. While half of the eggs belonged to *D. galeata*, 30 % were classified as *D. hyalina*. More than 10% of the samples were backcrossed *D. galeata x hyalina*.

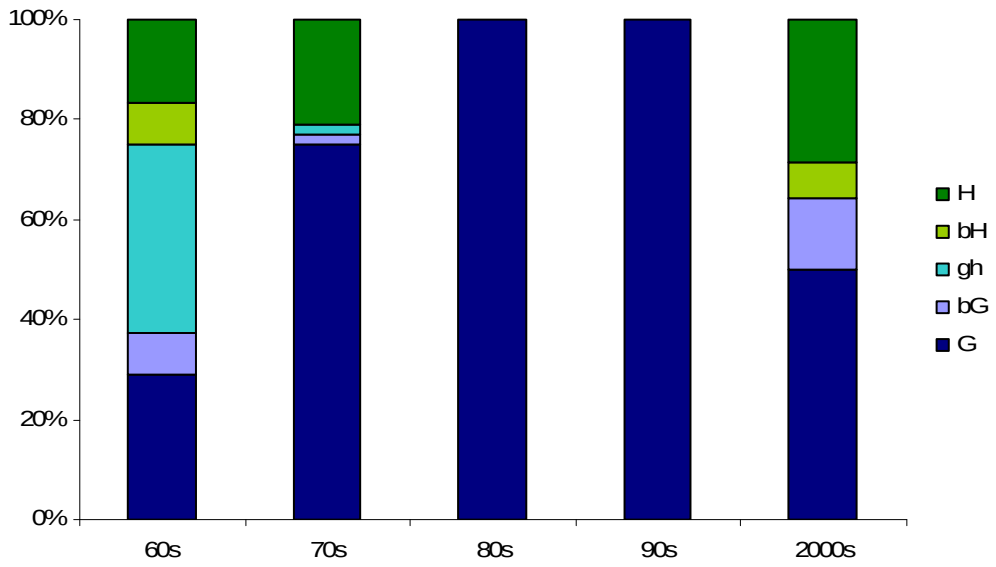

Figure 5. Taxonomic composition of resting eggs in Lake Constance including hybrid classes. dark green: *D. hyalina*, light green: backcrossed *D. hyalina x galeata*, turquoise: F_1 *D. galeata x hyalina*; light blue: backcrossed *D. galeata x hyalina*, dark blue: *D. galeata*

F_{st} values over time. We used F_{st} values as a parameter for taxon differentiation (between *D. hyalina* and *D. galeata*) over time (Figure 6). All hybrid genotypes were excluded from the analysis. Values in 1960-1970 and 1971-1975 range between 0.255 and 0.326. There was no

comparison possible between 1980 and 1995 since *D. galeata* resting eggs were the only ones present in the resting egg bank of Lake Constance. Those parental taxa of *D. hyalina* and *D. galeata* analyzed from the resting egg bank of 1999 to 2004 showed an F_{st} of 0.072.

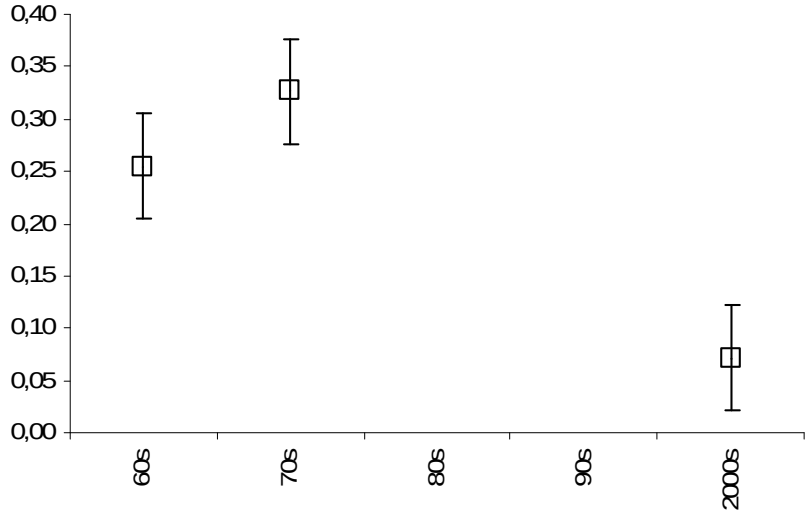

Figure 6. F_{st} values for *D. galeata* and *D. hyalina* populations within each time period. Values for 1980s and 1990s are missing since no resting eggs of *D. hyalina* were found.

A pairwise test of differentiation shows that multilocus genotypes of the 2000s sample are significantly different to those of the 1960s and to those of the 1970s ($P<0.001$, Fstat 2.9.3, Goudet & Keller, 2002).

Life history traits and the effects of selection. In a life history experiment we measured the somatic growth rate of *D. galeata* clones hatched from Lake Constance sediments representing different time periods: 1965-1970, 1970-1980 and 1999-2004. We compared *D. galeata* traits in high food quality (P+) and low food quality (P-). SGR is always higher in the high food quality treatment. In general SGR increases over time. While SGR in clones from 1965-1970 averages at 0.12, the SGR in the low food treatment of the most recent genotypes is 0.13 and 0.17 in P+. Over all, the SGR of *D. galeata* under high food conditions stays quite stable over time (between 0.1 - 0.17) but increases compared between the genotypes of different times significantly (Table 4): while all individuals from 1965 to 1970 had growth rates between 0.1 and 0.13, those individuals from 1999 to 2004 ranged in SGR between 0.09 and 0.17 - that represents the complete range of somatic growth rates measured in this experiment (Figure 7).

Table 4. The variation of the somatic growth rate in *D. galeata* clones hatched from the sediments of three different time periods (1960s, 1970s and 2000s). df = degrees of freedom, sq = sum of squares, all p values significant.

Effect	df	SQ	F	p
Quality	1	0.01	15.349	<0.001
Clone (Time)	14	0.07	7.14	<0.001
Clone (Time)*Quality	13	0.029	3.222	<0.001

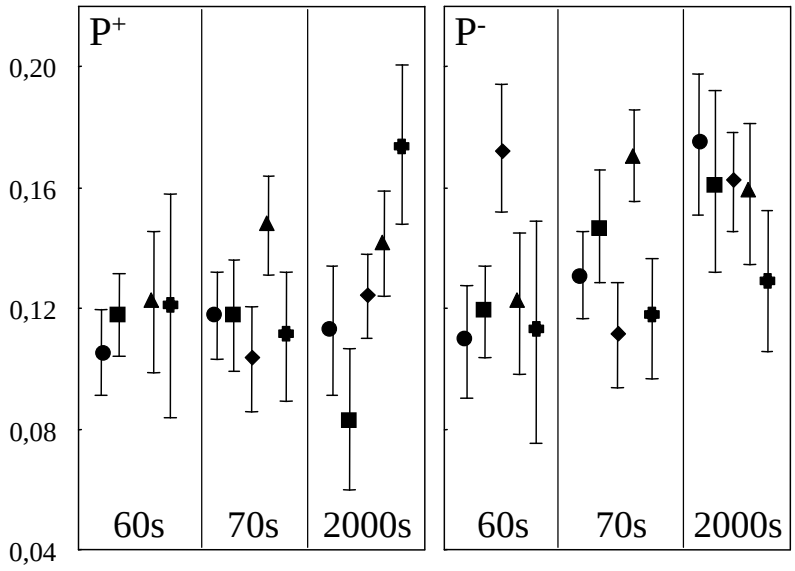

Figure 7. Somatic growth rates of *D. galeata* clones hatched from three different time periods exposed to two different algal diets. P^+: phosphorus enriched *Scenedesmus obliquus* diet; P^-: phosphorus deficient *Scenedesmus obliquus*. Bars represent 95% confidence intervals. Symbols reflect different clonal lineages within one time period.

Subsequently, we compared the F_{st} values calculated from microsatellite analysis with Q_{st} (derived from differentiation in SGR for high and low food quality between all time periods) to detect signals of selection between populations over time. For the time periods between 1960 to 1970 and since then (1960) until recent years (2000) selection has acted on the somatic growth rate (low as well as high food quality), the days until first reproduction and the size of the juveniles. All values decreased over time (Figure 8).

We also tested *D. galeata x hyalina* clones hatched from sediments of the time periods 1970-1975, 1975-1980 and 1999-2004 (Figure 9). The somatic growth rate of five clones in a multifactorial design was measured to distinguish between the role of importance of food quality and food quantity. While there is no significant difference in the SGR of parental *D.*

galeata and recombinants, hybrids show a wider range of SGR in the recent genotypes. Most recent genotypes (1999-2004) displayed the lowest SGR in all treatments but P+Q+. In the latter treatment they also had the highest SGR rate (0.13). Over all, *D. galeata x hyalina* has the highest SGR under good food quality and high amounts of food. Clones had the lowest SGR not only in low food quality and quantity but also in qualitatively good algae in low quantity. *Daphnia* showed a higher SGR given high amounts of food of low phosphorus content. In those treatments with a limiting factor (either P-, Q- or both) the clones hatched from sediments of 1970-1975 had the highest SGR. In general recombinant genotypes do not show any fitness deficiencies according to the recorded life history traits.

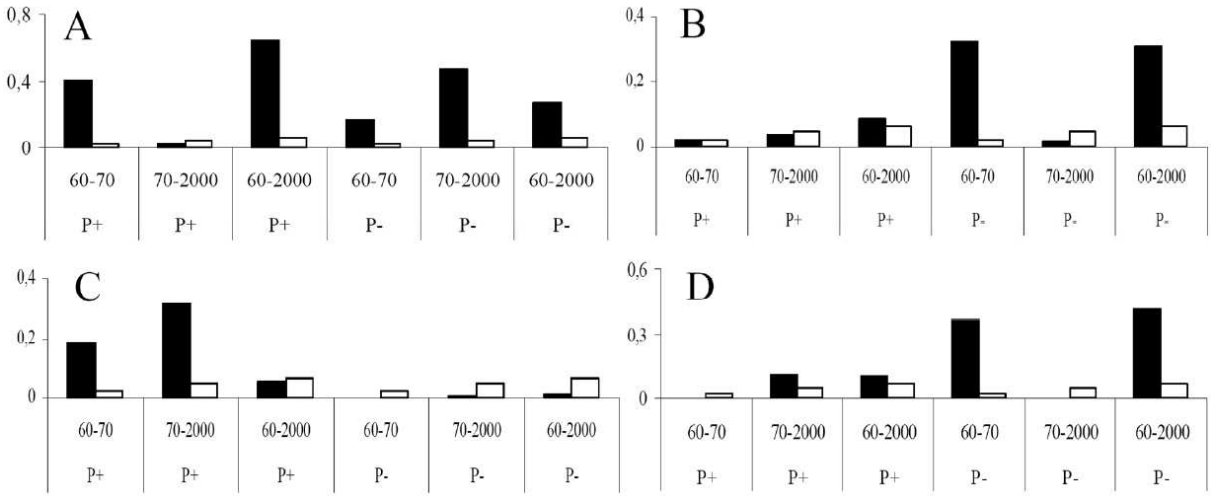

Figure 8. Comparison of F_{st} (white bars) and Q_{st} (black bars) values of *D. galeata* clones hatched the sediments of different time periods related to four life history traits: somatic growth rates (A), days until first reproduction (B), number (C) and size (D) of juveniles.

3.4. Discussion

The taxonomic composition of *Daphnia* in Lake Constance has changed severely over the last 60 years. Since *D. galeata* invaded the lake in the mid 1950s it hybridized freely with the indigenous *D. hyalina*. Interspecific hybridization occurred at its maximum mainly between 1954 and 1979 and then again during the latest decade (Jankowski & Straile, 2004). In between, *D. hyalina* inhabited the lake as clonal lineages but almost no sexual reproduction could be detected probably as a result of the altered ecological quality caused by eutrophication. In chapter 2 I show that interspecific hybridization between *D. galeata* and *D. hyalina* was most frequent during times of ecological change in this habitat. Here, I found evidence for introgression on the nuclear level due to ongoing recombination of the two taxa. Although I was not able to characterize multilocus genotypes of *D. hyalina* before *D. galeata*

invaded Lake Constance, allele frequencies that were formerly only detected in this species are found in *D. galeata* resting eggs of recent years.

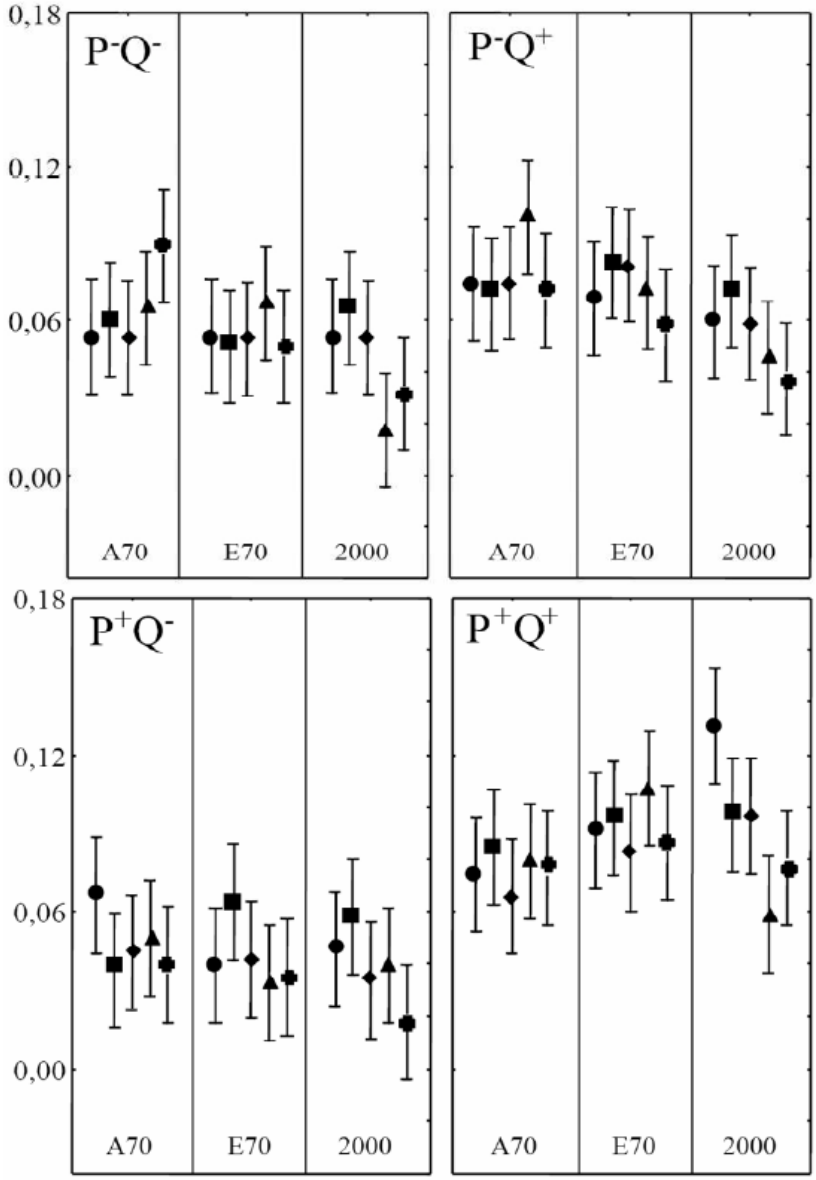

Figure 9. Somatic growth rates of *D. galeata x hyalina* clones hatched from three different time different time periods under four different food qualities and quantities: A70: 1970-1975, E70: 1975-1980, 2000: 1999-2004; P^+: C:P 120, P^-: C:P 1000, Q^+: 1.0 mg C/L, Q^-: 0.1 mg C/L. Symbols reflect different clonal lineages within one time period.

I detected based on RFLP analysis of mitochondrial DNA that interspecific hybridization is unidirectional for *D. galeata* (chapter 2). Still, another study investigating recent (2005 and 2006) planktonic samples showed that 6.25% of all *D. hyalina* identified with ITS RFLP and microsatellite analysis carried a *D. galeata* mitochondrial haplotype (chapter 5). Interspecific hybridization is therefore not unidirectional and has lead to introgression. F_{st} values derived from the conducted microsatellite analysis have shown that *D. galeata* and *D. hyalina*

converge over the observed decades. The genetical differentiation between the two taxa decreased significantly during the last 30 years. This development is yet another evidence for ongoing gene flow in the observed *Daphnia* taxa.

The conducted life history experiments showed that *D. galeata x hyalina* recombinant individuals hatched from sediments of different age are able to compete with the parental taxon *D. galeata* of the same time period. Tested for life history parameters like somatic growth rate, number of juveniles and size of juveniles no significant differences could be detected between both taxa. Although all hybrid clonal lineages were characterized with microsatellite analysis and chosen for the experiments according to their intermediate multilocus genotype in a factorial correspondence analysis (GENETIX v. 4.01) of genotype frequencies, it is most probable that younger recombinants originate from more than just one generation of hybridization. It seems that the genomes of both species combined are well compatible and do not cause fitness deficiencies in hybrid offspring.

Lake Constance has undergone severe ecological changes during the last 30 years (Correll, 1998; Jankowski, 2002). Reoligotrophication has altered practically all biological factors relevant for *Daphnia*: predators, vegetation, secchi depth, oxygen content and food (Schindler, 1978; Schindler, 2006). The life history experiments on *D. galeata* clones have shown that individuals deriving from 1999-2004 display a higher variability in SGR in both treatments with low and high quality algal diets. The results from the life history experiment indicate that diversifying selection has acted on *Daphnia galeata* over time – due to partial introgression of *D. hyalina* alleles into individuals assigned to the parental taxon *D. galeata*, clonal lineages might now be adapted to alternative ecological niches – those where *D. galeata* were originally able to persist and those typical for *D. hyalina* (Flößner, 1972).

Comparisons of quantitative trait measurements and population differentiation values at neutral genetic loci (i.e. Q_{st} vs. F_{st}) confirm the results of the life history experiment. Q_{st} values higher than F_{st} values confirm the hypothesis of diversifying selection within populations over time. Although F_{st} values decreased over time due to introgression, intensifying the difference between the quantitative and the molecular traits the results from this study show that selection for different optima has been acting on life history traits in *D. galeata* over time. While decreasing genetic differentiation at the first sight might be interpreted as a merging effect caused by ongoing hybridization and introgression,

comparisons of quantitative and genetic traits reveal the opposite: ‘Validated’ genetic traits originating from the one parental taxon can cause the enrichment of the other’s genome.

In future studies *D. hyalina* and *D. galeata* from today’s planktonic population of Lake Constance need to be tested with an emphasis on their life history traits. With this a more detailed insight into the ecological niche represented by each taxon today would help to understand the competitive and coexisting traits of this hybridizing population.

Chapter 4: The Contribution of Differential Hatching Success to the Fitness of Species and Interspecific Hybrids

Published in Hydrobiologia: N. Brede, together with D. Straile, B. Streit and K. Schwenk (2007)

4.1. Introduction

Dormant egg banks of microcrustaceans have been generally recognised as biological archives that allow to reconstruct microevolutionary and ecological changes (reviewed in: Brendonck & De Meester, 2003). Paleogenetic data of resting eggs have been used to understand changes of species assemblages due to invasive species (Kerfoot et al., 2004), morphological differentiation associated with variation in predation levels (Kerfoot & Weider, 2004) and natural selection for grazer resistance to toxic cyanobacteria (Hairston et al., 1999b; Hairston et al., 2001). Recent studies, however, revealed notable discrepancies between species assemblages derived from dormant eggs and species assemblages of "active" pelagic populations (Jankowski & Straile, 2003; Keller & Spaak, 2004). This discrepancy was attributed to differential levels (and timing) of sexual reproduction among taxa.

In general, species assemblages of "active" pelagic populations will only reflect the taxon composition of resting egg banks if the following assumptions are met: No differential (species specific) rates of (1) hatching, (2) survival of hatchlings and (3) reproductive output of ex-ephippial adults. In addition, successfully hatched individuals should not differ in their (4) level of clonal propagation, (5) induction of males and sexual females, and (6) mating success (numbers correspond to those in Figure 9).

Although we have ample information on the differential levels of selection which directly affect clonal propagation (4; Pfrender & Lynch, 2000), taxon specific levels of sexual reproduction (5; Spaak et al., 2004) and mating success (6; Keller & Spaak, 2004), we lack information on the initial steps of the life cycle (1-3; Figure 9). In order to bridge this gap, we compared the taxon composition of "non developing" resting eggs and hatchlings isolated from sediments of Lake Constance. In addition, we measured the reproductive success of ex-ephippial adults for each taxon (*D. galeata*, *D. hyalina* and the interspecific hybrid *D. galeata x hyalina*).

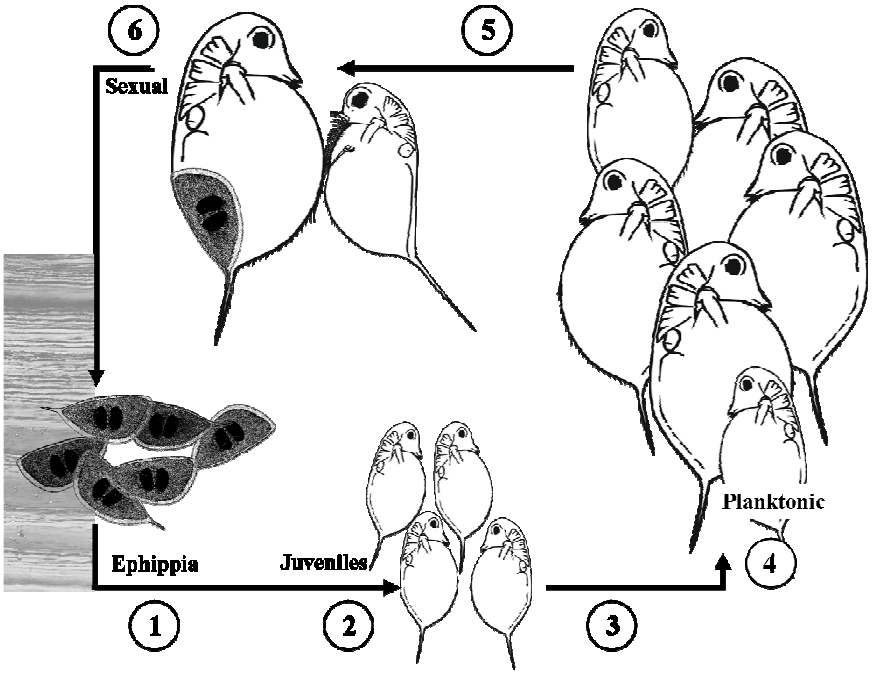

Figure 9. Reproductive cycle of natural and laboratory populations of *Daphnia* illustrating the different components of fitness. 1: Hatching from ephippia, 2: survival of juveniles, 3: reproductive mode of ex-ephippial adults, 4: level of clonal propagation, 5: induction of males and sexual females, 6: mating success.

D. hyalina represents the indigenous *Daphnia* taxon of Lake Constance; Lake Constance is the type locality for *D. hyalina* (Flößner, 2000). *D. galeata* invaded Lake Constance successfully in the early 1950s associated with a continuous shift in habitat quality through the long term process of increasing eutrophication. During the following decades multiple hybridization events and introgression altered the genetic structure of the species complex (Jankowski & Straile, 2004). During peak eutrophication (1970s) *D. galeata* was the most abundant taxon found in the resting egg bank whereas in the 1980s *D. hyalina* was present only in the plankton population and could not be found in the resting egg bank of that time (Jankowski & Straile, 2003; N. Brede, unpublished data). Due to effective pollution control of Lake Constance inflows, the lake recovered in the subsequent years and regained its characteristic oligotrophic conditions.

The aim of our study was to determine the level of differential hatching among *Daphnia* taxa and to identify the life history stages explaining the discrepancy between “active” pelagic and the dormant populations. Specifically, we addressed the question whether the relative frequencies of taxa found in the resting egg bank differ from the relative proportions of successfully reproducing individuals. To do so, we measured the i) hatching rate, ii)

proportion of individuals reaching maturity and iii) reproductive fitness of ex-ephippial females of the three *Daphnia* taxa inhabiting Lake Constance.

4.2. Material and Methods

Sampling. Sediment cores were sampled in Lake Constance in Germany. Cores were recovered in December 2002 from 220 m depth close to the deepest point of the lake between Konstanz and Langenargen (47° 34' 46'' N, 9° 27' 54'' E) and in September 2004 from 180 m depth close to the Langenargener Bucht (47° 37' 21'' N, 9° 26' 24'' E). Sediments were dated by lamination counting (Wessels et al., 1995) and prepared as in previous studies (Weider et al., 1997). In general, the sediments are well laminated and reference cores have been dated by ^{137}Cs dating before (Wessels et al., 1995). Ephippia were isolated by washing the sediments through a 220 µm mesh sieve.

Hatching experiments. Four hatching experiments were conducted in which ephippia from three different time periods were exposed to hatching stimuli (1970s, 1990s and 2000s; Table 1). All experiments were carried out in a 16:8 h light dark cycle at 18°C (pers. comm. T. Jankowski; Vandekerkhove et al., 2005a). Two different media were used for the experiments, pond water and Lake Constance water in order to obtain maximum hatching success. Both, the Lake Constance (drawn in winter right before use) and the pond water were filtered (0.45 µm Whatman filters) and autoclaved. Pond water originates from small artificial (concrete) pools filled with rainwater which are cleaned once a year. All ephippia isolated from the sediments were subjected to the hatching experiments. To avoid any damage of viable resting eggs we did not open ephippia to determine the presence or absence of eggs. The experiments "1970/1" and "1990" as well as the experiments "1970/2" and "2000" were conducted simultaneously.

Each plate was checked in the morning and if necessary also in the afternoon in search of neonates. Hatching started after two to six days and each hatchling was transferred to a 10 ml vessel and fed with *Scenedesmus obliquus* suspension containing ~ 1 mg C l^{-1} to guarantee a food supply above the incipient limiting level. Animals were controlled by eye until reaching maturity to monitor developmental differences between individuals. Experiment "1970/1" was carried out with two times 96 ephippia extracted from sediments of 1971-74 and placed

individually in 96 well plates with filtered Lake Constance water. After transferring hatchlings to 100 ml jars animals were controlled by eye until reaching maturity in this experiment to monitor developmental differences between individuals: All hatched individuals were observed daily and categorized in three groups. “Asexually reproducing” categorizes ex-ephippial adults establishing a clonal lineage, “not reproducing” represents animals that did not reproduce at all (after max. 43 days) and “ephippium producing” accounts for the observed fraction of hatchlings that built up an ephippial shell (without depositing eggs) right after molting to maturity. The definition of sexual females is imprecise and usually connected to the visibility of promoted ovary activity. The generation of an ephippial structure on the carapace is an indication, but not the ultimate proof of the status of a female. Experiment “1970/2” (same time period as in “1970/1”) was replicated four times; each replicate was carried out by exposing 96 ephippia in well plates with filtered pond water to hatching stimuli. For experiment “1990” 192 ephippia (two replicates) from the sediments of 1994-98 were exposed to hatching stimuli in filtered Lake Constance water. Experiment “2000” was carried out with ephippia extracted from sediments of 1999-2004 in filtered pond water in six replicates (one replicate = 96 ephippia).

Table 5. Absolute numbers of analyzed individuals and eggs of all experiments. Numbers refer to the amount of resting eggs or individuals subjected to a RFLP analysis of G: *D. galeata*, GH: *D. galeata x hyalina* H: *D. hyalina*. Hatching experiments were conducted either in filtered lake water from Lake Constance (1970/1 and 1990; L) or in filtered pond water (1970/2 and 2000; P) experiments. The experiments name is followed by the number of replicates with every replicate containing 96 ephippia (in brackets). The number of eggs or individuals was corrected with the number of genetically not analysed data. First row: “Non developed”: Eggs that did not hatch, Hatched: number of hatchlings, Sum: number of eggs or individuals per taxon and per experiment, Sum all: Total amount of experimental eggs per experiment. The total number of “non developed” eggs and hatchlings (uncorrected values, see text) are provided in the last column (Total*).

	1970/1 (2, L)			1970/2 (4, P)			1990 (2, L)			2000 (6, P)			Total*
	G	GH	H	G	GH	H	G	GH	H	G	GH	H	
“Non developing”	25	4	1	117.8	21.4	4.8	51	0	0	125.5	11.7	3.8	299
Hatched	106	5	0	167.8	13.2	0	242	0	0	64	7.1	0	501
Sum	131	9	1	285.6	34.5	4.8	293	0	0	189.5	18.7	3.8	
Sum all	141			325			293			211.6			800

In the two replicated experiments “1970/2” and “2000” a minor fraction of eggs and individuals was not identified genetically (“1970/2” total N = 384, “2000” total N = 576; Table 5). In order to correct the observed frequencies of taxa we multiplied the number of unidentified eggs or individuals with the observed proportion of each taxon. Hatching success

per taxon was calculated by dividing the total number of eggs (per taxon) over the whole experiment or per replicate by the number of hatched individuals. The total number of eggs was calculated by summing the number of genetically identified hatchlings and "non developing" eggs. In order to test whether the observed hatching frequencies of taxa are explained by the initial taxon composition of the exposed resting eggs we conducted a goodness-of-fit G-test with a Williams correction for small sample sizes (Sokal and Rohlf 1995).

Genetic analysis. Resting eggs were isolated from their ephippial shells and DNA was prepared separately in 35 µl H3 buffer (1x: 10 mM Tris-HCl; pH 8.3 at 25°C, 0.05 M potassium chloride, 0.005 % Tween 20 and 0.005 % NP-40) and 1.2 µl proteinase K (Sigma; 10 mg / ml). Adults were directly transferred to 70 µl H3 buffer and 2 µl proteinase K. After an incubation time of 12 hours proteinase K was deactivated by heating the sample for 12 min at 95°C. An ITS fragment was amplified using a total reaction volume of 14 µl. 2 µl of template and 3 mM $MgCl_2$, 1x PCR buffer, 0.2 mM dNTP, 0.3 µM of each primer (ITS2-5.8S: 5´-GGA AGT AAA AGT CGT AAC AAG G-3´; 10 µM; ITS1-18S: 5´-CGG TGG TCG ACG ACA CTT CGA CAC GC-3´; 10 µM) and 1 U *Taq* DNA polymerase (all chemicals and primers: Invitrogen) in 94 °C for 3 min, five cycles at 94 °C for 1 min; 52 °C for 1 min; 72 °C for 1.5 min; 35 cycles: 94 °C for 1 min; 50 °C for 30 sec; 72 °C for 1 min; final synthesis step at 72 °C for 5 min.

A restriction fragment length polymorphism analysis (RFLP) was used for taxon identification (Billiones et al., 2004). Amplicons of the ITS region were digested with the restriction enzyme *Mwo* I (5´-GVNNNNNN↓NNGC-3´; NEB) for 2.5 hours at 60 °C in a total reaction volume of 9.6 µl containing 8 µl PCR product and 10x NEBuffer for *Mwo* I, 5 U of the restriction enzyme and autoclaved dH_2O.

The digestion products were transferred to a 2 % agarose gel and bands were separated by applying 115 volts. Specific banding patterns allow identification of the taxa: *D. galeata* (100, 320, 380 and 490 bp), *D. hyalina* (100, 520 and 680 bp) and their hybrid who displays an additive banding pattern in the RFLP analysis. ITS RFLP analyses were compared with microsatellite analyses (six loci: DaB 10/15, DaB 17/17, DaB 17/16, DaB 10/14, Dp512 and Dp519; see Brede et al. 2005) and resulted in similar taxon classifications.

4.3. Results

Our genetic analyses of the resting egg bank revealed a very low frequency of *D. hyalina* and the interspecific hybrid compared to *D. galeata* ephippia (1 : 6.5 : 88; s.d. 0.8 : 4.6 : 4.4; Figure 10).

In all cases, hatching peaked within two days and slowly decreased over the next one or two weeks. In the experiments "1970/1" and "1970/2" with ephippia exposed to different water characteristics (filtered Lake Constance and pond water respectively) the hatching success for the lake water experiment was 78.7 % whereas for the pond water experiment the average hatching success was 55.7 %. Hatching success between lake and replicated, pond water experiments could not be tested because of large variation within treatments among replicates (hatching success differed over four replicates between 37.6 and 77.6 %). Eggs from the sediments of the 1990s hatched most successfully with 82.3 %. The hatching success for recent ephippia (experiment "2000") was lower at 33.5 %. We found no differences in hatching success among ephippia isolated from different sediment layers.

Hatching success among parental taxa did not differ significantly in any of the experiments ("1970/1": χ^2, df = 2, p = 0.48; "1970/2": replicated goodness-of-fit test, G (Williams) p = 0.1168; "1990": χ^2, df = 2, p = 0.66; "2000": replicated goodness-of-fit-test, G (Williams) p = 0.6090; Figure 10). Apart from experiment "2000", *D. galeata* eggs developed with 80.9 % hatching success in experiment "1970/1", an average success of 60.3 % in experiment "1970/2" (replicated experiment) and a hatching success of 82.3 % in the "1990" experiment. Further observations within this experiment showed that 28.7 % of all hatchlings died before the first molt. In recent times (experiment "2000"), *D. galeata* hatched with an average success of 34.7 % (ranging between 23.1 and 47.2 %). *D. galeata x hyalina* hybrid eggs were almost as successful: In both 1970s experiments hybrid eggs hatched in lake water with 55.6 %, whereas hatching success in pond water was 55.4%. No eggs of the interspecific hybrid were found in the sample from the 1990s but in recent times (experiment "2000"), the hybrid hatched with 38% success. In all three experiments in which we found *D. hyalina* eggs (N = 7, uncorrected value; see also Table 5) none hatched (Figure 10).

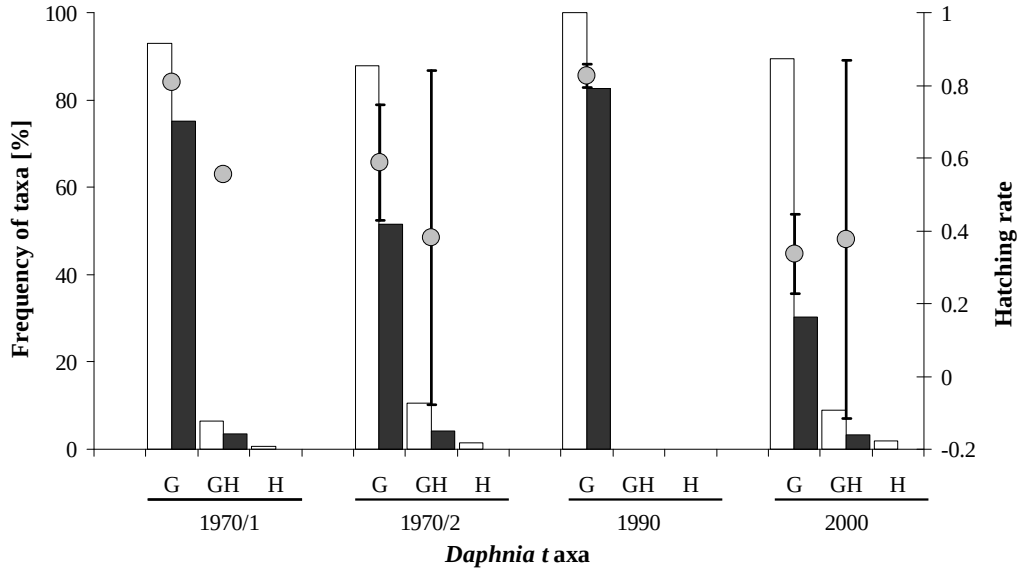

Figure 10. Comparison of hatching rate, taxon composition of resting eggs present in the experiment (white bars) and those that hatched (black bars) in percent (left y axis). G: *D. galeata*, GH: *D. galeata x hyalina*, H: *D. hyalina*. Dots represent the hatching rate (right y axis) and error bars standard deviation among replicates.

As mentioned before, the one egg of *D. hyalina* found in experiment "1970/1" did not hatch. Within the three categories observed, only *D. galeata* and the interspecific hybrid *D. galeata x hyalina* were detected. Within the category "asexually reproducing" all hatchlings turned out to be *D. galeata* (N = 74). All interspecific hybrids (N = 5) divided among the two other categories with 33.3 % "ephippium producing" and 22.2 % "not reproducing". All categories differed significantly ($p < 0.001$) compared to the taxon composition of all hatched individuals.

4.4. Discussion

The overall observed hatching rates are comparable with those found in a previous study (Weider et al., 1997). In addition, the variation of hatching success shows a similar pattern and confirms the tendency of reduced hatching rates of eggs recovered from recent sediments (experiment 2000).

The proportion of *D. hyalina* to *D. galeata x hyalina* to *D. galeata* eggs is on average 1 : 6.5 : 88 (s.d. 0.8 : 4.6 : 4.4; Figure 10). In all four experiments conducted in this study no significant frequency difference between the taxon composition within the resting eggs and

those that hatched and established a clonal lineage was observed. The discrepancy between the resting egg bank and the pelagic population can not be explained by differential hatching of taxa. In this study no *D. hyalina* resting eggs developed in three experiments representing two time periods (containing corresponding resting eggs). The most likely explanation for the lack of *D. hyalina* hatchlings is a stochastic effect due to the low number of resting eggs. Other studies showed that *D. hyalina* resting eggs hatch under natural conditions (Carvalho & Wolf, 1989; Wolf & Carvalho, 1989; Jankowski, 2002). For Lake Constance it has been shown that hatching success of *D. hyalina* may depend on lake depth; Jankowski (2002) showed that *D. hyalina* hatched in the littoral (25%) but did not hatch from the profundal. Caceres and Tessier (2003) found a similar pattern of spatial variation in hatching experiments on North-American *D. pulicaria*. Furthermore, several authors describe that some *Daphnia* species´ ephippia are buoyant, e.g. through spines, lipid drops or gas chambers.). This differential buoyancy may result in a spatial separation of *D. hyalina* resting eggs floating ashore whereas *D. galeata* ephippia sink mainly to the profundal. Recent population genetic studies of resting eggs isolated from Lake Constance sediments show that *D. hyalina* was present as dormant stages before the 1970s (Jankowski & Straile, 2003; N. Brede, unpubl. data). Based on these findings we conclude that spatial effects have to be taken into account when the resting egg bank and current populations are compared.

In the experiment “1970/1” we studied the development of *Daphnia* from the juvenile stage to maturity. Only ex-ephippial individuals of the taxon *D. galeata* reproduced parthenogenetically (Figure 11). Among those animals which failed to reproduce and those carrying an ephippium we found *D. galeata* and all interspecific hybrids. Still, some of the hatchlings that primarily produced an ephippium later on built up a clonal lineage. Although we cannot exclude the possibility that these observations are due to a differential response of taxa to laboratory conditions, we do not expect that our standardized laboratory conditions have such detrimental effects on basic developmental processes of hybrids. The observed patterns indicate that hybrids do experience fitness deficiencies, in particular, after reaching the adult stage. This reduced reproductive success of interspecific hybrids (and recombinant genotypes) and some *D. galeata* individuals might be caused by genetic incompatibilities of recombinant hybrid genomes. Some *D. galeata* individuals within the two categories are probable to be backcrosses of the parental species. Studies have shown that backcrossing occurs in Lake Constance (Jankowski & Straile, 2004; Löffler et al., 2004).

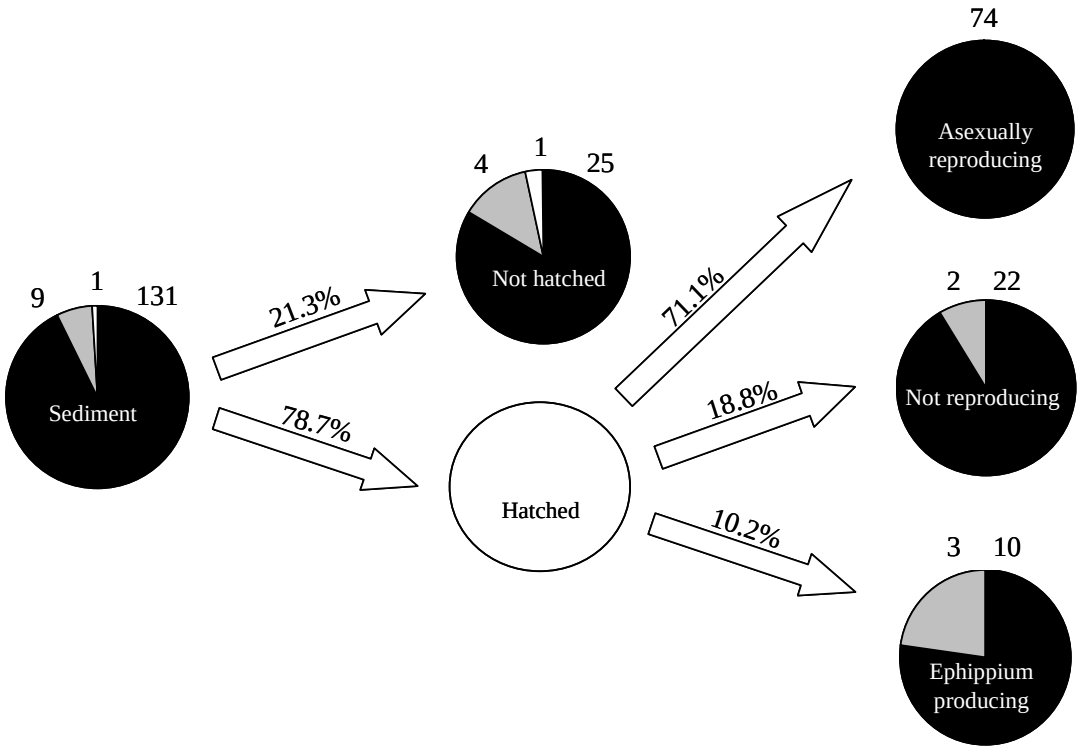

Figure 11. Relative taxon composition of *Daphnia* species and hybrids at two life history stages, after hatching (juveniles) and after first reproduction (experiment "1970/1"). Black area = *D. galeata*, grey area = *D. galeata x hyalina*, white area = *D. hyalina*. The total number of identified taxa is provided above each pie in the order *D. galeata x hyalina*, *D. hyalina*, *D. galeata*. Numbers above arrows describe the percent of undeveloped eggs versus hatchlings (left arrows) and represent the percentage of individuals attributed to different modes of reproduction (asexual reproduction, no reproduction and production of ephippial females; right arrows).

In Jankowski´s Ph.D. thesis (2002) hatching experiments in the laboratory and in the littoral zone of Lake Constance showed that *D. galeata* and *D. hyalina* hatch in different zones of the lake. After reconstructing the taxon composition over time using spininess of the ephippia, historical records, and genetically (allozymes) determined hatchlings, Jankowski and Straile (2003) concluded that the resting egg bank of *Daphnia* does not represent the "active" pelagic population. Similar results were obtained by comparing egg banks and "active" populations by Keller and Spaak (2004).

Both published results and our data suggest that the components of reproductive success in *Daphnia* contribute differentially to the fitness of species and interspecific hybrids. We found no species-specific (1) hatching rates and (2) no differential survival of juveniles. We observed in one experiment ("1990") a 28.7% mortality rate among juveniles, however, since they could not be subjected to genetic analyses we were not able to determine taxon specific survival rates. Species and hybrids differed in their mode of reproduction and in their level of

clonal propagation (3, 4; Figure 9). In addition, taxa varied in their rate of sexual reproduction (Jankowski & Straile, 2003; Keller & Spaak, 2004).

The observed differences between resting egg banks and pelagic populations might also be explained by the heterogeneous spatial distribution of resting eggs (Jankowski, 2002) and the comparison of pelagic populations representing the entire population with a non representative sample of the profundal resting egg bank. Furthermore, we have very little information on the level of random mating within and among taxa (Keller & Spaak, 2004; 6, Figure 9). All these observations suggest that it is highly unlikely to find "active" planktonic populations that reflect dormant populations.

In general, resting egg banks represent a conglomerate of recombinants sexually produced by "successful" parental genotypes. Speaking in evolutionarily relevant terms, the resting egg bank forms a large archive of genetic variation which results in *Daphnia* populations that can change rapidly following to ecological changes, i.e. predation levels, food quality or quantity (Hairston et al., 1999b; Cousyn et al., 2001). Long term evolutionary changes, like adaptations to novel environments, the consequences of interspecific hybridization or successful invasions of species and lineages will be reflected in resting egg banks (Duffy et al., 2000; Jankowski & Straile, 2004). Since several aspects of current populations are determined by their history, future ecological studies may profit from an interdisciplinary approach using both population genetic data over time and life history studies.

Chapter 5: Detecting Genetic Responses to Environmental Change: Mitochondrial DNA and Temperature

5.1. Introduction

Mitochondrial introgression. In general, mitochondria are maternally inherited (matrilines) although cases of paternal inheritance (*Mytilus*: Ladoukakis & Zouros, 2001), biparental inheritance (*Drosophila*: Kondo et al., 1990; *Mus*: Gyllensten et al., 1991; birds: Kvist et al., 2003) and parental leakage in combination with interspecific hybridization (Kvist et al., 2003) are known. The latter is a quite common process in *Daphnia*. Many species within one of the different subgenera *Daphnia*, *Ctenodaphnia* and *Hyalodaphnia* tend to hybridize. Although the genetic differentiation between species can be quite high *(7.6% between D. galeata mendotae* and *D. dentifera* on the 12S rRNA gene: Colbourne & Hebert, 1996), interspecific hybrids do not suffer from severe fitness deficiencies (Hobaek et al., 2004; Spaak & Boersma, 2006; Keller et al., 2008). Studies have shown that interspecific hybrids are even capable to replace the parental taxa from a certain habitat because their fitness is shown to be highest under intermediate conditions (Schwenk & Spaak, 1997).

Due to hybridization and later generation backcrossing introgression of mitochondrial and nuclear information occurs. As this gene flow between species proceeds, individuals may exhibit the nuclear information of one parental taxon but the mitochondrial information of the other parental taxon (mitochondrial introgression). This is evidence for successful introgression if other effects like, e.g. lineage sorting can be excluded by applying species specific markers on the mitochondrial level (for *Daphnia* see: Schwenk et al., 1998). Detected haplotypes are specific on the species level but introgress readily when interspecific hybridization occurs due to maternal inheritance. Many studies have shown that mitochondrial introgression may not be accompanied by apparent nuclear introgression (Doi et al., 1999; Mishmar et al., 2003). In their review, Ballard and Whitlock (2004) state the following: "… in taxa with some level of hybridization or migration, there is a non-negligible probability of introgression of mtDNA from one taxon into another. This can happen just by chance (because of the low effective size of mtDNA), by selective pressure (because of local adaptation of the mitochondria), or by selective introgression following mutational meltdown in small populations."

Temperature dependency. The question of temperature tolerance became more prevalent in the course of ongoing global climate change. In this frame work it is predicted that the natural range of species will change severely since many organisms are adapted to specific climate zones and not very tolerant to permanent temperature shifts (Somero, 2002; Sommer & Portner, 2002). Heat stress is known to affect not only membrane permeability and protein synthesis – it also affects the productivity of mitochondria. Although species adaptations are complex considering multiple cellular interactions and restricted in certain physiological constraints (mitochondria for example will stop activity at 55 to 60°C), temperature seems to be an important selective pressure on mtDNA (Somero, 2002; Sommer & Portner, 2002). Still, the influence of different mitochondrial haplotypes has not been investigated thoroughly, even in studies testing adaptation to different temperature ranges conducted on *Daphnia* species (Dufresne & Hebert, 1998; Mitchell & Lampert, 2000; Paul et al., 2004; Pinkhaus et al., 2007). In the present study our aim is to assess the response of different cytonuclear genotypes to variation in temperature regimes.

History of hybridization in Lake Constance. Lake Constance, the largest German waterbody, was originally inhabited by the planktonic crustacean *Daphnia hyalina* (Einsle, 1966). As grazers *Daphnia* are an important part of the ecological dynamics of a freshwater habitat. *D. hyalina* is known to inhabit mainly large, oligotrophic lakes. In comparison to *D. hyalina*, *D. galeata* is known to inhabit large lakes of eutrophic to hypereutrophic character with a pronounced sexual phase to survive nutrient poor times in winter (Flößner, 2000). The latter species invaded Lake Constance in the mid 1950s due to massive ecological changes induced by an anthropogenically induced eutrophication (Muckle & Dillmann-Vogel, 1976). During the periods of massive ecological changes, abundances of both parental taxa were more or less equal and a lack of reproduction isolation causes massive interspecific hybridization including introgression (see chapter two).

With this study we aim to show the importance of physiological constraints related to mitochondrial gene expression as a mechanism of adaptating to different seasonal temperatures and , therewith, the potential of mitochondrial introgression. While Nagao et al. (1998) and Burton et al. (1999) found fitness deficiencies in interspecific hybrids due to defective expression of interacting nDNA and mtDNA genes, we hypothesize that in introgressed *D. galeata* and *D. hyalina* gene complexes are cooperating and enabling individuals of one taxon to fill ecological niches of the opposite taxon. We used ncDNA and

mtDNA RFLP analysis as well as microsatellite markers to identify genetic characteristics in *D. galeata*, *D. hyalina* and the recombinant *D. galeata x hyalina*. In a life history experiment we tested the influence of haplotyes on temperature stress simulating winter and summer season.

5.2. Material and Methods

Sampling. Planktonic samples were drawn from Lake Constance Überlinger basin in May, July and August of 2005 and March, August and September of 2006. A plankton net with 140 µm mesh size was used to draw over 100 m a quantitative *Daphnia* sample. While one part of the samples was alive the other was preserved in 70% ethanol.
Live samples were transported to the University of Frankfurt and *Daphnia* were selected randomly and reared individualy to establish clonal lineages. 50 separate clonal cultures were grown in 250 ml jars.

Preparation of DNA and RFLP analysis.
Nuclear. A restriction fragment length polymorphism analysis (RFLP) was used for taxon identification. Amplicons of the ITS region were digested with a restriction enzyme and taxon specific banding patterns were displayed on an agarose gel and evaluated according to (Billiones et al., 2004).

Microsatellite analysis. A set of eleven primers amplifying microsatellites was used for identification of taxa and interspecific hybrids. From Brede et al. (2006, chapter one) we applied DaB 10/14, DaB 17/17, Dgm 105, Dgm 112, Dp 196NB, Dp 281NB, Dp 519, SwiD 6, SwiD 12, SwiD 14 and SwiD 18. ITS-RFLP and microsatellite analyses were used to destinguish between parental and hybrid taxa (Table 7).

16S rDNA. To distinguish between *D. galeata* and *D. hyalina* mitochondrial haplotypes, a digestion of a 16S rDNA fragment with restriction enzymes was conducted (nomenclature of mitochondrial haplotypes follows Schwenk et al., 1998). An additional hyplotype (*x7)* could be assigned to *D. hyalina* via DNA sequencing analysis of 12S rDNA.

Temperature experiment. The experiment was conducted with twelve clonal lineages of *Daphnia* selected from those individuals sampled from Lake Constance with the following characteristics: three different clonal lineages of *D. galeata* with haplotype g1 (g-g1, Schwenk et al., 1998), three of *D. galeata* with haplotype x7 (g-x7, see last paragraph), three of *D. hyalina* with haplotype g1 (h-g1) and three of *D. hyalina* with haplotype x7 (h-x7). Juveniles ≤ 24 hours of age were kept in five replicates of five to six individuals in climate chambers tempered at 5°C, 12.5°C and 20°C. Each chamber was illuminated for 16 hours with an 8 W penlight. *Daphnia* were kept in modified ADaM medium (Aachener Daphnien Medium: Klüttgen et al., 1994); 1 l: 0,333 g synthetic sea salt, 2.3 ml $CaCl_2$ solution (0.8 mol L^{-1}: 117.6 g L^{-1} $CaCl_2$ * 2 H_2O), 2.2 ml $NaHCO_3$ solution (0.3 mol L^{-1}: 22.5 g L^{-1} $NaHCO_3$) and 0.1 ml SeO_2 solution (0.013 mol L^{-1}: 14 mg L^{-1} SeO_2) in 100 ml jars and fed with 250 µl of *Scenedesmus obliquus* solution. Individuals were transferred to fresh jars every day at the same time. The size of individuals was determined after three days under a light microscope.

Table 7. Comparison of taxonomic assignment with ITS-RFLP and microsatellite NewHybrids analysis. N displays the number of individuals that was assigned equally with both methods.

ITS		*NewHybrids*			
	N	*D. galeata*	*D. hyalina*	F_1 hybrid	F_2 hybrid
D. galeata	142	120 (92 %)	5 (4 %)	3 (2 %)	4 (3 %)
D. hyalina	191	17 (9 %)	168 (88 %)	2 (1 %)	4 (2 %)
D. galeata x hyalina	42	23 (53 %)	5 (12 %)	2 (5 %)	13 (30 %)

The somatic growth rate was calculated with:

$SGR = [\ln(S_t) - \ln(S_0)] / t$

with S_0 being the mean of body size at the beginning of the experiment and S_t the mean of body size. *Daphnia* were measured (base of spine to top of head without the potential helmet) under a light microscope.

Results were tested generalized linear models based on an ANOVA (analysis of variance). Tested parameters were tested individually, hierarchically (parentheses) or nested (asterics): taxon, haplotypes, temperature, taxon*haplotypes*temperature, taxon*haplotypes, haplotypes*temperature, taxon*temperature, clone (taxon*haplotypes), clone (taxon*haplotypes).

5.3. Results

The planktonic population of Lake Constance. The analysis of the taxonomic composition of Lake Constance revealed dynamic changes in *Daphnia* taxon abundances.
D. galeata was found in the planktonic samples of May 2005 resembling a little less than one forth of the population. *D. hyalina* is the most dominant species in this month, whereas *D. galeata x hyalina* hybrids were detected with 27.7%. In July, the population is dominated by *D. galeata* (76.8%) and only every tenth individual belongs either to *D. hyalina* or the interspecific hybrid. In October *D. hyalina* represents 86.5% of the sample while 2.7% belong to *D. galeata*. In March of 2006 *D. hyalina* has an abundance of 86.4% and *D. galeata* 10.6%. The recombinants are present with 3%. The average water temperature in the particular months was as following: in May 14.8°C, in July 19.8°C, in October 13.9°C and in March of the following year 6.4°C (Figure 12).

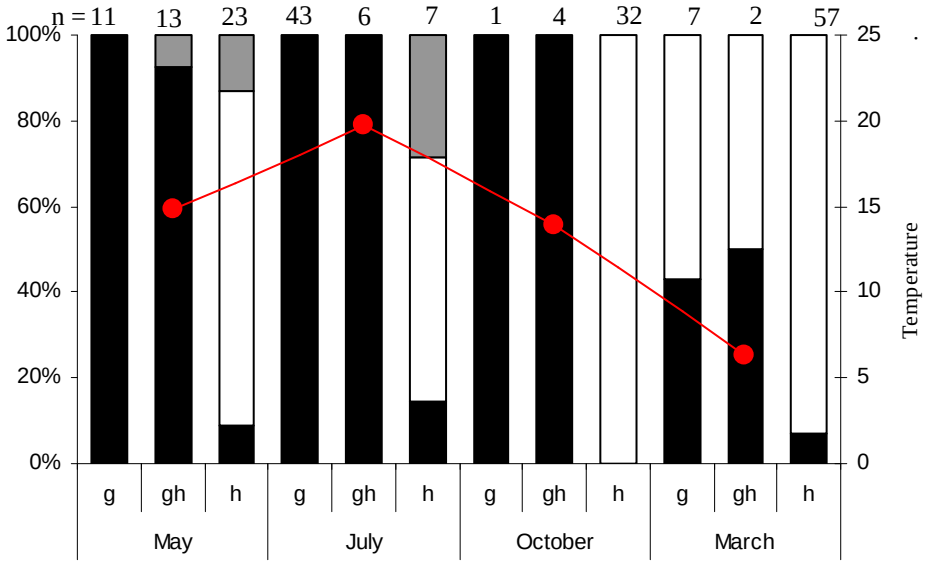

Figure 12. Distribution of mitochondrial haplotypes with nuclear taxa according to RFLP analyses sampled between 2005 and 2006. black: g1, white: x7, grey: h1; g: *D. galeata*, h: *D. hyalina*, gh: *D. galeata x hyalina*

All *D. galeata* individuals belonged to the g1 matriline, but in March four out of seven individuals exhibited the haplotype x7. *D. hyalina* individuals showed only in October the x7 haplotype. Individuals showing mitochondrial information of the *D. hyalina* typical haplotype h1 were found in the months May (n = 3) and July (n = 2). In the months May, July and March an average of 10% of all *D. hyalina* individuals carried the g1 haplotype.

Recombinants usually have mitochondria originating from g1 matrilines. Hybridization in Lake Constance is almost unidirectional as shown in other studies before (Brede et al., 2007). Only in May in one individual (7.7%) the haplotype h1 was detected and in October one of the two found was analysed as x7.

Life history experiment on selected *Daphnia* clones. In a life history experiment we show that both taxa, *D. galeata* and *D. hyalina*, have similar somatic growth rates under different temperatures (Figure 13). At 5°C the SGR was around 0.01, rising to 0.06 and above at 12.5°C and approximately 0.16 at 20°C.

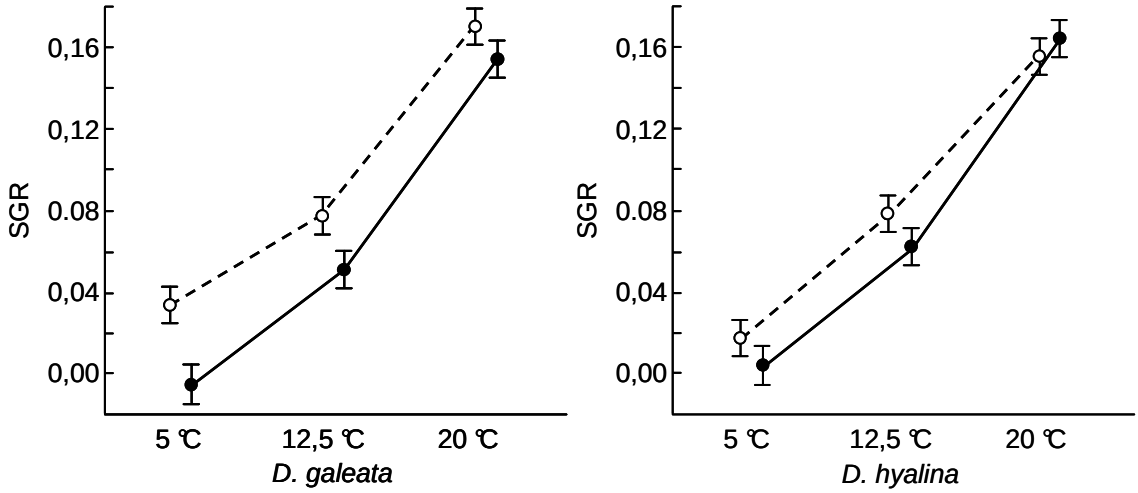

Figure 13. Somatic growth rates of the studied taxon-hyplotye-combinations at different temperatures; white: mitochondrial haplotypes x7, black: g1

At 5°C and 12.5°C individuals containing the haplotype x7 grew faster independently of their nuclear status. At cold and medium temperatures the difference between x7 matrilines and g1 matrilines was above 0.02. At 20°C individuals with g1 haplotypes grew almost as fast as x7 matrilines. At 20°C *D. hyalina* carrying the g1 haplotype had a slightly higher SGR. The haplotype * temperature interaction was tested significant at $p = 0.009$ whereas the interaction between taxon * temperature was not significant (Figure 13 and Table 8).

Table 8. Analysis of variance with generalized linear model testing the increase in body size of the four taxon-haplotype-combinations. Bold lines indicate significant values.

	df	SQ	MQ	F	p
Clone (taxon*haplotype)	**8**	**0,049**	**0,006**	**16,301**	**< 0,001**
Taxon	1	< 0,001	< 0,001	0,012	0,914
Haplotype	1	0,015	0,015	2,492	0,153
Temperature	**2**	**0,783**	**0,391**	**1046,379**	**< 0,001**
Taxon*haplotype*temperature	2	< 0,001	< 0,001	1,000	0,389
Taxon*haplotype	1	0,005	0,005	0,781	0,403
Haplotype*temperature	**2**	**0,005**	**0,002**	**6,428**	**0,009**
Taxon*temperature	2	0,001	< 0,001	1,245	0,314
Clone (taxon*temperature)	16	0,006	< 0,001	0,975	0,486
Error	172	0,066	< 0,001		

5.4. Discussion

Seasonal abundance shifts in *Daphnia*. The planktonic population of Lake Constance is dynamic with its taxon composition changing over the season. The abundances of *D. galeata*, *D. hyalina* and the interspecific hybrid *D. galeata x hyalina* seem to follow saisonal patterns (no significant correlation). In general, *D. galeata* dominates the planktonic population during warm times – in this study the average monthly temperature was 20°C in July. In contrast, *D. hyalina* occurs in its highest densities during colder temperatures, here in October (14°C) and March (6°C). It is known, that *D. hyalina* individuals of this taxon survive winter as parthenogenetic females. This change in reproductive strategy in *D. hyalina* might be associated with the eutrophication history of Lake Constance (Jankowski, 2002). Analyses of the *Daphnia* resting egg bank show that *D. hyalina* reproduced also sexually until end of 1950s. With exceeding eutrophication and increased food availability during winter *D. hyalina* was able to survive without relying on dormant stages. *D. galeata* on the other side strongly relies on sexual reproduction (Jankowski & Straile, 2003). While abundances are high during summer, the parthenogenetic population of this taxon almost completely breaks down and disappears in winter. Both taxa seem to display a differential adaptation to the changing ecological conditions in Lake Constance. This ecological differention might also explain the maintenance of both taxa despite high rates of hybridization.

The effect of haplotypes. We found significant differences in growth between clones within a taxon and, moreover, the life history experiment showed that mitochondrial haplotypes affect the somatic growth rate at different temperature regimes. *D. galeata* (with the haplotype g1) generally grow slower at 5°C and 12.5°C than *D. hyalina* (with the haplotype x7). For the mitochondrially introgressed clones, *D. galeata* carrying the x7 haplotype (originating from *D. hyalina*) grew larger under low temperatures compared to those of this taxon with g1 haplotypes. A similar pattern was observed for *D. hyalina* introgressed by the g1 haplotype – under warm conditions (20°C) the SGR was higher compared to x7. Studies on mice (Felclair et al., 1998; Nagao et al., 1998) and the copepod *Tigripus californicus* (Edmands & Burton, 1999) have shown, that interspecific hybridization and resulting introgression can lead to a reduction in physical performance caused by a defective coordination of the expression in nDNA- and mtDNA-encoded genes. Pinkaus et al. (Pinkhaus et al., 2007) report in their study on clones of D. galeata, D. hyalina and interspecific hybrids from Saidenbach Reservoir no differences in temperature dependent occurrence between species but between different multilocus genotypes (distinguished by allozyme analysis). In a series of physiological tests, the authors find a significant correlation between physiological parameters and multilocus genotype. Although later generation interspecific hybridization was apparent, mitochondrial genotyping was not conducted.

In this study, we were not able to detect such an effect. Introgressed individuals did not show abnormal SGRs. Moreover, recombinant genotypes seem to be capable of competing in the natural population as the analysis of the planktonic population of 2005 and 2006 indicates.

Mitochondrial introgression. We observed a significant interaction between haplotypes and temperature on somatic growth rate in individuals mitochondrially introgressed facilitating the co-existence with a parental taxon at times exceptional of the observed range of the other parental taxon. The effect of mitochondrial haplotypes with temperature is significant while genotypes based on nuclear analysis did not differ in SGR. Differential mitochondrial gene expression is a physiological trait susceptible to selective pressures like environmental temperature change. Species specific adaptations, i.e. temperature preferences, are easily permeated if later generation interspecific hybridization leads to mitochondrial introgression. Therefore, ecological preferences of *Daphnia* species can be seen as instable factors if those species hybridize frequently. Ballard & Whitlock (2003) remind in their review that mitochondrial introgression has not only occurred when observed by researchers but probably

many times in the phylogenetic history in species. Only recent events can be detected while old incidences stay unidentified.

We propose that mitochondrial introgression may have a greater impact on natural populations than originally expected since it can cause changes in the physiological adaptation of organisms. In further studies the impact of different haplotypes on the organism needs to be explored to better understand the potentials and limits of physiology and ecological adaptation deriving from this former endosymbiont.

General Discussion

In this last part of my thesis I will develop a more general view on the effects of interspecific hybridization based on the results presented. Initially, I will summarize the main answers to those questions asked at the beginning of this project: 1) How did the genetic architecture of the species complex change after a hybrid sweep? 2) Are levels of interspecific hybridization and introgression associated with environmental changes? 3) What effect did introgression have on the current *Daphnia* populations of Lake Constance? I develop general findings of my study hereafter and show that the analysis of biological archives allows not only to interpret processes of the past but also predict the effect of ecological change in the future. Finally, I will suggest questions to be answered prospecitively and show the scientific potential of resting egg banks with upcoming molecular genetic applications.

Daphnia Populations of Lake Constance and Greifensee

My findings of the taxonomic composition of *Daphnia* in Lake Constance and Greifensee (Chapter 2) are to a great extent in concordance with those presented by Jankowski and Straile (2003) based on allozyme analysis. I was able to show that *D. galeata* invaded Lake Constance in the beginning of the 1950s and established successfully in the upcoming decades. Massive hybridization could be observed within the first decade after its arrival probably because of random mating apart from any reproductive barrier with *D. hyalina*. The production of recombinants follows the massive eutrophication of this large water body until the late 1980s. By then, the modernization of environmental controls and implementing of sewage treats initiated its re-oligotrophication. I found very similar patterns in Greifensee where the inhabiting *Daphnia* species (i.e. *D. hyalina* and *D. cucullata*) encountered the same challenges (with a slight shift in time and a massive difference in the extent of ecological relevant charge in the sense of hypereutrophication and re-mesotrophication).

In Lake Constance, *D. hyalina* almost stopped sexual reproduction during times of maximum eutrophication – the most probable explanation for this change in reproductive strategy is the species' adaptation to limited food. With the increase in phosphorus load, even during winter times algae were abundant and, since *D. hyalina* shows a higher resistance to cold temperatures, there was no need to invest in the production of dormant stages for this taxon

anymore. Thus, the almost exclusive unidirectionality of hybridization originating from *D. galeata* probably explains the limited extent of gene flow directed to *D. hyalina* (see chapter 2, but see also chapter 3 and 5). Analyses of the recent planktonic population conducted in the last decades and those presented here (chapter 5) have shown that *D. hyalina* was always present and even abundant in the pelagic of Lake Constance (for example in shallow littoral regions: Jankowski, 2002). In contrast, *D. galeata* is almost not present during winter times suggesting that this species strongly relies on the production of resting eggs. While todays *D. galeata* can be seen as an assemblage of recombinant genotypes resulting from indiscriminate and highly frequent sexual reproduction, *D. hyalina* genotypes have preserved their clonal diversity. It remains unclear whether clonal selection has or does influence *D. hyalina* lineages negatively or if hatching of *D. hyalina* does occur, but in technically undetectable frequencies or in a certain area of Lake Constance which was not part of this study (2006).

For *D. galeata*, in Chapter 3 I describe the change in allelic composition of those genotypes that inhabited Lake Constance in the past and today. A life-history experiment showed, that *D. galeata* individuals hatched from resting eggs produced in the 1960s, 1970s and 2000s tested for their capability of coping with food limitations (C:P 1000), indicating that diversifying selection has affected *D. galeata* populations over time, which can be connected to altering phosphorus levels in Lake Constance.

Although not connected to Lake Constance we found very similar patterns in *Hyalodaphnia* population dynamics in Greifensee. In this shallow lake we were able to observe the same patterns as in Lake Constance. In the course of ecological change due to anthropogenically induced eutrophication a species may be able to invade the habitat of another. Because local adaptations of indigenous species are compensated by the altered environment, establishment of immigrants becomes more probable. In this case, the invading species is also capable of hybridizing with the local species. In general, this study shows that invadors may not only be a threat to local species because they outcompete the former one, they may also hybridize with the other species and thus induce a severe change in the genetic architecture of the population. Introgression is a process that is not reversible in the sense that the genetic architecture of each species will include some alleles of the other species. Keller et al. (2008) conducted a study on the geographical distribution of *D. galeata*, *D. hyalina* and *D. cucullata* north and south of the Alps. All but three out of the 43 analysed lakes were also (although not always predominantly) inhabited by interspecific hybrids of at least two of the mentioned

species. While the authors found a strong correlation with geography and habitat temperature and the occurrence of parentals, another factor explaining the distribution was the trophic level of the habitat. *D. hyalina* and *D. cucullata* preferred large and less phosphorus containing lakes, and *D. galeata* was more often found in southern and warmer tempered lakes. The authors propose that the former distribution of *D. hyalina* and *D. galeata* along a geographic gradient were altered by anthropogenic impact on the habitats of *Daphnia*.

Interspecific Hybridization over Time

The findings of this study are applicable to more general dicussions in science. In 1985, Barton & Hewitt presented the tension zone model stating that a hybrid zone is only maintained by the dispersal of parental taxa and interspecific hybridization occurring in a region of overlap. While the assumptions of this model are based solely on endogenous factors (i.e. incompatibility of parental genomes in recombinant genotypes) susceptible to selection processes, gradient models incorporate ecological factors (Endler, 1977). Although mentioned before by Moore (1985) a clear definition of the bounded hybrid superiority model was proposed in the same year as the former by Moore and Buchanan (1986): While the relative fitness of interspecific hybrids may be low in those habitats parental communities are adapted to, they are more successful in intermediate habitats or disturbed environments in the range of migration to the parental species. With the Mosaic Hybrid Zone model Harrison (e.g. Rose et al., 2002) described geographical characteristics of some hybrid zones as a patchwork of parental and recombinant populations with an emphasis on the importance of environment-dependant selection. One of the reasons many scientist underestimate the extent of genetic exchange through interspecific hybridization probably lies in the assumption that hybrids are generally of minor fitness. While studies of plants have shown, that hybrids can even be of higher fitness (i.e. heterosis, mostly observed in the F_1 generation of recombinants), many zoologists still believe that later generation backcrosses and gene flow through introgression are rare events in animals since hybrids are often of reduced vigor (Seehausen, 2004). Quite recent studies on hybridizing animal taxa like cichlids have even lead to new hypothesis of adaptive radiation via hybrid swarms (Seehausen, 2004). Most important to the conducted study here is the emphasis on the ecological factors influencing the generation of interspecific hybrids. In chapter 2 I show, that this process is directly connected to the rapid and severe

alteration of ecological characteristics of the natural habitat for *Daphnia* species (i.e. eutrophication of Lake Constance and hypereutrophication of Greifensee).

I have attempted to summarize the underlying processes in the temporary hybrid cline hypothesis for sexually reproducing and hybridizing taxa which are subjected to major and fast environmental variation before (Brede, 2003). It allows comprehending the origin and fate of interspecific hybrid lineages over evolutionary relevant time periods including potential gene flow among species, recombination and natural selection. Rapid environmental changes are the premise to enable the successful invasion of a species, since under stable environmental conditions local adaptation prevents immigration (De Meester et al., 2002). Hybrid fitness is expected to be superior to the parental fitness during the phase of ecological change when the habitat is intermediate or perturbed. If the system returns to a stable – but different – ecological phase, the indigenous species will be out-competed by the invading taxon, and consequently hybridization will cease. If, however, the habitat returns to its original state, our hypothesis predicts more than one possible outcome: (1) the taxon composition will also return to its original state including a second phase of interspecific hybridization during a qualitative habitat shift; or (2) gene flow will cause the merging of taxa, establishment of hybrid swarms, reinforcement of parental taxa, or (3) the stabilization of introgressants. The conclusions of the temporary hybrid cline hypothesis effect current attempts to preserve pristine habitats and restore disturbed transitional zones.

Arnold states (2006, p. 152): “In some regards then, introgressive hybridization between rare and more common forms is merely another example of what is occurring continuously through evolutionary time. Yet with the whole-scale modification of the biosphere by humans, genetic exchange-mediated effects on taxa are likely to become more pronounced and, in concert with the loss of habitat, have affects on conservation efforts. (…) Likewise, I will argue that if evolutionary diversification is indeed a web-like process we should not use the occurrence of genetic exchange alone to determine either (i) a taxonomic unit for the hybridizing forms or (ii) a value for their conservation.”

Especially, the results of the chapters 2, 3 and 5 show, that interspecific hybridization is facilitated by anthropogenic alterations of habitats. Hybridization may leed to gene flow between species – also of genetic information relevant for fitness traits. This way, hybridizing *Daphnia* populations are capable of a rapid evolutionary reaction to perturbed habitats.

Consequences of Interspecific Hybridization and Introgression

At this point, I would like to emphasize the unique opportunity to study hybridizing species over a time period longer than most long-term studies accomplished by analyzing *Daphnia* resting egg banks. Studies like the 30 year long observation of Galapagos finches conducted by Peter and Rosemary Grant have given new insights in the fate of recombinant genotypes and their effect on the parental taxa (Grant & Grant, 2002; Grant et al., 2003). In the study on Darwin's finches as well as in my doctoral work the ecological change of the habitat is a key factor influencing the natural populations occurring there. Both cases document the success of stabilized introgressants (see also cases reviewed in: Mallet, 2005) as a response of species to rapid ecological changes causing severe selective pressure on morphological or genetic adaptations (e.g. seed size acting selectively on Darwin's finches beak morphology or dietary and temperature differences affecting *Daphnia*).

It has been hypothesized that interspecific hybridization and therewith allopolyploidy may lead to parthenogenesis or cyclic parthenogenesis in the subgenera *Daphnia* (*pulex*) and *Ctenodaphnia* but also elsewhere: "All parthenogenetic vertebrates (Avise et al., 1992), and many parthenogenetic lineages in other taxa (Bell, 1982), have arisen via interspecific hybridisation and carry the nuclear genomes of two or more sexual species. (...) Just how hybridisation leads to parthenogenesis is not clear, but parthenogenesis can potentially maintain heterotic, highly heterozygous genotypes (...)" (1998). Obligate parthenogenesis is often interpreted as an evolutionary 'dead end' because taxa are not able to respond to selective pressures via sexual recombination of present allelic composition. On the contrary, cyclic parthenogenesis not only facilitates the fast colonization of new habitats (like the former referring to the capability of rapidly establishing large population sizes), it also maintains genetic variability and permits interspecific hybridization.

With ongoing and omnipresent genetic exchange and the dynamics between *Daphnia* belonging to different species, a more appropriate description of this group would be 'dynamic taxa'. This term seems more suitable taking into account the frequency of recombinants found in natural habitats and with the obviously low reproductive barriers at present. All taxa are of more or less distinct genetic integrity (but see also: Schwenk et al., 2000; Petrusek et al., 2008). Although hybrids generated by *Hyalodaphnia* species in some cases exhibit low fitness, many studies have shown that recombinants can also exhibit high

fitness levels and dominate (or take over) whole habitats (Schwenk, 1997; Keller et al., 2008; Petrusek, 2008). It seems that an important component of reproductive isolation in *Daphnia* is based on ecological preferences and monopolization effects when similarly adapted genotypes of different taxonomic origin compete (De Meester et al., 2002). The establishment of hybrid lineages might be successful if ecological niches of intermediate character to those possessed by parental taxa can be filled – cases of tension zones or mosaic patterns are conceivable as well as shown in this study and described within the temporary hybrid cline hypothesis during periods of ecological change and – with this – the loss of competitiveness of the inhabiting parental lineages.

Reticulate evolution in *Daphnia* may not only describe the taxonomic past of this genus but also the very present of genetic exchange among species. For the argument that *Daphnia* species are still distinguishable on the genetic and (in some cases) morphological level I propose the following: The frequency of hybrids and introgressants found at present in many European lakes does not seem to fit the observed 'tidiness' of species (Keller et al., 2008; Rellstab, 2008). The severe changes in taxonomic and genetic composition in *Daphnia* that were caused by the anthropogenic change of ecology of Lake Constance and Greifensee have been shown to be no singularities in Europe (Keller et al., 2008; Rellstab, unpubl.). In concordance with the cultural and industrial development in human society many freshwater habitats have been changed severely. To *Daphnia*, interspecific hybridization and gene flow between species opens the opportunity to respond to rapid ecological change with maximal possible genetic recombination: "Furthermore, even hybrids with lower fitness than their parents can act as the foundation for much evolutionary innovation." (Arnold, 2006; p. 83). In chapter 5 I analyzed the planktonic population of Lake Constance and show, that mitochondrial introgressed individuals are now present in seasonal niches (depending on temperature adaptations of the parental taxon) which has been made possible by the "acquisition" of alien genetic information (in terms of mitochondrial genome).

Arnold states in his book *Evolution Through Genetic Exchange* (2006) the following: "Rather, adaptive trait transfer produces introgressants that are by definition hybrid. Thus, in considering such transfers we should recognize that the highly fit products, some of which are the founders of new taxonomic lineages, are hybrids." Especially species like *D. galeata* which reproduce sexually more often than others (see chapter 4 and Jankowski and Straile, 2003) may hybridize more frequently. This study shows that in ecologically changing habitats

interspecific hybridization and introgression may be a key factor for the survival of *Daphnia* since these processes allow fast adaptation to new challenges.

Future perspectives

Biological archives like *Daphnia* resting egg banks allow observing biological processes over many generations in natural populations within a scientist's life-time. The questions applied to biological archives are manifold. Not only those questions adressed here – anthropogenically induced alteration of habitats or interspecific hybridization in natural populations, but also ecotoxicological effects (Brede, not published) and host-parasite interactions (Decaestecker et al., 2007) in these species have been answered. By now, the genome of *D. pulex* is sequenced (http://wfleabase.org/) and more are up to come: *D. pulicaria* and *D. magna* are in progress. In future projects also *D. galeata*, a species of the *Hyalodaphnia* subgenus, will be sequenced. With the known genome, modern applications like SNP analyses, microarrays and different libraries are available. BAC and cosmid libraries for microbiological approaches and cDNA libraries for gene expression studies are already downloadable from the homepage of the *Daphnia* Genomics Consortium (http://daphnia.cgb.indiana.edu).

Decaestecker et al. (2007) conducted a study on host-parasite interactions in *D. magna* clones hatched from a resting egg bank. The authors exposed *Daphnia* hosts deriving from the recent past with parasites extracted from sediments older, of same age and younger than the *Daphnia* individuals. With this, clones suffered from parasitic infections of different evolutionarily adaptive states. Decaestecker and her co-authors exposed *Daphnia* to parasites of their past, presence and future. This wonderful approach best resembles the great opportunity diapausing stages offer to scientists: In recent years, the need to predict nature's reaction to global changes becomes more urgent. Global warming and the melting of the polar caps influences the ecology of almost all habitats. Rather than observing the changes as they happen in natural populations when habitats alter in their characteristics, scientists can observe the effects past perturbations had on populations and species and above that analyse the outcome of these.

Synopsis

Commonly, interspecific hybridization is a phenomenon observed in nature over ecological gradients. This study adds new insights into the effect of interspecific hybridization and the fate of recombinants. I have showed that species are able to invade habitats more easily if these are perturbed. The invasion of a species may be possible, because habitat disturbances reduced the competition abilities of the indigenous species. The invading species initially occurs in low densities, resulting in asymmetrical and basically unidirectional introgression if recombinant individuals do not suffer from significant fitness deficiencies. Gene flow among species may be detected more easily on the mitochondrial level, because this part of the genome is inherited maternally and therefore not diluted when entering the invading species via meiotic separation and fusion of two parental genomes. In this study, the distribution of haplotypes indicates that introgression on the mitochondrial level was bidirectional. By reconstructing the history of the *Daphnia* populations inhabiting Lake Constance I was able to show that the anthropogenically induced alteration of habitat ecology comprising an eutrophication event and, lateron, an oligotrophication event, resulted in severe changes of the taxon composition. I found that hybridization was mainly of unidirectional character due to random mating probabilities and species densities and complete intermixing occurred when human efforts initiated the re-oligotrophication of Lake Constance. The analysis of the population history revealed, that the indigenous species stopped sexual reproduction almost completely caused by altered ecological conditions making it possible for *D. hyalina* to survive harsh times as parthenogenetic female. With re-oligotrophication, the opposite scenario (excluding the actual invasion) took place and gene flow was directed to *D. hyalina*. Today's individuals are of introgressed nature – with some interbred genotypes obviously positively selected und persisting among the other taxon.

Summary

In this study I analysed past and recent *Daphnia* populations from Lake Constance and Greifensee. Herefore, I first established a set of microsatellite markers applicable to European *Hyalodaphnia* species (chapter 1). Primers were also identified for species specific fragment lengths. 32 markers were then available to characterize the resting egg banks of *Daphnia galeata* and *D. hyalina*. Chapter 2 presents the reconstruction of the taxonomic composition in these two ecologically different lakes. This part of my work shows that the eutrophication that occurred in both lakes in the mid of the last century has strongly influenced the *Daphnia* populations. In both lakes *Daphnia galeata* established and hybridized with the indigenous *D. hyalina*. Interspecific hybridization resulted in introgression on the mitochondrial and nuclear level. In chapter 3 resting eggs from the sediments of the 1960s, 1970s, 1980s, 1990s and 2000s were characterized with microsatellite markers. The aim was to specify the extent of interspecific hybridization and nuclear introgression assuming that the genetic exchange between both species has an impact on their adaptation to their habitat. In life history experiments *D. galeata* and *D. galeata x hyalina* clones hatched from different time periods showed significant differential responses to food quality. Therefore, the question had to be answered how the *Daphnia* resting egg bank and the planktonic population are connected. In chapter 4 hatching experiments were conducted to bridge this gap of scientific knowledge in the life cycle of cyclic parthenogenetic waterfleas. Only *D. galeata* individuals were able to establish a clonal lineage after maturity. All observed recombinant individuals did not reproduce at all or firstly went through another sexual phase of reproduction i.e. produced resting eggs. In order to compare the findings of chapter 4 with the taxon composition of the recent planktonic population of *Daphnia* in Lake Constance, samples were taken over one season (between May 2005 and September 2006). During the season, the taxonomic composition of *Daphnia* changes severely with *D. galeata* being most abundant during the warm season and *D. hyalina* in the cold season. Moreover, some individuals were detected, that did not follow this pattern. With mitochondrial analysis those individuals were identified as mitochondrial introgressants and processed to life history experiments. Significant differences in the somatic growth rate under different temperatures (5°C, 12.5°C and 20°C) were related to the origin of the mitochondrial genome rather than the nuclear taxonomic assignment of the individual.

The findings of this study show that all organisms exposed to rapid ecological changes and their microevolutionary reaction to those.

Reference list

ANDERSON, E. C. & THOMPSON, E. A. (2002). A model-based method for identifying species hybrids using multilocus genetic data. — Genetics 160, 1217-1229.

ARMOUR, J. A., NEUMANN, R., GOBERT, S. & JEFFREYS, A. J. (1994). Isolation of human simple repeat loci by hybridization selection. — Human Molecular Genetics 3, 559-565.

ARNOLD, M. L. (1997). Natural hybridization and evolution. — Oxford University Press, New York, U.S.A.

—. (2006). Evolution Through Genetic Exchange. — Oxford University Press, Oxford.

AVISE, J. C., QUATTRO, J. M. & VRIJENHOEK, R. C. (1992). Molecular clones within organismal clones - mitochondrial DNA phylogenies and the evolutionary histories of unisexual vertebrates. — In: M.K. Hecht B. Wallace & R.J. Macintyre, 225-246,.

BALLARD, J. W. O. & WHITLOCK, M. C. (2004). The incomplete natural history of mitochondria. — Molecular Ecology 13, 729-744.

BELKHIR, K., BORSA, P., CHIKHI, L., RAUFASTE, N. & BONHOMME, F. (1996-2004). GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France); http://www.univ-montp2.fr/~genetix/genetix/genetix.htm —.

BELL, G. (1982). The Materpiece of Nature. — University of California Press, San Francisco.

BENZIE, J. A. H. (2005). Cladocera: the genus *Daphnia* (including *Daphniopsis*). — Kenobi Productions & Backhuys, Ghent & Leiden.

BILLIONES, R., BREHM, M., KLEE, J. & SCHWENK, K. (2004). Genetic identification of *Hyalodaphnia* species and interspecific hybrids. — Hydrobiologia 526, 43-53.

BOERSMA, M., SPAAK, P. & DE MEESTER, L. (1998). Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: The uncoupling of responses. — American Naturalist 152, 237-248.

BREDE, N. (2003). Rekonstruktion populationsgenetischer Prozesse einer Invasion - Molekulare Analyse von *Daphnia* Dauereiern aus Sedimentablagerungen des Bodensees. — Diploma thesis, J. W. Goethe University.

—. (2004). The history of interspecific hybridisation in Lake Constance *Daphnia* as revealed by genetic analysis on the resting egg bank. — Diploma Thesis J.W. Goethe-University Frankfurt am Main.

BREDE, N., STRAILE, D., STREIT, B. & SCHWENK, K. (2007). The contribution of differential hatching succes to the fitness of species and interspecific hybrids. — Hydrobiologia 594, 83-89.

BREDE, N., THIELSCH, A., SANDROCK, C., SPAAK, P., KELLER, B., STREIT, B. & SCHWENK, K. (2006). Microsatellite markers for European *Daphnia*. — Molecular Ecology Notes 6, 536-539.

BRENDONCK, L. & DE MEESTER, L. (2003). Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. — Hydrobiologia 491, 65-84.

BURTON, R. S., RAWSON, P. D. & EDMANDS, S. (1999). Genetic architecture of physiological phenotypes: Empirical evidence for coadapted gene complexes. — American Zoologist 39, 451-462.

CÁCERES, C. E. (1998). Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. — Ecology 79, 1699-1710.

CÁCERES, C. E. & HAIRSTON, N. G. (1998). Benthic-pelagic coupling in planktonic crustaceans: the role of the benthos. — Archiv für Hydrobiologie, Beiheft Ergebnisse der Limnologie 52, 163-174.

CACERES, C. E. & TESSIER, A. J. (2003). How long to rest: The ecology of optimal dormancy and environmental constraint. — Ecology 84, 1189-1198.

CARVALHO, G. R. & WOLF, H. G. (1989). Resting eggs of lake-*Daphnia*. I. Distribution, abundance and hatching of eggs collected from various depths in lake sediments. — Freshwater Biology 22, 459-470.

COLBOURNE, J. K. & HEBERT, P. D. N. (1996). The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. — Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 351, 349-360.

COLBOURNE, J. K., ROBISON, B., BOGART, K. & LYNCH, M. (2004). Five hundred and twenty-eight microsatellite markers for ecological genomic investigations using *Daphnia*. — Molecular Ecology Notes 4, 485-490.

CORRELL, D. L. (1998). The role of phosphorus in the eutrophication of receiving waters: A review. — Journal of Environmental Quality 27, 261-266.

COUSYN, C., DE MEESTER, L., COLBOURNE, J. K., BRENDONCK, L., VERSCHUREN, D. & VOLCKAERT, F. (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., GOMEZ, A., OKAMURA, B. & SCHWENK, K. (2002). The Monopolization Hypothesis and the dispersal-gene flow paradox in aquatic organisms. — Acta Oecologica 23, 121-135.

DECAESTECKER, E., GABA, S., RAEYMAEKERS, J. A. M., STOKS, R., VAN KERCKHOVEN, L., EBERT, D. & DE MEESTER, L. (2007). Host-parasite 'Red Queen' dynamics archived in pond sediment. — Nature 450, 870-873.

DOI, A., SUZUKI, H. & MATSUURA, E. T. (1999). Genetic analysis of temperature-dependent transmission of mitochondrial DNA in *Drosophila*. — Heredity 82, 555-560.

DOOLITTLE, W. F. (1999). Phylogenetic classification and the universal tree. — Science 284, 2124-2129.

DUFFY, M. A., PERRY, L. J., KEARNS, C. M., WEIDER, L. J. & HAIRSTON, N. G. (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.

DUFRESNE, F. & HEBERT, P. D. N. (1998). Temperature-related differences in life-history characteristics between diploid and polyploid clones of the *Daphnia pulex* complex. — Ecoscience 5, 433-437.

EDMANDS, S. & BURTON, R. S. (1999). Cytochrome C oxidase activity in interpopulation hybrids of a marine copepod: A test for nuclear-nuclear or nuclear-cytoplasmic coadaptation. — Evolution 53, 1972-1978.

EINSLE, U. (1966). Einige Beobachtungen und Hypothesen zur Taxonomie der Gattung *Daphnia*. — Schriften des Vereins für Geschichte des Bodensees und seiner Umgebung 84, 1-17.

ELSTER, H. J. & SCHWOERBEL, I. (1970). Beiträge zur Biologie und Populationsdynamik der Daphnien im Bodensee. — Archiv für Hydrobiologie, Supplement 38, 18-72.

ENDER, A., SCHWENK, K., STÄDLER, T., STREIT, B. & SCHIERWATER, B. (1996). RAPD identification of microsatellites in *Daphnia*. — Molecular Ecology 5, 437-441.

ENDLER, J. A. (1977). Geographic variation, speciation, and clines. — Princeton University Press, Princeton.

FELCLAIR, F., CATALAN, J., LENORMAND, T. & BRITTONDAVIDIAN, J. (1998). Centromeric incompatibilities in the hybrid zone between house mouse subspecies from denmark: evidence from patterns of NOR activity. — Evolution 52, 592-603.

FLÖßNER, D. (1972). Kiemen- und Blattfüsser, Branchiopoda, Fischläuse, Branchiura. — VEB Gustav Fischer Verlag, Jena.

—. (2000). Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas. — Backhuys Publishers, Leiden.

FOX, J. A. (2004). New microsatellite primers for *Daphnia galeata mendotae*. — Molecular Ecology Notes 4, 544-546.

GAUTSCHI, B., TENZER, I., MÜLLER, J. P. & SCHMID, B. (2000). Isolation and characterization of microsatellite loci in the bearded vulture (*Gypaetus barbatus*) and cross-amplification in three Old World vulture species. — Molecular Ecology 9, 2193-2195.

GIEßLER, S., MADER, E. & SCHWENK, K. (1999). Morphological evolution and genetic differentiation in *Daphnia* species complexes. — Journal of Evolutionary Biology 12, 710-723.

GOUDET, J. & KELLER, L. (2002). The correlation between inbreeding and fitness: does allele size matter? — Trends in Ecology & Evolution 17, 201-202.

GRANT, P. R. & GRANT, B. R. (2002). Unpredictable evolution in a 30-year study of Darwin's finches. — Science 296, 707-711.

GRANT, P. R., GRANT, B. R., KELLER, L. F., MARKERT, J. A. & PETREN, K. (2003). Inbreeding and interbreeding in Darwin's finches. — Evolution 57, 2911-2916.

GRANT, P. R., GRANT, B. R. & PETREN, K. (2005). Hybridization in the recent past. — Americhan Naturalist 166, 56-67.

GRANT, R. & GRANT, P. (2008). Fission and fusion of Darwin's Finches populations. — Philosophical Transactions of the Royal Society B - Biological Sciences.

GREEN, A. J. & FIGUEROLA, J. (2005). Recent advances in the study of long-distance dispersal of aquatic invertebrates via birds. — Diversity and Distributions 11, 149-156.

GUILIZZONI, P., LAMI, A., MANCA, M., MUSAZZI, S. & MARCHETTO, A. (2006). Palaeoenvironmental changes inferred from biological remains in short lake sediment cores from the Central Alps and Dolomites. — Hydrobiologia 562, 167-191.

GYLLENSTEN, U., WHARTON, D., JOSEFSSON, A. & WILSON, A. C. (1991). Paternal Inheritance Of Mitochondrial-DNA In Mice. — Nature 352, 255-257.

GYLLENSTRÖM, M., HANSSON, L. A., JEPPESEN, E., GARCIA-CRIADO, F., GROSS, E., IRVINE, K., KAIRESALO, T., KORNIJOW, R., MIRACLE, M. R., NYKANEN, M., NOGES, T., ROMO, S., STEPHEN, D., VAN DONK, E. & MOSS, B. (2005). The role of climate in shaping zooplankton communities of shallow lakes. — Limnology and Oceanography 50, 2008-2021.

HAIRSTON, N. G., HANSEN, A. M. & SCHAFFNER, W. R. (2000). The effect of diapause emergence on the seasonal dynamics of a zooplankton assemblage. — Freshwater Biology 45, 133-145.

HAIRSTON, N. G., HOLTMEIER, C. L., LAMPERT, W., WEIDER, L. J., POST, D. M., FISCHER, J. M., CACERES, C. E., FOX, J. A. & GAEDKE, U. (2001). Natural selection for grazer resistance to toxic cyanobacteria: Evolution of phenotypic plasticity? — Evolution 55, 2203-2214.

HAIRSTON, N. G., JR., , PERRY, L. J., BOHONAK, A. J., FELLOWS, M. Q., KEARNS, C. M. & ENGSTROM, D. R. (1999a). Population biology of a failed invasion: Paleolimnology of *Daphnia exilis* in upstate New York. — Limnology and Oceanography 44, 477-486.

HAIRSTON, N. G., LAMPERT, W., CACERES, C. E., HOLTMEIER, C. L., WEIDER, L. J., GAEDKE, U., FISCHER, J. M., FOX, J. A. & POST, D. M. (1999b). Rapid evolution revealed by dormant eggs. — Nature 401, 446.

HAIRSTON, N. G., VANBRUNT, R. A., KEARNS, C. M. & ENGSTROM, D. R. (1995). Age and survivorship of diapausing eggs in a sediment egg bank. — Ecology 76, 1706-1711.

HARRISON, R. G. (1986). Pattern and process in a narrow hybrid zone. — Heredity 56, 337-349.

HAVEL, J. E. & SHURIN, J. B. (2004). Mechanisms, effects, and scales of dispersal in freshwater zooplankton. — Limnology and Oceanography 49, 1229-1238.

HERZIG, A. (1985). Resting eggs, a significant stage in the life cycle of crustaceans *Leptodora kindtii* and *Bythotrephes longimanus*. — Verhandlungen Internationale Vereinigung für theoretische und angewandte Limnologie 22, 3088-3098.

HESSEN, D. O., FAAFENG, B. A. & ANDERSEN, T. (1995). Replacement of herbivore zooplankton species along gradients of ecosystem productivity and fish predation pressure. — Canadian Journal of Fisheries and Aquatic Sciences 52, 733-742.

HOBÆK, A. & LARSSON, P. (1990). Sex determination in *Daphnia magna*. — Ecology 71, 2255-2268.

HOBAEK, A., MANCA, M. & ANDERSEN, T. (2002). Factors influencing species richness in lacustrine zooplankton. — Acta Oecologica-International Journal Of Ecology 23, 155-163.

HOBAEK, A., SKAGE, M. & SCHWENK, K. (2004). *Daphnia galeata x D. longispina* hybrids in western Norway. — Hydrobiologia 526, 55-62.

Elber, F., HÜRLIMANN, J. & NIEDERBERGER, K. (2004). Entwicklung des Gesamtphosphors im Greifensee anhand der im Sediment eingelagerten Kieselalgen. — AWEL, Amt für Abfall, Wasser, Energie und Luft des Kanonts Zürich:

http://www.labor.zh.ch/internet/bd/awel/gs/gq/de/doku/doku_seen.html.

ISHIDA, S. & TAYLOR, D. J. (2007). Quaternary diversification in a sexual Holarctic zooplankter, *Daphnia galeata*. — Molecular Ecology 16, 569-582.

JANKOWSKI, T. (2002). From Diapause to Sexual Reproduction. Evolutionary Ecology of the *Daphnia* Hybrid Complex from Lake Constance. —, Ph.D. thesis Universität Konstanz.

JANKOWSKI, T. & STRAILE, D. (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.

—. (2004). Allochronic differentiation among *Daphnia* species, hybrids and backcrosses: the importance of sexual reproduction for population dynamics and genetic architecture. — Journal of Evolutionary Biology 17, 312-321.

JEPPESEN, E., JENSEN, J. P., JENSEN, C., FAAFENG, B., HESSEN, D. O., SONDERGAARD, M., LAURIDSEN, T., BRETTUM, P. & CHRISTOFFERSEN, K. (2003). The impact of nutrient state and lake depth on top-down control in the pelagic zone of lakes: A study of 466 lakes from the temperate zone to the arctic. — Ecosystems 6, 313-325.

JEPPESEN, E., JENSEN, J. P., SONDERGAARD, M., LAURIDSEN, T. & LANDKILDEHUS, F. (2000). Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient. — Freshwater Biology 45, 201-218.

JEPPESEN, E., SONDERGAARD, M., JENSEN, J. P., HAVENS, K. E., ANNEVILLE, O., CARVALHO, L., COVENEY, M. F., DENEKE, R., DOKULIL, M. T., FOY, B., GERDEAUX, D., HAMPTON, S. E., HILT, S., KANGUR, K., KOHLER, J., LAMMENS, E., LAURIDSEN, T. L., MANCA, M., MIRACLE, M. R., MOSS, B., NOGES, P., PERSSON, G., PHILLIPS, G., PORTIELJE, R., SCHELSKE, C. L., STRAILE, D., TATRAI, I., WILLEN, E. & WINDER, M. (2005). Lake

responses to reduced nutrient loading - an analysis of contemporary long-term data from 35 case studies. — Freshwater Biology 50, 1747-1771.

KELLER, B. & SPAAK, P. (2004). Non-random sexual reproduction and diapausing egg production in a *Daphnia* hybrid species complex. — Limnology and Oceanography 49, 1393-1400.

KELLER, B., WOLINSKA, J., MANCA, M. & SPAAK, P. (2008). Spatial, environmental, and anthropogenic effect on the taxon composition of hybridizing *Daphnia*. — Philosophical Transactions of the Royal Society B - Biological Sciences in press.

KERFOOT, W. C., MA, X., LORENCE, C. S. & WEIDER, L. J. (2004). Toward resurrection ecology: *Daphnia mendotae* and *D. retrocurva* in the coastal region of Lake Superior, among the first successful outside invaders? — Journal of Great Lakes Research 30, 285-299.

KERFOOT, W. C., ROBBINS, J. A. & WEIDER, L. J. (1999). A new approach to historical reconstruction: Combining descriptive and experimental paleolimnology. — Limnology and Oceanography 44, 1232–1247.

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

KLEIVEN, O. T., LARSSON, P. & HOBÆK, A. (1992). Sexual reproduction in *Daphnia magna* requires three stimuli. — Oikos 65, 197-206.

KLÜTTGEN, B., DÜLMER, U., ENGELS, M. & RATTE, H. T. (1994). ADaM, an artificial freshwater for the culture of zooplankton. — Water Research 28, 743-746.

KONDO, R., SATTA, Y., MATSUURA, E. T., ISHIWA, H., TAKAHATA, N. & CHIGUSA, S. I. (1990). Incomplete Maternal Transmission Of Mitochondrial-DNA In *Drosophila*. — Genetics 126, 657-663.

KVIST, L., MARTENS, J., NAZARENKO, A. A. & ORELL, M. (2003). Paternal leakage of mitochondrial DNA in the great tit (*Parus major*). — Molecular Biology And Evolution 20, 243-247.

LADOUKAKIS, E. D. & ZOUROS, E. (2001). Direct evidence for homologous recombination in mussel (*Mytilus galloprovincialis*) mitochondrial DNA. — Molecular Biology And Evolution 18, 1168-1175.

LAFORSCH, C. & TOLLRIAN, R. (2004). Extreme helmet formation in *Daphnia cucullata* induced by small- scale turbulence. — Journal of Plankton Research 26, 81-87.

LAMPERT, W. (1988). The relative importance of food limitation and predation in the seasonal cycle of two *Daphnia* species. — Verhandlungen Internationale Vereinigung für theoretische und angewandte Limnologie 23, 713-718.

LAMPERT, W., SCHMITT, R. D. & MUCK, P. (1988). Vertical migration of freshwater zooplankton: test of some hypotheses predicting a metabolic advantage. — Bulletin of Marine Science 43, 620-640.

LAMPERT, W., TOLLRIAN, R. & STIBOR, H. (1994). Chemical induction of defense mechanisms in freshwater animals. — Naturwissenschaften 81, 375-382.

LEWONTIN, R. C. & BIRCH, L. C. (1966). Hybridization as a source of variation for adaptation to new environments. — Evolution 20, 315-336.

LIMBURG, P. A. & WEIDER, L. J. (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.

LÖFFLER, A., WOLINSKA, J., KELLER, B., ROTHHAUPT, K. O. & SPAAK, P. (2004). Life history patterns of parental and hybrid *Daphnia* differ between lakes. — Freshwater Biology 49, 1372-1380.

LOTTER, A. F., STURM, M., TERANES, J. L. & WEHRLI, B. (1997). Varve formation since 1885 and high-resolution varve analyses in hypertrophic Baldeggersee (Switzerland). — Aquatic Sciences 59, 304-325.

LYNCH, M. & SPITZE, K. (1994). Evolutionary genetics of *Daphnia*. — In: Ecological genetics (L. Real, ed). Princeton University Press, Princeton, p. 109-128.

MALLET, J. (2005). Hybridization as an invasion of the genome. — Trends In Ecology & Evolution 20, 229-237.

MANCA, M. & ARMIRAGLIO, M. (2002). Zooplankton of 15 lakes in the Southern Central Alps: comparison of recent and past (pre-ca 1850 AD) communities. — Journal of Limnology 61, 225-231.

MANCA, M. & RUGGIU, D. (1998). Consequences of pelagic food-web changes during a long-term lake oligotrophication process. — Limnology and Oceanography 43, 1368-1373.

MARCUS, N. H., LUTZ, R., BURNETT, W. & CABLE, P. (1994). Age, viability and vertical distribution of zooplankton resting eggs from an anoxic basin: Evidence of an egg bank. — Limnology and Oceanography 39, 154-158.

MARTENS, K. E. (1998). Sex and Parthenogenesis. — Backhuys, Leiden.

MAYR, E. (1963). Animal Species and Evolution. — Belknap Press of Harvard University Press, Cambridge, Massachusetts.

MEIER, N. (2006). Die Bedeutung von Nahrungsqualität und -quantität für die Fitness und Hybridisierung bei *Daphnia*. — Diploma thesis, J. W. Goethe University.

MERILA, J. & CRNOKRAK, P. (2001). Comparison of genetic differentiation at marker loci and quantitative traits. — Journal of Evolutionary Biology 14, 892-903.

MISHMAR, D., RUIZ-PESINI, E., GOLIK, P., MACAULAY, V., CLARK, A. G., HOSSEINI, S., BRANDON, M., EASLEY, K., CHEN, E., BROWN, M. D., SUKERNIK, R. I., OLCKERS, A. & WALLACE, D. C. (2003). Natural selection shaped regional mtDNA variation in humans. — Proceedings of the National Academy of Sciences of the United States of America 100, 171-176.

MITCHELL, S. E. & LAMPERT, W. (2000). Temperature adaptation in a geographically widespread zooplankter, *Daphnia magna*. — Journal of Evolutionary Biology 13, 371-382.

MOORE, W. S. & BUCHANAN, D. B. (1985). Stability of the northern flicker hybrid zone in historical times: implications for adaptive speciation theory. — Evolution 39, 135-151.

MOSS, B., STEPHEN, D., BALAYLA, D. M., BECARES, E., COLLINGS, S. E., FERNANDEZ-ALAEZ, C., FERNANDEZ-ALAEZ, M., FERRIOL, C., GARCIA, P., GOMA, J., GYLLSTROM, M., HANSSON, L. A., HIETALA, J., KAIRESALO, T., MIRACLE, M. R., ROMO, S., RUEDA, J., RUSSELL, V., STAHL-DELBANCO, A., SVENSSON, M., VAKKILAINEN, K., VALENTIN, M., VAN DE BUND, W. J., VAN DONK, E., VICENTE, E. & VILLENA, M. J. (2004). Continental-scale patterns of nutrient and fish effects on shallow lakes: synthesis of a pan-European mesocosm experiment. — Freshwater Biology 49, 1633-1649.

MUCKLE, R. & DILLMANN-VOGEL, H. (1976). Die bisexuelle Fortpflanzung in der *Daphnia longispina* Gruppe des Bodensee-Obersees. — Beiträge zur Naturkundlichen Forschung in Südwestdeutschland 35, 81-94.

NAGAO, Y., TOTSUKA, Y., ATOMI, Y., KANEDA, H., LINDAHL, K. F., IMAI, H. & YONE-KAWA, H. (1998). Decreased physical performance of congenic mice with mismatch between the nuclear and mitochondrial genome. — Genes and Genetic Systems 73, 21-27.

PAUL, R. J., LAMKEMEYER, T., MAURER, J., PINKHAUS, O., PIROW, R., SEIDL, M. & ZEIS, B. (2004). Thermal acclimation in the microcrustacean *Daphnia*: a survey of behavioural, physiological and biochemical mechanisms. — Journal of Thermal Biology 29, 655-662.

PEAKALL, R. & SMOUSE, P. E. (2001). GenAIEx V5: Genetic analysis in Excel. — In. Australian National University, Canberra, Australia.

—. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. — Molecular Ecology Notes 6, 288-295.

PETRUSEK, A., HOBAEK, A., NILSSEN, J. P., SKAGE, M., CERNY, M., BREDE, N. & SCHWENK, K. (2008). A taxonomic reappraisal of the European *Daphnia longispina* complex (Crustacea, Cladocera, Anomopoda). — Zoological Scripta.

PETRUSEK, A. S., J; MACHÁCEK, J; RUTHOVÁ, S; SMILAUER, P. (2008). *Daphnia* hybridization along ecological gradients in pelagic environments: the potential for the presence of hybrid zones in plankton. — Philosophical Transactions of the Royal Society B - Biological Sciences, doi: 10.1098/rstb.2008.0026.

PFRENDER, M. E. & LYNCH, M. (2000). Quantitative genetic variation in *Daphnia*: Temporal changes in genetic architecture. — Evolution 54, 1502-1509.

PINKHAUS, O., SCHWERIN, S., PIROW, R., ZEIS, B., BUCHEN, I., GIGENGACK, U., KOCH, M., HORN, W. & PAUL, R. J. (2007). Temporal environmental change, clonal physiology and the genetic structure of a *Daphnia* assemblage (*D. galeata-hyalina* hybrid species complex). — Freshwater Biology 52, 1537-1554.

PRITCHARD, J. K., STEPHENS, M. & DONNELLY, P. (2000). Inference of population structure using multilocus genotype data. — Genetics 155, 945-959.

PROCTOR, V. W. (1964). Viability of crustacean eggs recovered from ducks. — Ecology 45, 656-658.

PROCTOR, V. W. & MALONE, C. R. (1965). Further evidence of the passive dispersal of small aquatic organisms via the intestinal tracts of birds. — Ecology 46, 728-729.

RAUTIO, M. & KORHOLA, A. (2002). UV-induced pigmentation in subarctic *Daphnia*. — Limnology and Oceanography 47, 295-299.

REID, V. A., CARVALHO, G. R. & GEORGE, D. G. (2000). Molecular genetic analysis of *Daphnia* in the English Lake District: species identity, hybridisation and resting egg banks. — Freshwater Biology 44, 247-253.

RELLSTAB, C. (2008). Life at low food - population dynamics and genetic structure of *Daphnia* in ultra-oligotrophic and turbid Lake Brienz. — PhD thesis, ETH Zürich.

ROSE, M. R., DRAPEAU, M. D., YAZDI, P. G., SHAH, K. H., MOISE, D. B., THAKAR, R. R., RAUSER, C. L. & MUELLER, L. D. (2002). Evolution of late-life mortality in *Drosophila melanogaster*. — Evolution 56, 1982-1991.

SALINGER, M. (2007). Dauereier der europäischen Daphnien: Taxonomie, Paläolimnologie und Merkmalsevolution. — Diploma thesis, J. W. Goethe University.

SCHINDLER, D. W. (1978). Factors regulating phytoplankton production and standing crop In world´s freshwaters. — Limnology and Oceanography 23, 478-486.

—. (2006). Recent advances in the understanding and management of eutrophication. — Limnology and Oceanography 51, 356-363.

SCHWENK, K. (1993). Interspecific hybridization in *Daphnia*: distinction and origin of hybrid matrilines. — Molecular Biology and Evolution 10, 1289-1302.

—. (1997). Evolutionary genetics of *Daphnia* species complexes - hybridism in syntopy. — Ph.D. thesis, University of Utrecht, The Netherlands.

SCHWENK, K., BIJL, M. & MENKEN, S. B. J. (2001). Experimental interspecific hybridization in *Daphnia*. — Hydrobiologia 442, 67-73.

SCHWENK, K., BREDE, N. & STREIT, B. (2008). Interspecific hybridization in animals - extent, processes and evolutionary impact. — Philosophical Transactions of the Royal Society B - Biological Sciences, doi: 10.1098/rstb.2008.0055 .

SCHWENK, K., POSADA, D. & HEBERT, P. D. N. (2000). Molecular systematics of European *Hyalodaphnia*: the role of contemporary hybridization in ancient species. — Proceedings of the Royal Society of London Series B-Biological Sciences 267, 1833-1842.

SCHWENK, K., SAND, A., BOERSMA, M., BREHM, M., MADER, E., OFFERHAUS, D. & SPAAK, P. (1998). Genetic markers, genealogies and biogeographic patterns in the Cladocera. — Aquatic Ecology 32, 37-51.

SCHWENK, K. & SPAAK, P. (1995). Evolutionary and ecological consequences of interspecific hybridization in cladocerans. — Cellular and Molecular Life Sciences (CMLS) 51, 465-481.

—. (1997). Ecology and genetics of interspecific hybridization in *Daphnia*. — In: Evolutionary Ecology of Freshwater Animals (B. Streit, T. Städler & C. M. Lively, eds). Birkhäuser Verlag, p. 199-229.

SEEHAUSEN, O. (2004). Hybridization and adaptive radiation. — Trends in Ecology and Evolution 19, 198-207.

SEIDENDORF, B., BOERSMA, M. & SCHWENK, K. (2007). Evolutionary stoichiometry: The role of food quality for clonal differentiation and hybrid maintenance in a *Daphnia* species complex. — Limnology and Oceanography 52, 385-394.

SKAGE, M., HOBAEK, A., RUTHOVA, S., KELLER, B., PETRUSEK, A., SEDA, J. & SPAAK, P. (2007). Intra-specific rDNA-ITS restriction site variation and an improved protocol to distinguish species and hybrids in the *Daphnia longispina* complex. — Hydrobiologia 594, 19-32.

SOMERO, G. N. (2002). Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living. — Integrative and Comparative Biology 42, 780-789.

SOMMER, A. M. & PORTNER, H. O. (2002). Metabolic cold adaptation in the lugworm *Arenicola marina*: comparison of a North Sea and a White Sea population. — Marine Ecology-Progress Series 240, 171-182.

SPAAK, P. (1996). Temporal changes in the genetic structure of the *Daphnia* species complex in Tjeukemeer, with evidence for backcrossing. — Heredity 76, 539-548.

—. (1997). Hybridization in the *Daphnia galeata* complex: are hybrids locally produced? — Hydrobiologia 360, 127-133.

SPAAK, P. & BOERSMA, M. (2006). Predator mediated coexistence of hybrid and parental *Daphnia* taxa. — Archiv für Hydrobiologie 167, 55-76.

SPAAK, P., DENK, A., BOERSMA, M. & WEIDER, L. J. (2004). Spatial and temporal patterns of sexual reproduction in a hybrid *Daphnia* species complex. — Journal of Plankton Research 26, 625-635.

SPAAK, P. & HOEKSTRA, J. R. (1995). Life history variation and the coexistence of a *Daphnia* hybrid with its parental species. — Ecology 76, 553-564.

SPITZE, K. (1993). Population structure in *Daphnia obtusa* - quantitative genetic and allozymic variation. — Genetics 135, 367-374.

STARK, J. D. & BANKS, J. E. (2003). Population-level effects of pesticides and other toxicants on arthropods. — Annual Review of Entomology 48, 505-519.

SYMONDS, V. V. & LLOYD, A. M. (2004). A simple and inexpensive method for producing fluorescently labelled size standard. — Molecular Ecology Notes 4, 768-771.

TAYLOR, D. J. & HEBERT, P. D. N. (1993). Habitat-dependent hybrid parentage and differential introgression between neighboringly sympatric *Daphnia* species. — Proceedings of the National Academy of Sciences of the United States of America 90, 7079-7083.

TAYLOR, D. J., HEBERT, P. D. N. & COLBOURNE, J. K. (1996). Phylogenetics and evolution of the *Daphnia longispina* group (Crustacea) based on 12s rDNA sequence and allozyme variation. — Molecular Phylogenetics and Evolution 5, 495-510.

TAYLOR, D. J., SPRENGER, H. L. & ISHIDA, S. (2005). Geographic and phylogenetic evidence for dispersed nuclear introgression in a daphniid with sexual propagules. — Molecular Ecology 14, 525-537.

TEMPLETON, A. R. (1989). The meaning of species and speciation: A genetic perspective. — In: Speciation and its consequences (D. Otte & J. A. Endler, eds). Sinauer, Sunderland, MA, p. 3-27.

TESSIER, A. J., LEIBOLD, M. A. & TSAO, J. (2000). A fundamental trade-off in resource exploitation by *Daphnia* and consequences to plankton communities. — Ecology 81, 826-841.

TOLLRIAN, R. (1990). Predator-induced helmet formation in *Daphnia cucullata* (Sars). — Archiv für Hydrobiologie 119, 191-196.

VAKKILAINEN, K., KAIRESALO, T., HIETALA, J., BALAYLA, D. M., BECARES, E., VAN DE BUND, W. J., VAN DONK, E., FERNANDEZ-ALAEZ, M., GYLLSTROM, M., HANSSON, L. A., MIRACLE, M. R., MOSS, B., ROMO, S., RUEDA, J. & STEPHEN, D. (2004). Response of zooplankton to nutrient enrichment and fish in shallow lakes: a pan-European mesocosm experiment. — Freshwater Biology 49, 1619-1632.

VANDEKERKHOVE, J., DECLERCK, S., BRENDONCK, L., CONDE-PORCUNA, J. M., JEPPESEN, E. & DE MEESTER, L. (2005a). Hatching of cladoceran resting eggs: temperature and photoperiod. — Freshwater Biology 50, 96-104.

VANDEKERKHOVE, J., DECLERCK, S., BRENDONCK, L., CONDE-PORCUNA, J. M., JEPPESEN, E., JOHANSSON, L. S. & DE MEESTER, L. (2005b). Uncovering hidden species: hatching diapausing eggs for the analysis of cladoceran species richness. — Limnology and Oceanography-Methods 3, 399-407.

WEIDER, L. J., LAMPERT, W., WESSELS, M., COLBOURNE, J. K. & LIMBURG, P. (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.

WESSELS, M., LENHARD, A., GIOVANOLI, F. & BOLLHÖFER, A. (1995). High resolution time series of lead and zinc in sediments of Lake Constance. - Aquatic Sciences 57, 291-304.

WOLF, H. G. & CARVALHO, G. R. (1989). Resting eggs of lake-*Daphnia*. II. In situ observations on the hatching of eggs and their contribution to population and community structure. — Freshwater Biology 22, 471-478.

WOLINSKA, J., BITTNER, K., EBERT, D. & SPAAK, P. (2006). The coexistence of hybrid and parental *Daphnia*: the role of parasites. — Proceedings of the Royal Society B-Biological Sciences 273, 1977-83.

Zusammenfassung der Doktorarbeit

Viele Seen in Europa waren in den letzten Jahrzehnten starken anthropogenen, ökologischen Veränderungen ausgesetzt, wobei die Einschleppung von Neobiota, Einbringung von Medikamenten-Rückständen und Chemikalien und die Eutrophierung dieser Habitate einen besonders gravierenden Einfluss auf deren natürliches Gleichgewicht haben. Die weit verbreitete Eutrophierung der Gewässer durch den Eintrag von phosphorhaltigen Waschmitteln und Düngemitteln wurde in den letzten Jahrzehnten durch Umweltschutzmaßnahmen und Kläranlagen grösstenteils annähernd rückgängig gemacht, um in diesen Habitaten einen ursprünglichen ökologischen Status wieder herzustellen.

Für die Erforschung der durch Eutrophierung ausgelösten Prozesse wurden Arten aus der Familie Daphniidae (Untergattung *Hyalodaphnia*) zur Untersuchung ausgewählt. Während sich Daphnien unter optimalen Lebensbedingungen, wie sie im Frühjahr und Sommer herrschen, asexuell vermehren, werden zu anderen Zeiten nicht nur sexuelle Weibchen sondern auch Männchen gebildet. Im sexuellen Reproduktionszyklus bilden Daphnien Dauereier, die in eine cuticuläre Hülle eingebettet sind. Diese Ephippien sind resistent gegen Austrocknung, Frost und Druck und können so Zeiten überstehen, in denen das Überleben für ein adultes Tier nicht mehr möglich ist. Die Dauereier sind auch nach mehreren Jahrzehnten noch in der Lage, sich zu entwickeln und bilden in ihrer Gesamtheit im Sediment eine *resting egg bank* (Dauerstadienbank), in der das genetische Material der vergangenen Jahre konserviert vorliegt.

In dieser Doktorarbeit werden die Prozesse innerhalb von Populationen mehrerer Hyalodaphnien-Arten rekonstruiert, die anthropogenen Einflüssen ausgesetzt waren. Im Bodensee (Deutschland, Schweiz und Österreich) und Greifensee (Schweiz) kommen die drei untersuchten Arten *Daphnia galeata*, *D. hyalina* und *D. cucullata* (nur in letzterem) vor, die, seit sie zusammen vorkommen, hybridisieren. Die Prozesse, die sich innerhalb dieser Populationen abgespielt haben, wurden anhand der Dauerstadienbanken dieser Arten rekonstruiert, teils durch direkte Anwendung von molekular-genetischen Methoden, teils

durch *Life-History*-Versuche an Tieren, die aus Dauerstadien schlüpften und bis zu 40 Jahre alte Genotypen repräsentieren. Auf der einen Seite wurde besonderer Wert auf die Analyse der Auswirkungen der Eutrophierung und Reoligotrophierung dieser physikalisch unterschiedlichen Seen auf die *Daphnia* Populationen gelegt. Auf der anderen Seite wurde verfolgt, welchen evolutionären Effekt interspezifische Hybride und Introgression zwischen den Arten über die Zeit haben.

Hierzu wurden sechs spezifische Fragen gestellt, die im Rahmen der oben präsentierten fünf Kapitel aufgearbeitete werden:

1. Wie hat sich die genetische Zusammenstellung des Arten-Komplexes nach einer Phase der Hybrdisierung verändert?
2. Steht das Ausmaß interspezifischer Hybridisierung und Introgression in Zusammenhang mit ökologischen Veränderungen?
3. Wie effektiv ist die postzygotische Isolierung zwischen den Arten, und wie genau gibt die Dauerstadienbank die genetische Zusammensetzung der geschlüpften Tiere wieder?
4. Wie deutlich ist die genetische Differenzierung der Arten an neutralen genetischen Loci sichtbar?
5. Woraus setzen sich die rezenten *Daphnia* Populationen des Bodensees zusammen?
6. Welchen Effekt hatte Introgression auf die aktuellen *Daphnia* Populationen des Bodensees?

Um diese Fragen beantworten zu können, wurde ein Set von Mikrosatelliten-Markern entwickelt, die sich für die genetische Charakterisierung von Hyalodaphnien eignen (Kapitel 1). Hierfür wurden Mikrosatelliten-Marker für die Arten *Daphnia galeata*, *D. hyalina*, *D. cucullata* und *D. curvirostris*, sowie für interspezifische Hybride der ersten drei Arten getestet, damit die Marker später sowohl für die Bestimmung der Arten und Hybrid-Klassen verwendet werden können, als auch klassische Anwendungen im Bereich der Populationsgenetik finden. Das Set umfasst insgesamt 32 Primer für deren Nutzung Multiplex-PCR vorgeschlagen wird, um den Arbeits- und Kostenaufwand für eine solche Analyse zu minimieren.

Mit Kapitel 2 beginnt die Vorstellung des eigentlichen Projektes, das sich mit der Rekonstruktion von Populationsdynamiken der Wasserflöhe im Bodensee über die letzten 100 Jahre beschäftigt. Beide Seen sind in den letzten Dekaden, beginnend in den 1950er Jahren, durch eine Phase der Eutrophierung (im Fall des Greifensees eine Hypereutrophierung) gegangen. Zu dieser Zeit wanderte *D. galeata* in die Seen ein und etablierte sich über die kommenden Jahre. Darüber hinaus hybridisierte die Art ausserdem mit der indigen vorkommenden Art *D. hyalina*. Während *D. hyalina* heute im Greifensee nicht mehr zu finden ist, beträgt der Anteil der Individuen dieser Art im Plankton des Bodensees ca. 50%. In Kapitel 2 wird die Artzusammensetzung von *Daphnia* in den beiden Seen mithilfe molekular genetischer Methoden rekonstruiert und gezeigt, dass es phasenweise zu vermehrter interspezifischer Hybridisierung kam und es Hinweise auf Introgression zwischen den Arten gibt. Das Ausmass an Hybridisierung erklärt sich durch fehlende Isolationsmechanismen, da Analysen ergaben, dass die Häufigkeit der Hybridisierungsereignisse direkt mit den Abundanzen sympatrisch vorkommender Parentalarten korreliert ist. Mikrosatelliten-Analysen liessen darüber hinaus den Schluss zu, dass sich die *Daphnia* Populationen der beiden Seen aufgrund derer ökologischen Veränderung und der einhergehenden interspezifischen Hybridisierung in ihrer genetischen Zusammensetzung so verschoben haben, dass eine Wiederherstellung der ursprünglichen ökologischen Verhältnisse nicht den einstigen Zustand bei *Daphnia* herbeiführt.

In Kapitel 3 wird nicht nur die Dauerstadienbank des Bodensees mit Mikrosatelliten-Analysen genauer untersucht, sondern *Life-History*-Experimente mit Daphnien präsentiert. Hierfür wurden Dauereier aus Sedimenten verschiedenen Alters extrahiert und zum Schlupf gebracht. Nachdem Individuen aus den 1960er, 1970er und 2000er Jahren als klonale Linien herangezogen wurden, wurde getestet, wie sich zwei unterschiedliche Nahrungsqualitäten und -quantitäten auf die somatische Wachstumsrate von *D. galeata* und *D. galeata x hyalina* Klonen aus verschiedenen Dekaden auswirken.

Es konnte gezeigt werden, dass sich die *D. galeata* und *D. hyalina* Populationen – repräsentiert durch ihre Dauerstadienbank in den Sedimenten des Bodensees – über die Zeit nicht nur signifikant unterschiedlich sind, sondern sich auch einander annähern. Die *Life-*

History-Experimente konnten nachweisen, dass es eine signifikante Interaktion zwischen Klonen aus verschiedenen Zeiten und der Nahrungsqualität gibt. Ein Vergleich von genetischer und ökologischer Differenzierung zeigt, dass gerichtete Selektion auf mehreren Ebenen gewirkt hat.

Im Weiteren wurde sich auf die Verbindung zwischen Dauerstadienbank und Planktonpopulation der Daphnien des Bodensees konzentriert (Kapitel 4). Es galt zu beantworten, inwieweit die Proportion der geschlüpften Tiere die Gesamtheit der zum Schlupf angesetzten Dauereier repräsentiert. Es ist bekannt, dass sich unterschiedliche Arten unterschiedlich stark sexuell vermehren – so ist das Verhältnis der Dauerstadien von *D. hyalina* zu *D. galeata* 1 : 88. In mehreren Replikaten wurden Dauerstadien aus verschiedenen Jahrzehnten zum Schlupf angesetzt und im Verlauf sowohl die Artzugehörigkeit etablierter klonaler Linien als auch die nach Beendigung des Versuches nicht entwickelten Eier überprüft. Es konnte gezeigt werden, dass *D. hyalina* aufgrund seiner geringen Häufigkeit nicht schlüpft, es aber keinen Unterschied im Schlupferfolg zwischen *D. galeata* und *D. galeata x hyalina* gibt, unabhängig vom Alter der Dauerstadien. Dennoch konnte gezeigt werden, dass alle geschlüpften interspezifischen Hybriden nicht in der Lage waren, sich normal zu vermehren – ein Zeichen für Fitness-Defizite.

Im fünften Kapitel wird eine ausführliche Analyse der *Daphnia* Planktonpopulation im saisonalen Verlauf eines Jahres vorgestellt. Hierfür wurden die Planktonproben mit molekular genetischen Methoden (ITS-RFLP und Mikrosatelliten) auf ihre Art hin bestimmt. Darüber hinaus wurde für jedes Tier der mitochondriale Haplotyp mittels Restriktionfragment-Längenpolymorphismus bestimmt.

Die Studie konnte zeigen, dass sich über die Saison die Taxon-Zusammensetzung ändert. Während *D. galeata* im Sommer am häufigsten ist, dominiert *D. hyalina* während der kühleren Jahreszeit. Hybride lassen sich nur während des Frühjahrs und Sommer in den Proben nachweisen. Es konnte ausserdem nachgewiesen werden, dass *D. hyalina* Klone dann im Sommer auftreten, wenn sie mitochondrial einen Haplotypen von *D. galeata* tragen – und anders herum. Mitochondriale Introgression durch interspezifische Hybridisierung konnte in

mehreren Fällen nachgewiesen werden. In einem *Life-History*-Versuch, bei dem die somatische Wachstumsrate in verschiedenen Hälterungs-Temperaturen gemessen wurde. Dabei ergab sich eine signifikante Interaktion zwischen mitochondrialem Haplotypen und Temperatur.

Diese Arbeit konnte zeigen, dass sich in beiden Seen, Bodensee und Greifensee, die Taxon-Zusammensetzung der vorkommenden *Hyalodaphnia* Arten innerhalb weniger Jahrzehnte verändert hat. Die ökologische Veränderung eines Habitates hat dazu geführt, dass einwandernde Arten mit den einheimischen und lokal angepassten Arten konkurrieren können. Die invasive Art kann sich etablieren und einheimische Arten verdrängen oder mit ihnen koexistieren. Wenn es zwischen den Arten keinen prä- oder postzygotischen Isolationsmechanismen gibt, kann es aufgrund von Wahrscheinlichkeiten dazu kommen, dass interspezifische Hybride gebildet werden. In einem ökologisch alternierenden Habitat, können interspezifische Hybridisierung und anschliessender Genfluss zwischen den Arten von Vorteil sein, weil der Austausch von „bewährten" Allelen und Genen unter starkem Selektionsdruck eine schnellere Anpassung ermöglicht. Genfluss zwischen den Arten bedingt allerdings auch eine andere Schlussfolgerung: die Wiederherstellung von anthropogen veränderten Habitaten durch Umweltschutzmassnahmen kann nicht den Schutz und die Erhaltung ursprünglicher Habitate ersetzen.

Die Nutzung biologischer Archive durch molekular-genetische Rekonstruktionen der ablaufenden Prozesse ermöglicht es, in einer konzentrierten Studie Prozesse, die sich über mehrere Dekaden hinziehen, zu analysieren. Im vorliegenden Fall wurde der Schluss gezogen, dass es sich bei Arten wie *D. galeata* und *D. hyalina* eher um „dynamische Taxa" handelt, die unterschiedliche ökologische Anpassungen haben, diese aber teilen können, auch wenn interspezifische Hybride Fitness-Defizite aufweisen. Weil auch interspezifische Hybride die Möglichkeit haben, sie asexuell zu vermehren, reichen relativ wenige Tiere, um zu einer Rückkreuzung mit den Parentalarten zu führen, die wiederum die Voraussetzung für Introgression ist.

In der Zukunft können biologische Archive wie die Dauerstadienbanken von *Daphnia* zu einem Modellsystem in den Wissenschaften werden. Das Genom von *Daphnia pulicaria* ist vor kurzem sequenziert worden. Daraus ergibt sich eine Vielfalt neuer Analyse-Möglichkeiten mit denen immer detailliertere Fragen gestellt und beantwortet werden können. Forscher haben nun die Möglichkeit, in konzentrierten Studien Prozesse und Dynamiken natürlicher Populationen über mehrere Dekaden zu rekonstruieren und hieraus Erkenntnisse über die Reaktion auf Stressoren, anthropogene Einflüsse und Temperaturveränderungen zu erlangen. Diese einmalige Kombination aus physiologischen Eigenschaften und molekular-genetischen Methoden bietet ausserdem vielversprechende Ansätze, um Themen im Bereich der Biodiversitätsforschung zu bearbeiten.

Curriculum Vitae

Nora Brede
Ausserdorf 6
CH-8322 Madetswil
Schweiz
email: nora.brede@eawag.ch

Geburt:		18. August 1977, Frankfurt am Main
Staatsangehörigkeit:		Deutsch
Schulbildung:	1986-88	Lessing Gymnasium – Humanistisches Gymnasium, Frankfurt a. M.
	01-05/1989	St. Hilda's & St. Hugh's School, private high school, New York, New York, U.S.A.
	1989-93	Ziehen-Schule, Gymansium, Frankfurt a. M.
	1993-94	Swifton High School, public high school, Swifton, Arkansas, U.S.A.
	1994-97	Odenwaldschule Ober-Hambach, Landschulerziehungsheim, Heppenheim
	1997	Allgemeine Hochschulreife (1,9)
Studium:	09/1997	Studium Biologie, J. W. Goethe-Universität, Frankfurt a. M.
	2000	Vordiplom (gut)
	2003	Diplom (sehr gut)
		Diplomarbeit: Rekonstruktion populationsgenetischer Prozesse einer Invasion – Molekulare Analyse von *Daphnia*-Dauereiern aus den Sedimentablagerungen des Bodensees
	seit 05/2004	Promotionsstudium mit Unterstützung des Hessischen Ministeriums für Wissenschaft und Kunst (Stipendium zur Förderung von Frauen in den Naturwissenschaften)

Nora Brede CH-Madetswil, den ……

Erklärung

Ich erkläre hiermit, dass ich mich bisher keiner Doktorprüfung unterzogen habe.

CH-Madetswil, den

.....
(Unterschrift)

Eidesstattliche Versicherung

Ich erkläre hiermit an Eides Statt, dass ich die vorgelegte Dissertation über

The Reconstruction of Evolutionary Patterns from *Daphnia* Resting Egg Banks

selbständig angefertigt und mich anderer Hilfsmittel als der in ihr angegebenen nicht bedient habe, insbesondere, dass aus Schriften Entlehnungen, soweit sie in der Dissertation nicht ausdrücklich als solche mit Angabe der betreffenden Schrift bezeichnet sind, nicht stattgefunden haben.

CH-Madetswil, den ..... (Unterschrift)

Acknowledgements

I deeply thank Klaus Schwenk who inspired me in innumerable occasions and who hung on with tremendous patience, understanding, energy and friendship. Thank you for teaching me science – the one way or the other.

I thank Prof. Dr. Bruno Streit for the support and insights into the administrative world of science.

I thank my cooperation partners Dietmar Straile, Piet Spaak and Adam Petrusek for this productive cooperation.

Special thanks to all of you diplomas contributing to this project: Anne Thielsch, Christoph Sandrock, Nadine Meier, Katja Heubach, Christian Claßen and Moritz Salinger.

I want to thank all those people – researchers and others - I discussed with, talked and listened to and profited from during the last years: All motivated practicum students helping me with my project, Christian Claßen, Susi Denzau, Outi Dove, Carsten Färber (above all – for the wonderful time we had), Holger Geupel, Katja Heubach, Thomas Jankowski, Barbara Keller, Kirsten Klappert, Kerstin Kuhn, Carsten Nowak, Adam Petrusek, Markus Pfenninger, Susi Rausch, Tanja Ruch, Moritz Salinger, Christoph Sandrock, Gisela Schmiedeskamp, Katrin Stapput, Sid Staubach, Dirk Steinke, Anne Thielsch, Elke Völker, Sönke 'Minc' von den Berg and Eva Wörner. It's alphabetically of course... but special thanks to Piet Spaak for extra assistance.

For those chapters submitted or published already, my thanks go to Michael Arnold, John Colbourne, Nelson Hairston Jr., Erik Jeppesen, Luc de Meester, Michael Reid, Alfred Sulger, Derek Taylor and Martin Wessels.

I thank more than anyone else my mother, Karola Brede, for supporting me in all possible ways. I wish there would be a way to express how thankful I am. I love you.

Markus und Vivian 'Jula' Kleinbölting – a fixed star and its micro satellite – I thank you for the support here and now. I love you and can't wait to share all this future with you.