From Diapause to Sexual Reproduction
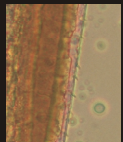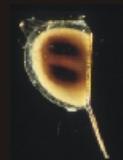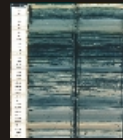
Evolutionary Ecology of the *Daphnia* Hybrid Complex from Lake Constance

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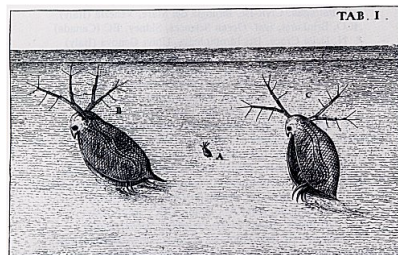
cover photos (top to bottom):
- Sexual (ephippia carrying) female of *Daphnia galeata* (by Kerstin Bittner)
- Emergence trap in the littoral zone of Lake Constance (by Martin Mörtl)
- Laminated sediment core from a deep part of Lake Constance (220m)
- Silhouette of Lake Constance
- Diver (me) taking a littoral sediment core (by Martin Mörtl)
- Littoral sediment core from Lake Constance
- Ephippium from Lake Constance sediments (by Kerstin Bittern)
- Dorsal margin of a spineless ephippium isolated from Lake Constance sediments
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FROM DIAPAUSE TO SEXUAL REPRODUCTION

EVOLUTIONARY ECOLOGY OF THE *DAPHNIA* HYBRID COMPLEX FROM LAKE CONSTANCE

Dissertation

zur Erlangung des akademischen Grades

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an der Universität Konstanz

- Fachbereich Biologie -

vorgelegt von

Thomas Jankowski

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Chapter 1

GENERAL INTRODUCTION

.

*Die Vielfalt und Geschichte
lebender Dinge zu beschreiben,
Wissen als Selbstzweck zu erlangen
bedeutet eine Bereicherung
der Humanität.*

DJ FUTUYMA 1990

Sexual activity, hybridization and diapause are important mechanisms influencing ecological and evolutionary processes among populations (e.g. Charnov, 1993; Roff, 1997; Arnold, 1997), because these mechanisms are known to influence the genetic composition and adaptive traits of populations. Hence, organisms inhabiting these three components offer the unique possibility to study not only each of these components in isolation, which reveals insights in the outcomes of a single factor, but also their interplay. Cyclically parthenogenetic *Daphnia* (Crustacea: Anomopoda) hybrid populations offer all these three components: i) The cyclically parthenogenetic life-cycle provides a model system for the investigation of sexual reproduction, as under parthenogenesis males and sexual eggs are normally absent from the population and were only produced during sexual phase (Hebert, 1978). ii) In some groups within the genus *Daphnia*, e.g. in the *Daphnia-longispina* species complex, hybridization and stable hybrid populations are very common (Schwenk & Spaak, 1995). And iii) diapause is an essential component of their life-cycle, in particular for temporary pond *Daphnia* populations (Hebert, 1978).

This thesis deals with the interplay between sexual reproduction, hybridization and diapause and their ecological and evolutionary consequences for the *Daphnia hyalina-galeata* complex from Lake Constance. In the following paragraphs of this chapter I will outline these components in more detail and with special emphasis on *Daphnia*. I will briefly introduce my model system: Lake Constance and its *Daphnia* populations.

Sexual reproduction and the life cycle of *Daphnia*

The variation that exists in a population is the resource with which natural selection works (Ridley, 1996). Recombination by sexual reproduction is one main important factor producing new variants which are again exposed to natural selection, the evolutionary force most responsible for adaptation to the environment. Hence, sexual reproduction is a main feature in the life-history of an individual.

In cyclically parthenogenetic *Daphnia*, the sexual phase is restricted to distinct periods of the year, while during the rest of the year parthenogenesis is the common mode of reproduction (Hebert, 1978). Under favourable environmental conditions, a parthenogenetic *Daphnia* female produces female broods (Fig. 1.1). However, under stress conditions she can switch to production of males and sexual eggs. Fertilization of sexual eggs results in the production of resting-eggs encapsulated in an ephippia. These sexually produced resting-stages sink to the sediment. After a dormancy phase new parthenogenetic females hatch to rebuild the next year generation. A critical point in this life-cycle is the switch to sexual reproduction. *Daphnia* is believed to exhibit environmental sex determination (ESD, Korpelainen, 1990). This does not preclude genetic involvement in the expression of sex nor in the control of sex ratios. Indeed, clones among one *Daphnia* population showed strong genetic variation in the allocation to sexual reproduction (e.g. Innes & Singleton, 2000).

Many laboratory studies were conducted to ascertain the factors inducing sexual reproduction in *Daphnia*, but up to now the induction of sexual activity is not a trivial task in this group (Lynch pers. com.), and in particular in lake dwelling daphnids (Spaak, 1995, pers. obs.). The switch to sexual activity *in-situ* is not well understood. It appears to be related to deterioration in the environment due to increased population density and reduced food (Hebert, 1978). Other factors influencing sexual activity in *Daphnia* are temperature (Stross, 1987), photoperiod (Stross & Hill, 1968), fish exudates (Slusarczyk, 1999) and other chemicals (Dodson et al., 1999) or combinations of these factors (Kleiven et al., 1992). It is not clear, whether high population density is a cue indicating on future unbenign conditions or if it indicates increasing mating success (Innes & Singleton, 2000).
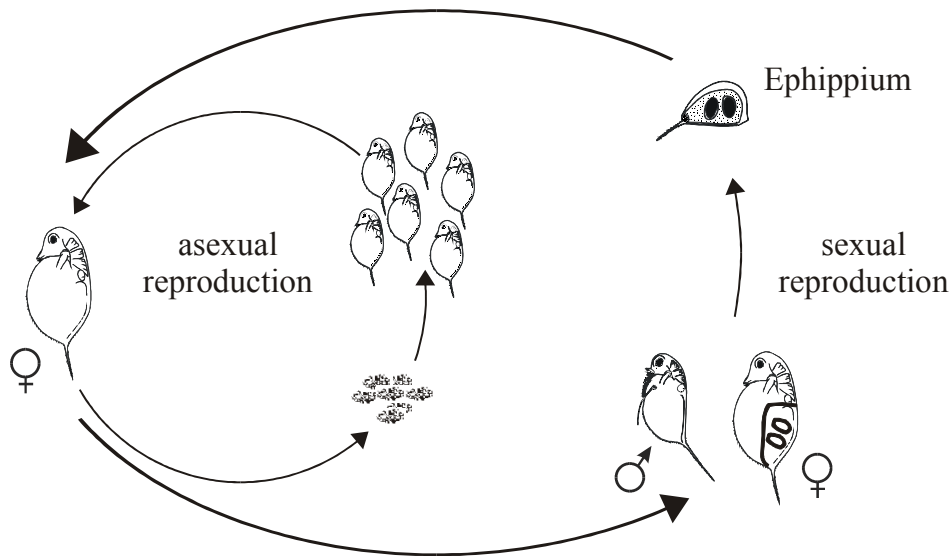
FIGURE 1.1 Reproduction in *Daphnia*. The left cycle represents asexual reproduction during which parthenogenetic eggs are produced. Parthenogenetic females hatch from these eggs. Under unfavorable environmental conditions female produce sexual eggs and males. Sexual reproduction results in the formation of diapausing eggs that are enclosed in a ephippia. Only parthenogenetic females hatch from these ephippial eggs in *Daphnia* (*Daphnia* drawings from Flössner 2000).

Despite the high number of studies dealing with sexual reproduction in *Daphnia*, investigations on the influence of the timing of and allocation to sexual reproduction on ecological, e.g. competitive interactions and local species persistence (Caceres, 1997), and evolutionary consequences, e.g. genetic diversity and adaptive traits (Lynch, 1983; Pfrender & Lynch, 2000), for *Daphnia* populations of large permanent lakes are relatively sparse. In particular, their consequences for the seasonal and long-term population dynamics are less understood. The cyclical parthenogenetic life-cycle involves a trade-off between parthenogenetic and sexual reproduction, i.e., between short-term population growth and

and population persistence and long-term population persistence. High investment into sexual reproduction may cause short-term population declines even contributing to short term extinction (McCauley et al., 1999; Ciros-Perez et al., 2002). On the other side, high investment into sexual reproduction might increase the chances of long-term persistence of populations (Ciros-Perez et al., 2002).

Two important consequences of sexual reproduction in *Daphnia* are hybridization in sympatric populations and the production of resting-stages, both will be outlined in the next two paragraphs.

Hybidization and *Daphnia*

Hybridization is a common feature among *Daphnia* all over the world (Hebert, 1985; Taylor & Hebert, 1993; Schwenk & Spaak, 1995). The *Daphnia longispina* group, within the subgenera *Hyalodaphnia*, is one of the most diverse *Daphnia* groups in Europe (Flössner, 2000). This group comprises several species complexes known to form hybrids across large geographic areas (Wolf & Mort, 1986; Mort & Wolf, 1986; Hebert et al., 1989; Hebert et al., 1993; Schwenk & Spaak, 1995; Giessler, 1997; Giessler, 1997; Schwenk, 1997). Species and hybrids of this group frequently occur in syntopy. Recent analysis suggests that reproductive isolation in *Hyalodaphnia* seems to evolve significantly slower than genetic isolation (Schwenk et al., 2000). Population genetic investigations (Spaak, 1996; Giessler, 1997) and combined approaches of population genetics and morphological evolution (Giessler et al., 1999) revealed strong evidence for probably backcrossing mediated reticulate evolution within the *Daphnia longispina* species complex.

For decades hybridization in animals was viewed as an evolutionary dead end, due to, for example, genetic incompatibilities and fitness disadvantages (reviewed in Harrison, 1993; Arnold, 1997). In *Daphnia*, but also in other animal groups (Arnold, 1997), it could be shown not only that stable hybrid populations exist (Schwenk & Spaak, 1995), but that hybrids frequently dominate sympatric populations (Spaak et al., 2000) and that hybrids exhibit, under special circumstances, higher fitness than the parentals (Spaak & Hoekstra, 1997; Spaak & Boersma, 2001). This is in contrast to the earlier hypothesis that hybrids have a lower fitness than the parentals, which should be compensated by recurrent hybrid production (reviewed in Harrison, 1993; Arnold, 1997). Consequently, based on laboratory fitness estimates and field observations Spaak & Hoekstra (1995) formulated the "temporary hybrid superiority model", which assumes that during certain periods in the year, due to environmental conditions (e.g. predation, food), hybrids will have higher fitness compared to the parental species.

However, most studies on *Daphnia* hybrids focused on the asexual phase during the season and the F1-hybrids. Hence, there is a lack on information on both the importance of sexual reproduction (allocation and timing) and the importance of backcrosses in natural populations. Sexual reproduction of hybrids and parentals enables gene flow between

species, and therewith probably the "flow" of adaptive characters (Arnold, 1997), especially if the parentals are adapted to different environments. Furthermore, in a rapidly changing environment, hybridization was believed to result in a maximation of genetic diversity and introgression can lead to an elevated fitness and thus an increase in the frequency of individuals belonging to the introgressed form (Arnold, 1997). Both the knowledge of sexual reproduction of *Daphnia* hybrids and the fate of backcrosses as well as introgressed individuals in natural population is sparse. Hence, studies dealing with the timing of and allocation to sexual reproduction in natural sympatric hybrid populations, combined with an analysis of backcrossing and introgression, are necessary to understand their ecological and evolutionary influence on the hybrid complex, in particular, in a fluctuating environment.

Diapause and *Daphnia*

Diapause is a major trait in the evolution of plant and invertebrate life-histories (e.g. Roff, 1992). In zoological and ecological textbooks diapause has originally been regarded as a strategy for dispersal and survival during periods of seasonally unfavourable environmental conditions (e.g. Hebert, 1978), like drying in lakes or deserts. Of the hundreds of studies examining zooplankton population and community dynamics, all but a handful fail to consider the production of possibly long-lived resting stages, i.e. fail to consider the organism's true generation time (but see Caceres, 1997; McCauley et al., 1999). Freshwater zooplankton are considered to be short-lived species, with population and community dynamics proceeding on the time scale of a few weeks or months. In many species, however, the active stage represents only a small fraction of the total life span, and much of an animal's lifetime is spent in dormancy, e.g. because many dormant propagules can remain viable for many years or decades (also called "prolonged diapause", Hairston et al., 1996). It has been noted that storage commonly occurs in one of the two stages: either dormant propagules or iteroparous adult. It could be shown that, at least for crustaceans, species have either long adult lives or long-lived dormant eggs (Hairston et al., 1995). Because of their longevity resting-stages may substantially impact the ecological and evolutionary dynamics of the populations that produce them.

Recent advance in ecological and evolutionary research attribute the diapause phase a number of adaptive functions (Hairston, 1998), which will be considered in more detail in this paragraph. Theoretical models and empirical data provide evidence that diapause is not only an advanced strategy for seasonally fluctuating environments, but also for the persistence of species in stochastic and harsh environments, due to risk-spreading (bet-hedging) by prolonged diapause (Ellner et al., 1998; Callaghan, 1998; Danforth, 1999; Menu et al., 2000). Besides the avoidance of unbenign abiotic conditions, diapause might also be a strategy to escape from biotic interactions with predators or competitors. For example, the dinoflagellate *Peridinium* was suggested to produce resting stages to avoid a

predatory copepod (Rengefors et al., 1998). *Daphnia mendotae* seem not to be able to coexist with the competitor *Daphnia pulicaria* in Oneida Lake without prolonged dormancy (Caceres, 1997). Theory regarding this so-called "storage effect" was developed in the 1980s (see Hairston et al., 1996; Chesson, 2000). It suggests that overlapping generations, due to long-lived resting stages, in a temporally fluctuating environment, may allow the persistence of competitors, and therewith maintain species diversity. The storage effect promotes biodiversity in a fluctuating environment when the temporal fluctuations in recruitment of the competing species tend to be negatively correlated, but the long-lived stage allows each species to persist through periods when its recruitment is poor.

Additionally, to the maintenance of species diversity, the "storage effect" was also considered to maintain genetic diversity within populations in fluctuating environments (Ellner & Hairston, 1994). This points on another important role of long-lived diapause. Emergence from long-term diapause will release lineages into the population which have not experienced the most recent bout of selection. In fact, from crustaceans it is known that they are viable for several decades (e.g. Weider et al., 1997). This recruitment of "old" individuals (genotypes, phenotypes) might decelerate evolutionary processes considerably (Hairston & DeStasio, 1988; Hairston et al., 1999), but could also maintain variability to react to environmental changes.

The knowledge on the influence of such processes on *Daphnia* population development is sparse. In particular, in permanent, large and deep lakes such processes are not only influenced by the allocation to and timing of sexual reproduction, but also by the recruitment, i.e. hatching success from dormant eggs. As the environmental cues for breaking diapause, i.e. temperature increase and light (Stross, 1987; Pfrender & Deng, 1998), decrease with lake depth, emergence probability also decreases with depth, and is probably very low in deep lake areas. Therefore, resting-egg production in deep lakes does not seem to be a risk-free strategy and, hence, the deposition of resting-eggs, e.g. in the littoral zone, might play an important role for recruitment. On the other side, a prolonged parthenogenetic phase, i.e. overwintering in temperate lakes, might offer an alternative strategy, but probably with the consequences of no or rare sexual reproduction, thus subsequent consequences for genetic and phaenotypic variability and no prolonged diapause. The consequences of such different strategies have rarly been rarely investigated, but a comparison of temporary pond and permanent pond *Daphnia* populations revealed strong differences in the genetic structure (Lynch, 1983).

The longevity of dormant eggs offers a new approach for the investigation of population histories ("resurrection ecology", Kerfoot et al., 1999). Because these resting stages are strongly coupled to pelagic development certain stages offer insights into the magnitude of historic environmental perturbations and the time scale of population responses to ecosystem recovery (Kerfoot et al., 1999). Resting stages stored in the sediments can be revived and these hatchlings can be used for experimental investigations.

Studies on resting stages records ((Weider et al., 1997; Hairston et al., 1999; Hairston et al., 1999; Kerfoot et al., 1999; Cousyn et al., 2001) obviously rely on the assumption that sedimentary egg banks do really reflect past populations. Up to now, there is no study which critically examines this assumption.

Daphnia in Lake Constance

The research presented in this thesis is based on the *Daphnia hyalina-galeata* hybrid complex from Lake Constance as a model system. Lake Constance is a mesotrophic warm-monomictic lake at the northern fringe of the European Alps (47°39'N, 9°18'E). This lake consists of two basins: the upper basin with an area of about 470km² and a mean depth of 100m (max. depth 254m) and the lower, more eutrophic basin with an area of about 62km² and a mean depth of 13m (Wessels, 1998). The seasonal and long term dynamics of phytoplankton, zooplankton, and microbial populations have been studied in great detail during the last decades (Bäuerle & Gaedke, 1998).

The trophic state changed dramatically during the last century: originally an oligotrophic lake, it underwent massive eutrophication in the 1950s, with a maximum at the end of the 1970s, followed by re-oligotrophication (Güde et al., 1998). During eutrophication *Daphnia galeata* invaded Lake Constance in the 1950s, where originally only *Daphnia hyalina* occurred (Muckle & Muckle-Rottengatter, 1976). Morphological data suggests that there was a massive hybridization phase in the 1970s (Einsle, 1983). Allozyme investigations showed that the hybrid ratio during the late 1980s ranged between 20 and 30% (Weider & Stich, 1992). Lake Constance *D. hyalina* and *D. galeata* differ in several ecological aspects including antipredator- (Stich & Lampert, 1981; Stich, 1989) and overwintering strategies (Straile & Geller, 1998). In addition, *D. hyalina* exhibits higher growth and reproduction rates at low food concentrations (Stich & Lampert, 1984), which suggests that *D. hyalina* might be competitively superior as compared to *D. galaeta*, which invaded the lake during eutrophication.

Sexual reproduction and the subsequent production of diapause stages as well as hybridization might have important influences of the competitive interaction within this sympatric *Daphnia* population. Because, as pointed out above, these components might influence the genetic variability and the coexistence of species, due to for example the "storage effect" (Caceres, 1997) and/or "temporary hybrid superiority" (Spaak, 1995). Despite intensive investigations during the last decades in Lake Constance, the taxa specific differences of the timing of and allocation to sexual reproduction, recruitment pattern and seasonal occurrence within the hybrid swarm are less understood.

Research objectives

The research presented in this thesis aims to improve the understanding of evolutionary ecological processes, i.e. sexual reproduction, hybridization and diapause,

within the cyclic parthenogenetical *Daphnia* hybrid complex from Lake Constance. Therefore, this study covers the whole life-cycle of daphnids. This thesis starts with the parthenogenetic phase, continues with sexual reproduction and diapause and ends with the recruitment of new parthenogenetical females. Four main topics will therefore be addressed in this thesis. First, data on the seasonal pattern of population dynamics and population genetic structure of the hybrid swarm are provided (chapter 2). Second, taxa specific differences to the timing of sexual reproduction, sexual allocation and sex ratio were studied *in-situ* (chapter 2 and 3) as well as in mesocosm experiments (chapter 3). Third, the taxa specific differences in the potentials and limits of ecological and evolutionary studies using the ephippial record from datable sediment cores will be shown (chapter 4). And at least, to complete the life-cycle, both the recruitment from ephippial hatchlings using laboratory and *in-situ* experiments and the distribution of the resting-stages were investigated (chapter 5). Taken together this study will show that sexual reproduction, i.e. diapause formation, is a key process for a better understanding of the ecological and – possibly – microevolutionary development of the Lake Constance *Daphnia* population.

Chapter 2

ALLOCHRONIC DIFFERENTIATION AMONG *DAPHNIA* SPECIES, HYBRIDS AND BACKCROSSES: THE IMPORTANCE OF SEXUAL REPRODUCTION FOR POPULATION DYNAMICS AND GENETIC ARCHITECTURE

together with D. Straile, submitted to J evol Biol

Seasonal dynamics of the abundance, sexual reproduction and genetic architecture in a *Daphnia hyalina-galeata* hybrid complex were studied in the large and deep Lake Constance. We found evidence for the occurrence of first and second order hybridization. Our study revealed strong differences between the parental species regarding not only their seasonal dynamics, genetic architecture and diversity, but also their sexual reproductive behaviour. The overwintering *D. hyalina* showed low genetic diversity, no genetic differentiation during the season, and reproduced sexually in autumn, whereas *D. galeata* reached higher levels of genetic diversity, reproduced sexually in early summer, and exhibited changes in genetic structure during the season, but was only present from spring to autumn. Within all variables studied, F1 and F2 hybrids showed an intermediate pattern, whereas proposed backcross hybrids were more similar to their respective parentals. These differences in phenotype as well as significant differences in pairwise F_{st} values between parentals suggest that gene flow seems to be relative low in the Lake Constance hybrid system. We found evidence for unidirectional introgression by backcrossing from *D. galeata* to *D. hyalina* and found a decrease in at least one of the proposed introgressed alleles in the *hyalina*-backcross with ongoing season. Our findings suggest allochronic differentiation within this hybrid population and different microevolutionary trajectories of the parental species, which will be discussed in light of the ongoing reoligotrophication process of Lake Constance.

Introduction

Hybrid systems are often viewed as natural experiments that serve as windows on evolutionary processes (Harrison, 1993; Arnold, 1997). In particular, the highly recombinant genotypes found in hybrid systems offer a unique opportunity to dissect the architecture of gene flow between species. Several authors (e.g. Anderson & Hubricht, 1938; Anderson, 1949) have argued that the most common outcome of the formation of hybrids is the transfer of genes from one species of the hybridizing type to the other. During the last decade several studies showed evidence for introgression (for review see Dowling & Secor, 1997; Arnold, 1997) not only in plants, e.g. in *Populus* (Martinsen et al., 2001), *Helianthus* (Rieseberg et al., 1999; Rieseberg et al., 1999; Kim & Rieseberg, 2001) and *Rorippa* (Bleeker & Hurka, 2001), but also in animals, e.g. in *Daphnia* (Schwenk & Spaak, 1995; Spaak, 1996; Giessler, 1997; Giessler et al., 1999), *Bufo* (Malmos et al., 2001) and in *Drosophila* (Noor et al., 2001). Hybrids have been shown to be fitter than their parents, and the pursuit of this hybrid vigor has been important in agriculture and animal breeding for over a century (Darwin, 1876). In particular, hybridizing species might be better in quickly adapting to changing situations since they can pick up genes of both species. For example, Galápagos finch hybrids appear to have higher fitness than parental species during unusual stressful conditions (Grant & Grant, 1996). For Louisiana irises it could be shown that the *I. fluva*-like hybrid was superior in the colonization of new habitats as compared to the parental species (Cruzan & Arnold, 1993, but see Hercus & Hoffmann, 1999). Most studies on the fitness of interspecific and intraspecific crosses have examined only the F1 hybrids, whereas effects of genetic mechanisms are typically most pronounced in F2 and backcross hybrids (Lynch 1991). The genome rearrangements (e.g. in F2 and backcrosses) may disrupt epistatic interactions that confer fitness in specific environments (local adaptation), as well as gene interactions that are independent of the environment. Studies on the success and fitness of F2 and backcross hybrids in animals are sparse (Arnold, 1997; Edmands, 1999), but there is evidence that their fitness could also be high (McMillan et al., 1997; Hatfield & Schluter, 1999). Typically, studies on hybrid fitness/success are carried out in laboratory settings, but the success of genealogical classes and introgressed individuals in a hybrid complex depends on their fitness (compared to the parentals) in a fluctuating environment over several generations.

The *Daphnia longispina* group is one of the most diverse *Daphnia* groups in Europe. This group comprises several species complexes known to form hybrids across large geographic areas (Wolf & Mort, 1986; Mort & Wolf, 1986; Hebert et al., 1989; Hebert et al., 1993; Schwenk & Spaak, 1995; Giessler, 1997; Giessler, 1997; Schwenk, 1997). Species and hybrids of this group frequently occur in syntopy. Genetic data on hybrid swarms support backcrossing towards one parental species (Spaak, 1996), suggest nonrandom mating of the parental species (Spaak, 1996) and suggest that unidirectional (Schwenk, 1993) as well as bidirectional hybridization (Giessler et al., 1999) occurs in this

group. In addition, recent molecular genetic analysis information on many *Daphnia* species has provided data on phylogenetic relationships and the age of species complexes (Schwenk, 1993; Taylor & Hebert, 1993; Coulbourne & Hebert, 1996; Schwenk et al., 2000) and suggest that reproductive isolation in *Hyalodaphnia* seems to evolve significantly slower than genetic isolation (Schwenk et al., 2000).

The success of *Daphnia* lineages is determined by parthenogenetic as well as sexual reproduction. Sexual reproduction is an important component of the population dynamics of cyclic parthenogenetic daphnids, because it results not only in the formation of new genotypes but also in the production of long-lived resting stages. Sexual reproduction is both under genetic and environmental control (Larsson, 1991; Innes & Singleton, 1994) and is induced by environmental conditions like crowding, food limitation and day length (Stross, 1969b; Kleiven et al., 1992; Slusarczyk, 1999; Slusarczyk, 2001; Alekseev & Lampert, 2001). The seasonal timing of sexual reproduction shows strong intra- and interspecific variability (Wolf, 1987; Larsson, 1991; Innes & Singleton, 1994; Spaak, 1995) depending not only on the habitat, e. g. temporary pond or lake (Lynch, 1984; Carvalho, 1994), but also on the geographic location (Deng, 1997). In addition to environmental control of sexual reproduction, environmental conditions determine the success of genotypes during parthenogenetical reproduction. Environmental conditions, i.e., food availability and predation pressure as well as physical and chemical parameters, change dramatically between lakes and within a season (Sommer et al., 1986). Hence, both modes of reproduction have to be adapted to the special conditions of the respective habitat including its seasonal variability. However, studies dealing with *Daphnia* population biology usually concentrate on parthenogenetically produced daphnids (e.g. Spaak, 1996; Giessler, 1997; MacKay & Elser, 1998; Straile, 2000; Grover et al., 2000; Straile & Adrian, 2000). However, knowledge on the sexual reproductive behaviour of lineages is mandatory to understand the population biology and population genetics of daphnids.

Here we analyse the population dynamics and population genetics of a 50-year-old *Daphnia* hybrid complex (Einsle, 1978) in large and deep Lake Constance focussing on sexual reproduction of daphnids. In this lake, *D. hyalina* and *D. galeata*, two closely related and hybridizing species, occur in syntopy (Weider & Stich, 1992). Earlier studies suggest, that in Lake Constance both species are still distinct and show differences in several traits, such as diel vertical migration (Stich & Lampert, 1981; Stich & Lampert, 1984; Stich, 1989) and overwintering behaviour (Straile & Geller, 1998), with traits of F1 hybrids being more similar to *D. hyalina* (Weider & Stich, 1992). Allozymes were used for two decades to distinguish between parentals and hybrids in the *Daphnia hyalina - galeata* hybrid system (Wolf & Mort 1986). Recently, Giessler (1997) suggested a second diagnostic marker. Using two species-specific markers allows us for the first time to provide a detailed field analysis estimating the seasonal dynamics, sexual reproduction, and success of F1, possible F2, and backcross hybrids compared to the parentals in this *Daphnia* hybrid complex. Specifically, we ask whether the *Daphnia* population represents one hybrid

swarm or distinct species, respective genotype groups? Do F1 hybrids reproduce sexually? Is there evidence for higher order hybridization? If so, what are the dynamics of abundance and sexual reproduction of higher order hybrids compared to the parental species in a seasonally fluctuating environment?

Methods

Study site and organism: *Daphnia* in Lake Constance

The mesotrophic, large and deep prealpine Lake Constance, located on the northern fringe of the Alps (47°39' N), offers a unique system for the study the genetic architecture in the *Daphnia hyalina-galeata* hybrid complex. Lake Constance has been intensively investigated during the 20th century (Bäuerle & Gaedke, 1998). The trophic state changed dramatically during the last century: originally an oligotrophic lake, it underwent massive eutrophication in the 1950[s], with a maximum at the end of the 1970[s], followed by re-oligotrophication (Güde et al., 1998). During eutrophication *Daphnia galeata* invaded Lake Constance in the 1950s, where originally only *Daphnia hyalina* occurred (Muckle & Dillmann-Vogel, 1976). Morphological data suggested massive hybridization during the 1970s (Einsle, 1983) and allozyme investigations showed that the hybrid ratio was between 20 and 30% in the late 1980s (Weider & Stich, 1992). Lake Constance *D. hyalina* and *D. galeata* differ in several ecological aspects including overwintering (Straile & Geller, 1998) and antipredator strategies, such as diel vertical migration and helmet formation (Stich & Lampert, 1981; Stich, 1989). These strategies are responses to a highly seasonal environment, with seasonal changes in temperature, food availability and predation regimes (Sommer et al. 1986). Briefly, winter conditions are characterised by low temperatures and food concentrations. During spring, food is abundant and predation pressure is low resulting into exponential population growth controlled by temperature (Straile 2000). In summer predation and food limitation are probably important selection factors for daphnids (Sommer et al. 1986).

Sampling

Daphnia were sampled weekly to bi-weekly in the central part of the fjord-like northwestern part of Lake Constance (Überlinger See) from January to December 2000 with a Clark-Bampus sampler (mesh size 140µm) by vertical hauls from 50m depth. At each sampling date up to six vertical net hauls were taken, depending on *Daphnia* abundance, in order to get a minimum number of 100 adults, if possible egg carrying females. These samples were pooled and stored cool and brought to the lab within 3 hours until subsequent preparation in the laboratory. In addition, two extra net hauls were taken; one was frozen immediately after sampling in liquid nitrogen as backup sample and the other was fixed in formol for subsequent determination of abundance. Living individual

adult females, males and ephippia carrying females were picked randomly from the sample and were frozen in a −80°C freezer for subsequent electrophoretic analysis.

Allozyme analysis

The seasonal genetic architecture among *D. galeata-hyalina* hybrid complex populations was determined using allelic variation at allozyme loci, using cellulose acetate electrophoresis. Four enzyme loci were screened for polymorphism in the *Daphnia* population of Lake Constance: aspartate amino transferase (AAT; E.C. 2.6.1.1.), aldehyde oxidase (AO; E.C. 1.2.3.1.), phosphoglucomutase (PGM; E.C. 5.4.2.2.) and phosphoglucose isomerase (PGI; E.C. 5.3.1.9.). Protocols for electrophoresis were as described by Hebert & Beaton, 1993, except that electrophoresis was run at 300V. Both, AAT (Wolf & Mort, 1986) and AO loci (Giessler, 1997) are considered to be diagnostic for *D. galeata* and *D. hyalina*. Using two marker loci it is possible to distinguish six possible genotype groups. We classified these genotype groups as proposed genealogical classes which can occur in case of two hybridizing species: parentals (*D. hyalina* = P_{hya}, *D. galeata* = P_{gal}; with two homozygous species specific alleles at each loci), first (F1; both loci heterozygous) and secondary (F2; both loci homozygous, but one for species 1 and the other for species 2) hybrids and first generation backcrosses (BP_{hya}, BP_{gal}; one locus is homozygous for one species and the other is heterozygous). This classification assumes only first and second order hybridization. Hence, it provides a conservative estimate of the relative frequency importance of higher order hybridization, including backcrosses, as, for example, 25% of the offspring of a parental x F1 hybrid cross can show the same genotype as the F1 hybrid class or the parental class, respectively (for discussion see: Nason et al., 1992, Nason & Ellstrand, 1993). The two polymorphic loci PGI and PGM were used to differentiate between Multi-Locus-Genotypes (MLG's) within each genotype class.

Data analysis

MLG diversity was calculated for all classes. Samples were pooled monthly to get representative sample sizes, in particular for winter samples and rare genotype classes. Calculations were performed only at a sample size > 5 individuals per class. We used the genotype diversity measure, G_0 (Carvalho, 1994), which was quantified as:

$$G_0 = \frac{1}{\sum_{i=1}^{k} p_i^2}$$

where p_i is the relative frequency of the ith MLG and k is the number of genotypes. G_0 will vary from a minimum of 1, where there is only a single genotype, to a maximum of k, when genotypes are evenly distributed.

F-statistics and deviation from Hardy-Weinberg equilibrium (HWE) were calculated with FSTAT (Goudet, 2000) and GENETIX (Belkhir, 2001). Within-population and

subpopulation deviations from Hardy-Weinberg equilibrium (HWE) were estimated by F_{is} values (small f) calculated using FSTAT (Gouldet, 2000) and GENETIX (Belkhir, 2001). To determine the degree of genetic differentiation between genotype classes (all sampling dates pooled) pairwise F_{st} (θ) values (Weir 1984) were calculated using FSTAT (Goudet, 2000). Additionally, pairwise F_{st} (θ, Weir & Cockerham, 1984) values were calculated for 'subpopulations' of three seasons differing in food availability for daphnids: S1 (samples of January to April), S2 (samples of May to July) and S3 (samples of August to December) within each class, to detect potential differences in the genetic architecture resulting from recruitment of sexual produced resting eggs (differences between 'subpopulation' 1 and 2) and clonal selection (differences between 'subpopulations' 2 and 3). Probability values (p) of the F_{is}- and pairwise F_{st}-values were adjusted for multiple table-wide test using Bonferroni correction to minimise type-I errors. Genetic distances between the three classes were calculated as

$$D_{st} = -\ln(1-F_{st}) \qquad \text{(Reynolds et al., 1983)}$$

and plotted using the unweighted pair-group averages (UPGMA). The tree does not imply phylogenetic relationships but genetic dissimilarities.

Results

Screening more than 1800 individuals at four loci, and using AAT and AO as markers we found all 6 genetical classes, i.e. both parentals (P_{hya}, P_{gal}), the F1 hybrid as well as the F2 and backcross (BP_{hya}, BP_{gal}) hybrids. Abundance of all genotype classes increased exponentially during spring (Fig. 2.1a), with P_{gal} showing the fastest increase resulting in a relative abundance of more than 70 % in May (Fig. 2.1b). After obtaining peak abundances P_{gal} decreased in abundance and was absent from plankton samples from November onwards. In contrast, spring increase of P_{hya} was slowest and the contribution of P_{hya} to overall *Daphnia* abundance was lowest during May. However, P_{hya} maintained high abundances until autumn. Seasonal dynamics of backcross hybrids were similar to the respective parentals. Hence, P_{hya} and BP_{hya} occurred during the whole year and contributed in winter and autumn more then 95% of the population size. F1 and F2 hybrids showed a intermediate pattern: their spring increase was lower than the spring increase of P_{gal}, and their drop in abundance during summer was intermediate between P_{gal}, BP_{gal} on one hand and P_{hya} and BP_{hya} on the other hand (Fig. 2.1a). Consequently relative abundance of hybrids was highest from end of May to mid of August, and abundance of hybrids at the end of the year was intermediate between P_{gal}, BP_{gal} and P_{hya}, BP_{hya}.

The occurrence of ephippial females and males was restricted to two distinct periods (early summer and autumn) with a tendency for the start of male production to be just before the peak of ephippial females (Fig. 2.2). Even within these two periods, ephippial females contributed less than 1 % and males less than 5 % to overall abundance. The timing of sexual production differed between the two parental species (Fig. 2.3): *D. galeata*
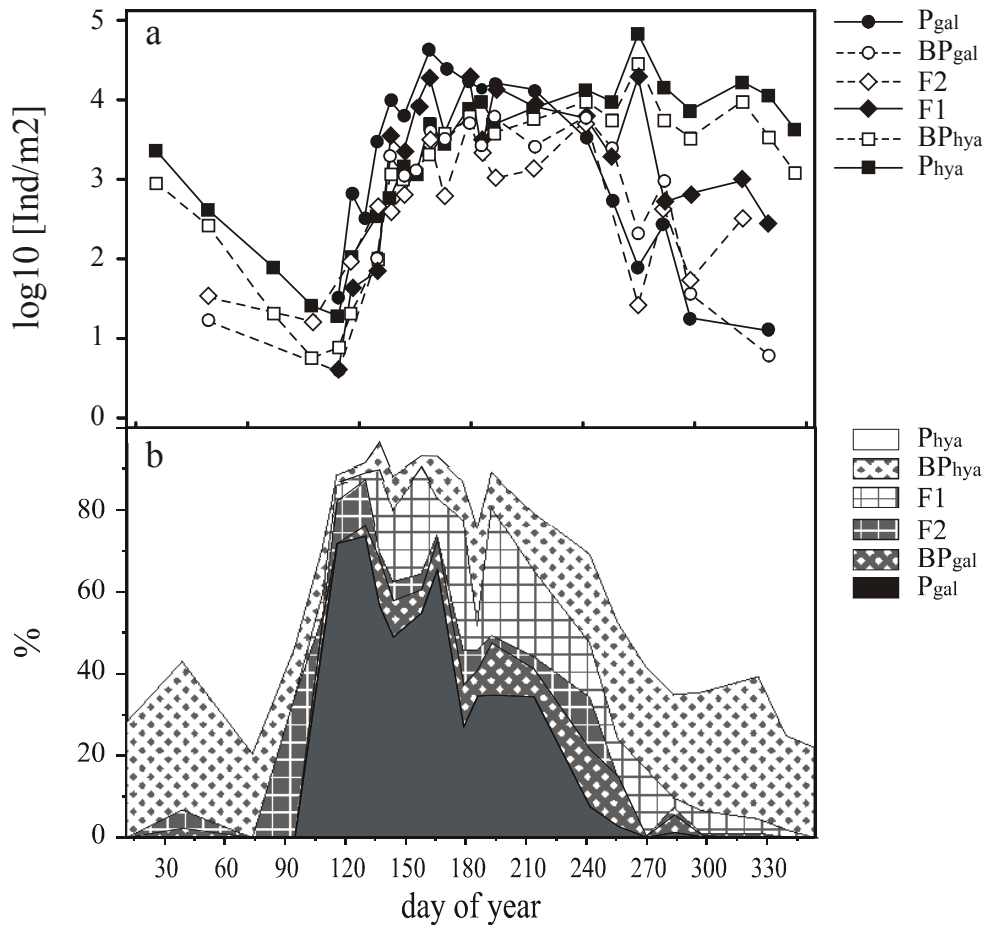
FIGURE 2.1 Seasonal abundance (a) and proportion (b) of the six genotype classes (identified with the two marker loci AAT and AO) in the *Daphnia* population of Lake Constance in the year 2000. Genotype classes represent parental species (P_{hya} = *D. hyalina* and P_{gal} = *D. galeata*) F1 hybrids and proposed F2 and backcross (BP_{hya}, BP_{gal}) hybrids.

produced ephippia only after the spring peak, whereas, *D. hyalina* produced ephippia only in autumn. Hybrids were observed to produce ephippia during both periods. The abundance of ephippial females was 10 times higher in spring than in autumn, i.e., *D. galeata* produced more ephippia than *D. hyalina*. Male production of *D. hyalina* was 10 times higher in autumn than in spring, and *D. galeata* produced males nearly exclusively in spring.

Genetic differentiation between parentals, hybrids and backcrosses

F_{st} values between the six genotype classes (all samples pooled) ranged from 0.001 to 0.245 showing significant differences between most of these classes (Tab. 2.1). Both parentals (P_{hya}, P_{gal}) were significantly different from all other classes, except from their backcrosses (BP_{hya}, BP_{gal}). The F1 hybrid differed significantly from all other classes. It was more closely related to P_{hya} and BP_{hya} than to F2, P_{gal} and BP_{gal}. In contrast, the F2 hybrid was more closely related to P_{gal} and BP_{gal} and was not significantly different from $BP_{gal.}$ The UPGMA analysis of the genetic distance supports this pattern of genetic similarities (Fig. 2.4).
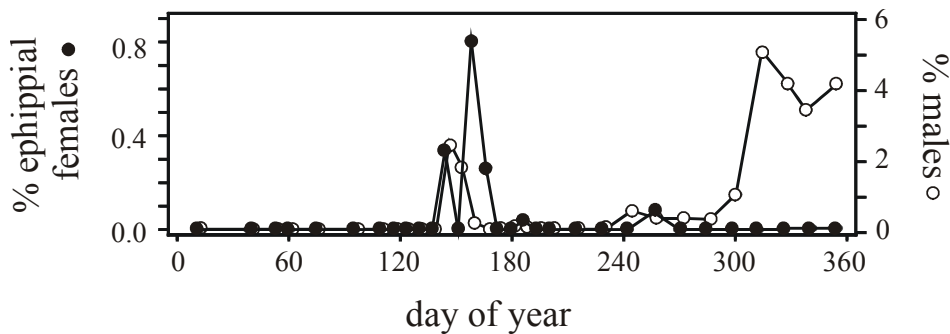

FIGURE 2.2 Seasonal proportion of sexual (ephippial) females (●) and males (○) of the *Daphnia* population in Lake Constance.

Diversity and seasonal pattern of the six genotype classes

The six genotype classes differed in respect to a) their clonal diversity, b) changes in clonal diversity and clonal composition during the season. MLG diversity (G_0) based on PGI and PGM were calculated for monthly-pooled samples. Overall we found 7 P_{hya}, 8 BP_{hya}, 10 F1, 12 F2, 13 BP_{gal} and 23 P_{gal} MLG's. G_0 was highest for P_{gal} (max. 8) and lowest for P_{hya} (max. 2) with intermediate diversities for hybrids and backcrosses. G_0 of F1 was close to the G_0 of P_{hya} whereas G_0 of F2 was close to the G_0 of P_{gal} (Fig. 2.5). G_0 of P_{gal} and the F2 hybrid showed a distinct seasonal pattern (Fig. 2.6). G_0 was highest in May and June and decreased towards the end of the season. G_0 of the other genotype classes exhibited no clear seasonal pattern. Pairwise F_{st} (θ) values calculated for the 'subpopulations' of the season S1, S2 and S3 (Tab. 2.2) revealed no significant differences between seasons for P_{hya}, F2 and BP_{gal}, while BP_{hya} populations differed significantly between S1 vs. S2 and S2 vs. S3, and F1 hybrid populations between S1 and S2, P_{gal}, populations from all three seasons were significantly different from each other.
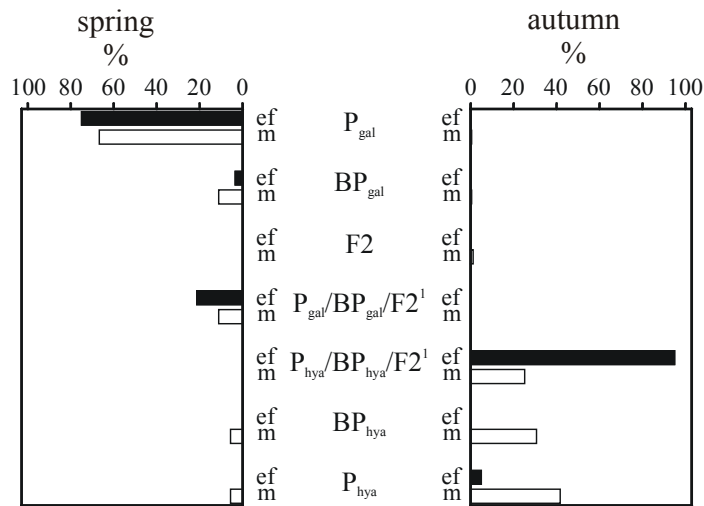
FIGURE 2.3 Proportion of genotype classes among sexual (ephippial) females (ef = black) and males (m = white) in spring (right panel) and autumn (left panel). Deviation between spring (January to July) and autumn (August to December) were done on the basis of the seasonal proportion of sexual individuals (Fig. 2.2), which lacking in summer. Abbreviations of genotype classes as in Fig. 1. [1] = Because of technical problems we failed to screen sexual (ephippial) females and males for one of the species specific marker (AO) for some of the samples. But the AAT locus indicated that all of these ephippial females and males in spring belong to the genotype classes P_{gal}, BP_{gal} or F2 hybrid, whereas in autumn to P_{hya}, BP_{hya}, F1 or F2 hybrid.

Evidence for selection against introgressed individuals

Within the polymorphic locus PGI allele "2" was only present in one parental population, but not in the other (Table 2.3). The frequency of this allele decreased from P_{gal} (0.3) to BP_{gal} (0.2) to F2 (0.1) to F1 (0.015) to BP_{hya} (0.004) to P_{hya} (0). At the PGM locus two alleles showed a similar pattern (Table 2.3). Alleles "2" and "3" decreased from a frequency of 0.2/0.3 in P_{gal} to 0.006/0.017 in P_{hya}. Moreover, the monthly pooled frequencies of allele "3" indicated a seasonal shift within P_{gal}, PB_{gal} and BP_{hya} (Fig. 2.7). In P_{gal} and PB_{gal} the PGM "3" allele was most frequent in late spring, i.e. after hatching, and its frequency decreased towards the end of the season. PGM "3" allele carrying individuals within the BP_{hya} class did not contribute to the overwintering population of BP_{hya}, reached their maximum relative frequency during May, and declined thereafter in relative abundance (Fig. 2.7).

Discussion

Our study revealed strong differences between the parental species regarding their seasonal dynamics, genetic architecture and diversity, and their sexual reproductive

behaviour. Within all variables studied, F1 and F2 hybrids showed an intermediate pattern, whereas proposed backcross hybrids were more similar to their respective parentals. These differences in phenotype as well as significant differences in pairwise Fst values suggest that gene flow seems to be relative low in the Lake Constance hybrid system.

Frequency of hybridization and introgression

Our results are in agreement with other studies on *Daphnia*, which show that hybrids live in syntopy with one or both parental species (Schwenk & Spaak, 1995; Spaak, 1996; Giessler, 1997; Spaak & Hoekstra, 1997; Giessler, 1997; Reid et al., 2000). Furthermore, our results show that hybrids reproduced sexually during the same seasonal periods as their parental species, i.e. backcrossing and introgression is
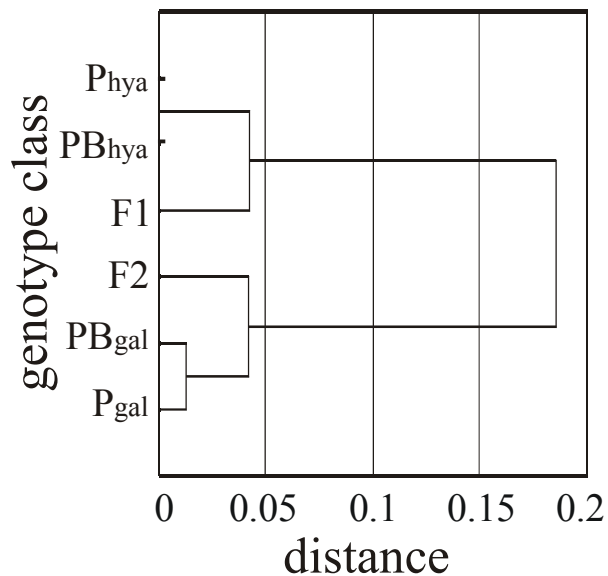

FIGURE 2.4 UPGMA clustering of the genetic distances (all samples pooled) of the six genotype classes, calculated for the two polymorphic loci PGI and PGM. Abbreviations of genotype classes as in Fig. 1.

possible. Using two species-specific markers we identified six genotype groups, including BP_{hya} and BP_{gal}, which possibly represent back-crossed individuals of parental species. Although daphnids belonging to genotype groups BP_{hya} and BP_{gal} can also result from sexual reproduction between hybrids, genetic distance analysis and diversity pattern suggest that this taxa result from backcrossing between hybrids and parentals. F_{ST} values and diversity patterns – both calculated without using the species specific markers – suggest that BP_{hya} and BP_{gal} are more closely related to the respective parental species than to the F1 and F2 hybrids, and hence, represent backcrosses. This supports recent studies on *Daphnia* hybrids, which suggests that backcrossing and introgressive hybridization might be much more common than previously expected (Giessler, 1987; Taylor & Hebert, 1993; Streit et al., 1994; Giessler, 1997; Giessler et al., 1999; Spaak et al., 2000).
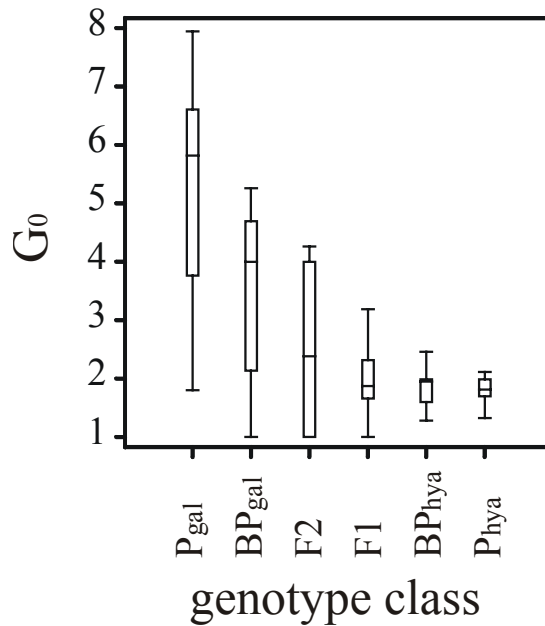
FIGURE 2.5 Genetic diversity (G_0) calculated with the two polymorphic loci PGI and PGM for the six genotype classes. Shown are the mean, the 50% (box) and the 95% (bar) confidence limits of monthly pooled samples over the year. Abbreviations of genotype classes as in Fig. 1.

However, significant differences in F_{st} values between parentals and hybrids suggest that the daphnid population does not represent one large and homogenous hybrid swarm despite parentals do co-occur in Lake Constance since more than 4 decades (Straile & Geller 1998). Consequently gene flow seems to be rare. Furthermore, we found rather low hybrid frequency in Lake Constance compared to other lakes (Tab. 2.4). Hybrids reached their peak abundance between the maximum abundance of the parental species. This supports the 'temporary hybrid superiority model' (Spaak & Hoekstra, 1995),

which assumes that during certain periods in the year, due to environmental conditions (predation, food), hybrids will have higher fitness compared to the parental species. However, "temporal hybrid superiority" in Lake Constance seems not to be as strong as in e.g., Lakes Greifen, Tjeukemeer and Maarsseveen (Tab. 2.4), where hybrids reached maximum frequencies up to 60-100 %. as the hybrid ratio in Lake Constance never exceeded 50 % and was considerably lower throughout most of the season (Fig. 2.1b).

TABLE 2.1 F_{st} values calculated for two polymorphic loci (PGI, PGM) between all genotype classes over all samples. Abbreviations of genotype classes as in Fig. 1.

	P_{hya}	BP_{hya}	F1	F2	BP_{gal}
BP_{hya}	<-0.001				
F1	0.045*	0.038*			
F2	0.127*	0.096*	0.066*		
BP_{gal}	0.246*	0.193*	0.159*	0.021	
P_{gal}	0.245*	0.202*	0.172*	0.060*	0.012

* significant after Bonf. ($p<0.003$); 1500 permutations.

A possible selective advantage of parentals – at least of one parental species, *D. hyalina* – is suggested by the seasonal occurrence of possibly introgressed alleles (Tab. 2.3). We found evidence for selection against genotypes carrying proposed introgressed alleles at the PGI and PGM loci (Fig. 2.7). Earlier studies suggest that PGI and PGM loci might be important markers to characterise the genetic structure of *Daphnia* populations: Spaak, 1996), for example found also evidence for backcrossing suggesting introgression at the PGI loci in a *Daphnia galeata-cucullata* hybrid system. Additionally, Weider et al., 1997), who investigated the long-term genetic changes in the *Daphnia* population from Lake Constance using ephippial hatchlings from datable cores, suggest that significant
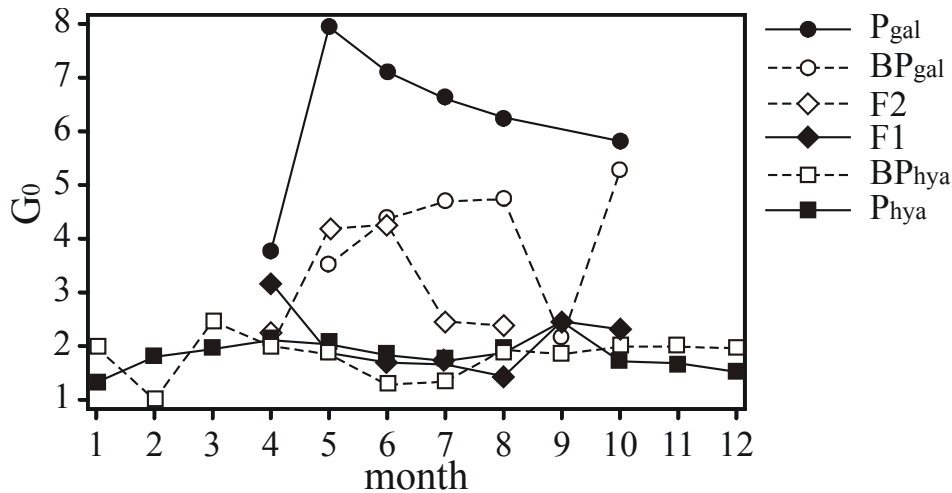

FIGURE 2.6 Seasonal changes in the genetic diversity among the six genotype classes calculated as G_0 for monthly pooled samples. Abbreviations of genotype classes as in Fig. 1.

shifts have occurred at the PGM and PGI loci within the last 40 years. Unfortunately experiments concerning the fitness of daphnid backcrosses compared to parentals are not conducted up to now, and those experiments are also rare for other species (Arnold, 1997; Schluter, 2000). Fitness differences of backcross hybrids, F2 hybrids and introgressed individuals to their parental species have been examined for *Drosophila* species (MacRae & Anderson, 1988; Kilpatrick & Rand, 1995; Hutter & Rand, 1995), *Helicornius* butterflies (McMillan et al., 1997), *Tigriopus* copepods (Edmands, 1999) *Geospiza* Darwin finches (Grant & Grant, 1992) and *Gasterosteus* sticklebacks (Hatfield & Schluter, 1999). These studies show that the relative fitness of backcrosses or introgressed individuals depends on the specific environmental (experimental) conditions (Arnold, 1997; Schluter, 2000). These are likely to change during the seasonal course, but also during the oligotrophication process of Lake Constance.

Seasonal pattern of recruitment and clonal selection in parental taxa

The parental species showed strong differences in their seasonal occurrence and genetic architecture (Fig. 2.1a + b and 2.6, Table 2.2). These differences were not specific to the year 2000 as similar patterns were observed in 1999 and 2001 (own unpubl. data). In contrast to other investigations (Wolf, 1987; Weider & Stich, 1992; Spaak, 1996) *D. galeata* was not found in late fall and winter. Hence, our allozyme study supports morphological data (Straile & Geller, 1998) indicating different overwintering strategies for the two parental *Daphnia* species, i.e., *D. galeata* seemed to rely more strongly on resting stages than *D. hyalina* to get through severe winter conditions, while for *D. hyalina* overwintering in the plankton is more important (Fig. 2.8). These differences in the importance of resting stages for the seasonal cycle are supported by our data on the abundance of sexual individuals and additionally by the increase in G_0 during the hatching period within the *D. galeata* population, but not within *D. hyalina*. This increase in G_0 and the higher frequency of sexual reproduction indicates that hatching from resting eggs was more frequent for *D. galeata*. Furthermore, there was no significant difference in F_{st} values between the spring and autumn population of *D. hyalina* suggesting that hatching from ephippia did not alter the *D. hyalina* clonal composition of individuals that were successful to overwinter. Hence, the life cycle of *D. galeata* resembles to some extant the life cycle of pond daphnids, which typically show a massive recruitment from resting eggs during spring, resulting into a high diversity during this time period followed by clonal selection and a decline in diversity (Lynch, 1984; Weider, 1985; Wolf, 1988). At least partially these changes in population structure (Fig. 2.6, Table 2.2) might result from selection against introgressed genes (Fig. 2.7), as pointed out above. However, compared to pond daphnids (Korpelainen, 1984; Korpelainen, 1986; Innes, 1997), the frequency of sexual reproduction was low in Lake Constance even for *D. galeata*.

Consequences of the differences in the timing of sexual reproduction for seasonal clonal selection and diversity

Differences in clonal selection and diversity between the two parental species can be at least partially explained by the differences in the timing and frequency of sexual reproduction, which is both under genetical and maternal control (Larsson, 1991; Innes & Singleton, 1994; Alekseev & Lampert, 2001). Because of species differences in sexual reproduction, the seasonal origin of the spring cohorts of *Daphnia* populations differed between *D. hyalina* and *D. galeata.* As *D. galeata* did not overwinter in the plankton and did not produce resting eggs in autumn, the spring cohort stems from hatching of resting eggs produced after previous spring blooms, i.e. in May and June of previous years. Furthermore, this suggest that *D. galeata* clones which were successful in summer did not contribute to the next spring generation either because they failed to produce resting eggs and/or failed to survive winter conditions. Hence, we expect differences in genetic architecture between seasons for *D. galeata* populations. In contrast, the spring cohort of *D.*

hyalina stems from the clones that either successfully produced resting eggs in autumn or successfully got through the winter in the plankton. *D. hyalina* clones especially adapted for rapid growth during benign spring conditions would only contribute to the next spring generation, if they were also successful during summer. As *D. hyalina* performs diurnal vertical migration (DVM) to avoid predation by visually oriented predators during summer (Stich & Lampert, 1981; Stich, 1989; Weider & Stich, 1992), *D. hyalina* clones have to cope with a low food and low temperature environment in their day-time refuge during summer. That is, they experience in summer during day time conditions which at least approach winter conditions. Hence, DVM behaviour of *D. hyalina* might contribute to the lacking difference in genetic architecture between summer and winter populations of *D. hyalina*.

TABLE 2.2 Population genetic characterization between seasons (S) within each genotype class. The left side shows the number of individuals (N), the number of Multi-Locus-Genotypes (MLG's) and the F_{is} values for each season (S). The right side shows the pairwise F_{st} values calculated for two polymorphic loci (PGI, PGM) between three seasons. Abbreviations of genotype classes as in Fig. 1.

class	S	N	MLG's	F_{is}		S	F_{st}	
P_{hya}	1	110	7	0.004		1 vs. 2	-0.001	
	2	72	4	-0.125		2 vs. 3	-0.003	
	3	306	5	-0.058		1 vs. 3	-0.001	
BP_{hya}	1	40	4	-0.152		1 vs. 2	0.045	*
	2	60	4	0.125		2 vs. 3	0.043	*
	3	145	7	-0.092		1 vs. 3	-0.006	
F1	1	13	5	-0.220		1 vs. 2	0.056	*
	2	151	7	-0.067		2 vs. 3	-0.001	
	3	56	5	-0.095		1 vs. 3	0.021	
F2	1	10	4	0.250		1 vs. 2	0.010	
	2	39	11	-0.028		2 vs. 3	0.051	
	3	16	6	-0.134		1 vs. 3	-0.010	
BP_{gal}	1	5	5	0.200		1 vs. 2	-0.005	
	2	65	11	-0.194	*	2 vs. 3	<-0.001	
	3	37	10	-0.193		1 vs. 3	-0.005	
P_{gal}	1	57	10	-0.453	*	1 vs. 2	0.031	*
	2	332	21	-0.199	*	2 vs. 3	0.012	*
	3	97	13	-0.233	*	1 vs. 3	0.030	*

* = Significant different after Bonf. correction (p=0.008 for F_{is} and p=0.017 for F_{st}); 1000 permutations; S = seasons: 1 = month 1-4; 2 = month 5-7; 3 = month 8-12.
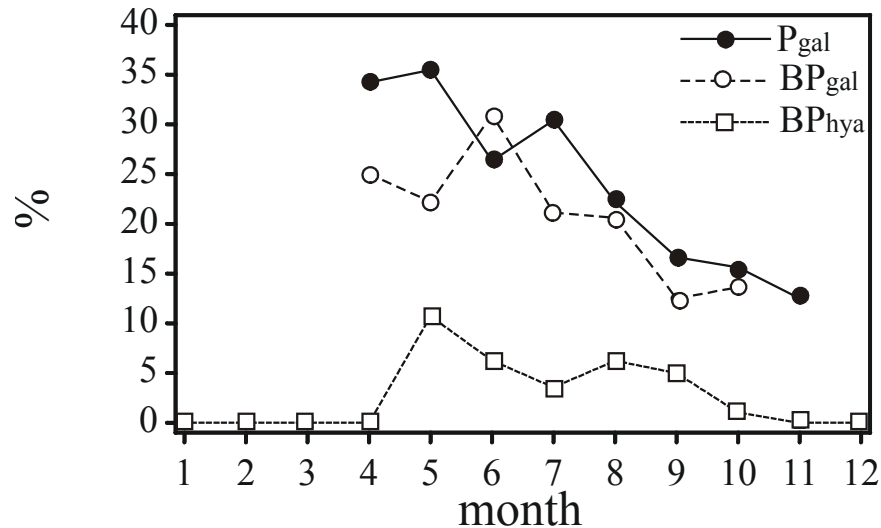
FIGURE 2.7 Seasonal frequencies of the PGM allele "3" for three genotype classes P_{gal}, BP_{gal} and BP_{hya} for monthly pooled samples. Abbreviations of genotype classes as in Fig. 1.

The timing of sexual reproduction hence should strongly contribute to different microevolutionary trajectories of the two parental *Daphnia* populations on an interannual scale. *D. galeata* clones should be selected which are able to cope with the benign spring conditions, i.e. high food concentrations and low predation pressure, whereas *D. hyalina* clones should be selected which can best cope with summer conditions, i.e. higher predation pressure and lower food concentrations at least in their day- time hypolimnetic refuge. This line of argument is further supported by the decrease of allele frequency "3" at PGM during summer, which is most prominent in *D. galeata*. The frequency of this allele within various genotype groups decreases during summer (Fig. 2.7). As hatching from ephippia was only observed during spring (own unpubl. data), this suggests that clonal selection might have decreased the relative contribution of 'pure' *D. galeata* and backcrosses with a high amount of '*galeata*'-alleles during the growing season. In contrast 'pure' *D. hyalina* and backcrosses with a high amount of '*hyalina*'-alleles seemed to be better adapted to the conditions after the spring bloom. This is supported by life-history experiments, where *D. hyalina* grew and reproduced better at low food conditions than *D. galeata* (Stich & Lampert, 1984). As there seems to be a general trade-off in daphnids between abilities to exploit high vs. low resource richness (Dudycha & Tessier, 1999; Tessier et al., 2000), adaptation of daphnids to spring conditions, i.e. to exploit high resource levels, might have even detrimental effects for summer conditions, i.e. to exploit efficiently low resource levels. It is worth noting that F1 and F2 hybrids seem to have rather intermediate success during both, spring and summer conditions. In evolutionary

terms, adaptation to spring versus summer conditions might represent two adaptive peaks (Schluter, 2000). If this is the case, disruptive ecological selection might also contribute to the rather low hybrid ratio observed in Lake Constance. Hence, the timing of sexual reproduction in combination with increasingly unfavourable conditions in summer in Lake Constance with ongoing oligotrophication, might contribute or even promote different microevolutionary pathways for the two parental *Daphnia* species.

TABLE 2.3 Allele frequencies of the two polymorphic loci PGI and PGM over all samples. Abbreviations of genotype classes as in Fig. 1.

locus	allele	P_{hya}	BP_{hya}	F1	F2	BP_{gal}	P_{gal}
PGI	1	1.000	0.995	0.984	0.900	0.789	0.686
	2	-	**0.004**	**0.015**	**0.100**	**0.210**	**0.313**
PGM	1	-	-	-	0.007	-	0.001
	2	0.006	0.012	0.011	0.084	0.186	0.171
	3	**0.017**	**0.026**	**0.129**	**0.184**	**0.214**	**0.293**
	4	0.852	0.840	0.850	0.669	0.570	0.517
	5	0.120	0.120	0.006	0.053	0.028	0.016
	6	0.003	-	0.002	-	-	-

Frequencies of proposed introgressed alleles are in bold.

Consequences of the differences in the timing of sexual reproduction for long-term clonal selection and persistence

As many other European lakes, Lake Constance experienced strong changes in trophic status during the last century resulting from anthopogenic eutrophication and oligotrophication (Güde et al., 1998). Currently, nutrient levels in the lake decrease strongly from year to year with subsequent changes of phytoplankton biomass and production and now approach levels typical for the time period when *D. galeata* invaded Lake Constance (Güde et al., 1998). This immediately poses the question on how long *D. galeata* will be able to persist in the lake given a further reduction of nutrient concentrations. So far, changes in phytoplankton biovolume and production have been only observed during summer and autumn because only then were nutrients strongly limiting (Gaedke, 1998). In contrast, spring phytoplankton biovolume and production was not affected by the oligotrophication of the lake (Gaedke, 1998) as nutrients are still available during this time of the year. That is, so far, the resting egg production of *D. galeata* and of hybrids after the spring bloom was not likely to be influenced by oligotrophication and there should be no influence of oligotrophication on *D. galeata* resting egg production until phytoplankton production during the spring bloom is reduced by oligotrophication. In other words, this suggests that resting egg production early in the season seems to be a "short –cut" of the seasonal dynamics which should allow *D. galeata* and hybrids to persist in the lake despite of unfavourable conditions during late summer and autumn. The draw-back of this "short-

cut", however, is, that *D. galeata* will be less able to adapt to decreasing food concentrations during summer as clones which are successful in summer do not produce resting eggs in autumn and also fail to overwinter and hence do not contribute to the next spring generation. On first sight, these findings are in conflict with recent suggestions that *D. galeata* evolved the capability to cope better with cyanobacteria in Lake Constance during summer (Hairston et al., 1999; Hairston et al., 2001). However, we cannot exclude that *D. galeata* reproduced sexually also in autumn in previous years.

TABEL **2.4** Hybrid frequencies (AAT locus) of the *Daphnia galeata-hyalina-cucullata* complex from different European lakes.

Lake	taxa	hybrid frequency	sampled	Ref.
Greifensee	hya, gal	h x g: 70-90%	Feb – Jul 1998	1
Tjeukemeer	gal, cuc	c x g: 10-70%	Apr 1989 – Apr 1992	2
Lake Maarsseveen	(hya,) gal	h x g: 50-100%	May 1989 – Aug 1991	3
Kellersee	hya, gal, cuc	c x g: 0-20% h x g: 10-60%	May 1984 – Feb 1985	4[1]
Schöhsee	hya, gal, cuc	h x g: 40-90%	May 1984 – Feb 1985	4[1]
Lake Constance	hya, gal	h x g: 10-50%	Nov 1989 – Oct 1990	5
Lake Constance	hya, gal	h x g: 10-40%	Jan – Dec 2000	this study

hya=h = *D. hyalina*, gal=g = *D. galeata*, cuc=c = *D. cucullata*, taxa in parenthesis means taxa not found.

[1]= Wolf, 1987 sampled also several other lakes, but for this comparison we used only lakes which were sampled during at least one year.

1.= Spaak et al., 2000; 2.= Spaak, 1996; 3.= Spaak & Hoekstra, 1993; 4.=Wolf, 1987; 5.= Weider & Stich, 1992.

Given the observed timing of sexual reproduction we should expect that the duration of the seasonal occurrence of *D. galeata* will decrease with oligotrophication in Lake Constance. This is supported by morphological data, which show that during oligotrophication, the time of occurrence of *D. galeata* in fact declined (Straile & Geller, 1998). Additionally, allozyme data from 1989/90 using only AAT as species specific marker revealed a frequency of *D. galeata* of about 10 % during winter (Weider & Stich, 1992). However, we found no '*galeata* genotypes' in the overwintering *Daphnia* population in 1999/2000, which suggests that overwintering of these genotypes was considerably lower than in the winter population of 1989/90 (Weider & Stich, 1992). Hence, the seasonal window of occurrence of *D. galeata* already seems to shrink in Lake Constance. However, persistence of this species is likely as long as population growth and subsequent resting egg production following the spring bloom of phytoplankton is possible. The timing of sexual reproduction events hence seems to have a major impact on seasonal clonal selection and microevolutionary dynamics of *Daphnia* populations. In the case of *D. galeata* the strategy of resting egg production in early summer may represent an ecological
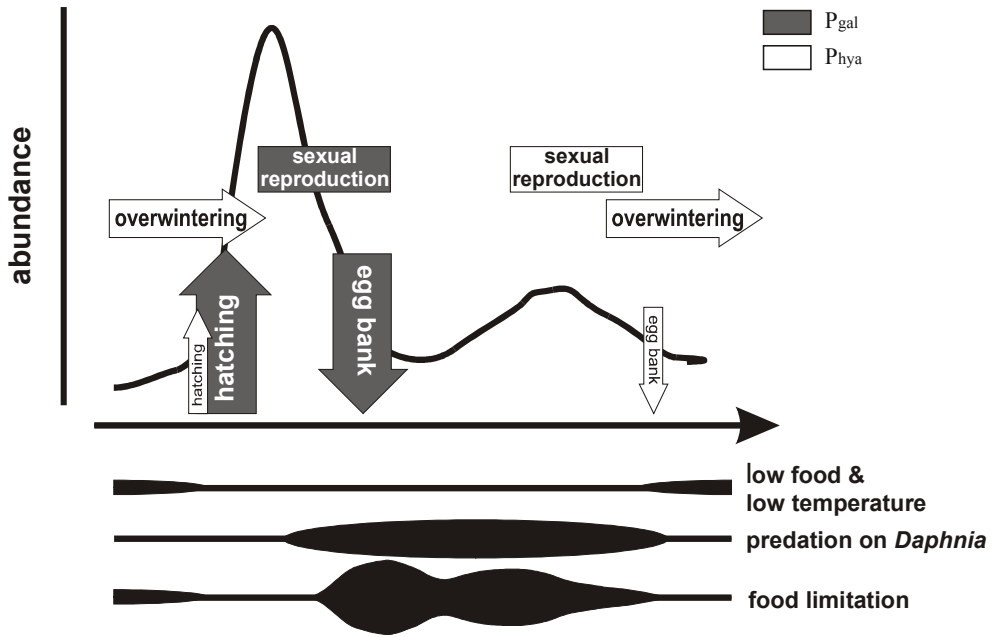
FIGURE 2.8 Model of the seasonal differences in the importance of sexual reproduction for the two parental species *D. hyalina* (white) and *D. galeata* (grey). Black line represent idealized seasonal *Daphnia* abundance. The horizontal diagrams at the bottom indicate the periods when major environmental constrains are important (adapted from Sommer et al., 1986).

short-cut which might allow persistence of this species also in a oligotrophic lake; however, it also presents to some extent an evolutionary "one-way street" hindering an adaptation to reoligotrophied conditions.

Chapter 3

VARIATION IN ALLOCATION TO SEXUAL REPRODUCTION WITHIN THE CYCLICALLY PARTHENOGENETIC *DAPHNIA HYALINA-GALEATA* HYBRID COMPLEX – EVIDENCE FROM MESOCOSM AND LAKE POPULATIONS

together with D. Straile

Seasonal dynamics in allocation to and timing of sexual reproduction were studied over three years in a *Daphnia hyalina-galeata* hybrid population of large and deep Lake Constance. These results were compared to a multispecies mesocosm experiment carried out under natural conditions. In all three years we observed two distinct periods of sexual activity. In early summer, no D. hyalina sexual females were found and 90 % of the sexual females and males were D. galeata. In autumn, however, no *D. galeata* sexual females were found and more than 60 % of the sexual females and males were D. hyalina. The D. hyalina-galeata hybrid were sexual during both periods. Despite these seasonal differences all three taxa produced ephippia and males during an enclosure experiment conducted in July 2001, i.e., a time when no sexual activity was observed in the lake. The parentals showed in the lake as well as in the enclosures similar allocation to sexual females and males. In contrast, the hybrid showed a much higher allocation to sexual females during the enclosure experiment. In the field, *D. galeata* contributed the most to ephippia production. *D. hyalina* ephippia production was relative sparse, and the hybrid showed, at least in the lake population, an intermediate allocation. Our findings indicate a species x environment interaction for the induction of and allocation to sexual activity. This will be discussed in the light of different overwintering strategies and their consequences for the maintenance of genetic variation within populations.

Introduction

The sexual reproduction of Daphnia resulting in the production of long-lived resting-eggs called ephippia, has recently provided new insights into ecological and evolutionary theories (e.g. Hairston, 1998). In population ecology theory, for example, the switch to resting-egg production is dynamically important as it prevents population explosion, and thus turning-off predator-prey cycles (McCauley et al., 1999; Ciros-Perez et al., 2002). The "storage effect", i.e. the effect of individuals hatching from resting eggs of different lengths, can allow for the long-term coexistence of competitors (Hairston et al., 1996; Caceres, 1997; Chesson, 2000), hence, maintain genetic diversity within populations (Ellner & Hairston, 1994). Therefore, sexual reproduction, stretches the timescale of ecological dynamics and blurs its boundary with evolutionary dynamics (Hairston et al., 1999; Grover, 1999; Hairston et al., 2001).

Despite the important role of sexually produced resting-stages in ecological and evolutionary dynamics, sexual activity, i.e. the ecology of sexual reproduction, is not well understood in *Daphnia* populations. Here we present data from a three year lake survey and a mesocosm study of the sexual activity of a sympatric *Daphnia* hybrid population. In *Daphnia*, the sexual phase is restricted to a distinct window of time (Stross, 1987); during the rest of the year, parthenogenesis is the common mode of reproduction (Hebert, 1978). Normally a parthenogenetic *Daphnia* female produces female broods. However, under stressful conditions she can switch to the production of males and sexual eggs. Fertilization of sexual females results in the production of resting-eggs encapsulated in an ephippia. These sexually produced resting-stages sink to the sediment and, after a dormancy phase, new parthenogenetic females hatch to rebuild the next year's population. Because sexual reproduction results in the production of resting-stages, sexual activity was supposed to be more pronounced in temporary-pond *Daphnia* populations (Hebert, 1978). However, sexual reproduction is also important for permanent lake populations when conditions are unfavorable (Jankowski & Straile, submitted). The life-cycle of cyclical parthenogens involves a trade-off between parthenogenetic and sexual reproduction (Roff & Bradford, 2000). Delaying sexual reproduction allows parthenogenetic reproduction to increase the size of a clone and hence make a greater contribution to the pool of sexual propagules.

An early and higher investment in males has often been observed in lake and pond populations (e.g. Lynch, 1983). This could be interpreted as a strategy to have enough males for mating when conditions become unfavorable and females switch to sexual reproduction (Zaffagnini, 1987; Stross, 1987), since males need several days after being released from the brood chamber to mature (Yampolsky, 1992). This, however, assumes that the environmental cues inducing male production should be different from cues inducing the switch to sexual females.

Many laboratory studies have been conducted to invesigate the factors inducing sexual reproduction in *Daphnia*, but, up to now, the induction of sexual activity is not a

trivial task in this group (Lynch pers. com.), and in particular in lake dwelling daphnids (Spaak, 1995, pers. observ.). The switch to sexual activity appears to be related to deteriorating environmental conditions and is probably triggered by an increase in population density (Stross 1987). Whether high population density is a reliable indicator of future unbenign conditions or increasing mating success is still under discussion (Innes & Singleton, 2000; Innes et al., 2000). Other environmental factors such as food limitation (Kleiven et al., 1992), temperature (Stross, 1969b), photoperiod (Stross & Hill, 1965; Stross & Hill, 1968), fish exudates and alarm chemicals (Slusarczyk, 1999) and "other chemicals" (Zhang 2000 1243 1565) can also influence the switch from asexual to sexual reproduction. Usually a combination of different factors is necessary to induce sexual reproduction (Kleiven et al., 1992), but it seems that the combinations might not be fix. It was shown that temperature uncouples photoperiod and density sensitivity (Stross, 1987) at least in some clones. This suggests that the sum of unbenign conditions is important for the induction of sexual reproduction rather than one special combination of factors which is logical since environmental factors inducing sexual reproduction independently vary from year to year. The switch from the production of parthenogenetic eggs to resting eggs in *Daphnia* is influenced by a maternal food effect (LaMontagne & McCauley, 2001) and a maternal photoperiod effect (Alekseev & Lampert, 2001) and a subsequent model predicts two distinct periods of sexual reproduction, one in early summer and the other in autumn (Alekseev & Lampert, 2001). From these studies it becomes apparent that conditions stimulating the production of males and those inducing the switch to sexual females could be different (Stross, 1987; Kleiven et al., 1992), and that the cues for the switch could vary between species of *Daphnia* (Stross, 1987; Deng, 1997) and between populations and clones of the same species (Carvalho & Hughes, 1983; Larsson, 1991). For example, Arctic populations tend to be more responsive to variation in photoperiod than density compared to temperate populations (Ferrari & Hebert, 1982). In another study using several clones of *D. pulicaria*, significant interaction between genotype, photoperiod and food was found suggesting that clones react differently to the environments offered (Deng, 1996). The presence of this genetic variation suggests that studies of the cues for sexual expression using a single clone should be interpreted with caution (Innes & Singleton, 1994). The transferability of this laboratory results to natural populations is also questionable. For example, in the *D. galeata-cucullata* hybrid system from Lake Tjeukemeer, Spaak (1995) found significantly more sexual females and a significantly lower male:sexual female ratio in the field than in the laboratory.

Here we present a study of the *Daphnia hyalina-galeata* hybrid complex from Lake Constance with the intent to explore the taxa specific differences in the allocation to sexual females under natural conditions. One of the parental species (*D. galeata*) seems not to be able to overwinter as a pelagic, parthenogenetic population (Jankowski & Straile, submitted). This suggests that *D. galeata* is more dependent on sexual reproduction and subsequent resting-egg production than is *D. hyalina*. In 2000, we found two distinct periods of sexual activity, each dominated by one parental species, and we found species specific differences in allocation to sexual reproduction and in their expressed sex-ratio (Jankowski & Straile, submitted). The purpose of the present study was to verify the taxa specific differences in the switch from parthenogenetic to sexual reproduction observed in the preliminary study. For this we investigated the natural lake population an additional two years and conducted a mesocosm experiment. Because it is difficult to obtain information on sex allocation for individual genotypes of *Daphnia* under natural
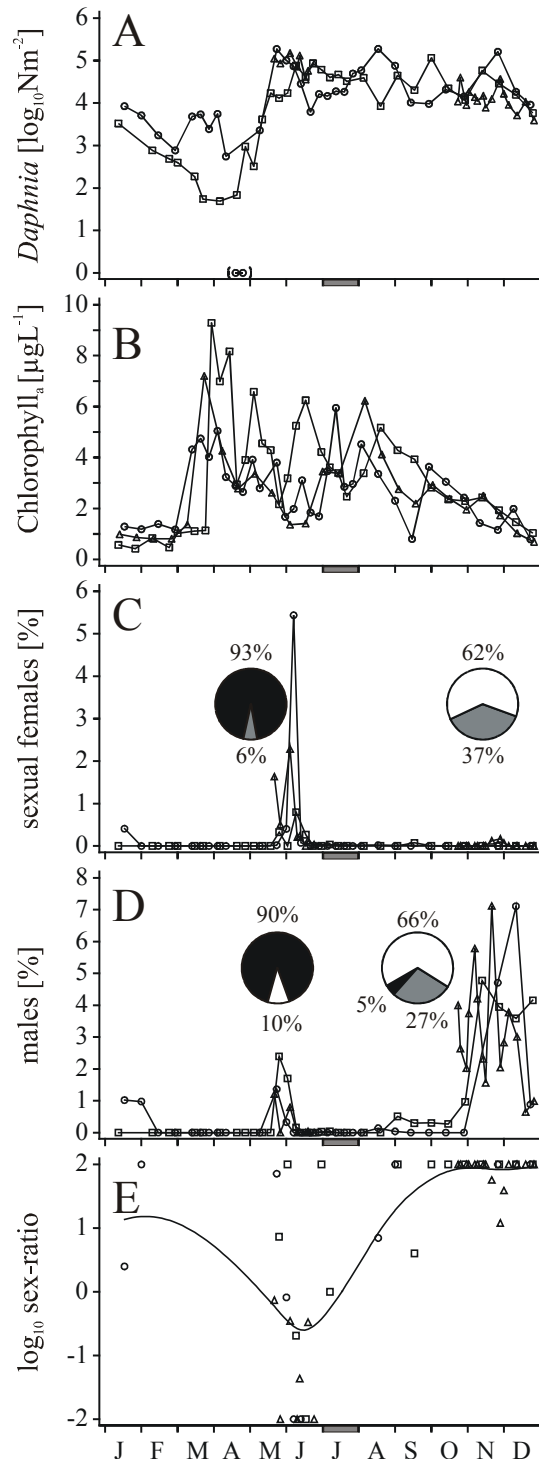
FIGURE 3.1 Seasonal course of *Daphnia* abundance (A), chlorophyll-a concentration (B), percent sexual females (C), percent males (D) and sex-ratio (E, $\log_{10}$ transformed male/sexual females) of Lake Constance for the three years investigated (triangle = 1999, square = 2000, circle = 2001). A sex-ratio of –2 indicates that no males were present, whereas +2 indicates the opposite. The two dates in parenthesis were removed because the sampling net were blocked by a *Conochilus* bloom and subsequently we found nearly no daphnids in the sample. Inlets in (C) and (D) represent the contribution of each taxa (white = *D. hyalina*, gray = hybrid, black = *D. galeata*) to sexual females (C) and males (D) in early summer (left) and autumn (right) over all three years as revealed by the allozyme electrophoresis (AAT as species specific marker). Grey boxes in all graphs indicate the time in 2001 of the enclosure experiment.

conditions (Innes, 1997), most studies have been conducted in the laboratory, often using only a single clone (Stross, 1969b; Stross, 1971; Hobaek & Larsson, 1990; Kleiven et al., 1992). Interactions between individuals and between taxa of a hybrid system may influence the allocation to sexual reproduction. For this reason we have attempted to mimic this in lake-enclosure experiments. Mesoscale enclosure experiments can be replicated and have proven useful for exploring a number of ecological and evolutionary questions (Hatfield & Schluter, 1999; Tessier et al., 2000; Urabe et al., 2002). The present experiment was designed to investigate variation in the allocation to sexual reproduction between the three taxa (*D. hyalina*, *D. galeata* and the hybrid), and to determine the relationship between the allocation to sexual females and that to males. The following specific questions were addressed: Is the pattern of sexual activity observed in the first year a characteristic pattern in all three years? Is it possible to induce sexual activity in mesocosm experiments even in a time when no sexual reproduction is occurring in the natural *Daphnia* population? Do taxa within a hybrid system differ in their relative investment in sexual reproduction and is there a relationship between the investment in males and sexual females?

Methods

Study site and organism

Lake Constance is located at the northern fridge of the Alps (47°39'N, 9°18'E). The mesotrophic large (500 km²) and deep (z_{max} = 254 m, z_{mean} = 100 m) lake has been intensively investigated during the last century (reviewed in Bäuerle & Gaedke, 1998). The trophic state changed dramatically during the last century: originally an oligotrophic lake, it underwent massive eutrophication in the 1950s, with a maximum at the end of the 1970s, followed by re-oligotrophication (Güde et al., 1998). During the eutrophication period *Daphnia galeata* invaded Lake Constance in the 1950s, where originally only *Daphnia hyalina* had occurred (Muckle 1976). The subsequent hybridization process resulted in a stable hybrid population, contributing 20 to 40 % to the total population (Weider & Stich, 1992, Jankowski & Straile, submitted). Lake Constance *Daphnia* differ in several
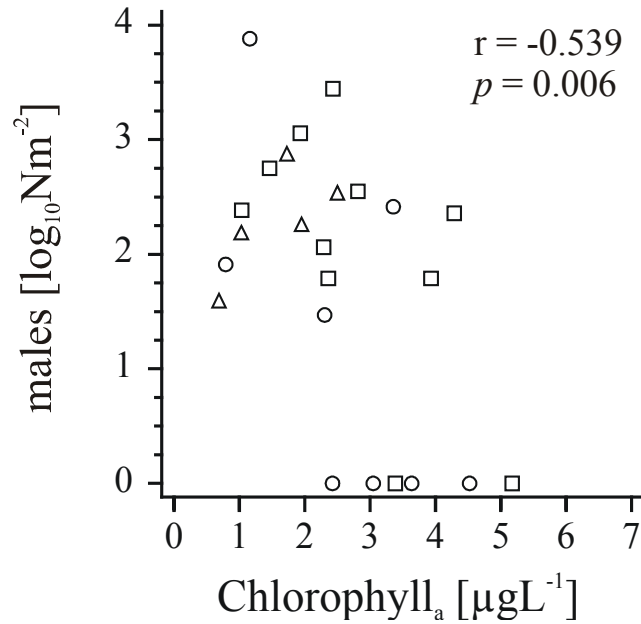
FIGURE 3.2 Association of male abundance and chlorophyll-a concentration in Lake Constance for autumn of all three years investigated (triangle = 1999, square = 2000, circle = 2001).

ecological aspects, for instance antipredator strategies (Stich 1981 Stich 1989): whereas *D. hyalina* and the hybrid perform pronounced diel vertical migrations (Weider & Stich, 1992), *D. galeata* is a non-migrating, but helmet producing species. Furthermore, it was suggested that *D. hyalina* overwintered as parthenogenetical females, whereas nowadays *D. galeata* depend on forming resting-stages for overwintering (Straile & Geller, 1998, Jankowski & Straile, submitted). Despite intensive studies on the Lake Constance *Daphnia* populations the knowledge on its sexual activity and subsequent resting-egg production is sparse.

Sexual reproduction *in-situ*

In order to get a better understanding of the sexual reproductive activity of the *Daphnia galeata-hyalina* hybrid complex in Lake Constance under natural conditions we investigated the population for three years. *Daphnia* were sampled weekly to bi-weekly in the central part of the fjord-like northwestern part of Lake Constance (Überlinger See) from May to June 1999 and from October 1999 to December 2001 with a Clark-Bampus sampler (mesh size 140 μm) by vertical hauls from 50 m depth. At each sampling date up to six vertical net hauls were taken, depending on *Daphnia* abundance, in order to get a minimum number of 100 adults (if possible egg carrying females), and as much sexual females and males as possible. The samples were pooled, stored cool and brought to the lab within 3
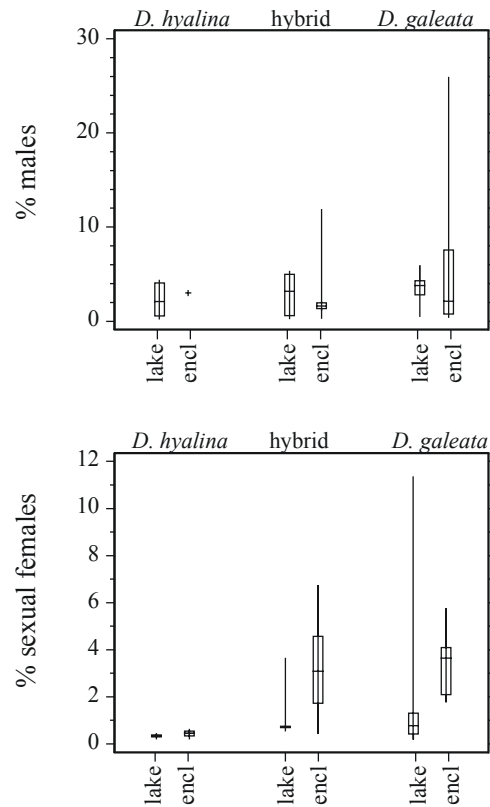
FIGURE 3.3 Comparison of male (upper graph) and sexual female (lower graph) percentages for the enclosure-experiment and the three year lake survey. Shown are the mean and minimum and maximum percentages.

hours until subsequent preparation. In addition, two extra net hauls were taken; one was frozen immediately after sampling in liquid nitrogen as a backup sample, the other was fixed in formol for subsequent determination of *Daphnia* abundance. Living individual adult females were picked randomly, and all males and ephippia carrying females were picked from the sample and kept frozen at -80°C for subsequent electrophoretic analysis. Chlorophyll-a data, as an estimation for food availability, were obtained by a Pauli-Sampler sampled at different depths from 1 to 20m. For subsequent analysis mean values of 1-20m were used.

Sexual reproduction in mesocosms

On the basis of the results gained by field investigations, we conducted a lake-mesocosm experiment to induce sexual reproduction during a time of rare or low sexual reproduction of daphnids in the lake. The experiment was performed in the Obere Güll Bay,

in the upper part of Lake Constance, in July 2001. We used 12 enclosures measuring 1 m diameter, which consisted of 4 m deep clear polyethylene tubes The tubes were closed at the bottom and had a total volume of about 3 m³. The enclosure were filled by pumping water from the Obere Güll Bay from a depth of 2 m through a 200 μm mesh. To mimic potential effects of variation in trophic state (= food availability), 8 enclosures were enriched by adding inorganic phosphorous (as NaH_2PO_4) to an end-concentration of 50 μgPL^{-1}, representing the trophic state of the late 1980s, after re-oligotrophication was initiated, or early 1970s, before eutrophication reached maximum, respectively (Güde et al., 1998). In each half of the fertilised and unfertilised enclosures a natural *Daphnia* population was added (lake-enclosures). Before lake zooplankton had been collected by vertical hauls (0 to 20 m) with a 200 μm mesh net (diameter: 1 m) which was filtered through 1 mm net before added to each enclosure to remove invertebrate predators (*Bythotrephes*, *Leptodora*) and fish larvae. The second half was enriched with a mixture of clones of *Daphnia hyalina*, *D. galeata* and the hybrid, which had been cultured for several generations in the laboratory. It was made sure that initial daphnid populations represented natural lake abundances (Straile & Geller, 1998). In total there were four treatments consisting of three replicates.
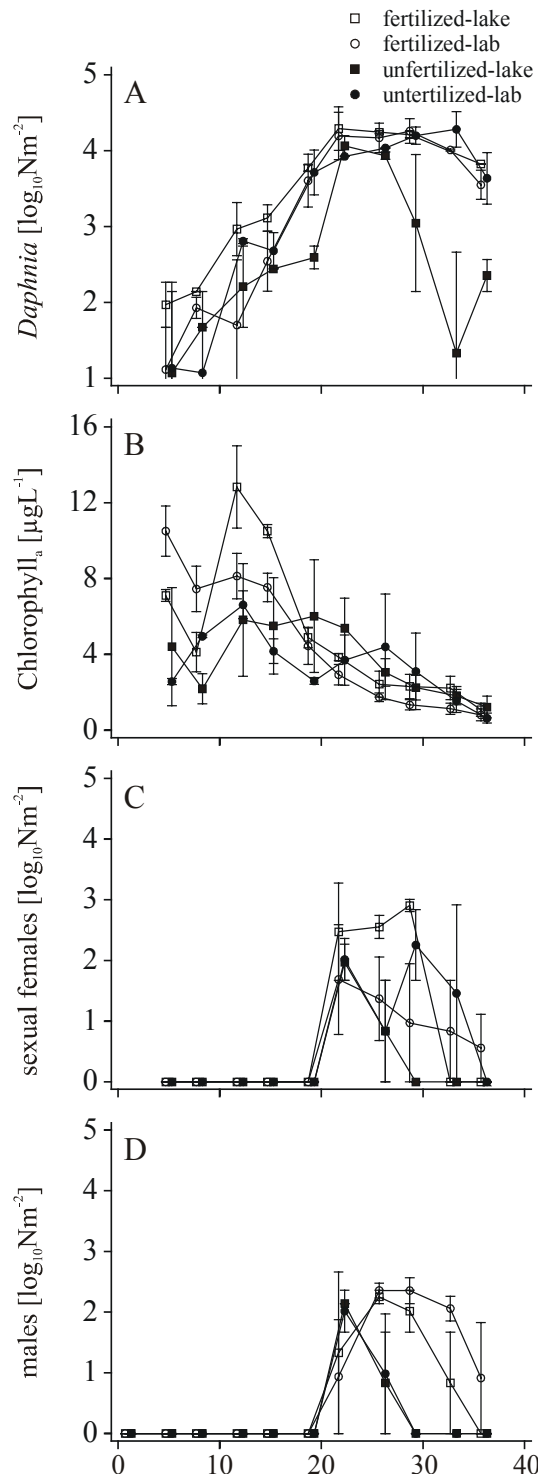
The enclosures were sampled each third to fourth days over a period of 36 days. At each sampling enclosures were first mixed, then daphnid abundance, number of sexual females and males and chlorophyll-a concentration were monitored. Daphnids were sampled by vertical net hauls from the bottom to the surface. Daphnid samples were transported to the laboratory within 2 h. Fifty parthenogenetical females as well as the total amount of sexual females and males were removed from each sample and stored at -80°C for subsequent electrophoretic analysis. The rest of the sample was preserved in formol and later on used for determining daphnid abundance. Chlorophyll-a samples were taken in 1 m depth.

Allozyme analysis

Both taxa composition and genetic variation of the lake as well as the enclosure populations were determined by allelic variation at allozyme loci, using cellulose acetate electrophoresis. Three enzyme loci were screened: aspartate amino transferase (AAT; E.C. 2.6.1.1.), phosphoglucomutase (PGM; E.C. 5.4.2.2.) and phosphoglucose isomerase (PGI; E.C. 5.3.1.9.). Protocols for electrophoresis were as described previously (Hebert & Beaton, 1993) except that electrophoresis was run at 300V. AAT is considered to be diagnostic for *D. galeata* and *D. hyalina* (Wolf & Mort, 1986). The two polymorphic loci PGI and PGM were used to differentiate between Multi-Locus-Genotypes (MLG's) within each genotype class.

Results

Sexual reproduction *in-situ*

Lake daphnid population showed the typically seasonal pattern (Fig. 3.1a, Straile & Geller, 1998) in all three years: It reached minimum abundance in March/April ($\sim 10^2$ individuals m^{-2}) followed by an exponential increase in April/May. After daphnids had reached peak abundance of about 10^4 to 10^5 individuals m^{-2} in June their abundance decreased towards the end of the season. Mean chlorophyll-a concentration of the upper 20 m never exceeded 10 µgL^{-1} during the three year survey and reached most of the time not more than 5 to 6 µgL^{-1} (Fig. 3.1b), except the spring bloom in March. With an increasing daphnid abundance in spring, chlorophyll-a concentration decreased to a summer minimum (clear-water phase). After an additional increase chlorophyll-a concentration decreased from August onwards. In winter food availability for daphnids remained low (< 2 µgChl-aL^{-1}). Within the three years investigated we found two distinct periods of sexually

reproductive activity (Fig. 3.1c), one in early summer shortly after daphnids had reached peak abundance and chlorophyll-a concentrations reached summer minimum, the other in autumn, when chlorophyll-a concentration declined to a winter minimum. Sexual females reached 1 to 6 % of the total population size in early summer and less than 0.2 % in autumn, i.e., ephippial production was 10 to 50 times higher in early summer. In contrast, male abundance was more intense in autumn than in early summer. Maximum male proportion reached 5 to 8 % in autumn, compared to 2 to 3 % in early summer. We found a 2 to 3 times higher abundance of males in autumn than in early summer. Male abundance in autumn showed a significant negative correlation with chlorophyll-a concentrations (r = 0.539, p = 0.006, Fig. 3.2). No males were present in autumn when chlorophyll-a concentrations exceeded 5 μgL^{-1}. In spring, male data were too sparse to reveal any significant association.

TABLE 3.1 Results of Repeated Measurement Analysis (SAS 1987) for the character Chlorophyll$_a$ concentration for the first 20 days during the enclosure experiment. First 20 days were taken because after *Daphnia* reached peak abundance Chlorophyll$_a$ concentration depends more on grazing pressure of daphnids than on fertilization.

Source	df	Type III error	MS	F	p
treatment	1	1.99	1.99	0.17	0.693
fertilization	1	118.43	118.43	10.23	0.019
day	4	83.92	20.98	5.69	0.002
day x treatment	4	49.76	12.44	3.38	0.025
day x fertilization	4	33.52	8.38	2.27	0.091

TABLE 3.2 Results of Repeated Measurement Analysis (SAS 1987) for the character male abundance ($\log_{10}$ transformed) for the period when males were present during the enclosure experiment (from day 22 onwards).

Source	df	Type III error	MS	F	p
treatment	1	0.635	0.635	0.78	0.410
fertilization	1	9.45	9.45	11.68	0.014
day	4	11.83	2.96	4.39	0.008
day x treatment	4	1.16	0.29	0.43	0.785
day x fertilization	4	12.99	3.25	4.82	0.005

TABLE 3.3 Results of Repeated Measurement Analysis (SAS 1987) for the lake-enclosures for the character sexual female abundance ($\log_{10}$ transformed) for the period when sexual females were present during the enclosure experiment (from day 22 onwards).

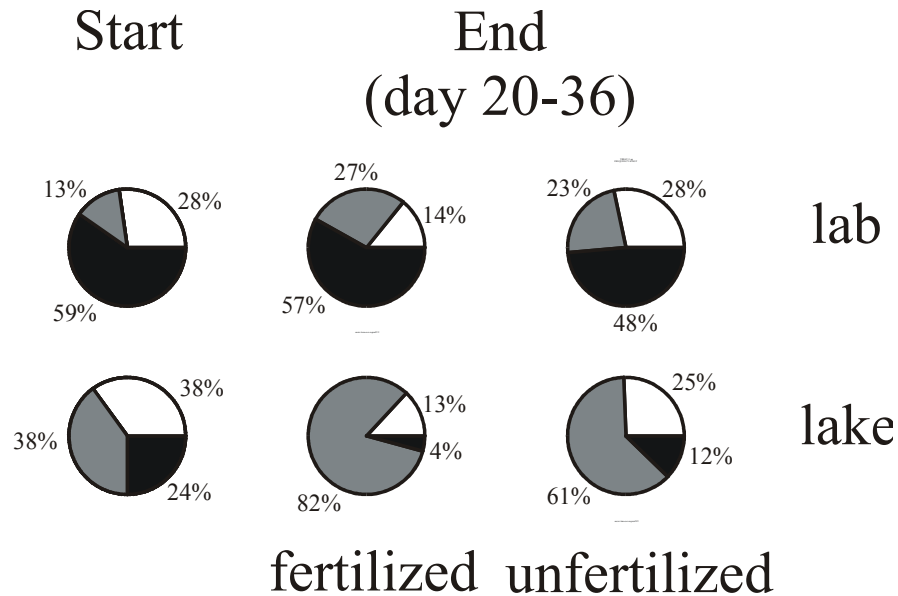| Source	df	Type III error	MS	F	p
fertilization	1	5.26	5.26	47.47	0.020
day	4	16.60	4.15	12.17	0.002
day x fertilization	4	6.39	1.60	4.68	0.031

Start   End
(day 20-36)

FIGURE 3.5 Taxa composition of *D. hyalina* (white), *D. galeata* (black) and the hybrid (grey) at the beginning (left) and the end (mean of day 20 to 36) during the enclosure-experiment in the for treatments (mean over replicates). Taxa determination based of allele composition at the AAT locus.

Seasonal differences in the abundance of sexual females and males resulted in pronounced seasonal differences in sex-ratio (ratio of males to sexual females). Whereas in early summer the sex-ratio never exceeded 0.3, we found in autumn a ratio of more than 10 (Fig. 3.1e).

The analysis of allozymes revealed strong taxa specific differences (Fig. 3.3). *Daphnia galeata* switched to sexual females only in early summer. They contributed up to 10 % to their population. Sexual females of *D. hyalina* were present only in autumn, which contributed to less than 1 %. The hybrid was found to produce sexual females during both periods (1 to 4 %). We observed 100 times more sexual females of *D. galeata* as of *D. hyalina*. *D. galeata* produced more males in early summer and *D. hyalina* more in autumn. For the three taxa male percentage reached less than 3 % of their respective population. During early summer males of *D. galeata* were 10-fold more abundant than males of *D. hyalina*, whereas in autumn the opposite abundance ratio was found. Hybrid males only occured in autumn. During the observation period *D. galeata* showed a much higher allocation to sexual females (males:females = 1:4) than *D. hyalina* (35:1) and the hybrid (4:1), respectively.

Sexual reproduction in mesocosms

Within all enclosures daphnids reached similar peak abundances within the experimental phase of 20 days (about 10^{-4} individuals m^{-2}, Fig. 3.4). Abundances did not differ significantly neither between fertilized and unfertilized nor between lake-clones and lab-clones. At the end of the experiment in all but one treatment, average abundance decreased slightly. The composition of *Daphnia hyalina*, *D. galeata* and the hybrid changed during the experiment. The lake-enclosures, originally composed of similar amounts of the three taxa were dominated by hybrids at the end of the experiment (Fig. 3.5). In contrast, the lab enclosures were dominated by *D. galeata* at the beginning as well as at the ending (Fig. 3.5). In the first half of the experiment chlorophyll-a concentrations were relatively high reaching average maximum levels between 6 to 13 μgL^{-1}, but decreased towards the end of the experiment to about 3 μgL^{-1} (Fig. 3.4). We found a significant positive effect of fertilizing on chlorophyll-a concentrations (Repeated Measurement Analysis, RMA: $F_{1,6}$ = 10.23, *p* = 0.02, Tab. 3.1). Although sexual reproduction was not observed in the lake population during the experiment, daphnids produced ephippia in the enclosures. Sexual reproductive activity was first observed around day 20, when daphnids reached a peak abundance together with a chlorophyll-a decline to around 3 to 6 μgL^{-1}. Mean maximum male abundances in the treatments was between 10^{2} to 10^{3} individuals m^{-2}, representing 1 to 4 % of the total daphnid abundance. The timing of male production was significantly influenced by fertilization (RMA: $F_{1,6}$ = 0.014, *p* < 0.01, Tab. 3.2), male abundance peaked later in the fertilized treatments than in the unfertilized treatments. Sexual females numbered around 10^{2} to 10^{3} individuals m^{-2}, representing 2 to 5 % of the total daphnid population. Within the lake-enclosures we found a significantly positive effect of fertilizing on the abundance of sexual females (RMA: $F_{1,2}$ = 47.47, *p* = 0.02, Tab. 3.3).

Male and sexual female percentages showed large species specific differences. In *D. galeata* males contributed on average about 4 % to the total population, but reached also up to 26 %, whereas sexual females contributed 2 to 6 %. In contrast, male and sexual female percentages in *D. hyalina* were less than 2 %. In the hybrid population males contributed on average less than 2 % to total population, with one exception where males reached 12 %. Sexual females contributed between 1 and 7 % to the hybrid population.

The regression analysis of the maximum sexual female abundance showed a strong association to the total maximum *Daphnia* abundance (r = 0.562, *p* = 0.003) but no correlation to the minimal chlorophyll-a concentration. *Daphnia* taxa differed significantly in the abundance of ephippial females (ANCOVA: $F_{2,5}$ = 6.46, *p* = 0.007, Tab. 3.4). These strong taxa specific differences and the association to taxa abundance were also found in maximum male abundance (ANCOVA: $F_{2,5}$ = 4.36, *p* = 0.027, Tab. 3.4). In contrast to maximum male abundance which was not related to the total maximum daphnid abundance achieved in distinct mesocoms (ANCOVA: $F_{1,5}$ = 0.6, *p* = 0.45, Tab. 3.4), maximum sexual

female abundance was related to the total daphnid abundance (ANCOVA: $F_{1,5} = 6.16$, $p = 0.022$, Tab. 3.4). Neither maximum sexual females abundance nor maximum male abundace was related to minimum chlorophyll-a concentration (Tab. 3.4). Taxa abundance was included into the models to adjust for differences in taxa abundance between mesocosms. After accounting for differences in taxa abundance, taxa was still a significant factor in the model. Hence, the taxa specific differences in ephippial females or male abundance are not due to overall abundance differences of the taxa, but due to species specific allocation into sexual reproduction and the sexes.

Overall, in the enclosures *D. galeata* and *D. hyalina* showed a similar sex ratio (1:2), while in the hybrid sexual females dominated (sex-ratio = 15:1). *D. galeata* produced 100 fold more males and 33 fold more sexual females than *D. hyalina*.

Discussion

We present, to our knowledge, for the first time data on sexual reproduction in *Daphnia* using multispecies mesocosms under natural conditions. Additionally, we compared our results with data of a three-year survey of the natural lake population. In the lake population we found two distinct periods of sexual activity. *Daphnia galeata* dominated the time frame of early summer and *D. hyalina* the second one in autumn, respectively, whereas the hybrid was sexually active during both periods. In the enclosures, all three taxa produced both ephippia and males, while no sexual activity was observed in the lake population during the same time. This indicates, together with the lake pattern observed taxa x environment interactions for the timing of sexual reproduction. Both total male and sexual female percentage reached in the mesocosms were similar to the lake population in early summer, but showed species specific differences in the allocation to and the timing of sexual reproduction as well we the sex-ratio.

As in our preliminary study in 2000 (Jankowski & Straile, submitted), we also found two distinct periods of sexual activity in 1999 and 2001, each dominated exclusively by one parental species (*D. galeata* in early spring and *D. hyalina* in autumn). This suggests it might be representative for Lake Constance daphnids. Comperable other studies on sympatric *Hyalodaphnia* populations (Wolf, 1987; Giessler, 1987; Spaak, 1995) and other lake *Daphnia* populations (Caceres 1998), respectively, indicating the pattern here described is not only characteristic for Lake Constance, but a common feature of permanent lake *Daphnia* populations. These species specific differences in the timing of sexual reproduction indicates that species react in a different way to varying environments, either between habitats (Stross, 1987; Deng, 1997) or between seasons.

The actual cues initiating sexual reproduction are complex, and the study presented here does not explain why daphnids switch from parthenogenetic to sexual reproduction. However, the cues discussed in the literature (e.g. temperature, photoperiod, daphnid density and food limitation) differ between early summer and autumn in Lake Constance.

FIGURE 3.6 Correlation of maximum abundance of sexual females (upper graphs) and maximum abundance of males (lower graphs) with taxa abundance (left graphs) and total *Daphnia* abundance (right graphs) in the enclosure experiment.

During ephippia production of *D. galeata*, i.e. in early summer, daphnid population density and food concentrations were higher, and day length exceeded that of autumn and early winter, the time period when *D. hyalina* sexually reproduces. As abundance of *D. galeata* in autumn/early winter was at or below the detection limit, except for 2001, we cannot exclude the possibility that *D. galeata* would have responded to the specific environmental conditions prevalent in autumn with sexual reproduction.

Environmental conditions in the mesocosm differ in several aspects from *in-situ* conditions, both, during the experimental period and during the periods of *in-situ* ephippial production. This suggests that specific combinations of cues might be responsible for the induction of sexual activity in Lake Constance daphnids. All *Daphnia* taxa produced ephippia in the mesocosms although the photoperiod differed from the photoperiod of *in-situ* sexual reproduction. This clearly demonstrates that a specific photoperiod is neither a necessary cue for Lake Constance daphnids to produce ephippia nor the reason for interspecific differences in the timing of ephippia production.

Chlorophyll concentrations in the mesocosm were comparable to chlorophyll concentrations during the early summer period of ephippial production, and higher than during the autumn period of sexual reproduction (Fig. 3.7). For vertically migrating *Daphnia hyalina* (Stich 1989) food concentrations during early summer might nevertheless be too high, at least in the upper strata night time refuge, to switch to sexual reproduction. However, *D. hyalina* produced ephippia in the mesocosms, which suggests that sexual activity might be inducable at higher chlorophyll concentrations, if other cues, e.g. crowding, are strong enough.

The density of daphnids was higher in the mesocosms than *in-situ* even when assuming that all daphnids concentrate in the upper 20 m of the water column. Mean *Daphnia* abundance, converted to densities per liter, amounted to about 5 individuals L^{-1} (maximum < 10 ind. L^{-1}) during the time of sexual activity in early summer and in autumn about 1 ind. L^{-1} (max. 2 ind. L^{-1}). In contrast, in the mesocosms daphnids reached mean abundances of 6 ind. L^{-1}, with average maximum values of more than 20 ind. L^{-1}. The crowding effect in the mesocosms is strengthened, in particular for *D. hyalina*, because due to *in-situ* diel vertical migration (DVM) of *D. hyalina* and hybrids (Weider & Stich 1992), which is blocked in the mesocosms. This high density in the mesocosms might induce sexual activity in *D. hyalina*, even when food concentrations are relatively high. On the other hand, *D. hyalina* may needs a stronger foot limitation to switch to sexual activity during conditions of low crowding. The fact that the maximum percentage of ephippial females increased with increasing maximum density in the mesocosms, supports the role of crowding conditions as a cue of sexual reproduction.

TABLE 3.4 Analysis of variance (SAS 1987) of the traits maximum sexual female abundance ($\log_{10}$ transformed) and maximum male abundance ($\log_{10}$ transformed) for the enclosure experiment.

Trait	Source	df	Type III error	F	*p*
Maximum sexual female abundance ($\log_{10}$)	taxa	2	5.60	6.46	0.007
	max. taxa abundance ($\log_{10}$)	1	4.40	10.15	0.005
	max. *Daphnia* abundance ($\log_{10}$)	1	2.66	6.15	0.022
	min. Chlorophyll$_a$	1	0.13	0.31	0.586
Maximum male abundance ($\log_{10}$)	taxa	2	6.53	4.36	0.027
	max. taxa abundance ($\log_{10}$)	1	2.55	3.40	0.08
	max. *Daphnia* abundance ($\log_{10}$)	1	0.45	0.6	0.45
	min. Chlorophyll$_a$	1	0.70	0.94	0.35

However, *in-situ D. hyalina* switched to sexual activity at lower food concentrations than *D. galeata*. This is concordant with the observation that *D. hyalina* might generally

better cope with low food conditions, e.g. in laboratory experiments *D. hyalina* showed higher growth rates at low food concentrations than *D. galeata* (Stich & Lampert, 1984; Geller, 1989). Furthermore, it is in accordance with a recent study, indicating that *D. hyalina* and *D. galeata* might have different overwintering strategies in Lake Constance (Jankowski & Straile, submitted). The non-overwintering *D. galeata* depend strongly on production of resting stages. Hence, their allocation to sexual reproduction is higher and consequently showed a higher genetic diversity than in *D. hyalina*. These differences between the overwintering *D. hyalina*, with low level of genetic diversity and seasonal genetic differentiation, and the non-overwintering *D. galeata*, with high genetic diversity and seasonal genetic differentiation, have different ecological and evolutionary consequences: The *D. hyalina* strategy favors generalist genotypes (those that can survive all seasons), while selecting against highly specialized clones. On the other hand, the *D. galeata* strategy, favors genotypes that frequently produce ephippia. As ephippia production showed strong genotype x environment interaction (Deng 1996) and environments changes temporally and varies between years, this strategy facilitate the selection of more specialized genotypes. These two strategies were previously interpreted as differences between temporary pond and permanent lake populations (Lynch, 1983). The consequences of these overwintering strategies might also explain the low allocation of *D. hyalina* to ephippia production. Lynch (1983) suggests that successful overwintering in the plankton will cause large fitness benefits as compared to resting egg production. Hence, clones exhibiting a high frequency of sexual reproduction might have been outcompeted in the Lake Constance *D. hyalina* population.

Abundance of ephippial females and of males in the water column is a function of production and loss processes. Both, production and losses differ between the sexes. Production of ephippia is more expensive than production of males. For example, Lynch (1983) estimated that in *D. pulex* the production of one ephippium, i.e. two eggs, is energetically equivalent to the production of 9 males. Evolutionary theory suggests that parents should show an approximately energetically equal investment in male and female progeny (Seger & Stubblefield, 2002). Hence we expect to find higher abundances of males compared to females in the water column. The expected difference in abundance between males and sexual females should be further increased by the maximum "life expectancy" of males and ephippial females. Which is for ephippial females, the duration of ephippium development until the ephippium is shed, i.e., a few days corresponding to one molt, and which is the life expectancy of males, i.e. up to a few weeks. This expectation is not met for the early summer sexual reproduction period suggesting that additional factors might contribute to the observed sex ratios. One of these factors is size specific predation. Due to the dark ephippia, ephippial females are highly conspicuous and should suffer from visually oriented predators selecting for large and conspicuous prey (Mellors 1975). In contrast, small males might be more attractive to gape-limited predators, i.e., juvenile fish and invertebrate predators. The analysis of long-term data sets in Lake Constance suggests that
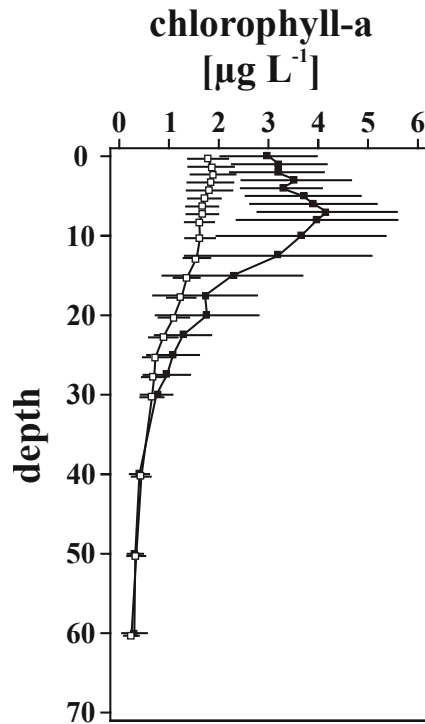
chlorophyll-a [μg L⁻¹]

FIGURE 3.7 Vertical profile of chlorophyll-a concentrations during sexual activity of *D. galeata* (early summer, black squares) and *D. hyalina* (autumn/early winter, white squares) pooled over 1999 to 2001.

during early summer, predation by invertebrate predators, e.g., copepods, *Leptodora kindtii* and *Bythotrephes longimanus*, and juvenile fish is more important than predation by non-gape-limited predators (Straile, unpublished data). Higher mortality of males hence might contribute to the excess of ephippial females observed during early spring in *D. galeata*. Consequently in the predator-free mesocosm the sex ratio of *D. galeata* was shifted to males. However, contrary to our expectations the sex ratio of hybrids was female biased in the mesocosm experiment.

In conclusion, our study revealed taxa specific differences in the timing on and allocation to sexual reproduction in *Daphnia hyalina-galeata* hybrid population of Lake Constance. However under specific environmental conditions all three taxa reproduced sexually simultaneously. Clearly much more has to be learned about sexual reproductive activity in *Daphnia* to understand the pattern of sexual activity in natural populations.

Chapter 4

A COMPARISON OF EGG-BANK AND LONG-TERM PLANKTON DYNAMICS OF TWO *DAPHNIA* SPECIES, *D. HYALINA* AND *D. GALEATA*: POTENTIALS AND LIMITS OF RECONSTRUCTION

together with D. Straile, submitted to Limnol Oceanogr

Resting eggs of planktonic organisms from datable sediment cores are increasingly used to reconstruct historical information on the abundance, size, genetic composition and microevolution of planktonic organisms. All these studies rely on the up to now mostly untested assumption that the resting egg bank in the sediment will indeed allow an accurate reconstruction of past populations. Here we test the performance of the egg bank to reconstruct historical data of the *Daphnia* population of Lake Constance, which has been thoroughly investigated throughout the last century. We show that it is possible to reproduce variability in abundance, size, and genetic composition of *Daphnia galeata* within a period of approximately two decades. Furthermore, resting egg data allowed us to reconstruct the timing of the invasion of *Daphnia galeata* into Lake Constance. However, the egg bank failed to reconstruct a) the dynamics of the native *Daphnia* species of Lake Constance, *D. hyalina*, and b) the relative importance of the two *Daphnia* species. We argue and present some evidence that the latter is caused by differences between the two species as to sexually reproductive activity and in the buoyancy of ephippia. The failure to reconstruct the long-term dynamics of *D. hyalina* in the lake is most probably due to a change during sexual activity and possibly also of ephippia buoyancy in the course of eutrophication.

Introduction

The existence of long-lived dormant eggs in lake sediments offers aquatic ecologists powerful opportunities to reconstruct past population abundances (Yan et al., 1996), population genetics (Weider et al., 1997), food web structure (Jeppesen et al., 2001; Jeppesen et al., 2001) and microevolutionary changes (Hairston et al., 1999; Kerfoot et al., 1999). The occurrence and abundance of resting stages were used to investigate past population abundances, species invasions and the species recovery following the restoration of lakes (Verschuren & Marnell, 1997). The size of daphnid ephippia was used to infer fish predation pressure in shallow lakes and to reconstruct past fish predation pressure (Verschuren & Marnell 1997, Jeppesen et al. 2002). Genetic analysis of resting stages (Duffy et al., 2000; Limburg & Weider, 2002) and of hatchlings from resting stages (Weider et al., 1997; Hairston et al., 1999) revealed information about the genetic composition of past populations. As cladocerans and calanoid copepods produce resting stages that remain viable for several decades and more, hatching of such resting stages from sediments of known ages and conducting experiments with those past populations can yield important insight into the mechanisms causing microevolutionary changes. This promising new research agenda, termed "resurrection ecology" (Kerfoot et al., 1999), was used to study, for instance, microevolutionary changes in the phototactic behavior of a *D. magna* population in response to differences in fish predation (Cousyn et al., 2001), and microevolutionary changes in the copper sensitivity of Lake Superior daphnids in response to mining (Kerfoot et al., 1999). Using the methods of resurrection ecology, it was suggested that the *D. galeata* population of Lake Constance showed microevolutionary responses to increased abundances of toxic cyanobacteria resulting from eutrophication (Hairston et al., 1999, Hairston et al., 2001).

Obviously, most of these applications rely on the assumption that sedimentary eggs banks do really reflect past populations. Up to now, there is no study which critically examines this assumption. Here we use the long-term data set of *Daphnia* in Lake Constance to check the reliability of the egg bank record. Zooplankton populations of Lake Constance have been studied since the beginning of the 20th century (Straile & Geller, 1998). In particular the *Daphnia* population has been studied in great detail (Stich & Lampert, 1984; Stich, 1989; Weider & Stich, 1992; Straile & Geller, 1998; Straile & Geller, 1998; Straile, 2000). This enables us to compare various characteristics of the *Daphnia* population with their egg bank reconstruction. The findings from historical observations, which can be used to test the possibilities of egg bank reconstructions, are as follows:

Daphnia invasion and change in Daphnia species composition - In the course of the eutrophication of Lake Constance, *D. galeata* invaded the lake in the 1950s and produced hybrids with the native *D. hyalina* population (Einsle, 1978; Weider & Stich, 1992). We expect to find this pattern in the egg bank record.

Change in Daphnia abundance – *Daphnia* abundance in Lake Constance increased strongly during the last century as a result of eutrophication (Straile & Geller, 1998; Straile & Geller, 1998). We expect a strong increase in the ephippia abundance.

Daphnia body size - Body size of planktonic *Daphnia* during 1979 – 1998 exhibited interannual variability, possibly associated with interannual variability in the predation pressure of juvenile fish (Straile, in preparation). As large *Daphnia* produce large ephippia (Boersma et al., 2000; Jeppesen et al., 2002) we expect to find interannual variability in ephippia size in the sediment core to be associated with *Daphnia* size.

Daphnia clonal composition - Allozyme studies of Lake Constance *Daphnia* were conducted during 1989/90 (Weider & Stich, 1992) and during 1999 – 2001 (Jankowski, unpubl.; Jankowski & Straile, submitted) and revealed distinct allozyme frequencies. We finally expect to find these allozyme frequencies in the respective sediment layers.

Hence, our test covers most aspects of *Daphnia* egg bank reconstructions, i.e., *Daphnia* invasion, species composition, clonal composition, abundance and size. We will discuss our results in order to improve our understanding of the factors which control the production and deposition of ephippia.

Methods

Lake Constance is located on the northern fringe of the Alps (9°18'E, 47°39' N). It is a large (476 km²) and deep (z_{max} = 254m, z_{mean} = 100m) lake of warm-monomictic character. The trophic state changed dramatically during the last century, as indicated by $P_{tot, \ mix}$ concentration (Fig. 4.1): originally an oligotrophic lake, it underwent massive eutrophication beginning in the 1950s, with a maximum at the end of the 1970s, followed by re-oligotrophication (Güde et al., 1998).

Palaeo-data of ephippia

Ephippia were isolated from 15 laminated and dateable cores (acrylglas tube, 1m long, ⌀ 5.8cm inner cross-section) sampled in 2000 (7 cores) and 2001 (8 cores) from 220m water depth (between Friedrichshafen and Romanshorn) in the main basin of Upper Lake Constance. Layers were dated, like in previous studies (Weider et al., 1997; Hairston et al., 1999), by counting of laminae (Wessels, 1995). Each core was cut in half lengthwise, photographed and the layers were dated. Sediment samples were taken from different layers representing 1 to 5 years in the 2000 cores and 1 to 4 years in the 2001 cores from each half of the core. The 1 year samples represent prominent high water markings. To avoid the transfer of ephippia between different layers the outer edge (~ 0.5 cm) of each section was not sampled. Ephippia were isolated either by sieving through a 250µm sieve. The ephippia of the 2000 cores were cleaned with filtered lake water (0.45µm) and then subdivided into two parts. One part was transferred into clean 6x6 microtiter plates and incubated in a walk-in environmental chamber at constant light and constant temperature for subsequent

allozyme analysis of the hatchlings. Ephippia were monitored daily to every other day for a period of 35 days. Newly hatched daphnids were removed and carefully transferred to culture jars to grow up for 3 to 5 days. The hatchlings were frozen and stored at –80°C for subsequent allozyme analysis. The second part was counted and measured (without the spine) with a dissecting microscope (mag. 2x10). The ephippia of the 2001 cores were measured (without spine) with a dissecting microscope (mag. 2-5x10). *Daphnia* ephippia were distinguished by the occurrence (*D. hyalina*) or absence (*D. galeata*) of small spines at the dorsal edge of the ephippia (Flössner, 2000). In addition to these two categories, we found mixed type ephippia with only few dorsal spines and classified them as potential hybrids.
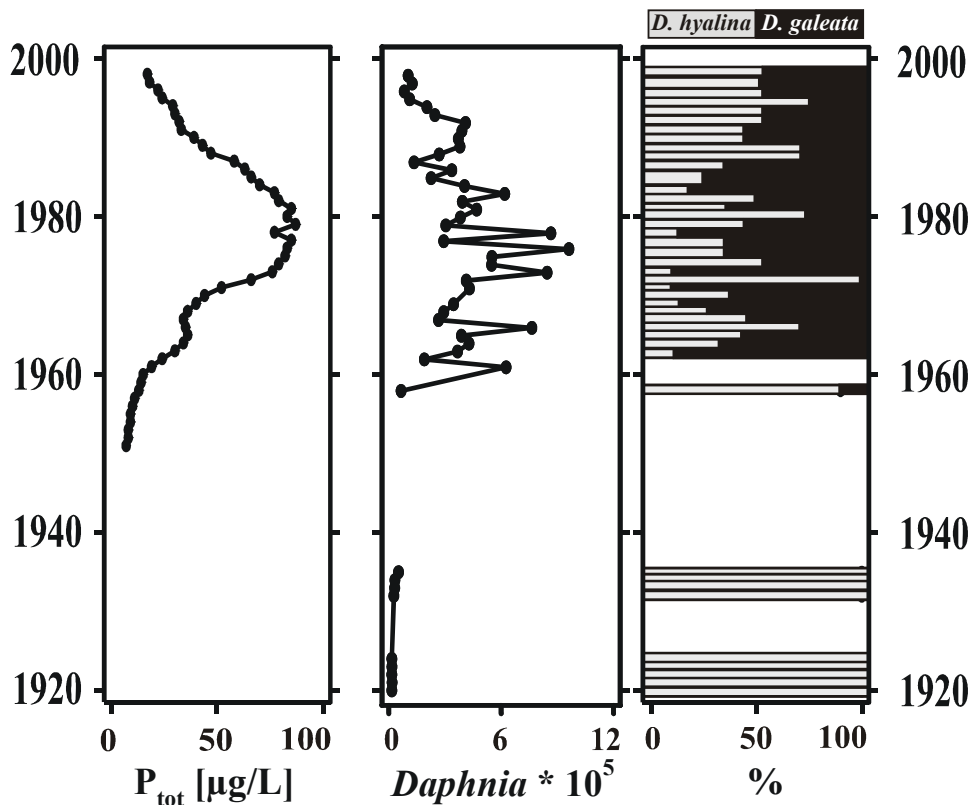
Figure 4.1 Long-term data set from Lake Constance. Shown are the average winter total phosphorous concentration (left graph, data from Güde et al., 1998), mean yearly *Daphnia* abundance (mid graph) and taxa composition (right graph) of *Daphnia hyalina* (grey) and *D. galeata* (black). *Daphnia* data were taken from several studies

The genetic architecture of planktonic daphnids and of hatchlings was determined using allelic variation at allozyme loci, using cellulose acetate electrophoresis. Three enzyme loci were screened for polymorphism: aspartate amino transferase (AAT; E.C. 2.6.1.1.), phosphoglucomutase (PGM; E.C. 5.4.2.2.) and phosphoglucose isomerase (PGI; E.C. 5.3.1.9.). Protocols for electrophoresis were as described by others (Hebert & Beaton, 1993), except that electrophoresis was run at 300V.

Long-term data set of daphnids

Daphnia plankton data were taken from the long-term data set of the Limnological Institute of the University of Constance and the Seenforschungsinstitut in Langenargen (partly published in Elster & Schwoerbel, 1970; Muckle & Muckle-Rottengatter, 1976; Muckle & Dillmann-Vogel, 1976; Einsle, 1978; Einsle, 1983; Straile & Geller, 1998). From 1979 to 1995 *Daphnia* were collected at weekly or biweekly intervals by vertical net hauls from 140m depth at the sampling station of the Limnological Institute in the fjord like branch of the Upper Lake Constance (Überlingersee) and data on the abundance, frequency of *D. hyalina* and *D. galeata*, and size are available. Data from 1920-24 (Auerbach et al., 1924; Auerbach et al., 1926), 1932-35 (Elster & Schwoerbel, 1970), 1952-62 (Muckle & Muckle-Rottengatter, 1976; Muckle & Dillmann-Vogel, 1976) and 1962-93 (partly published in Einsle, 1978; Einsle, 1983) were taken at different sampling stations in the main basin of the Upper Lake Constance. Data on the abundance, the percentage of males and the frequencies of *D. hyalina* and *D. galeata* for the years 1999, 2000 and 2001 were taken from Jankowski unpubl. and Jankowski & Straile (submitted). Plankton allozyme data of daphnids for 1989/90 were taken from Weider & Stich (1992), for 1999 from Jankowski (unpubl.) and for 2000 from Jankowski & Straile (submitted).

For statistical comparison of the ephippia data from the sediment cores and the long-term data sets we calculated the mean value for each time period sampled in the cores and (for the same time periods) in the plankton samples. We used correlation analysis to relate the egg bank reconstruction with the historical data. Data were plotted using the midpoint of the sampled intervals. Data analysis was performed using SAS (SAS Institute, 1988).

Because electrophoretical data of the ephippial hatchlings suggested that most ephippia produced belong to *D. galeata*, we used only the long-term data sets for size of adults of *D. galeata* for the statistical analysis of size differences. Additionally, as *D. galeata* produced ephippia between May and July (Jankowski & Straile, submitted), only size data from this time of the year were analyzed.

Results

Continuos measurement of total phosphorus concentrations during winter mixis started in the 1950s (Fig. 4.1). These measurements show an exponential increase until the 1970s and a fast decrease of total phosphorus starting in the early 1980s. The abundance of
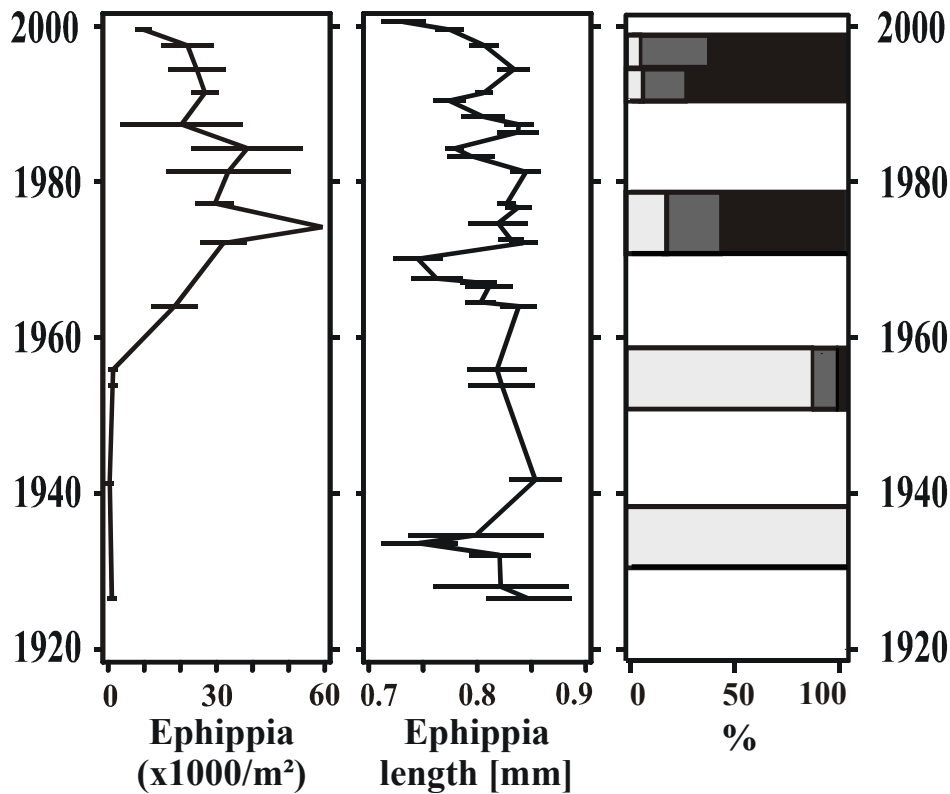
FIGURE 4.2 Data obtained from the ephippial record. Shown are the ephippia densities (left graph), the ephippia size (mid panel) and the composition of the ephippial record using the presence or absence of small dorsal spines (right graph). Ephippia with dorsal spines indicates *D. hyalina* (light grey), ephippia without spines *D. galeata* (black). Additionally, we found ephippia with few spines, probably indicating hybrids.

daphnids showed a similar development with abundances less then 0.4×10^5 individuals m^{-2} in the 1920s and 1930s. Following eutrophication, abundances increased towards more than 1×10^6 individuals m^{-2} in the 1970s and decreased markedly again especially in the late 1990s. In the course of eutrophication *Daphnia galeata* invaded the lake in the 1950s and soon reached a relative abundance of ≥ 50 %. In more recent years, the relative contribution of both *Daphnia* species to overall *Daphnia* abundance was roughly similar (Fig. 4.1).

The ephippia abundance in the egg bank ranged between 1000 to 60000 m^{-2} y^{-1} (Fig. 4.2). Before the 1950s ephippia abundance was very low (between 350 to 2800 m^{-2} y^{-1}), but increased from 1960 onwards with a maximum from the 1970s to the early 1980s (mean 32,000 $m^{-2}y^{-1}$). From the late 1980s onwards ephippia abundance decreased to the level of the late 1960s (Fig. 4.2). Mean ephippia abundance was positively associated with $P_{tot,mix}$ (r = 0.737, *p* = 0.010, n = 11) and with mean *Daphnia* abundance (r = 0.739, *p* = 0.006, n =

12) throughout the study period. The size of the egg bank ephippia (range: 720 – 880 μm) was highly variable within the last century. From the beginning of the 1990s mean ephippia size decreased to levels ranging between 700μm and 750μm. A similar decrease was also observed in the 1970s and the 1930s. Ephippia size was neither correlated with *Daphnia* abundance (r = 0.105, *p* = 0.589, n = 29) nor with total phosphorus concentration during winter mixis (r = 0.165, *p* = 0.402, n = 28). Ephippia size was, however, strongly correlated with the size of *D. galeata* within the period 1979 – 1998 (r = 0.702, *p* = 0.007, n = 13, Fig.
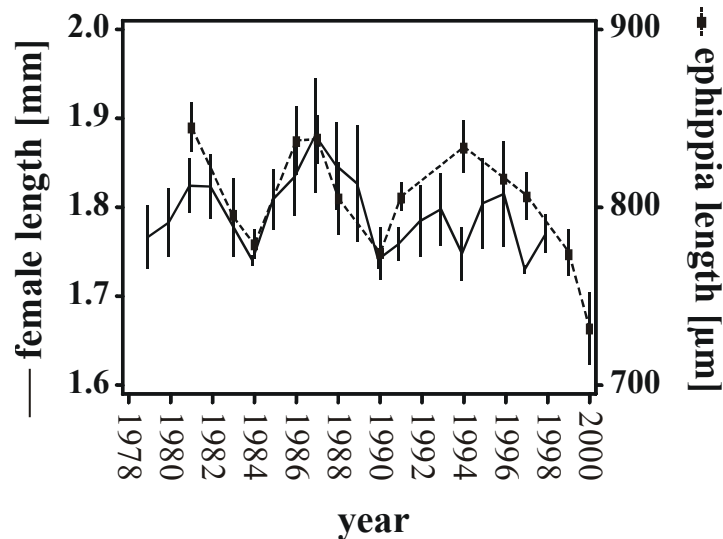
FIGURE 4.3 Size of ephippia isolated from the sediment record (squares and dashed line) and size of parthenogenetic adult females obtained by historical plankton record from the beginning of the 1980s to the end of the 1990s. Shown are the mean and standard error for the selected years of the ephippia record (n = 5 cores), and the mean and standard error from May to July for each year of the historical record.

4.3), i.e., the only period when we have size measurements of planktonic animals.

Using the absence or presence of small spines at the dorsal margin of the ephippia we distinguished between three types of ephippia: with spines, without spines and with few spines. The proportion of ephippia with dorsal spines (native *D. hyalina*) changed dramatically from the 1930s (~100%) to the 1990s (less the 10%). In contrast, the proportion of ephippia without spines (*D. galeata* like ephippia) increased from 0 to 80% during the same time period. Additionally we found ephippia with few spines, probably representing hybrids. This type of ephippia also occurred for the first time in the 1950s and their relative frequency also increased from the 1950s onwards, from 10% to 30% (Fig. 4.2).

A total of 304 animals hatched from ephippia were electrophoretically screened, sample size per layer varied between 10 to 60 animals; for the years of particular interest, the beginning of the 1990s and 1999/2000, we screened at least 30 individuals per year. All but two screened individuals belong to the AAT genotype "ff", i.e. *D. galeata*, whereas the the two individuals showed an "sf" genotype, i.e. the *D. hyalina x galeata* hybrid. The latter were found in the sample of the beginning of the 1970s. No "ss" genotype was found. At the PGI loci we found two alleles ("mm" and "ff"). The "f" alleledecreased in frequency from 90 – 100% in the 1960s and 1970s to approx. 80% in 1999/2000, whereas the "m" allele increased within this period (Fig. 4.4). At the PGM loci we found four different alleles. The "f" allele reached the highest frequencies, ranging between 40 and 80%. From the 1970s onwards the amount of this allele decreased from more than 75% to less then 60%. The alleles with the lowest contribution ("s" and "f+") at the PGM loci never reached more than 20% (Fig. 4.4).

A comparison of the allele frequencies of the hatchlings from the early 1990s and 1999/2000 with the plankton data of similar periods using a pairwise G-test showed no significant differences, neither between the parthenogenetical females of the plankton and the ephippial hatchlings nor between the ephippial females and the ephippial hatchlings (Table 4.1, Fig. 4.5), except for one comparison. At the PGI loci there were slightly significant differences (G = 7.725, *p* = 0.021) between the parthenogenetical plankton females and the ephippial hatchlings of the 1999/2000 samples.

FIGURE 4.4 Changes in the allele frequencies at the PGM (upper graph) and PGI (lower graph) loci from the 1960s to the end of the century obtained by the ephippial hatchlings from the sediment record

Discussion

The detailed comparison between sediment ephippia characteristics and long-term data revealed striking possibilities but also potential limitations of egg bank reconstruction which have to be taken
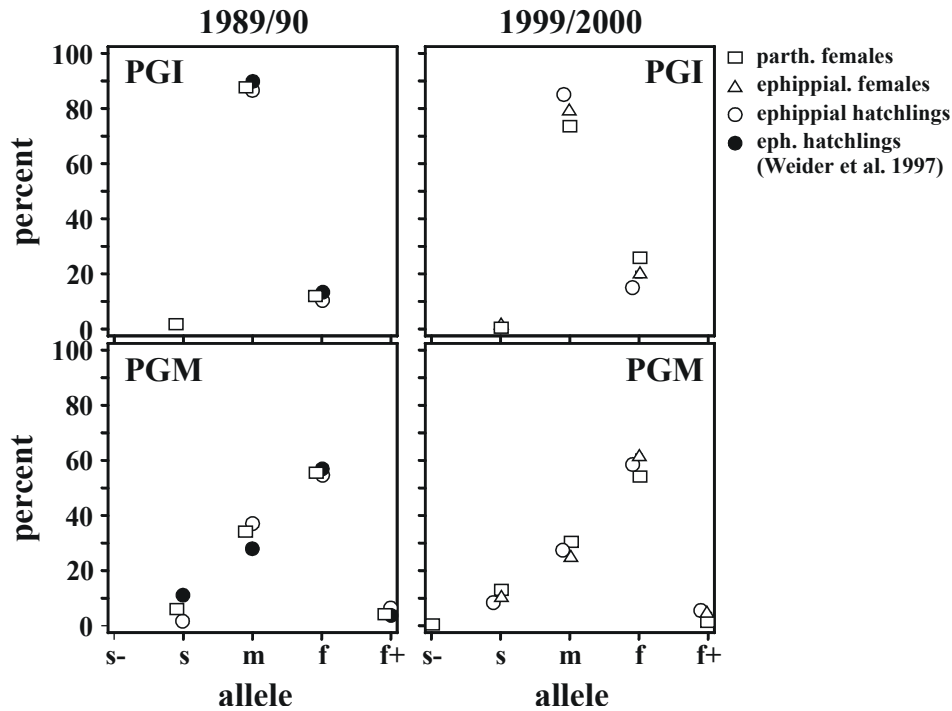
Figure 4.5 Allele frequencies at the PGI (upper graphs) and PGM (lower graphs) loci of ephippial hatchlings (circles) and parthenogenetical (squares) and ephippial (triangles) plankton females from 1989/90 (left graphs) and 1999-2000 (right graphs). Data on the parthenogenetical females of 1989/90 were taken from Weider & Stich (1992). Included are the allozyme frequency data of ephippial hatchlings from the beginning of the 1990s (black dots) levied by Weider et al. (1997).

into account when reconstructing palaeolimnological and historical trajectories. Ephippial data allowed the successful reconstruction of the invasion of *D. galeata* into Lake Constance, the abundance increase of *Daphnia* following the eutrophication of Lake Constance, the allozyme composition of *D. galeata* for selected years, as well as interannual variability of *Daphnia* body size during 1979 - 1998. In contrast, morphological and allozyme data did not allow a reconstruction of the relative abundance of *Daphnia* taxa especially in more recent years, and indicate also limitations in reconstructing *Daphnia* abundance.

Studies from the first half of the last century report only one *Daphnia* species in Upper Lake Constance, i.e. *D. hyalina* (Auerbach et al., 1924; Auerbach et al., 1926; Elster & Schwoerbel, 1970). This is supported by ephippia morphology by the exclusive occurrence of ephippia with spines (Fig. 4.2). As it is not possible to investigate allozyme composition of ephippia, and as we did not observe hatching of ephippia prior to the 1950s, we were not able to check species identity prior to the 1950s with allozymes. However, ephippial genetics can be studied directly with microsatellites, and the first analyses of

Lake Constance ephippia from the 1920s support our morphology data and shows that these ephippia were indeed *D. hyalina* (Schwenk, unpublished results). Morphological data also allowed a reconstruction of the *D. galeata* invasion into Lake Constance: the first occurrence of ephippia without spines in the 1950s corresponds to historical observations (Fig. 4.1 and 4.2, Muckle & Muckle-Rottengatter, 1976; Muckle & Dillmann-Vogel, 1976).

TABLE 4.1 Comparison of the allele frequencies at the PGM and PGI loci for the two periods (1989-90 and 1999-2000) when allele frequencies were available for the plankton parthenogenetical and ephippial females and ephippial hatchlings isolated from respective sediment depths (pairwise G-tests). Allele frequency data of parthenogenetical females of 1989/90 were taken from Weider & Stich (1992).

Periode	loci	df	value	*p*
1999-2000				
eph. females vs. eph. hatchlings	PGM	3	0.958	0.812
eph. females vs. eph. hatchlings	PGI	2	2.946	0.229
parth. females vs. eph. hatchlings	PGM	4	9.134	0.058
parth. females vs. eph. hatchlings	PGI	2	7.725	0.021
eph. females vs. parth. females	PGM	4	6.315	0.177
eph. females vs. parth. females	PGI	2	5.505	0.064
1989-90				
eph. hatchlings(early90) vs. parth. females	PGM	3	3.211	0.360
eph. hatchlings(early90) vs. parth. females	PGI	2	0.431	0.806

The genetic composition of the *D. galeata* population seems to be well represented by the ephippial record. As shown by a previous study (Weider et al., 1997), the data of 1989/90 are very similar and showed no bias between parthenogenetical plankton females and the ephippial record neither at the PGI nor at the PGM loci. This was also the case for the 1999/2000 data comparing sexual females and ephippial hatchlings (Table 4.1, Fig. 4.5). The differences at the PGI loci between parthenogenetical females and ephippial hatchlings suggest, that mating in this population may be not random. However, we cannot exclude that these differences were due to pooling the samples from different years, which was unavoidable as it was impossible to separate sufficiently thin sediment layers to represent single years from the sediment cores. The temporal shifts in genetic composition through time at the PGI and PGM loci (Fig. 4.4) were similar to those reported by Weider et al. (1997).

Population size of the two planktonic daphnid populations combined seems to be well reflected by the abundance of ephippia in the respective sediment layers. This is not only true regarding the large differences in population size, which occurred during one century of eutrophication, but also for more subtle differences during the period of
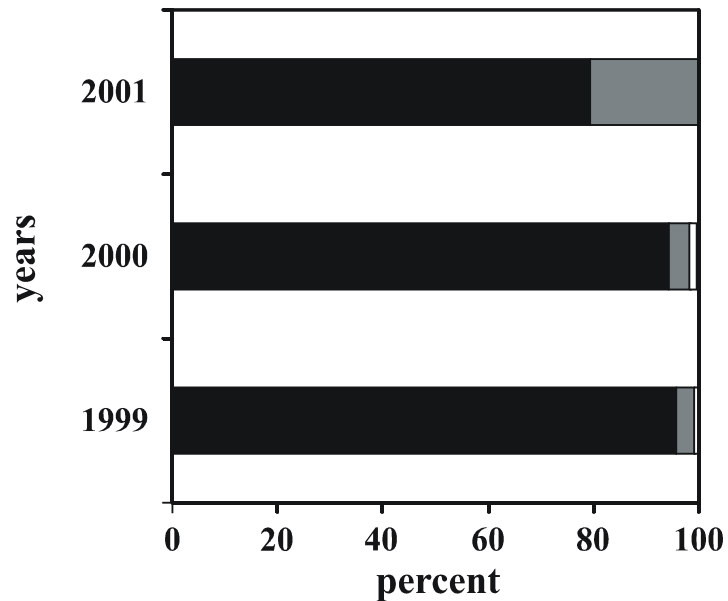
FIGURE 4.6 Relative contribution of *D. galeata* (black bars)*, D. hyalina* (white bars) and *D. galeata* X *D. hyalina* (grey bars) to total occurrence of ephippial females in three study years. Taxa were identified using AAT loci as marker (Wolf, 1987). Data from Jankowski & Straile (submitted) and Jankowski (unpublished).

reoligotrophication from 1979 to the present (r = 0.866, *p* = 0.026, n = 6). The ephippial record seems also to be a good archive to reconstruct the mean female size. Both female as well as ephippial size showed cyclic fluctuations during the 1980s and 1990s, with periods of larger and smaller ephippia/females (Fig. 4.3). Hence, the variability in size was not related to the trophic status of the lake as indicated by total phosphorus concentrations, which showed no fluctuations but decreased monotonically during this period. Variability in ephippia size between lakes (Jeppesen et al., 2002), but also between years within a lake (Cousyn et al., 2001; Jeppesen et al., 2001), was suggested to result from variability in fish predation pressure. This suggests that ephippia size variability in Lake Constance is also related to fish predation pressure, which in Lake Constance seems to be associated with climatic variability (Straile, unpublished).

Hence, significant variability in the egg bank is not associated with processes resulting from eutrophication and oligotrophication of Lake Constance. Consequently, studies of "resurrection ecology" (sensu Kerfoot et al., 1999) in Lake Constance should not only consider eutrophication and oligotrophication as the driving forces behind, for instance, microevolutionary changes (e.g. Hairston et al., 1999; Hairston et al., 2001), but have to consider also alternative explanations.
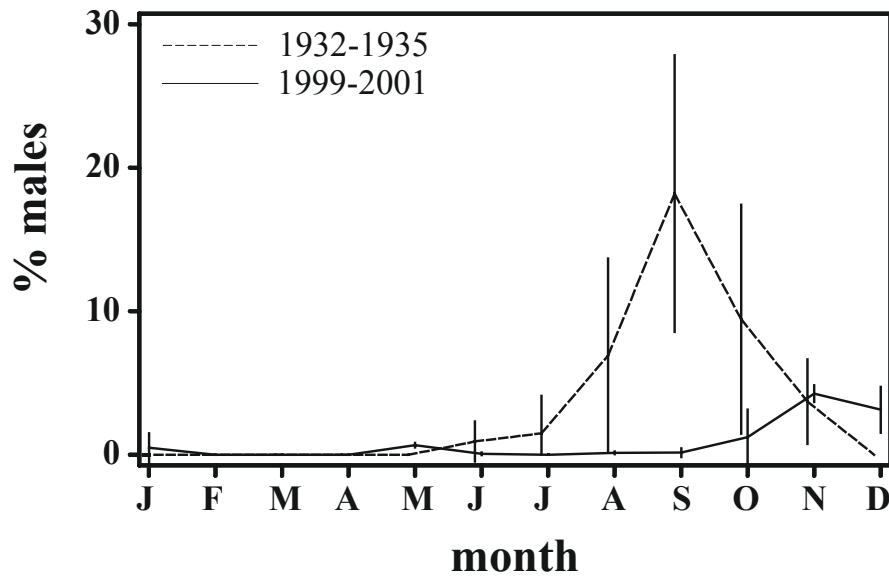
FIGURE 4.7 Mean (+/- se) monthly percentage of *D. hyalina* males prior to eutrophication (1932-1935, dashed line) and at the end of the century (1999-2001, line) of overall population size. Data from Elster & Schwoerbel (1970), Jankowski & Straile (submitted) and Jankowski (unpublished).

Our study revealed also important differences between egg bank reconstructions and historical data as discussed below. These differences suggest that egg bank reconstructions might be complicated by species specific differences in *Daphnia* life history, i.e., timing and frequency of sexual reproduction, the hydrodynamical conditions during the time of sexual reproduction, as well as by changes in *Daphnia* life history in response to changes in trophic status of a lake.

Whereas the morphological analysis of the ephippial record seems to reflect very well the taxa composition at the beginning of the 20th century and showed also the time of invasion by *D. galeata* around the 1950s, there seems to be a bias not only between ephippial record and plankton data but also between the allozyme and the morphological analysis of the ephippial record from the mid of the 20th century onwards. From morphology based historical records we would expect to find a ratio of *D. hyalina* to *D. galeata* of around 50:50 (Fig. 4.1), or if including hybrids of around 40 (*D. hyalina*) : 40 (*D. galeata*) : 20 (hybrid) for the 1990s (Weider & Stich, 1992; Jankowski unpublished). In fact, the morphological analysis of ephippia suggested a proportion of around 5 % *D. hyalina* and around 15 % hybrids. This bias is even more pronounced when looking at the allozyme composition of hatched individuals. From 300 individuals hatched, 298 *D. galeata*, no *D. hyalina* and only two hybrids (~0.6%) were identified. Nearly the same proportion (0.8%) of hybrid genotypes was found by Weider et al. (1997), and they also found no *D. hyalina*. Given the high number of hatchlings, 300 in our investigation and

1200 in the investigation of Weider et al. (1997), it is surprising that not a single *D. hyalina* was detected. Several factors might contribute to this discrepancy. First, *D. hyalina* might have a lower sexual reproductive activity, for instance, lower ephippial production, than *D. galeata*. This is supported by a three year study of sexual reproductive activities of daphnids in Lake Constance. We observed all three taxa to produce ephippia, but overall *D. galeata* produced more than 100 times more ephippia than *D. hyalina* and 30 times more than the hybrid (Fig. 4.6). Second, ephippia might differ in their buoyancy due to the presence of spines, gas chambers or lipid content (Wesenberg-Lund, 1939; Hebert, 1995; Weider et al., 1997; Flössner, 2000). With increasing buoyancy chances for ephippia to be transported to shallow water regions should increase. The presence of spines on *D. hyalina* resting eggs might indicate increased buoyancy. Indeed, the ephippial hatchlings caught in emergence traps put on the sediment in the littoral zone of Lake Constance revealed a different taxonomic composition of the littoral hatchlings than ephippia from our deep water cores (Jankowski, unpublished). We found considerably higher hatchling proportions of *D. hyalina* (25%) and hybrids (7%) from the littoral sediments, suggesting higher floating capabilities of *D. hyalina* ephippia. In sum, a 100-fold lower ephippial production and additionally a higher floating capability of *D. hyalina* ephippia as compared to *D. galeata* might explain why none of the hatched individuals in Weider et al.'s (1997) and in our study were *D. hyalina*.

However, these observations do not explain why there are *D. hyalina* ephippia in the deep sediment prior to the 1950's. The ephippia found in these sediment layers have to be *D. hyalina* because during this time only *D. hyalina* occurred in the lake which is supported by the first analysis of microsatellite DNA (Schwenk, unpublished results). Furthermore, as the abundance of *D. hyalina* was much lower prior to the 1950's we would expect even less ephippia in these sediment layers. This suggests a difference in sexual reproductive activity of *D. hyalina* and/or a change in the buoyancy of its ephippia during the course of eutrophication.

Increasing food levels during eutrophication changed the seasonal pattern of *D. hyalina* occurrence in Lake Constance (Straile & Geller, 1998). In contrast to the more eutrophic years, where *D. hyalina* occurred in all plankton samples, *D. hyalina* was a typical summer species and thus not found in the plankton during winter months in the 1920s (Auerbach et al., 1924; Auerbach et al., 1926) and only rarely in the 1930s (Elster & Schwoerbel, 1970). This suggests that *D. hyalina* in oligotrophic Lake Constance was more dependent on the production of resting eggs than it is in eu-/mesotrophic Lake Constance. Data on male occurrence as a proxy for sexual reproductive activity supports this hypothesis. In the 1930s the frequency of male production was 5 to 10 fold higher than it was between 1999 and 2001 (Fig. 4.7). Hence, the *D. hyalina* population seems to have responded to an increased possibility to overwinter in the plankton following eutrophication with a reduced sexual activity.

The change in sexual reproductive activity might have contributed to the occurrence of *D. hyalina* resting eggs in the sediment layers prior to the 1950s. However, why did these early ephippia sink down to the deep water sediment and did not end up in the littoral as *D. hyalina* ephippia do in recent times? The floating capabilities of ephippia are determined both by the characteristics of the ephippia, such as spininess and density, and by environmental conditions, like depth of the mixed zone. As a consequence, achieving optimal buoyancy of ephippia is not a trivial task. This might be especially true in a large and deep lake like Lake Constance which experiences pronounced seasonal changes in water level (seasonal range: 2 m) and mixing depth. In Lake Constance there is a strong seasonal variability in water levels with a minimum water level occurring during February and a subsequent rise in water level of on average 2 m towards a maximum in June and July due to melting snow in the Alp. From August onwards water levels begin to drop again (Kiefer, 1972). Due to the seasonal variations in mixing depth and water level, the timing of ephippia production will influence the deposition of ephippia. During June, i.e., during the time of ephippial production of *D. galeata,* mixing depth in Lake Constance is about 4 m (Bäuerle pers com.) and water levels are at their maximum, while during autumn, i.e., during the time of ephippial production of *D. hyalina,* mixing depth in Lake Constance is about 30 m and water levels are decreasing towards their minimum. Hence, during June ephippia in the upper 4 m of the water column will be kept in suspension, while in autumn this will be the case of ephippia within the upper 30 m of the water column. Obviously, ephippia of equal buoyancy will have a higher possibility of sinking down to the deep water sediments when produced in June as compared to autumn. This might also contribute to the fact that we find ephippia from *D. galeata*, i.e. ephippia produced in June, in the deep sediments but no autumnal produced ephippia of *D. hyalina*. Given the fact that Lake Constance is on average 100 m deep and the cues for hatching, i.e., light and temperature (Caceres & Schwalbach, 2001), are reduced at this depth, there may be selection to increase the buoyancy of ephippia produced in June, increasing the probability that ephippia will be transported to shallow water regions. However, as June is the period of maximum water level, transport of ephippia to very shallow water would also increase the probability of ephippia to fall dry with the decrease in water levels following June. As the water level during the time of the phytoplankton spring bloom is considerably lower than in June, these ephippia might miss the algal spring bloom. Furthermore, after a year of exceptionally high water level in June, ephippia with a high buoyancy could fall dry for several years. This suggests selection against a high buoyancy ephippia produced in June. As the water level in autumn is already considerably lower than in June, ephippia produced in autumn, even with a high floating capability, will not be in danger of missing the next spring bloom. This suggests that a high buoyancy poses no problems for autumn produced ephippia regarding falling dry, however, has the advantage to prevent them from sinking down. Hence, differences in the timing of sexual reproduction between *D. galeata* and *D. hyalina* might, at least partially, explain why their ephippia differ with respect to the presence of spines,

and also why there are much more *D. galeata* ephippia in deep water sediments. As the timing of sexual production of *D. hyalina* shifted from summer to autumn during eutrophication (Fig. 4.6), the same reasoning might also at least partially explain why *D. hyalina* ephippia sank down to deep –water sediments prior to the 1950s but not in recent years: all other things being equal, we should expect daphnids to produce ephippia with lower buoyancy in June as compared to autumn. Consequently, a change in the floating capability of ephippia might contribute to the pattern of occurrence of *D. hyalina* ephippia in Lake Constance deep water sediments. Hence, in deep lakes with seasonally varying water levels, optimal floating capability of *Daphnia* ephippia might be a trade-off between the dangers of sinking down to the depth of >100 m and to fall dry and consequently miss the next spring phytoplankton bloom. This trade-off will be influenced by the mixis depth and the water level at the timing of ephippia production. Further studies can test these hypotheses by comparing, for instance, the floating capabilities of ephippia produced in different seasons and in lakes differing in depth and water level fluctuations.

In conclusion, a precise reconstruction of *Daphnia* populations from sediment egg banks can be achieved if there is no clonal or taxa specific variability in the frequency of ephippia production and if there is no clonal or taxa specificity in ephippia buoyancy, i.e. in the probability that a produced ephippia will sink down to the sediment. Our study revealed that as for *Daphnia* both conditions are not met in Lake Constance. *D. hyalina* and *D. galeata* differ in their allocation into ephippia production and their ephippia also differ in spininess and probably other characters influencing their buoyancy. Furthermore, both the frequency of ephippia production and ephippia floating capability are most likely not taxa specific constants, but seem to be highly variable depending on environmental conditions and possibly also microevolutionary changes, even within a rather limited time period of less than a century.

The good correlation between ephippia density and *Daphnia* abundance is hence not a direct consequence of a constant allocation of daphnids into sexual reproduction, but rather the results of a variety of overlapping mechanisms. In fact, considering only *D. hyalina*, we would have found a negative relationship between planktonic females and egg bank ephippia. Hence, relying only on the sediment record, i.e., without knowing the actual historical population development from the analysis of plankton samples, we might have erroneously concluded that *D. galeata* was able to almost outcompete *D. hyalina* in Lake Constance. However, in fact, this was not the case and both species achieved abundances of similar magnitude during spring and summer in Lake Constance after the invasion of *D. galeata* (Straile & Geller, 1998). Consequently, a successful reconstruction of specific characteristics, i.e., abundance, variability in allozyme composition and body size, of the Lake Constance *Daphnia* population was only possible for one species, i.e. *D. galeata* within a limited period of time. Future research using *Daphnia* ephippia as palaeolimnological tools should note that ephippia production and deposition within a lake

might change during a rather limited period of time, especially when the lake is subjected to changes in trophic status.

Chapter 5

THE LITTORAL ZONE AS A NURSERY FOR ZOOPLANKTON? INSIGHTS FROM A *DAPHNIA* POPULATION OF A LARGE AND DEEP LAKE

The recruitment from resting-stages is a common feature of many planktic organisms in temporary ponds as well as in large lakes. Since the cues for breaking diapause, i.e., increases in temperature and light intensity, decrease with lake depth, we hypothesized that the littoral zone plays an important role in the recruitment of zooplankton in large lake populations. We investigated the recruitment of *Daphnia hyalina-galeata* in the large lake, Lake Constance. In laboratory, we examined emergence under 6 different temperature regimes, 5 different light intensity regimes and 6 different day-length regimes and compared these results with a 2-year survey of *in-situ* emergence patterns using emergence traps placed at varying depths. Additionally, we investigated the temporal and spatial ephippia distribution in Lake Constance using 114 core samples taken regularly from January and December and from depths of 1m to 220 m. The results from both our laboratory aand the *in-situ* emergence experiments indicate that emergence begins when temperatures exceed storage temperature (in Lake Constance this is between 4°C to 5°C), was restricted to a short period in February/March and was higher in deeper traps. Neither light intensity nor day-length had a significant effect on total emergence or on timing of emergence in the lab. In situ, we found no *D. hyalina* hatching from profundal sediments, but *D. hyalina* made up to 25% of littoral hatchlings. Ephippia densities increased with depth. In littoral sediments, ephippia were only found in the time period between ephippia production in early summer and late autumn. From these findings we conclude that the littoral might be more important for the recruitment of *D. hyalina*, however, the main recruitment of *D. galeata* seems to take place in rather deeper strata.

Introduction

A common feature among many organisms, plants and animals, is the presence of long-lived resting stages. The resting stage has been attributed a number of adaptive functions (Hairston, 1998), one of which is as a survival strategy during periods of unfavorable environmental conditions, like winter or a dry-season (DeStasio, 1989; Philippi, 1993).

In non-overwintering *Daphnia* populations from temperate lakes and ponds, diapause is an important component of the life-cycle (Hebert, 1978). Most *Daphnia* species are cyclical parthenogens; asexual reproduction is the dominant form of reproduction during periods of favorable conditions, but when conditions are unfavorable the daphnids switch to sexual reproduction which results in the formation of diapausing eggs, called ephippia (Hebert, 1978). Most ephippia sink to the bottom after a brief floating phase (Carvalho & Wolf, 1989) and after storage in the sediment for several months, the *Daphnia* hatch and build up the next population. Not all ephippia hatch at the beginning of the next growing season, as has become evident by studies quantifying the diapausing egg bank in natural *Daphnia* and other cladoceran populations (Moritz, 1987; Carvalho & Wolf, 1989; Weider et al., 1997; Cousyn & DeMeester, 1998). In many *Daphnia* populations there seems to be only a short time window from hatching to sexual reproduction, not only in fluctuating drying ponds (Lynch, 1983; Lynch, 1984) but also in lakes (Wolf & Carvalho, 1989; Caceres, 1998). Thus, this window of time is a valuable resource and diapause termination has to be adapted and optimized to best take advantage of it. Hatching should occur at a time when the conditions for somatic growth and parthenogenetic reproduction are favorable to build up populations as large as possible and, subsequently, to produce the maximal number of new diapause stages. There seems to be a trade-off between hatching early during and avoiding high competition while suffering poor diet and low temperatures and hatching just before the phytoplankton bloom to get optimal diet conditions with higher temperature but suffering higher competition and cutting short the time available for asexual reproduction.

The cues for terminating diapause have been intensively investigated in the genus *Daphnia* (reviewed in Stross & Hill, 1965; Stross, 1987). An increase in temperature and a light stimulus seems to be absolute requirements to break diapause (Stross, 1966) indicating the littoral zone plays an important role since temperature and light intensity decrease with water depth. Low O_2 and high CO_2 conditions (Stross, 1971), photoperiod (Stross & Hill, 1968; Stross, 1969b) and storage time as well as cooling, freezing and/or drying during storage (Schwartz & Hebert, 1987) have also been observed to influence hatchability in *Daphnia*. Daphnid species differ in their response to the different hatching stimuli (Schwartz & Hebert, 1987). Additionally, there is intraspecific genetic variation in hatching response at the micro-geographic (DeMeester & DeJager, 1993) and macro-geographic level (Schwartz & Hebert, 1987). The transferability of these results to the field is difficult
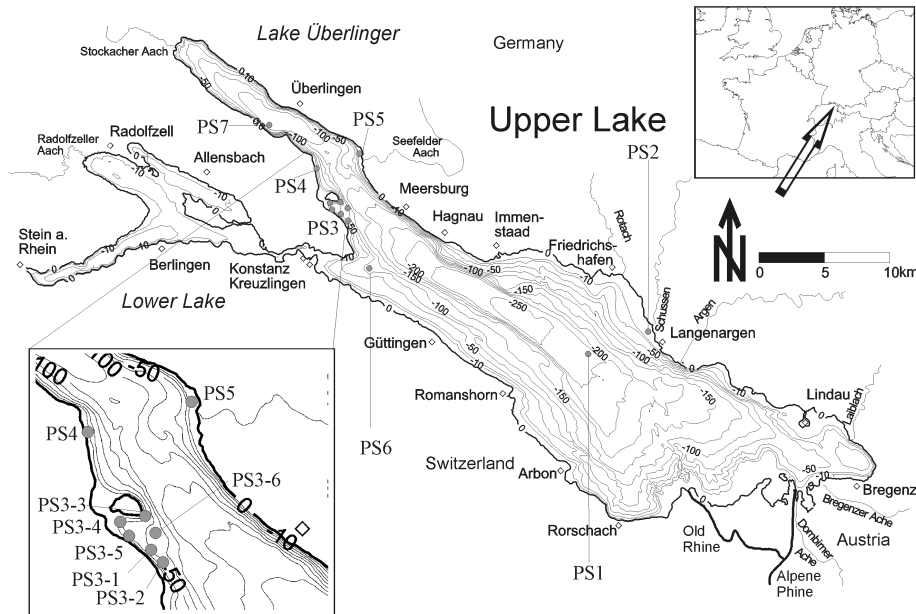
FIGURE 5.1 Map of Lake Constance with the sampling stations (see Table 1). Enlarged is the area of the Island of Mainau with the sampling stations round the island and the Limnological Institute.

since most studies have been conducted under artificial conditions in the laboratory and/or used daphnia clones which sometimes have been cultured in the lab for several years. Only a handful of studies have investigated cladoceran diapause termination in the field, which most of these lack to explain the mechanisms (but see Herzig, 1985; Caceres & Schwalbach, 2001). Unfortunately, the knowledge on the optimal hatching cues, in particular for deep lake daphnids, is sparse and the ties between the diapausing stage and the active plankton stage are not well understood.

Hatching rate per area depends not only on the availability of cues but also on the density of resting eggs. Despite a large number of studies on the spatial distribution of zooplankton resting eggs, to our knowledge, there is no detailed information on their temporal distribution available. This is true in particular for shallow littoral zones of deep lakes. The studies available indicate a positive correlation between water depth and both resting stage density (Herzig, 1985; Moritz, 1987; Carvalho & Wolf, 1989; Yurista, 1997; Caceres, 1998) and the density of viable eggs (Moritz, 1987). This suggests an accumulation of non-hatched eggs with depth and higher hatching rates in shallower areas. The fact that diapause termination in zooplankton was induced by temperature and light pointed also on the importance of the littoral as the main area for recruitment.

In this paper we focus on the role and function of the littoral zone of deep lakes for the recruitment of zooplankton. We hypothesize that the littoral zone is a nursery for zooplankton at the beginning of the season, due to high recruitment from diapausing eggs.

TABLE 5.1 Sampling sites (see Fig. 5.1), the date of sampling, water depth and the number of cores sampled. Additionally, the sampling station for the plankton samples are shown.

station	date	water depth [m]	No. cores
PS1	11.07.00	~220	5
PS2-1 to 6	25.01.-07.12.99	<3	54
PS3-1a	06.08.99	3, 5, 10	3
PS3-1b	08.08.-13.10.00	4	5
PS3-2	08.08.-13.10.00	4	5
PS3-3	08.08.-13.10.00	4	5
PS3-4a	19.08.99	2.5, 5.5, 9.5	9
PS3-4b	08.08.-13.10.00	4	5
PS3-5	08.08.-13.10.00	4	5
PS3-6	20.08.99	22, 24, 48, 49, 79, 81	6
PS4	26.11.99	3, 7.6, 10	9
PS5	26.11.99	3	3
PS6 (lab hatching exp.)	08.00	80	12
PS7 (plankton samples and temperature recording)			

To test this we studied the diapause termination of the *Daphnia* seed bank from Lake Constance, a large and deep lake in central Europe. The key questions are i) how large is the recruitment from resting stages, ii) when does it take place and iii) how important is the littoral zone as a nursery for pelagic zooplankton? To answer these questions we conducted hatching experiments in the laboratory under different abiotic conditions and followed the hatching success in *in-situ* emergence traps over two years. In addition field surveys to study temporal and spatial ephippia distribution in the sediments of Lake Constance.

Methods

Study site

Lake Constance is located on the northern fridge of the Alps (47°39'N), with a surface area of 472 km² and a maximum depth of 254 m (mean depth ~100 m). The littoral zone, estimated as the isobath area from 10 to 0 meters, covers 10 % of the total lake area. The seasonal dynamics of phytoplankton, zooplankton, and microbial populations have been studied in great detail during the last decades (Bäuerle & Gaedke, 1998). The trophic state changed dramatically during the last century: originally an oligotrophic lake, it underwent massive eutrophication in the 1950s, with a maximum at the end of the 1970s, followed by re-oligotrophication (Güde et al., 1998). Oxygen concentration in the deep water layer (sediment-water interface) was never anoxic during the last century and never fell below 4 mg/l during the last decade (Güde et al., 1998). In the 1950s, during eutrophication, *Daphnia galeata* invaded Lake Constance, where originally only *D. hyalina* had occurred (Muckle & Muckle-Rottengatter, 1976).

Laboratory emergence experiments

Core sampling: Using a research vessel, 12 cores (1m length, 6cm diameter) were taken from 80 m water depth in the "Konstanzer Trichter" of Upper Lake Constance (site PS6, Fig. 5.1). Immediately after sampling, the upper 10 to 15 cm of each core were pooled in a container. The container was stored cool and in the dark and brought to the lab within three hours. First, ephippia were isolated from the sediment using a 175 μm sieve, then cleaned with 0.45 μm filtered lake water in a 4°C walk-in chamber under red light. Isolated ephippia were stored at these conditions until subsequent experiments were conducted. Filtered water (0.45 μm) was used for all experiments.

The influence of three abiotic factors on hatching, i.e. temperature, photoperiod and light intensity was tested in different treatments. The temperature impact on hatching was tested under four constant temperatures (5°C, 8°C, 12°C and 20°C) plus two treatments with temperatures alternating every 12 hours between 5°C and 8°C (mean 6.5°C) and between 5°C and 12°C (mean 8.5°C), respectively. The alterating treatments were carried out because it is known that the temperatures can change dramatically within days in shallow lake areas, in particular on sunny days in early spring (Bäuerle, pers. com.). Temperature experiments were carried out at 24h constant light in temperature controlled water quenches, which were positioned in a 15°C walk-in chamber.

Day-length in southern Germany ranges from 7 h light in late December to 17 h light in June (Geller, 1989). Hence our light treatments were 8:16, 10:14, 12:12, 14:10 and 16:8 (light/dark). Additional to these treatments, we tested an "unnatural" photoperiod as a control 1:23 (light/dark), because in a completely dark treatment it was impossible to follow the continuous hatching success.

Light intensity decreases dramatically with water depth. Hence not only photoperiod and temperature, but also light intensity might influence hatching of ephippia in deeper water layers of a large lake. The light intensities tested were 100, 10, 9, 1.3 and 0.8 $\mu Em^{-2}s^{-1}$ with 24 h light. In February/March 0.8 $\mu Em^{-2}s^{-1}$ represents a depth of at least 35m (Häse, pers. com.). Day-length and light intensity experiments were carried out at 15°C.

Each treatment within each experiment had 6 replicates and each replicate contained up to 20 ephippia (each with 10 mL filtered lake water in a 50 mL jar). Every second day, hatchlings were counted with a dissecting microscope and water was changed. Allozyme analysis was carried out to investigate the taxonomic composition of the hatchlings in the temperature treatments. We used standard protocols (Hebert et al., 1989) using AAT loci as species specific marker (Wolf, 1987).
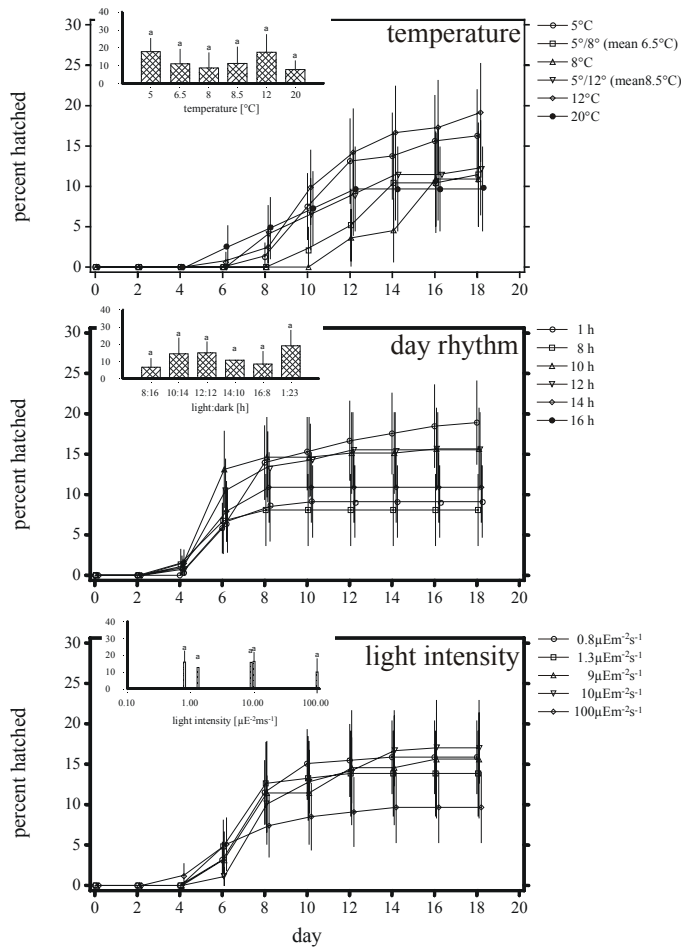
FIGURE 5.2 Results of the lab emergence experiments. Main graph show the time course of the emergence pattern (mean, +/- se) at the different abiotic factors (temperature = upper graph, day/night rhythm = middle graph, light intensity = lower graph). Inlets show the total percent of emergence for the treatments tested (mean, se). Different characters indicate significant ($P < 0.05$) differences between treatments (general linear model followed by Tukey's studentized range test with arc-sin transformed data).

In-situ emergence

sediment. The vertical sides of the frame were covered by 50µm mesh. The traps were deployed and checked by scuba, which assured a minimum of disturbance during deployment and sampling. To prevent introduction of animals from the water column during deployment, the open end of the trap was covered by 50 µm mesh just before it came in contact with the sediment. Then the mesh was removed and the trap was pushed into the sediment. We deployed sediment emergence traps in triplicates at three depths at

TABLE 5.2 Analysis of the influence of temperature on the time to emergence, using an accelerated failure time model with an underlying log-normal distribution (non-censored: 107; right-censored: 667). The maximized log-likelihood = -360.66.

variable [° C]	df	estimate (SE)	χ^2	p
			test statistical significance	
Intercept	1	4.267 (0.22)	383.12	0.0001
treat	5		7.51	0.19
5		-0.26 (0.22)	1.43	0.23
6.5		0.026 (0.25)	0.01	0.92
8		0.094 (0.25)	0.15	0.70
8.5		-0.089 (0.23)	0.15	0.70
12		-0.39 (0.21)	3.29	0.07
20	0	0	0	0
scale	1	1.154 (0.098)		

multiple comparisons of treatments based on accelerated failure time model:
comparisons: 5 vs. 6.5 : z=1.221 , p=0.111; 5 vs. 8 : z=1.559 , p=0.060; 5 vs. 8.5: z=0.802, p=0.211; 5 vs. 12: z=0.707, p=0.240; 6.5 vs. 8: z=0.261, p=0.397; 6.5 vs. 8.5: z=0.462, p=0.322; **6.5 vs. 12: z=1.798, p=0.036;** 8 vs. 8.5: z=0.755, p=0.225; **8 vs. 12 : z=2.156 , p=0.016;** 8.5 vs. 12 : z=1.439 , p=0.075

TABLE 5.3 Analysis of the influence of day/night-rhythm on the time of emergence, using an accelerated failure time model with an underlying log-normal distribution (non-censored: 159; right-censored: 1013). The maximized log-likelihood =-646.86.

variable (light [h])	df	estimate (SE)	χ^2	p
			test statistical significance	
Intercept	1	5.82 (0.37)	235.94	0.0001
treat	5		11.31	0.045
1	1	-0.86 (0.37)	5.36	0.02
8	1	0.12 (0.44)	0.07	0.79
10	1	-0.72 (0.38)	3.55	0.06
12	1	-0.68 (0.37)	3.38	0.07
14	1	-0.24 (0.39)	0.37	0.54
16	0	0	0	0
scale	1	2.27 (0.16)		

multiple comparisons of treatments based on accelerated failure time model:
comparisons: **1 vs. 8 : z=2.480 , p=0.007;** 1 vs. 10 : z=0.446, p=0.328; 1 vs. 12 : z=0.594 , p=0.276; **1 vs. 14 : z=1.849 , p=0.032;** 8 vs. 10 : **z=2.073 , p=0.019; 8 vs. 12 : z=2.033 , p=0.021;** 8 vs. 14 : z=0.862 , p=0.194; 10 vs. 12 : z=0.121 , p=0.452; 10 vs. 14 : z=1.380 , p=0.084; 12 vs. 14 : z=1.318 , p=0.094

TABLE 5.4 Analysis of the influence of light intensity on the time of emergence, using an accelerated failure time model with an underlying log-normal distribution (non-censored: 144; right-censored: 834). The maximized log-likelihood =-543.99

variable [μ Em²s⁻¹]	df	estimate (SE)	χ^2	p
			test statistical significance	
Intercept	1	5.095 (0.29)	307.95	0.001
treat	4		3.70	0.45
0.8	1	-0.47 (0.28)	2.84	0.09
1.3	1	-0.36 (0.30)	1.36	0.24
9	1	-0.43 (0.35)	1.52	0.22
10	1	-0.48 (0.27)	3.25	0.07
100	0	0	0	0
scale	1	1.79 (0.13)		

multiple comparisons of treatments based on accelerated failure time model:
comparisons: 0.8 vs. 1.3 : z=0.424 , p=0.334; 0.8 vs. 9 : z=0.124 , p=0.451; 0.8 vs. 10 : z=0.092 , p=0.463; 1.3 vs. 9 : z=0.217 , p=0.414; 1.3 vs. 10 : z=0.516 , p=0.303; 9 vs. 10 : z=0.194 , p=0.423

each of two stations (station 1, near to PS3-5: 2m, 5m and 20m; station 2, near to PS3-4: 1m, 8m and 11m, Fig. 5.1). On each sampling date the PE-bottles were exchanged by scuba
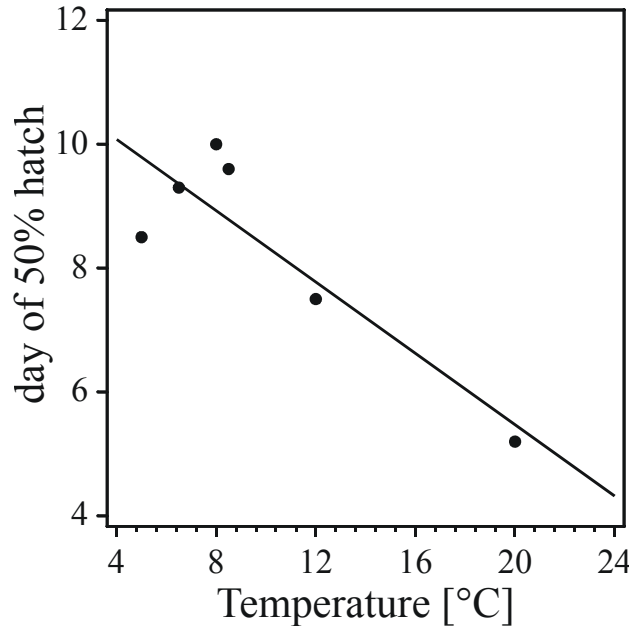
FIGURE 5.3 Time (day) of 50% hatch at the different temperatures tested. Results show a significant negative correlation (Pearson's r = -0.878, *P* = 0.022).

divers with new PE-bottles filled with 0.45μm filtered lake water. Bottles were transported to the lab within 2h and stored for subsequent analysis at 4°C. In 2000 the daphnids were removed and frozen by –80° for subsequent allozyme analysis. The remaining samples were preserved in 4% formalin which were counted in their entirety using a stereo-dissecting microscope. Due to the very small size of the hatchlings, allozyme analysis was difficult, therefore, in 2001 we cultured the animals for several days before subsequent allozyme analysis. Allozyme analysis was carried out to investigate the taxa composition of the hatchlings. We used the same methods as mentioned above. In the first year (2000) we started on March 21st and sampled weekly until April 30, afterwards fortnightly until August 2000. The mesh of some of the traps in the shallow sites ripped and were omitted from the analysis. For the year 2001 the mesh was replaced by metal plates. The preliminary results of 2000 allowed for a reduction of the sampling done in 2001 from March to May. The traps were deployed on March 5th 2001, and the first sampling started on March 13th.

  Temperature development in three different water depths (3.5 m, 7.5 m and 15 m) was recorded quasicontinously every 20 min using temperature loggers. Additional to the sediment near littoral temperature we recorded, the water temperatures of deeper strata (40 m, 50 m, 60 m, 80 m) using an automatic temperature logger. The logger is installed in the
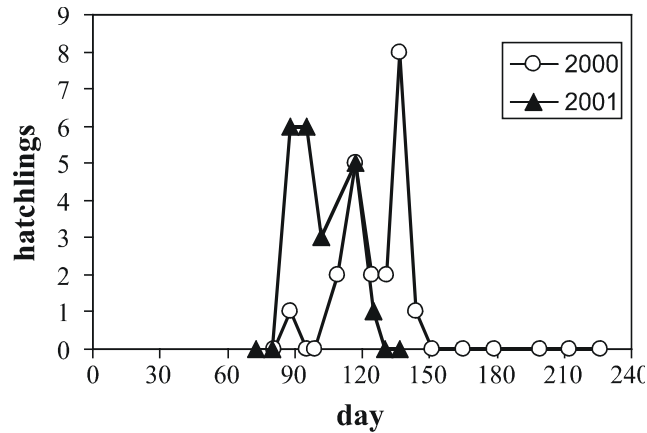
FIGURE 5.4 Time course of *in-situ* hatching experiments in 2000 and 2001. Hatchlings were pooled over all emergence traps.

fjord-like North-Western part of Upper Lake Constance (Überlinger See, PS7, Fig. 5.1) and is used for routine monitoring (Bäuerle & Gaedke, 1998). Temperature was recorded every 20 minutes (for more details see Ollinger, 1999).

Spatial and temporal ephippia distribution

For the estimation of the temporal and spatial distribution of ephippia we took 114 cores from different sites, depths and times. For details see Fig. 5.1 and Tab. 5.1. Four replicate cores from six shallow sites (0.5 to 3 m depth) of sampling station PS2 were taken from a small motor boat. Cores from 10 m and deeper (PS1, PS3-6) were taken with the research vessel 'Lauterborn'. All other cores (PS3-1 to PS3-5, PS4, PS5) were sampled by scuba divers.

For ephippia isolation, each core was cut lengthwise in half and photographed. Sediment samples were taken from different vertical layers, but for this study we used only the first two centimeters. To avoid transfering ephippia between different layers the outer edge (0.5 cm) of each section was not sampled. To isolate ephippia from the sediments we used a sugar floatation method (Onbe, 1978; Cromar & Williams, 1991). Ephippia were counted with a dissecting microscope (mag. 2 x 10). Because of the large number of cores and ephippia counted, we were not able to check for viable eggs in the ephippia.

Statistical analysis

Statistical analyses were performed using SAS (SAS Institute, 1988). Analysis of the laboratory hatching experiments: Absolute number of hatchlings were analyzed with variance analysis (general linear model) with treatment as class variable followed by Tukey's studentized range test. Percentage data were arc-sinus transformed prior to analysis. Different hatching times between treatments were analyzed using accelerated
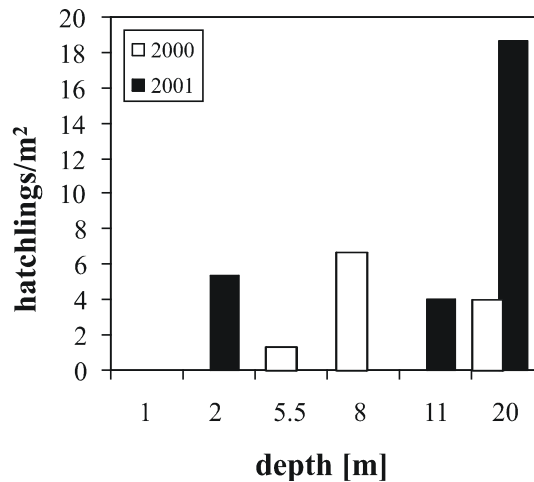
FIGURE 5.5 Number of hatchlings per square meter in the *in-situ* emergence experiments at different depths in 2000 and 2001. The 2000 data for 2 m and 1.5 m were removed (see text).

failure time models (Fox, 1993, PROC LIFEREG) rather than analysis of variance. There are two main problems associated with using variance analysis for "event time" data. First, data for traits like time until emergence are seldom normally distributed with normally distributed residuals, and transformations rarely help. Second, there is the problem of "censorship" – not all resting stages hatch. Variance analysis cannot account for this, which is especially a problem when treatment groups differ in their pattern of censorship. Accelerated failure time regression models take care of these problems by comparing "survivorship curves". In the case of emergence time, the "failure" time refers to the time of the first emergence, and "survivorship" cures describe resting stages that have not yet emerged ("failed", for more information see Fox, 1993 and Fox et al., 1995). We integrated treatment as a class variable in the analysis and assumed a log-normal distribution. Ephippial distribution patterns were analyzed using Pearson's correlation coefficient (r) and variance analysis (general linear model, SAS Institute, 1988).

Results

Laboratory emergence experiments

To investigate the hatching condition we tested several abiotic conditions (temperature, light intensity, day length). The mean hatching ratio ranged between 5 and 20% (Fig. 5.2).

Temperatures tested ranged from 5°C to 20°C and two treatments had alternating temperature regimes. Total number of hatchlings showed no significant differences between treatments ($F_5 = 1.03$, $P = 0.419$). Accelerated failure time models identified slightly

significant differences between the 12°C and the 5°C/8°C treatments (z = 1.798, *P* = 0.036) and between the 12°C and the 8°C treatments (z = 2.156, *P* = 0.016, Tab. 5.2). The time of 50% hatching success within each treatment was negatively correlated with incubation temperature (r = -0.878, *P* = 0.02, Q_{10} = 1.6, Fig. 5.3). Allozyme screening revealed that all hatchlings were *D. galeata* (AAT genotype "ff", Wolf, 1987).
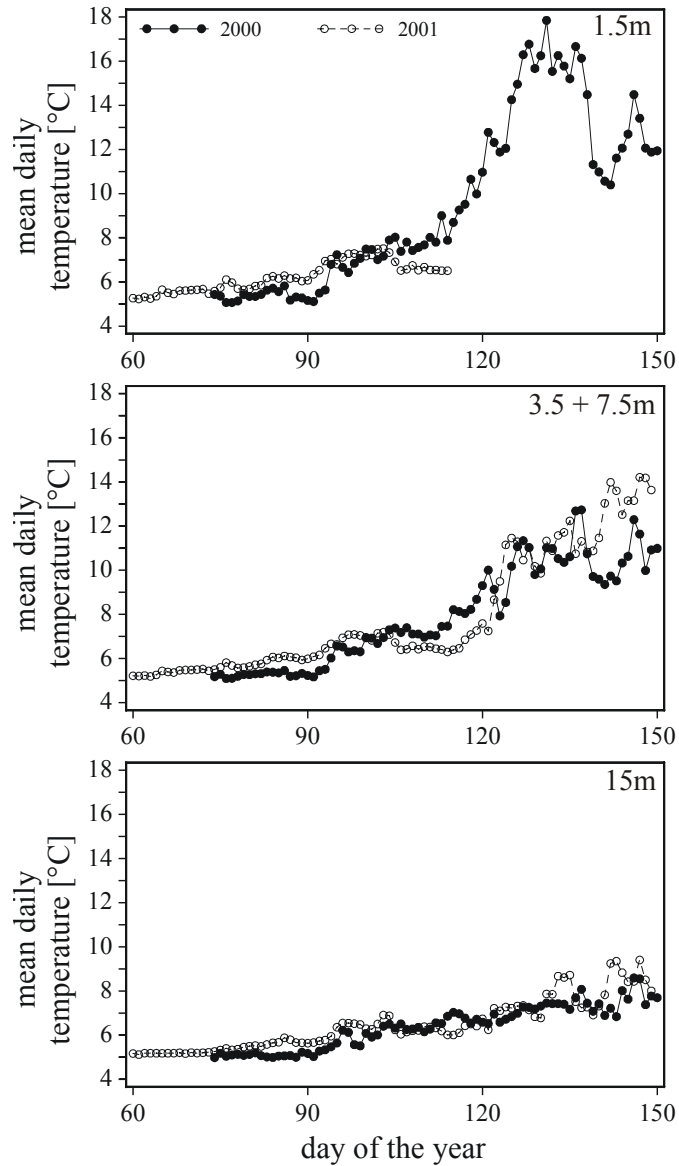

FIGURE 5.6 Average daily near bottom temperature in the littoral (PS3) at 1.5 m (upper graph), 3.5 m and 7.5 m (middle graph) and 15 m (lower graph).

Additional to natural day-lengths ranging from 8h to 16h light, a light/dark rhythm of 1:23 were tested. Total number of hatchlings showed slightly significant differences (F = 2.65, *P* = 0.044) between treatments, but Tukey's test did not identify one treatment to be significantly different from others. The total number of hatchlings (1 hour light excluded) showed a weak optimum function with an optimum between 10 and 12 hours. The accelerated failure time model indicated slightly significant differences between 1 h and 8 h (z = 2.48, p = 0.007) or 16 h respectively, and 8 h and 10 h (z = 2.07, *P* = 0.019) or 12 h (z = 2.033, *P* = 0.021) respectively, supporting an optimum between 10 to 12 h (Tab. 5.3).

Over the range of light intensities tested (0.8 to 100 $\mu Em^{-2}s^{-1}$), hatching was not influenced by light intensity neither regarding the total number of hatchlings (F_4 = 1.47, *P* = 0.246) nor the hatching time between treatments (Tab. 5.4).

In-situ emergence

Emergence of daphnids varied both within and between years. The overall hatching in the traps was very low. In each year we caught 21 individuals, representing a hatching rate of 1.5 daphnids $m^{-2}yr^{-1}$. In both years *Daphnia* hatched only from the end of March to May, with a slightly longer hatching period in 2000. In 2001, most of hatching occurred between the end of March and the end of April, whereas in 2000 most hatched between mid April to the end of May. Hatching, however, in both years started in the end of March. Daily hatching rates varied between 0.03 and 0.19 daphnids $m^{-2}d^{-1}$. 4 to 19 daphnids $m^{-2}yr^{-1}$
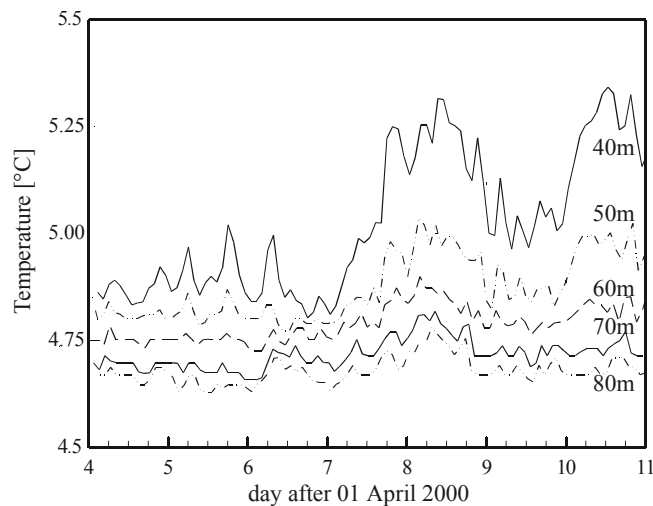

FIGURE 5.7 Average daily temperatures in the pelagial (PS7) at 40 m, 50 m, 60 m, 70 m and 80 m at the beginning of April 2000.

emerged in the deep traps (11 and 20 m), whereas only 0 to 6 daphnids hatched per square meter and year in the shallower traps (1 to 8 m).

Overall we conducted allozyme analysis for only 15 individuals because in 2000 most individuals were too small to give clear results. Moreover, in 2001, hatchling mortality was high in cultures. For the individuals screened, we found 68 % of them belonging to *Daphnia galeata*, 25 % to *D. hyalina* and 7 % to the hybrid.

Mean daily temperatures are shown for the period from March to end of May (Fig. 5.6), during the time when hatching was observed in the traps. In 2000, the temperature in the shallow littoral (3.5 and 7.5 m) was consistently around 5°C until the beginning of April and increased to 7°C in mid April followed by a strong increase above 10°C in May. In contrast, in 2001, temperatures increased continuously from March onwards, reaching temperatures greater than 6°C in the beginning of April and always maintaining temperatures higher than those observed in 2000. In April, the mean temperature was around 7°C and increased to more than 10°C in May. The pattern was similar at 15m, where the temperature was slightly higher in March and the beginning of April in 2001 (about 6°C) than in 2000. In deeper parts of the lake temperatures showed also a fluctuation within and between days (Fig. 5.7). In the beginning of April 2000, the temperature in 40 m to 80 m depth ranged between 4.6°C and 4.9°C but showed, particularly in the 40 m depth, a fluctuation of 0.15°C. From the beginning of April onwards temperatures increased slightly, especially of the 40 m and 50 m depth and reached values between 5°C and 5.25°C. In deeper parts the temperature increase was less pronounced and ranged between 0.1°C and 0.15°C.

Spatial and temporal ephippia distribution

Ephippia densities differed dramatically not only with sampling depth but also between sampling sites at the same time and between times at the same site. Overall, ephippia densities ranged from 0 to 100000 ephippia/m². The highest densities were found at the deepest stations, e.g. at depth greater than 80 m we found more than 30000 ephippia/m². In the littoral zone (less than 10 m, PS3-1 to PS3-4, PS4 and PS5), ephippia densities reached no more than 6000 ephippia/m². At depths between 20 and 80 m, densities ranged between 10000 and 60000 ephippia. Ephippia density increased significantly with increasing depth (r = 0.699, *P* = 0.0001, Fig. 5.8).

Ephippia showed patchy distribution on a large as well as on a small scale. Samples taken at the same depth (at 4 m) in a relatively small area (PS3-1b to PS3-5) and time interval (08.08.2000 to 13.10.2000) differed significantly between sites (ANOVA, F = 10.49, *P* = 0.0001) but not between sampling times (F = 0.15, *P* = 0.698). Using the photos taken from each core we estimated the thickness of the turbulent sediment layer of cores from the littoral zone (PS3-1 to PS3-5, PS4 and PS5 all taken at depths down to 10 m). This layer could be interpreted as an indicator for the disturbance of the sediment. Including turbulent layer depth as a covariate into the model yields a significant positive effect of the

turbulent sediment layer on ephippia density ($F_1 = 8.06$, $P = 0.02$), while sampling depth no longer contributed to the model significantly ($F_1 = 0.05$, $P = 0.828$, Tab. 5.5). This suggests that the observed differences between sites were particularly due to the thickness of the turbulent layer, which in turn suggests that sediment disturbance is an important factor influencing the ephippia distribution in the littoral zone.

TABLE 5.5 Results of the General Linear Model (site as class variable) testing for the effect of sample site (PS3, PS4 and PS5) and sampling depth (m) on ephippia density (first model). The second model includes turbulent sediment layer (cm); n = 62 cores.

Factor	df	F-value	*p*
depth	1	6.13	0.016
site	6	10.18	0.0001
turbulent sed. layer	1	5.66	0.021
depth	1	0.05	0.828
site	6	10.13	0.0001

The intensive sampling at PS2 allowed us an estimation of the temporal and vertical ephippia distribution in the littoral over one year (Fig. 5.9). From January to May, ephippia were very rare or absent in all layers of the littoral sediment. Only from May/June onwards did ephippia densities increase to around 10000 ephippia per square meter and most of them were found in the upper few millimeter of the sediment (Tab. 5.6). At a sediment depth > 2 cm, ephippia were rare or absent all year. The high abundance in the upper few millimeters persisted until the end of October when densities began to decrease again.

Discussion

Our investigation of *Daphnia* recruitment in a deep and large lake revealed the following results: 1) *in-situ* emergence showed that hatching occurred in early spring and was higher in traps from deeper sites (> 10 m) than in littoral sites (< 10 m). 2) Temporal and spatial ephippia distributions showed that ephippia density increased with water depth and, moreover, ephippia were rare in the littoral zone from January onwards, in particular during the hatching period up to the new production of ephippia. 3) temperature seems to be the main factor breaking diapause in this deep-lake *Daphnia* population. Finally, 4) we found considerable differences in the taxa composition between littoral and profundal hatchlings.

Although the number of *Daphnia* hatchlings in the 20 m *in-situ* emergence traps was comparable to the results of other studies (Caceres, 1998; Hairston et al., 2000), the observed hatching was low compared to the number of resting-eggs at this depth, e.g. we found more than 10000 ephippia m^{-2}, but only 20 hatchlings m^{-2}. Most likely, the *in-situ* traps changed the ephippia environment, e.g. chemical and physical factors, thereby
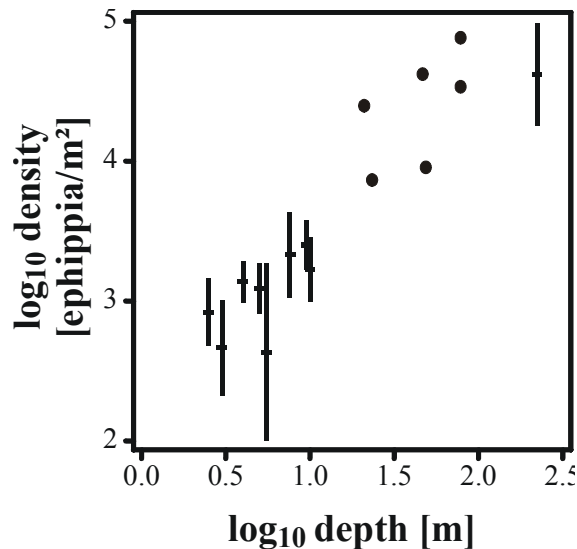
FIGURE 5.8 Number of ephippia (sum of 0-2 cm of sediment) per square meter at different depths (mean, +/- se). Ephippia density is positively correlated with lake depth (data $\log_{10}$ transformed, Pearson's r = 0.699, *p* = 0.0001).

reducing hatching induction and causing the underestimation of hatching rates. In particular the exclusion of currents with the subsequent mixing of upper sediment layers can reduce hatching substantially (Marcus, 1984; Marcus & Schmidt-Gengenbach, 1986; Kearns et al., 1996). Additionally, a mass development of mites, which are known to prey on daphnids (Wesenberg-Lund, 1939; Hirschmann, 1966), may have caused an underestimation of real hatching rates.

The strong differences in the taxa composition between littoral (25 % *D. hyalina*, 7 % hybrid and 68 % *D. galeata*) and profundal (100 % *D. galeata*) hatchlings indicate that the littoral might be more important for the recruitment of *D. hyalina* and the hybrid than for *D. galeata*. No *D. hyalina* hatchlings were found from profundal sediments of Lake Constance, neither from 80 m cores (this study) nor from sediments isolated from 200 m (Weider et al., 1997; Jankowski & Straile, in prep.). Such large differences in the observed distribution pattern between closely related taxa within one lake has never been observed before. This species specific distribution pattern suggests buoyancy differences between *D. hyalina* and *D. galeata* ephippia (Weider et al., 1997).

Temperature, rather than photoperiod and light intensity seems to be the most important hatching cue for *D. galeata* clones. The dependency of hatching time on temperature (Q_{10} = 1.6, Fig. 5.3) in *D. galeata* from our study was similar to that observed for *D. pulex* (Q_{10} = 1.8; calculated from Fig. 6 of Davison, 1969). Both our field and laboratory data suggest that hatching of the *Daphnia* population in Lake Constance can take place at low temperatures, i.e., when ever water temperatures exceed the storage

temperature (Fig. 5.2 and 4) of approximately 4 - 5°C which occurs *in-situ* from the end of March until May. This is supported by hatching experiments with daphnids from deep Mondsee. Also Herzig (1985) found in his experiments that with increasing temperature (2.5 to 4.5 and 2.5 to 6.5) for deep lake cladocerans, hatching starts when temperatures exceed the 4°C storage temperature. This suggests that hatching should also take place in deeper parts of the lake if there is a sufficient increase in temperature.

Our results suggest that emergence was largely independent from variations of day length and light intensity. This is in contrast to other studies which suggest that a light stimulus is required (Stross, 1969a; Stross, 1969b; Carvalho & Wolf, 1989) and that photoperiodic control is also involved (Stross, 1969b; Stross, 1971; Pfrender & Deng, 1998). For example, in some studies the hatching success was much lower in treatments incubated in the dark compared with light treatments at the same temperature (Stross, 1969a; Stross, 1969b; Carvalho & Wolf, 1989). However, Herzig (1985) found considerable hatching rates of *Leptodora* when in dark and cool conditions (2.5°C and 7.2°C). Furthermore, the length of photoperiod did not have a significant effect on the hatching success or development time of Lake Michigan *Bythotrephes* diapausing eggs (Yurista, 1997). Both investigations (Herzig, 1985; Yurista, 1997) were carried out with resting stages from deep lakes (> 60 m). Also Stross (1966, 1968) pointed out that "the absolute requirement for light persisted while the photoperiod effect disappeared after incubation at 4°C in the dark". This supports our own results; we could not find any significant response to the day rhythm offered (Fig. 5.2, Tab. 5.3). Investigations on the light intensity required for diapause termination are rare. Our results suggest that induction of hatching is broadly independent of the light intensity offered and can occur at very low intensities. Also, Davison (1969) found for *Daphnia pulex* that "the effect being independent of the intensity in the range examined" (3 to 36 foot-candle = 32.3 to 387.4 lx). This suggests that the physiological temperature dependence seems to be the most important factor for the termination of diapause in deep lake cladocerans. When temperatures in early spring exceed the storage temperature (~4°C) and ephippia are exposed to a light stimulus, hatching will occur independent of water depth (= light intensity) and day length.

TABLE 5.6 Results of the General Linear Model (site and layer as class variable) testing for the effect of sampling site, sampling layer (sediment depth, cm) and sampling date on ephippia density at PS2; n=54 cores.

Factor	df	F-value	*p*
site	5	8.06	0.0001
sampling layer	2	13.74	0.0001
date	1	5.50	0.019

Indeed, this was the pattern observed in the *in-situ* field traps (Fig. 5.4 and 5). Hatching already occurred during a relative short time period early in the season and differences in the hatching times between years match the respective differences in vernal warming (Fig. 5.6). In 2001, when temperatures were higher, hatching occurred earlier than in 2000. A rather short spring hatching period was also found in other lakes (Wolf & Carvalho, 1989; Caceres, 1998; Hairston et al., 2000). Increased hatching rates with
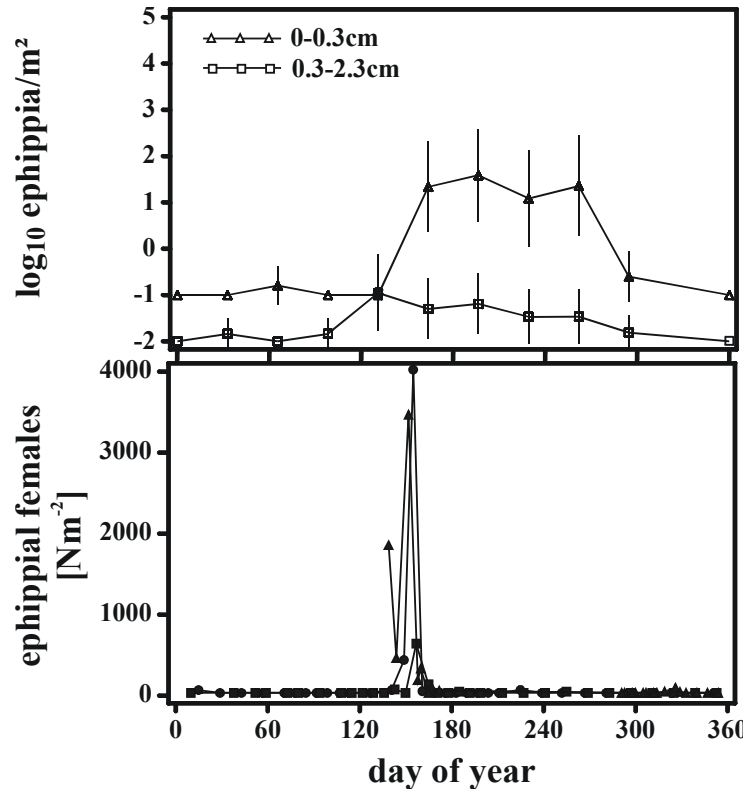

FIGURE 5.9 Time course of ephippia density in littoral sediments at PS2 in 1999 (mean, +/- se, upper graph) and timing of ephippia production (lower graph) during 1999 to 2001 in the pelagial (PS7). Data on ephippia production were taken from Jankowski & Straile (in prep.).

temperature were reported from Mondsee (Austria) and Schöhsee (Germany; Herzig, 1985; Wolf & Carvalho, 1989). The temperature dynamics in deeper water layers down to 50 – 80 m may be sufficient to induce hatching. A temperature increase in Lake Constance beyond 0.5°C was measured at 50 m depth (Fig. 5.7) and in some years 6°C were reached in up to 80 m meter depth during March (Bäuerle et al., 1998). This suggests that hatching can be induced at deeper parts of the lake. As the abundance of ephippia at deeper depth is much

higher than in the littoral zone in Lake Constance, and in other lakes as well (Carvalho & Wolf, 1989), it is possible that the main recruitment is occurring at depth greater than 20 m. This is concordant with the observations of Herzig (1985), who found considerable hatching of the cladocerans *Daphnia*, *Bythotrephes* and *Leptodora* at depths > 50 m.

The fact that the main recruitment does not occur in the littoral zone but in deeper parts, is supported by the temporal ephippia distribution. Our detailed analysis in littoral sediments suggests that ephippia were removed from littoral sediments in autumn and presumably accumulated in deeper lake regions (Fig. 5.9), possibly through sediment movements driven by wind events and partial water cooling effects (Wessels, 1999 Kaminski et al., 1998; Schröder pers. com.). Sediment movements are known to influence the egg deposition dramatically, as revealed for coregonid eggs (Ventling-Schwank & Livingstone, 1994). Ephippia were very rare or absent from the shallow littoral zone until new ephippia were produced in May/June shortly after the *Daphnia* spring peak. Ephippia were found during this time nearly exclusively in the upper strata, suggesting that these were newly produced. As ephippia need a dormant phase (low temperatures, darkness) before they can hatch (Moritz, 1987; Carvalho & Wolf, 1989; Weider et al., 1997; Cousyn & DeMeester, 1998), these newly produced ephippia could not have contributed to the plankton population of the same year.

In summary, we suggest that recruitment of *Daphnia galeata* in deep Lake Constance is higher from depth greater than 20 m than it is from the littoral zone (< 10 m). Even if the hatching rate is reduced in depths greater than 20 m, the much higher ephippia density should result in a hatching rate per area equivalent or greater than that in the littoral zone.

With this conclusion, the potential recruitment of daphnids from sediments can be estimated. As shown, in Lake Constance temperatures increased after winter circulation to as much as 5°C in depths of 50 m and day-length ranged during this period between 10 and 12 h (Geller, 1989). 30 % of the lake area is between 0 to 50 m deep, whereas only 10 % is between 0 - 10 m. Ephippia densities ranged from 6000 to more than 30000 ephippia m^{-2} at depth of 20 and 50 m and 0 to 10000 ephippia m^{-2} at depth of 0 and 10 m. Only *D. hyalina* overwintered in winter 1999/2000 and 2000/2001 and their minimum population density prior to their spring development was between 50 and 500 individuals m^{-2}. Assuming that hatching cues are available down to 50 m, a mean ephippia density between 10000 - 20000 ephippia/m² for 10 - 50m water depths, and complete hatching, we can expect a recruitment of 7000 - 14000 daphnids m^{-2} from the 10 - 50 m isobath area (= 20 % of the whole lake). For the whole of Lake Constance this will result in 1400 - 2800 daphnids m^{-2}. Of course not all ephippia will hatch in a specific year due to insuficient cues, survivability, and/or possibly bet-hedging strategies. However, even with a hatching as low as 10 %, abundance of *D. galeata* might be of a similar order of magnitude as the abundance of overwintering *D. hyalina*. Hence, both strategies, i.e. the overwintering of *D. hyalina* and the resting egg

production of *D. galeata*, have been roughly equally successful in providing a start population for the following spring.

In conclusion, this study questioned the role of the littoral zone as a nursery for deep lake daphnids. Due to the low number of ephippia in littoral sediments, overall recruitment from deeper parts are probably more important even with a reduced hatching success although species-specific differences might exist.

Chapter 6

CONCLUDING REMARKS AND PERSPECTIVES

*Biological evolution is a change over time
of the proportions of individual organisms
differing genetically in one or more traits*
DJ FUTUYMA 1990

Daphnia plays an important role in aquatic ecosystems (Carpenter & Kitchell, 1996) and due to their life-cycle they offer unique possibilities for research on evolutionary and ecological questions (Barker & Hebert, 1985; Hairston et al., 1999; Dudycha & Tessier, 1999; Innes & Singleton, 2000; Straile, 2002). This study focused on the ecological and evolutionary role of sexual reproduction in large lake Daphnia population. Sexual reproduction and the subsequent production of diapause stages plays an important role not only in temporary pond Daphnia populations (Lynch, 1983; Innes, 1997), but also in permanent lake populations (Caceres, 1997; Caceres, 1998). For pond daphnids resting-egg production was particularly believed to prevent population's extinction due to drying or freezing. In lakes, ephippia production has been shown to prevent the extinction of Daphnia species from lakes and hence to enable long-term coexistence of Daphnia species (Caceres, 1997).

To fully understand the ecological and evolutionary consequences of sexual reproduction in a *Daphnia* hybrid complex, there is not only a need to analyse sexual reproductive behaviour, but also the fate of sexual propagules, i.e. the fate and deposition of resting eggs and the hatching of *Daphnia*, in relation to the genetic structure of the hybrid complex. The previous chapters in this thesis have built the first comprehensive study which covered all these aspects.

Sexual reproduction of *Daphnia galeata*, *Daphnia hyalina* and *D. hyalina x galeata* was studied intensively *in-situ* (chapters 2 and 3) and in a mesocosm experiment (chapter 3). These studies revealed strong differences between the taxa in frequency, sex ratio as well as timing of sexual reproduction. Hence, *D. hyalina* and *D. galeata* do not only differ in antipredator behaviour, i.e. diel vertical migration (Stich & Lampert, 1981), cyclomorphosis (Stich, 1989), energetic requirements (Geller, 1989) and parasite prevalence (Bittner, 2001; Bittner et al., 2002), but also strongly in their sexual reproductive behaviour and their allocation into sexual reproduction. The latter difference might explain also differences in genetic diversity and differences in seasonal genetic differentiation between the two species. Additionally, sexual reproduction might be a key process for the long-term persistence and clonal structure of *Daphnia galeata* in Lake Constance (see below).

Large lake daphnid populations were generally considered to consist of 'general-purpose genotypes' occurring in permanent water bodies, overwintering in the water body and showing low genetic differentiation (reviewed in Mort & Wolf, 1986). Most studies on the fitness of large lake daphnids focused on the parthenogenetic reproductive stage and their interplay with predation and competition. This study is one of the first including sexual reproduction as a fitness component (see also Caceres, 1997; Caceres, 1998) and showed its significance also for large lake daphnid populations. The parental species, *Daphnia hyalina* and *D. galeata*, showed strong differences in their allocation to sexual propagules, seasonal occurrence and genetic architecture and suggest different strategies for

their persistence. *D. hyalina* showed the typical strategy of large lake daphnids (Wolf, 1987; Spaak, 1996), whereas the strategy of *D. galeata* resembles to some extent temporary pond populations (Lynch, 1983). Chapter 2 shows that *D. galeata* seemed to rely more strongly on resting stages than *D. hyalina* to get through severe winter conditions, while for *D. hyalina* overwintering in the plankton seems to be more important. The same pattern of sexual reproductive timing and frequency was observed within three stud years (chapter 3). The higher allocation to sexual propagules of *D. galeata* is also supported by a mesocosm study (chapter 3), carried out to investigate sexual reproduction in multi-species enclosures under natural conditions. Differences in genetic diversity between *D. hyalina* and *D. galeata* suggest that the *D. hyalina* population consists of few but generalist genotypes which occur throughout the season ('general-purpose genotypes'), whereas the *D. galeata* population was more diverse and showed a strong seasonal differentiation ('temporary specialised genotypes', Lynch, 1983). This life-cycle characteristic of *D. galeata* is also supported by the observed species specific differences in the emergence pattern (chapter 5). Recruitment from resting eggs was more frequent for *D. galeata* and suggests that it is, at least in recent times, more important for *D. galeata* to rebuild next years generations. But there seem to be differences between *D. hyalina* and *D. galeata* in the importance of different lake areas for emergence. For *D. hyalina* the littoral seems to be more important, whereas the main recruitment of *D. galeata* probably occurs in deeper lake areas (> 20 to < 50 m water depth; chapter 4).

The short cycle of *D. galeata* from hatching in February/March to sexual reproduction in June (chapter 2 and 3) in combination with non-overwintering (chapter 2) suggests that clones which were successful in summer did not contribute to the next spring generation. They failed either to produce resting stages and/or failed to survive winter conditions. This has enormous consequences for their long-term persistence, possible adaptations and hence evolutionary trajectories. Previous studies indicated a shift in genetic composition of *D. galeata* due to changes in the trophic state of Lake Constance (Weider et al., 1997) and suggest adaptation to summer blooms of toxic cyanobacteria (Hairston et al., 1999). This thesis offers evidence that the possibility for the last suggestion is relatively weak, due to the timing of sexual reproduction of *D. galeata* in early summer, i.e., before the cyanobacteria have reached maximum abundance. Assuming a non-overwintering strategy of *D. galeata*, as revealed in chapter 2 suggests that clones with an optimal timing of sexual activity have advantages in contrast to clones adapted to summer cyanobacteria blooms, because latter group fails to produce resting eggs and hence do not contribute to the next spring population. In contrast, clones with the optimal timing of sexual activity and subsequent resting egg production can establish a next year generation. Hence, there might be a strong selection pressure on the correct timing of sexual activity in *D. galeata*. This indicates that adaptation to the pronounced re-oligotrophication effect on summer food availability is more difficult or only restricted possible and, hence, suggests a disruptive process in Lake Constance daphnid population. *D. hyalina* might adapt to higher growth at

lower food levels, i.e. overwintering and rare ephippia production, whereas *D. galeata* might adapt to higher growth at spring benign food conditions, i.e. ephippia production and non-overwintering.

This assumed disruptive ecological selection process (Schluter, 2000) between the parental species might also explain the relatively low success of the hybrids in Lake Constance compared to other lakes (Wolf, 1987; Giessler, 1987; Spaak, 1996; Spaak et al., 2000). The relatively low contribution of the hybrids to total daphnid abundance, not only during the period of the present study (chapter 2), but also at the end of the 1980s (Weider & Stich, 1992), suggests that the hybrids might have some difficulties to adapt as well as the parentals to the environmental conditions. Nevertheless, during some times, i.e. late summer, and/or special environmental conditions, i.e. during the enclosure experiment, hybrids reached population growth rates higher than the parentals, supporting the "temporary hybrid superior model" (Spaak & Hoekstra, 1995). Hence, the long-term success of hybrids might depend on the special ecological conditions, i.e. low fish predation, as revealed by the mesocosm study.

A noticeable point was the observation that hybrids hatched only from sediment layers of the 1970s (chapter 4), during the period of maximum eutrophication. This is the same period for which Einsle (1983) observed an increasing morphological variability among parthenogenetical females. This may indicate a high(er) hybridisation frequency during the more eutrophic phase in the 1970s. However, sexual reproduction of the hybrids has also been found in recent years (chapter 2 and 3). The fact that they reproduce sexually during spring (as *D. galeata*) and autumn (as *D. hyalina*) combined with the presence of backcrosses suggest that gene flow might be possible. In contrast, the genetic data obtained indicate relatively distinct populations, but further investigations using more species specific marker are needed to confirm this assumption.

Egg-bank studies for investigating population history is a new and innovative field in ecological and evolutionary research ("resurrection ecology" Kerfoot et al., 1999). Several new outcomes arose using the combined approach of life-history and genetic studies of ephippial hatchlings isolated from different times of datable sediments (Jeppesen et al., 2001). Using neutral genetic markers and adaptive quantitative traits of ephippial hatchlings suggest rapid local adaptation to changes in predation pressure (Cousyn et al., 2001) and toxic cyanobacteria (Hairston et al., 1999). Furthermore, the occurrence and abundance of resting stages were used to investigate past population abundances, species invasions and species recoveries following the restoration of lakes (Verschuren & Marnell, 1997). The size of daphnid ephippia was used to infer fish predation pressure in shallow lakes and to reconstruct past fish predation pressure (Verschuren & Marnell, 1997; Jeppesen et al., 2002). Genetic analysis of resting stages (Duffy et al., 2000; Limburg & Weider, 2002) and of hatchlings from resting stages (Weider et al., 1997; Hairston et al., 1999) revealed information about the genetic composition of past populations. The detailed

comparison of the *Daphnia* egg-bank data, sexual behaviour and the historical plankton data set of the daphnids presented in this thesis (chapter 4) revealed the first time how reliable egg-banks for such studies are. Chapter 4 showed the potentials for the reconstruction of the population history of *D. galeata* over the last decades, but showed also the limitations for reconstructing the species composition, in particular the *D. hyalina* and hybrid history. The study revealed that, at least, two important conditions must be satisfied: 1) species must not differ in their sexual activity, i.e. ephippia production, and 2) they must also not show differences in ephippia buoyancy, i.e. different stock grounds of ephippia. In the Lake Constance daphnid hybrid complex both conditions are not satisfied. Only for one part of the population and in a specific time frame the ephippial sediment record revealed reliable data.

Hence, future research using *Daphnia* ephippia as a palaeolimnological tool should take into account that there might be species specific differences in sexual activity and ephippia buoyancy within a lake, which could influence the outcome of such studies. Furthermore, sexual activity and buoyancy might change during a rather limited period of time especially when the lake is subjected to environmental changing conditions. Therefore, ephippia illustrate not only a simple effigy of the plankton population, but it has to take into account that sexual reproduction, i.e. ephippia production, is an important fitness component and, hence, should be subjected to strong selection pressure.

The received picture highlights the importance of sexual reproduction, diapause and hybridization, linkage of the seasonal pattern as well as the long-term development for the understanding of the processes within large lake *Daphnia* populations. From these results several new questions and further research possibilities arise which will be partly outlined in the following paragraphs.

Analyzing possible consequence of a changing reproductive activity in *D. hyalina*

Based on the long-term survey from Lake Constance and the ephippial record it is suggested that during the last century *D. hyalina* has shifted to a lower allocation to sexual reproduction (chapter 4). This would suggest that the genetic diversity of daphnid populations in large lakes decreases with eutrophications. By analysing the molecular genetics of the resting-eggs (Duffy et al., 2000; Limburg & Weider, 2002; Schwenk, pers com.) from the beginning of the century in addition to recent females we would be able to test this hypothesis.

Going back to the hybrids origin

A combined approach using resurrection ecology (Kerfoot et al., 1999), population genetic as well as quantitative genetic methods (Lynch et al., 1999; Pfrender & Lynch, 2000; Pfrender et al., 2000) will enable us to go back to the origin of the hybrid population.

Hybrid systems are often viewed as natural experiments that serve as windows on evolutionary processes (Harrison, 1993; Arnold, 1997). In particular, the highly recombinant genotypes found in hybrid systems offer a unique opportunity to dissect the architecture of gene flow between species. The outcome of such hybridization processes depends at least in part on environmental conditions and, of course, on the relative fitness of the parental species as well as on the new arisen lineages. But not all new lineages might be successful. Beside the fact that new genotypes arise, also some branches break off, so probably only a few patterns emerge and remain constant. Although there are many studies on the evolution and ecology of interspecific hybridization, only few extend over a long enough time to track evolutionary changes or assess the ultimate outcome of interspecific hybridization (Carney et al., 2000). Using the combined approach of population and quantitative genetics will allow us to reconstruct the level of reproductive isolation, the impact of gene flow, genetic drift and selection on ecological relevant traits through time.

Predicting the future of *D. galeata* in Lake Constance

If it is true that *D. galeata* could not as good as *D. hyalina* cope with lower nutrient supplies, then arise consequently the question of the future of *D. galeata* in Lake Constance. Phosphorus concentrations in Lake Constance still decrease suggesting that both food quantity and quality will further decrease. As a consequence, successful overwintering in the plankton will be less likely. How will the *Daphnia galeata* population adapt to these environmental changes? This offers the unique opportunity to study the microevolutionary response of a daphnid population to long-term environmental changes.

Past investigations showed that *D. galeata* invaded Lake Constance with increasing eutrophication in the 1950s (Muckle & Muckle-Rottengatter, 1976), built up considerable abundance and hybridised with the native *D. hyalina* (Weider & Stich, 1992). The long-term population development (Straile & Geller, 1998) suggests that with increasing re-oligotrophication from the 1980s onwards the contribution of *D. galeata* to overwintering plankton females has decreased. Moreover, it seems that overwintering is very rare or absent, respectively, in recent times (chapter 2). This suggests that in recent times *D. galeata* depends more on the allocation to sexual reproduction than in the past. Additionally, the point that *D. galeata* reproduces sexually very early in the year, shortly after hatching from resting eggs, suggests that adapting to further reduced food supply will be complicated. If further re-oligotrophication affects the spring phytoplankton bloom, this in turn might affect *D. galeata*. Due to the hybridization of *D. galeata* and *D. hyalina* and the subsequent backcrossing of the hybrids with the parentals (chapter 2), even if it is considered to be low, it is possible that at least genes of *D. galeata* will persist in the Lake Constance *Daphnia* population (Giessler et al., 1999). Or in turn, gene flow from *D. hyalina* to *D. galeata* might influence the persistence of *D. galeata* in Lake Constance. If it is possible due to backcrossing and introgession that adaptive traits, like adaptation to lower

food supplies, can be transferred, it may influence the persistence of *D. galeata* in Lake Constance.

One possibility to reach a more detailed view of the future development of *D. galeata* might be continuing to investigate their egg-bank. Due to a higher allocation to sexual reproduction the genetic diversity might have increased during the last years, which would indicate the more important role of resting-egg production. In addition, the level of introgression and a possible association to the transfer of adaptive traits could be estimated using a combined approach of molecular genetic and quantitative genetic methods of resting-eggs,, ephippial hatchlings isolated from sediments and recent plankton females of the parental species and the hybrids.

To conclude, over the last years more and more attention has been focused on aspects of sexual reproduction, diapause and hybridisation for the understanding of seasonal and long-term development of large lake plankton populations. However, there are still many questions unsolved. For example, the role of benthic-pelagic coupling of resting-egg production for nutrient flows in ecosystems or the importance for community interactions due to diapause of major food resource. Also the significance of long-term environmental changes on the hybridisation process in sympatric populations is not well understood. A combined approach investigating natural population and laboratory populations, using limnological as well as population and quantitative genetic methods might improve our insights into the complex network of sexual reproduction, diapause and hybridisation.

ABSTRACT

This thesis deals with the interplay between sexual reproduction, hybridization and diapause and their ecological and evolutionary consequences for large lake *Daphnia* populations. Although the *Daphnia* population of Lake Constance was intensively investigated during the last century this thesis showed the first time a detailed analysis of temporal species specific differences in allocation to sexual reproduction and the importance of diapause. The results presented showed that the inclusion of these important life-history components is not only essential for the understanding for temporary pond, but also for permanent lake *Daphnia* populations. Therefore, this study cover the whole life-cycle of daphnids. This thesis starts with the parthenogenetic phase, continues with sexual reproduction and diapause and ends with the recruitment of new parthenogenetical females.

Seasonal dynamics of the abundance, sexual reproduction and genetic architecture in a *Daphnia hyalina-galeata* hybrid complex were studied in the large and deep Lake Constance (chapter 2). We found evidence for the occurrence of first and second order hybridization. The study revealed strong differences between the parental species regarding not only their seasonal dynamics, genetic architecture and diversity, but also their sexual reproductive behaviour. The overwintering *D. hyalina* showed low genetic diversity, no genetic differentiation during the season, and reproduced sexually in autumn, whereas *D. galeata* reached higher levels of genetic diversity, reproduced sexually in early summer, and exhibited changes in genetic structure during the season, but was only present from spring to autumn. Within all variables studied, F1 and F2 hybrids showed an intermediate pattern, whereas proposed backcross hybrids were more similar to their respective parentals. These differences in phenotype as well as significant differences in pairwise F_{st} values between parentals suggest that gene flow seems to be relative low in the Lake Constance hybrid system. The study presents evidence for unidirectional introgression by backcrossing from *D. galeata* to *D. hyalina* and found a decrease in at least one of the proposed introgressed alleles in the *hyalina*-backcross with ongoing season. The findings suggest allochronic differentiation within this hybrid population and different microevolutionary trajectories of the parental species, which will be discussed in light of the ongoing reoligotrophication process of Lake Constance.

Seasonal dynamics in allocation to and timing of sexual reproduction were studied over three years in a *Daphnia hyalina-galeata* hybrid population of large and deep Lake Constance (chapter 3). These results were compared to a multispecies mesocosm experiment carried out under natural conditions. In all three years we observed two distinct periods of sexual activity. In early summer, no D. hyalina sexual females were found and 90 % of the sexual females and males were D. galeata. In autumn, however, no *D. galeata* sexual females were found and more than 60 % of the sexual females and males were D. hyalina. The D. hyalina-galeata hybrid were sexual during both periods. Despite these

seasonal differences all three taxa produced ephippia and males during an enclosure experiment conducted in July 2001, i.e., a time when no sexual activity was observed in the lake. The parentals showed in the lake as well as in the enclosures similar allocation to sexual females and males. In contrast, the hybrid showed a much higher allocation to sexual females during the enclosure experiment. In the field, *D. galeata* contributed the most to ephippia production. *D. hyalina* ephippia production was relative sparse, and the hybrid showed, at least in the lake population, an intermediate allocation. Our findings indicate a species x environment interaction for the induction of and allocation to sexual activity. This will be discussed in the light of different overwintering strategies and their consequences for the maintenance of genetic variation within populations.

Chapter 4 deals with the egg-bank of the *Daphnia* population. Resting eggs of planktonic organisms from datable sediment cores are increasingly used to reconstruct historical information on the abundance, size, genetic composition and microevolution of planktonic organisms. All these studies rely on the up to now mostly untested assumption that the resting egg bank in the sediment will indeed allow an accurate reconstruction of past populations. Here the study tests the performance of the egg bank to reconstruct historical data of the *Daphnia* population of Lake Constance, which has been thoroughly investigated throughout the last century. The study shows that it is possible to reproduce variability in abundance, size, and genetic composition of *Daphnia galeata* within a period of approximately two decades. Furthermore, resting egg data allowed to reconstruct the timing of the invasion of *Daphnia galeata* into Lake Constance. However, the egg bank failed to reconstruct a) the dynamics of the native *Daphnia* species of Lake Constance, *D. hyalina*, and b) the relative importance of the two *Daphnia* species. The study present evidence that the latter is because the two species differ in the relative importance and timing of sexual reproductive activity and in the buoyancy of ephippia. The failure to reconstruct the long-term dynamics of *D. hyalina* in the lake is most probably due to a change in sexual activity and possibly also of ephippia buoyancy in the course of eutrophication.

Chapter 5 deals with species specific differences in recruitment. The recruitment from resting-stages is a common feature of many planktic organisms in temporary ponds as well as in large lakes. Since the cues for breaking diapause, i.e., increases in temperature and light intensity, decrease with lake depth, we hypothesized that the littoral zone plays an important role in the recruitment of zooplankton in large lake populations. We investigated the recruitment of *Daphnia hyalina-galeata* in the large lake, Lake Constance. In laboratory, we examined emergence under 6 different temperature regimes, 5 different light intensity regimes and 6 different day-length regimes and compared these results with a 2-year survey of *in-situ* emergence patterns using emergence traps placed at varying depths. Additionally, we investigated the temporal and spatial ephippia distribution in Lake Constance using 114 core samples taken regularly from January and December and from depths of 1m to 220 m. The results from both our laboratory aand the *in-situ* emergence

experiments indicate that emergence begins when temperatures exceed storage temperature (in Lake Constance this is between 4°C to 5°C), was restricted to a short period in February/March and was higher in deeper traps. Neither light intensity nor day-length had a significant effect on total emergence or on timing of emergence in the lab. In situ, we found no *D. hyalina* hatching from profundal sediments, but *D. hyalina* made up to 25% of littoral hatchlings. Ephippia densities increased with depth. In littoral sediments, ephippia were only found in the time period between ephippia production in early summer and late autumn. From these findings we conclude that the littoral might be more important for the recruitment of *D. hyalina*, however, the main recruitment of *D. galeata* seems to take place in rather deeper strata.

The presented studies revealed evidence not only for the importance of sexual activity for species specific differences in the seasonal pattern and seasonal genetic architecture, but also for species specific differences in the deposition of and emergence from resting stages. Furthermore, the results revealed evidence that sexual activity might not be constant, but changed over time, possibly influenced by eutrophication and competition. The received picture highlights the importance of sexual reproduction, diapause and hybridization and the linkage of the seasonal pattern and long-term development for the understanding of the processes within large lake *Daphnia* populations.

ZUSAMMENFASSUNG

In dieser Arbeit wird das Wechselspiel zwischen sexueller Reproduktion, Hybridisierung und Diapause und die daraus folgenden ökologischen und evolutiven Konsequenzen für die *Daphnia*-Populationen von großen Seen behandelt. Obwohl die *Daphnia*-Population des Bodensees im letzten Jahrhundert intensiv untersucht wurde, zeigt diese Arbeit erstmals eine detaillierte Analyse der zeitlichen artspezifischen Unterschiede in die Allokation in sexueller Reproduktion und die Bedeutung der Diapause. Die Ergebnisse zeigen, dass die Berücksichtigung dieser life-history Komponenten nicht nur für das Verstehen von Populationen aus temporären Gewässern wichtig ist, sondern auch für Daphnien Populationen von permanenten Seen. In der vorliegenden Arbeit wurde deshalb der gesamte Lebenszyklus von Daphnien, beginnend mit der parthenogenetischen Phase, gefolgt von sexueller Reproduktion und Diapause und bis hin zur Rekrutierung neuer parthenogenetischer Weibchen untersucht.

Die saisonale Dynamik der Abundanz, der sexuellen Reproduktion und der genetischen Architektur des *Daphnia hyalina-galeata* Hybridkomplexes wurde im Bodensee untersucht (Kapitel 2). Die Ergebnisse deuten daraufhin, dass Hybridisierung des ersten und zweiten Grades vorkommen kann. Es zeigten sich große artspezifische Unterschiede nicht nur in der saisonalen Dynamik, der genetischen Architektur und Diversität, sondern auch im sexuelle Verhalten. Die überwinternde *D. hyalina* Population zeigte eine geringe Diversität, keine saisonale genetische Differenzierung und sexuelle Reproduktion im Herbst, wohingegen *D. galeata* große genetische Diversität erreichte, sexuell im Frühsommer reproduziert, beträchtliche Veränderungen in der saisonalen genetischen Struktur aufwies, aber nur vom Frühjahr bis zum Herbst vorkam. In allen Variablen die untersucht wurden zeigten die F1 und F2 Hybride ein intermediäres Muster, wohingegen die Rückkreuzungshybride ihren Elternarten ähnlicher waren. Diese phänotypischen Unterschiede zusammen mit den signifikanten Unterschieden in den paarweisen F_{st}-Werten zwischen den Elternarten, deuten darauf hin, dass in dem Bodensee-Hybridkomplex Genfluss relativ gering ist. Es ergaben sich Hinweise für einseitige Introgression via Rückkreuzung von *D. galeata* zu *D. hyalina* und zeigt über die Saison eine Abnahme für mindestens eines dieser angenommenen introgressierten Allele in der *hyalina*-Rückkreuzungspopulation. Die Ergebnisse deuten auf eine allochronische Differenzierung innerhalb des Hybridkomplexes und unterschiedliche mikroevolutive Bahnen der Elternarten hin, welche im Hinblick auf die weitergehende Reoligotrophierung des Bodensees diskutiert werden.

Über einen Zeitraum von drei Jahren wurde die saisonale Dynamik der Allokation in und das zeitliche Auftreten von sexueller Reproduktion innerhalb des *Daphnia hyalina-galeata* Hybridkomplexes des Bodensees untersucht (Kapitel 3). Diese Ergebnisse wurden verglichen mit einem unter natürlichen Bedingungen durchgeführten Mehrarten-

Mesokosmosexperiment. In allen drei Jahren waren zwei distinkte Perioden der sexuellen Reproduktion zu beobachten. Im Frühsommer wurden kein sexuelles Weibchen von *D. hyalina* gefunden, während 90 % der sexuellen Weibchen und Männchen *D. galeata* waren. Im Herbst dagegen, wurde kein sexuelles Weibchen von *D. galeata* gefunden und mehr als 60 % der sexuellen Weibchen und Männchen gehörten zu *D. hyalina*. Der Hybrid war in beiden Zeiträumen sexuell aktiv. Trotz dieser saisonalen Unterschiede, reproduzierten sich in dem Mesokosmusexperiment alle drei Taxa sexuell, welches im Juli 2001 durchgeführt wurde, d.h. in einem Zeitraum, in dem keine sexuelle Aktivität im See beobachtet wurde. Die Elternarten zeigten sowohl im See als auch in den Mesokosmen eine ähnliche Allokation in sexuelle Weibchen und Männchen. Im Gegensatz dazu, zeigten die Hybride eine viel stärkere Allokation in sexuelle Weibchen in den Mesokosmen. Über alle drei Jahre trug die *D. galeata* Population den meisten Teil zu den produzierten Ephippien bei. Die Ephippienproduktion von *D. hyalina* war sehr gering und der Hybrid zeigte, zumindest in der Seepopulation, eine intermediäre Muster. Die Ergebnisse deuten auf eine "Art x Umwelt-Interaktion" für die Induktion von und Allokation in sexuelle(r) Aktivität. Diese Ergebnisse werden im Hinblick auf unterschiedliche Überwinterungsstrategien und deren Konsequenzen für den Erhalt der genetischen Variation innerhalb von Populationen diskutiert.

Kapitel 4 handelt von der Dauerstadienbank der *Daphnia* Population. Dauerstadien von planktischen Organismen aus datierbaren Sedimentkernen werden zunehmend zur Rekonstruktion historischer Informationen, wie Abundanz, Größe, genetische Zusammensetzung und Mikroevolution von planktischen Organismen, genutzt. Alle diese Studien vertrauen auf der bis heute weitgehend nicht getesteten Annahme, dass die Dauerstadien in den Sedimenten eine Rekonstruktion der vergangenen Population erlauben. Diese Studie testet die Leistung von Dauerstadienbanken für die Rekonstruktion historischer Daten anhand der *Daphnia* Population des Bodensees, welche im letzten Jahrhundert gründlich untersucht wurde. Die Arbeit zeigt, dass es möglich ist die Variabilität in der Abundanz, der Größe und der genetischen Zusammensetzung von *D. galeata* über einen Zeitraum der letzten zwei Jahrzehnte zu rekonstruieren. Darüber hinaus erlauben die Daten den Zeitpunkt der Einwanderung von *D. galeata* in den Bodensee zu rekonstruieren. Dagegen war die Rekonstruktion nicht möglich von a) der Dynamik der heimischen *D. hyalina* und b) der relativen Zusammensetzung dieser beiden Arten. Das Ausbleiben in der Rekonstruierbarkeit der Langzeitentwicklung der *D. hyalina* Population im Bodensee ist wahrscheinlich aufgrund einer Veränderung in der sexuellen Aktivität und vermutlich in der Schwimmfähigkeit der Ephippien im Zuge der Eutrophierung begründet.

In Kapitel 5 werden artspezifische Unterschiede in der Rekrutierung aus Dauerstadien behandelt. Die Rekrutierung aus Dauerstadien ist eine häufige Eigenschaft von vielen planktischen Organismen in temporären Gewässern, aber auch in großen permanenten Seen. Da die Signale, die die Diapause beenden, d.h. anstieg der Temperatur und Lichtintensität, mit zunehmender Wassertiefe abnehmen, scheint das Litoral eine

besondere Bedeutung für die Rekrutierung des Zooplanktons von großen und tiefen Seen zu haben. In dieser Arbeit wurde die Rekrutierung der *Daphnia hyalina-galeata* Population des Bodensees untersucht. In Laborversuchen wurde die Emergenz unter 6 verschiedenen Temperaturregimen, 5 verschiedenen Lichtintensitäten und 6 verschiedenen Tageslängen untersucht und mit einer 2-jährigen Studie zum *in-situ* Emergenzmuster verglichen, die mit Hilfe von Emergenzfallen durchgeführt wurde, die in unterschiedlichen Tiefen exponiert waren. Darüber hinaus wurde die zeitliche und räumliche Verteilung der Ephippien im Bodensee anhand von 114 Sedimentkernen untersucht, die regelmäßig von Januar bis Dezember in Tiefen von 1 m bis 220 m genommen wurden. Die Ergebnisse der Laborversuche und der *in-situ* Emergenzfallen zeigen, dass die Emergenz beginnt, wenn die Temperaturen die Lagerungstemperaturen (im Bodensee zwischen 4°C und 5°C) überschreiten. Sie ist auf eine kurze Periode im Februar/März beschränkt und war in den tiefer gelegenen Fallen höher. Weder Lichtintensität noch veränderter Tag/Nacht-Rhythmus beeinflussten die absolute Anzahl oder den Zeitpunkt der Emergenz im Labor. Es wurde keine *D. hyalina* gefunden, die aus profundalen Sedimenten geschlüpft ist, *D. hyalina* machte aber 25 % der geschlüpften Tiere aus dem Litoral aus. Die Ephippiendichten nahmen mit der Tiefe zu. Im Litoral wurden Ephippien nur in der Zeit von der Ephippienproduktion im Frühsommer bis in den Herbst gefunden. Diese Ergebnisse zeigen, dass das Litoral eine höhere Bedeutung für die Rekrutierung von *D. hyalina* hat, dagegen erfolgt die Hauptrekrutierung von *D. galeata* aus tieferen Bereichen.

In der vorliegenden Arbeit wurde nicht nur die Bedeutung der sexuellen Aktivität für artspezifische Unterschiede im saisonalen Muster und der saisonalen genetischen Architektur, sondern auch für die artspezifischen Unterschiede in der Lagerung von Dauerstadien und Emergenz aus diesen untersucht. Darüber hinaus, deuten die Ergebnisse darauf hin, dass sexuelle Aktivität nicht konstant ist, sondern sich über die Zeit ändert, möglicherweise beeinflusst durch die Eutrophierung und Konkurrenz. Dabei wird die besondere Stellung von sexueller Reproduktion, Hybridisierung und Diapause und die Verknüpfung von saisonalem Muster und Langzeitentwicklung für das Verständnis der Prozesse innerhalb von *Daphnia*-Populationen hervorgehoben.

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LIST OF PUBLICATIONS

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