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The dynamics of sexual reproduction and ephippia production of *Daphnia* in reservoirs

Ph.D. thesis

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Annotation

This thesis focused on the dynamics of the sexual reproduction of microcrustacean *Daphnia* (Cladocera, Anomopoda). Sex in *Daphnia* is induced environmentally after rounds of asexual parthenogenesis in favorable conditions. The onset of sexual reproduction and the occurrence of sexual individuals was investigated with respect to their distribution in the water column and clonal structure. The produced sexual eggs were genotyped and their density and distribution in sediment were analyzed. The impact of massive floods was investigated. The role of sexual reproduction in stable habitats and its relevance and importance for *Daphnia* communities is further discussed.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

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České Budějovice, 24.4. 2013

Mgr. Ivana Vaníčková

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List of papers and author's contribution

The thesis is based on the following papers and manuscripts (listed chronologically):

Vaníčková I, Seda J, Petrusek A (2010) The stabilizing effect of resting egg banks of the *Daphnia longispina* species complex for longitudinal taxon heterogeneity in long and narrow reservoirs. *Hydrobiologia* 643, 85-95. DOI 10.1007/s10750-010-0126-x. IF = 1.964

> *Ivana Vaníčková participated in field sampling and was responsible for sediment sample processing, the isolation of ephippia, evaluating the status of eggs, DNA isolation and subsequent ITS-RFLP analyses, data assembly, statistical evaluation, and writing the manuscript.*
> *RNDr. thesis of I. V. is based on this paper.*

Vaníčková I, Seda J, Macháček J, Petrusek A (2011) Effects of extreme floods on the *Daphnia* ephippial egg bank in a long narrow reservoir. *Journal of Limnology* 70, 369-377. DOI 10.3274/JL11-70-2-21. IF = 1.207

> *Ivana Vaníčková participated in field sampling, and was responsible for sediment sample processing, the isolation of ephippia, evaluating the status of eggs, data assembly, statistical evaluation, and writing the manuscript.*

Macháček J, **Vaníčková I**, Seda J, Cordellier M, Schwenk K (2013) Sexual reproduction of *Daphnia* in a deep temperate reservoir: the phenology and genetics of male formation. *Hydrobiologia*. In press DOI 10.1007/s10750-012-1419-z. IF (2011) = 1.784

> *Ivana Vaníčková participated in the field sampling of the* Daphnia *population, and was responsible for the isolation of animals of interest, for further DNA microsatellite analyses, the data assembly of the genetic part of the dataset, statistical evaluation, and writing the respective part of the manuscript.*

Vaníčková I, Macháček J, Seda J: Sexual reproduction of *Daphnia* in a deep temperate reservoir: seasonal patterns in the production and quality of resting eggs. *Manuscript (submitted).*

> *Ivana Vaníčková participated on project design, the field sampling of ephippia and sediment, and was responsible for sediment samples and sedimentation trap processing, the isolation of ephippia, DNA extraction from ephippia and its analyses, data assembly, statistical evaluation, and writing the manuscript.*

Vaníčková I, Macháček J, Seda J, Cordellier M, Schwenk K: Sexual reproduction of *Daphnia* in a deep temperate reservoir: avoidance of inbreeding through spatial segregation. *Manuscript (first draft).*

Ivana Vaníčková participated in the field sampling of the Daphnia *population and was responsible for the isolation of animals of interest for further DNA microsatellite analyses, data assembly, statistial evaluation, and writing the manuscript.*

▨ Co-authors agreement

The co-authors listed below fully acknowledge that Ivana Vaníčková is the first author of four papers or manuscripts and a significantly contributing co-author of one paper. Most of the processing as well as the analyses was performed by I.V. She also made a major contribution in writing to the manuscripts. All papers contain the original results. The first author of one paper J. Macháček, and supervisor J. Seďa consent to the publication in the PhD. thesis of I. V. and support this statement with their signature.

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The dynamics of sexual reproduction and ephippia production of *Daphnia* in reservoirs

�## Content

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Introduction

The microcrustacean *Daphnia* (Cladocera, Anomopoda)*, commonly referred to as the water flea, is a globally distributed keystone species in aquatic systems. It occurs in diverse freshwater habitats ranging from temporary puddles to large lakes, and from lowlands to high altitudes. It retains an intermediate position in the food web usually being the principal grazer of the primary production of algae, bacteria, protozoa, and is foraged by fish. *Daphnia* is widely used as an indicator species to assess the ecosystem response to various changes, but also serves as a model organism in various fields from parasitology, toxicology, and genetics, to ecology and population studies. *Daphnia* was the first crustacean to have its genome sequenced (Colbourne *et al.* 2011) and was introduced as the third invertebrate among the officially recognized model organisms for biomedical research.

The first known attention to *Daphnia* was paid by Jan Swammerdam (1591–1659). In his "Historia Insectorum Generalis," printed in Utrecht in 1669, Swammerdam gave an almost complete description of the species accompanied by a painting published later (Fig.1; Freyer 2008).
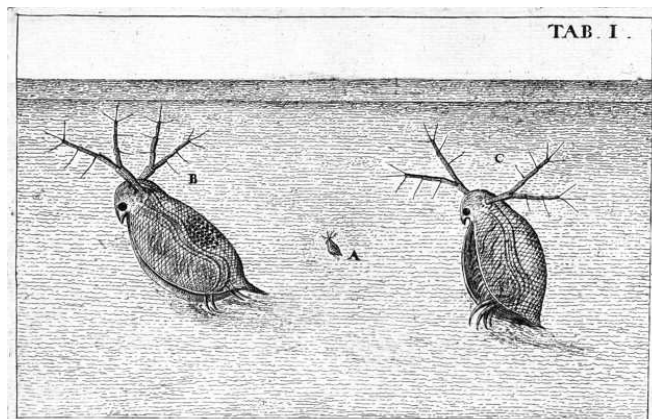
Fig. 1: The first illustrations of *Daphnia*, by Jan Swammerdam, 1669. The figure is reproduced from Freyer (2008).

The history of research on *Daphnia* reproduction

*Daphnia*s' complex life cycle involving amixis and diapause was tricky to reveal. The first relatively accurate studie on *Daphnia* was published by Jacob C. Schäffer in 1755 (Freyer 2008). He dealt with *D. magna* (a species later described by Straus in 1820). Schäffer was the pioneer researcher who described the fine but complex anatomical structures of the species body and revealed their function. Although some of his findings and conclusions were erroneous (Fig. 2), with respect to the limits of the microscopes of the day, and having no predecessor, his contribution was remarkable. Regarding reproduction, Schäffer noticed the formation of ephippium on the back of the adult female (Fig.2h), however he did not recognize its significance. To the contrary, he considered *Daphnia* to be hermaphrodite, as he detected no males (Freyer 2008). The role of ephippia remained unclear even a century later.

Baird (1837; 1850) identified the function of ephippium as a resistant stage for possible population recovery, and was aware of males. Nevertheless, he failed to comprehend the nature of *Daphnia* reproduction, either parthenogenetic or sexual, although he described an observation of switching between phases. He quoted males to be rare and found only at certain seasons, but wrongly concluded that males are able to fertilize females for life, as well as all their female progeny for several generations, including hatchlings from ephippia.

A significant insight into *Daphnia* reproduction was made by John Lubbock (Lubbock 1857), who provided an understanding of the basic differences of parthenogenetic or sexual eggs. He was aware of the finding of Charles Bonnet who discovered and described clonal propagation in his studies on aphids in the mid 18th century (Freyer 2008). Lubbock (1857) described the structure of ephippium, the process of formation, and its purpose for the renovation of the community. He also pointed out the mismatch in the occurrence of males and ephippial females: in his observation he noticed that the occurrence of males did not induce the formation of ephippia, and that ephippia were produced even without males. Although he still did not clearly understand the true difference in origin and formation of both mictic and amictic eggs, his remarkable contribution and mostly correct statements are still relevant.
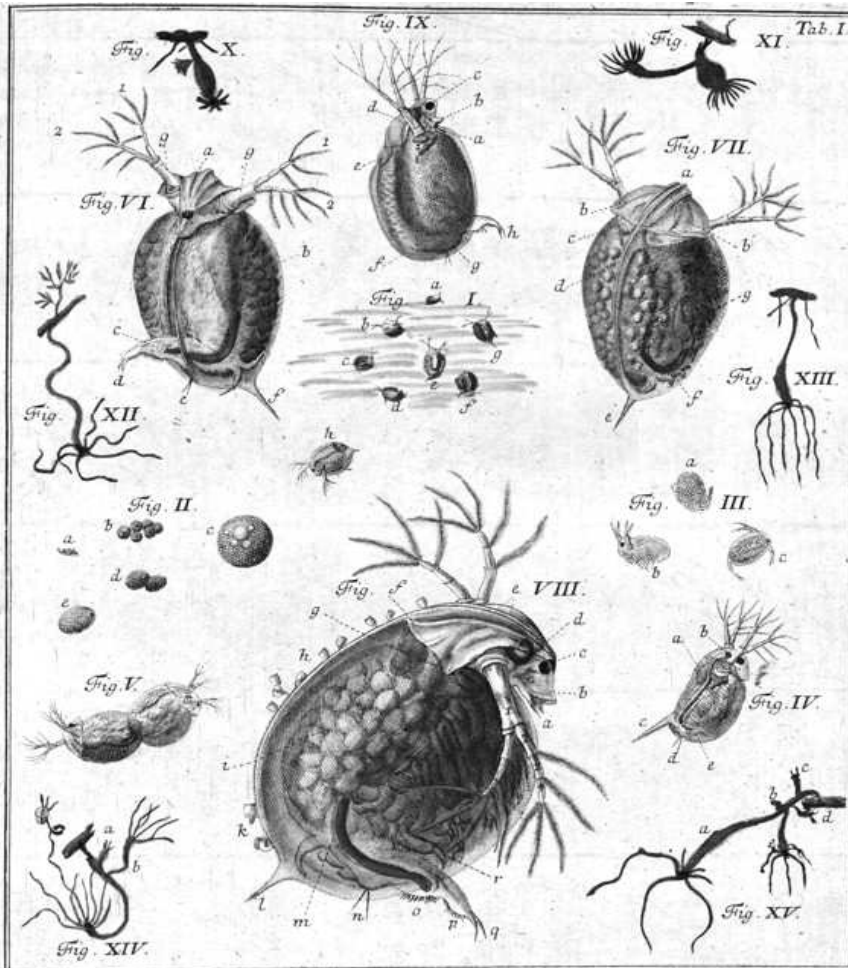
Fig. 2: Drawings of *Daphnia* by Schäffer. Figure and description reproduced from Freyer (2008): *Schäffer's Plate I. This shows (Fig. I, a–g) a group of Daphnia* magna*, about life size, swimming in various directions; (Fig. VI and Fig. VII) females more highly magnified, drawn from life, showing oblique ventral and oblique dorsal views respectively; (Fig. VIII) a female with its right carapace valve removed to reveal the trunk limbs and post-abdomen within the carapace chamber, and showing the minute protozoans and rotifers that sometimes frequent the carapace; (Fig. V) two females that have become entangled, which caused Schäffer to believe that mating was taking place and that Daphnia is a hermaphrodite; (Fig. I, h) an ephippial female; (Fig. II, Fig. III and Fig. IV) eggs and early development; and (Fig. IX)* Simocephalus vetulus*.*

Daphnia reproduction mode

Besides *Daphnia*, there are roughly 15,000 animal species that have kept an advantageous combination of asexual and sexual reproduction cycles (Hebert 1987), the most commonly mentioned being monogonont rotifers, aphids, and Cladocera (Schön *et al*. 2009). In *Daphnia* the plasticity in reproductive mode is viewed as an adaptive response to cope with seasonal changes. Generally speaking, sexual recombination generates extensive diversity and thus enlarges the potential for evolution by expanding the range of genetic variability on which selection can act. In contrast to this, during asexual propagation the maternal genotype is inherited, producing cohorts of genetically identical offspring utilizing favorable feeding conditions.

Despite parthenogenesis in *Daphnia* having a monophyletic origin (Taylor *et al.* 1999), the general schedule of cyclical parthenogenesis can be specific and we observe a wide range of frequency in sexual behavior (Decaestecker *et al.* 2009). Under normal and favorable conditions *Daphnia* reproduce clonal female offspring but differ in the way they produce dormant eggs stored in ephippia. *Daphnia* communities may be considered to be either obligatorialy of facultatively parthenogenetic.

Obligatory parthenogens

In this case, the *Daphnia* population consists of females that besides clonal propagating are able to produce ephippia completely asexually (Adamowicz *et al.* 2002; Colbourne *et al.* 2006; Dufresne & Hebert 1994, 1995, 1997; Hebert 1981; Mergeay *et al.* 2008). Asexual clones of *Daphnia* have been reported with the capability of ameiotic recombination during the production of resting stages (Omilian *et al.* 2006) showing that the loss of sexual reproduction is not necessarily accompanied by a complete loss of recombination.

Nevertheless, obligatory parthenogenetic *Daphnia* are also capable of spreading their asexuality via producing males (Paland *et al.* 2005). These are produced not to fertilize females of their kin but to spread asexuality into the offspring of the mated sexual females of normal, facultatively parthenogenetic clones. This sexually transmitted asexuality has been reported in various other taxa and is often associated with changes in ploidy (Decaestecker *et al.* 2009) or hybridization, similarly as in flowering plants (Richards 2003) or insects (Sandrock & Vorburger 2011).

Facultative parthenogens

In this case, dormant eggs are produced as haploids and require fertilization for their further development. Sex is induced environmentally when *Daphnia* strongly react to environmental stimuli suggesting a deterioration of suitable life conditions, such as changes in the photoperiod, food availability, population density or the presence of a predator (Carvahlo & Hughes 1983; Kleiven *et al.* 1992; Spaak & Boersma 2001; and many others). These signals lead to sexual determination in favour of males in the immature oocyte before final cleavage in the mother's ovary (Ignace *et al.* 2011) and the occurrence of male clutches in otherwise all-female *Daphnia* populations (Fig. 3).
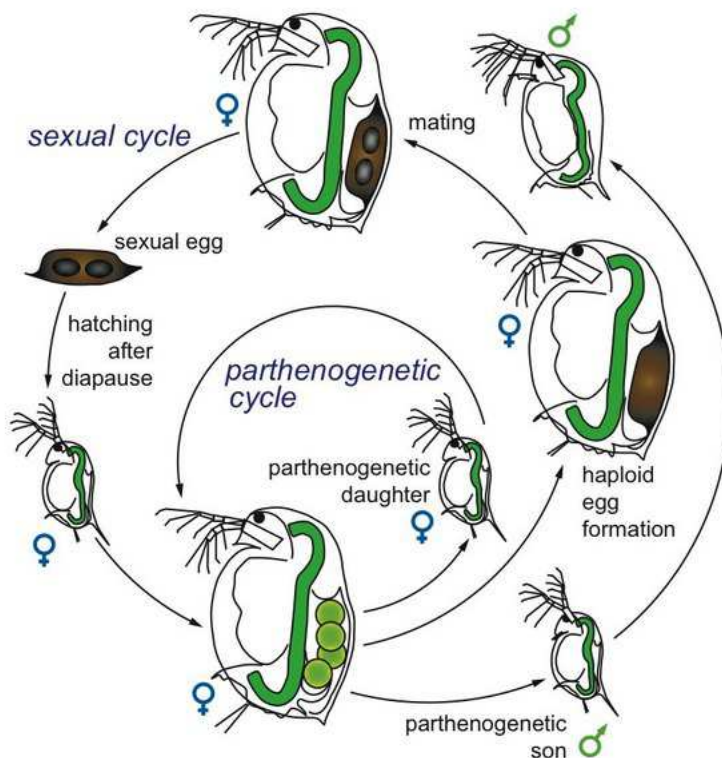

Fig. 3: *Daphnia* life cycle. Figure and its description reproduced from Ebert (2008): *This diagram depicts the sexual and the asexual (parthenogenetic) life cycle of a* Daphnia*. During the parthenogenetic cycle, females produce diploid eggs that develop directly into daughters.*

The same female may produce diploid asexual eggs that develop into sons. Male production is under environmental control. Furthermore, the same female may produce haploid eggs that require fertilization by males. These eggs are then enclosed in a protective shell (ephippia) and need to undergo a diapause before female offspring will hatch from them. Drawing by Dita B. Vizoso, Fribourg University.

After the males are released and left to mature freely in the water column, maternal *Daphnia,* with a certain delay, produce a pairs of haploid eggs that require successful mating to initiate the further development of the eggs. In natural habitats the peak of sexual reproduction of *Daphnia* occurs shortly after the abundance peak, which may be used as a mechanism maximizing meeting probability in the vast space of the water column (Lynch 1983). After fertilization, the development of the eggs is arrested in the gastrula stage enveloped by several protective membranes, including a modified part of the carapace of the mother *Daphnia*. The entire structure constitutes an ephippium (Lubbock 1857; Zaffagnini 1987). If a maternal *Daphnia* has not been fertilized, eggs degenerate while the ephippium is still a part of the mother's body or shortly after release (Zaffagnini 1987). The whole structure is molted and may float on the surface of the water or sink down to the sediment (Slusarczyk & Pietrzak 2008). In the event of fertilization, eggs wait for signals indicating the renovation of suitable conditions and genotypic unique female *Daphnia* hatch (Fig. 3). Maternal *Daphnia* may resume parthenogenesis and transit to sexual reproduction several times. Facultative alternation between asexual and sexual reproduction combines the benefits of both within the same lifecycle.

The ephippia and dormancy

Daphnia's wide distribution and persistence in occupied habitats is enabled, besides other things, by the production of dormant stages. In Crustacea there are several groups with a capacity for dormancy, but in Branchiopoda, in fairy shrimps, clam shrimps, tadpole shrimps, and Cladocera, this ability occurs in all taxa (Fryer 1996). Daphniid dormant eggs stored in ephippia are highly resistant to desiccation or ingestion by invertebrates (Frisch *et al.* 2007) and may stay viable for many decades or even a century (Cáceres 1998a). This high resistance to decay and the durability of resting stages provide a tool to overcome periods of unsuitable living conditions and the recovery of the *Daphnia* population when these periods pass.

Ephippia are recognized as the vectors of dispersal in both time (Hairston & De Stasio 1998) and space (Adamowicz *et al.* 2009).

Dormant eggs may be produced either asexually or sexually, depending on taxa or population (Decaestecker *et al.* 2009), but in most *Daphnia* populations dormancy and sexual reproduction are closely tied, which results in more variable dormant offspring in the changing habitat. After molting from maternal *Daphnia*, ephippial eggs become a part of the dormant community, an egg bank, ready to renovate the water column community (Brendonck & De Meester 2003). Egg banks are archives of dormant genotypes and continuous hatching from old ephippia may either restore genetic diversity (Cáceres 1998b) or slow down adaptation to rapidly changing conditions (Hairston & De Stasio 1998).

Dormancy and the emergence of hatchlings are crucial in habitats with the discrete distribution of suitable living conditions caused by seasonality. In the freshwater ecosystem, the main obstacles to overcome may be environmental constraints such as the drying-out (Crean & Marshall 2009) and freezing-out of the habitat (Edmondson 1955; Larsson & Wathne 2006), or community interactions such as competitive exclusion (Cáceres 1998b) and over predation (Hairston & De Stasio 1998). Under unsustainable conditions, dormant stages ensure the long term existence of particular taxa in its habitat (Brendonck & De Meester 2003; Caceres & Tessier 2003; Crean & Marshall 2009; Fryer 1996; Menu *et al.* 2000). Ephippial production is much less frequent in permanent environments where the overwintering of females is an optional mechanism for the maintenance of clonal groups (Hebert 1987; Lynch 1983).

Genetic diversity in permanent populations and its relevance to sex

The richness of communities, either specific or genotypic, is closely tied to the size of habitat (Storch *et al.* 2012). Contrary to the situation in small habitats, where isolation and strong priority effects may lead to assemblages of a few if not a single clone, in larger habitats there is an expectation of enhanced genetic diversity (Michels *et al.* 2003) due to environmental heterogeneity providing niches for the coexistence of ecologically different genotypes (De Meester 1996).

In *Daphnia* assemblages, usually only several taxa co-occur in a particular habitat. Diverse, but closely related taxa, that share habitat may mate interspecifically and thus produce hybrids (Schwenk

et al. 2000; Schwenk & Spaak 1995). The process of hybridization is necessarily tied to the occurrence sexually-produced ephippia and their subsequent hatching. Genotypes of the first hybrid generation may be maintained by parthenogenesis in the water column and at least temporarily succeed due to the advantageous combinations of parental traits (Repka *et al.* 1999; Spaak & Hoekstra 1995). As a result, the hybrids are commonly present in European lakes and reservoirs (Keller *et al.* 2008; Schwenk & Spaak 1995; Seda *et al.* 2007b) and later-generation hybrids and backcrosses can also be detected in the water column in lower proportions (Keller & Spaak 2004; Wolf 1987).

Stable habitats host populations for a long time (Duigan & Birks 2000; Jankowski & Straile 2003; Limburg & Weider 2002). On the level of a single species, habitat permanency and relative stability allows prolonged periods of clonality leading to the adaptation of clones to efficiently utilize resources and the long term maintenance of clonal lineages (Delmotte *et al.* 2002; Halkett *et al.* 2005; Taylor *et al.* 1999). The extent of clonal reproduction has a large impact on population structure (Xu *et al.* 2011). Temporal asexuality brings strong intra-specific competition when multiple clonal lineages clash over maintenance in the system, resulting in potential fixation (Gabriel & Bürger 2000) and the occasional or accidental die-outs of clonal lineages (De Meester *et al.* 2006; Vanoverbeke & De Meester 2010). Fitness differences among clonal lineages may rapidly change genotype frequencies. For instance, parasitism helps to maintain the richness of traits involved (Mitchell *et al.* 2005) due to particular clones hosting parasites to various extents (Yin *et al.* 2012). As a result, parasites alter the clonal composition in time and space (Buser *et al.* 2012; Wolinska *et al.* 2008; Wolinska & Spaak 2009; Yin *et al.* 2010).

Prolonged amixis may significantly erode the number of genetically unique clones within a population and thus decrease the effective population size available for later sexual reproduction (Frankham 1995). Mating between close relatives reduces the advantageous effect of sexual recombination and therefore is generally considered as detrimental to general fitness (Boersma *et al.* 2000; Caceres *et al.* 2009; Charlesworth & Willis 2009; Haag & Ebert 2007; Haag *et al.* 2002; Innes & Dunbrack 1993; Keller & Waller 2002; Salathé & Ebert 2003). Mate choice has been documented in copepod and rotifers several times (Ceballos & Kiorboe 2010; Gomez & Serra 1996; Palmer & Edmands 2000; Titelman *et al.* 2007), but in *Daphnia* there are no known mechanisms of mating avoidance (Brewer 1998) especially based on kin recognition (Winsor & Innes 2002).

From large water bodies with permanent *Daphnia* populations the data on the dynamics and patterns of sexual

reproduction and ephippia production are insufficient and suffer due to the size of the habitats resulting in methodological difficulties. Our knowledge of the intensity of sexual reproduction is usually constrained to the detection of sexual reproduction by sampling ephippial females or sampling the sediment ephippia. Most of the data regarding clonal structure is derived from laboratory experiments, or by using methods that do not enable the reliable and detailed determination of key players.

Aims of the study

In the present study we investigated large lake *Daphnia* and the dynamics of their sexual phase placed between rounds of parthenogenetic generations enabled by habitat permanency. We investigated the onset of sexual reproduction with a focus on the occurrence and clonal structure of males and sexual ephippial females. We then targeted the production of sexual offspring, i.e. dormant eggs stored in ephippia; we monitored the production within and over years and evaluated the quality of the resting eggs inside. We examined ephippia distribution within five reservoirs and, in a case study, we assessed the impact of massive floods on the ephippia, assessing the potential role of ephippia in the *Daphnia* community.

In order to investigate the role of sexual reproduction on reservoir *Daphnia*, the following detailed questions and study aims were set.

I. How are males produced at the onset of sexual reproduction?

We investigated the onset and duration of the production of male individuals in spring *Daphnia* population and compared their genetic composition with the genetic composition of male-producing and female-producing parthenogenetic females. We focused on the following questions:

i. The onset and dynamics of male production is closely connected to a specific combination of inducing (environmental) factors during the spring succession in the reservoir, and as such it is strongly delimited in space and time, unlike the alternative strategy of a more or less constant production of a certain proportion of males in the population.

ii. Male-producing females are not a random subgroup of the overall parthenogenetic population and thus there is

a significant difference in the clonal composition between male-producing females and the rest of the parthenogenetic population.

iii. The clonal structure of male-producing females and that of adult males later present in the reservoir is different due to differences in survival and other selective forces acting on the postembryonic development of males.

II. What is the clonal and spatial structure of the sexual part of the population and its relation to the sexual reproduction and the production dormant offspring?

We investigated the dynamics of sexual reproduction of the cyclical parthenogen *Daphnia galeata* in a natural permanent population during the spring. In two consecutive years, we focused on the population structure after surviving the winter and on the formation of sexual individuals. Additionally, in connection with previously published data the production and genetic structure of the male individuals (Macháček *et al.* 2013) and with background data on the asexual females' pool, we analyzed the sexual population. Its spatial distribution, the probability of selfing and the parentage of sexually produced eggs were inferred using 12 microsatellite loci. We tested the following hypotheses:

i. Clones producing males and sexual ephippial females belong to different clonal groups.

ii. Clones of sexual females and males show a homogeneous vertical depth distribution and the pattern of distribution of sexual individuals is similar to the distribution of parthenogenetic females.

iii. Clones of *Daphnia* sexual individuals are homogeneously vertically distributed.

iv. The clonal composition of mictic individuals of both sexes is reflected in the genotypic composition of dormant eggs produced in the corresponding period.

III. What is the course of ephippia production within a season and between years? Is there any variability in the number of ephippia and the content of healthy eggs?

The aim of this study was to investigate the dynamics of the ephippia production of a permanent natural population of *Daphnia galeata* within one year and to evaluate the variation among years. By checking the eggs inside, we aimed to reveal the

fertilization success and quality of the eggs. We tested the following hypotheses:

i. The population of *Daphnia galeata* investigated has only one main peak of ephippia production and that it occurs in the spring. If ephippia occur in other periods, they are of a negligible number.

ii. During the main peak of production, the number of ephippia produced is in relation to the size of the *Daphnia* population in the water column. If the size of the *Daphnia* population is stable, inter-annual changes in the total number of ephippia produced are insignificant.

iii. Irrespective of the main peak of *Daphnia* sexual reproduction occurring in spring, the fertilization success is not period dependent but stable throughout the year.

IV. What is the distribution of ephippia in sediment along the longitudinal axis of a reservoir and what is this distribution like in various reservoirs? Is there a correspondence between the dormant and active community?

In this study we analyzed the spatial distribution of ephippia of the *Daphnia longispina* complex and the preservation state and taxonomic structure of the eggs within them. We focused on five long narrow reservoirs in which a well-pronounced tributary-to-dam gradient in taxonomic composition of the *D. longispina* complex has been observed in zooplankton samples, and compared data on the dormant *Daphnia* community in ephippia with the taxonomic composition of *Daphnia* adults. We worked with two hypotheses:

i. Ephippia densities differ between the upstream and dam regions, reflecting variations in the sedimentation rate, *Daphnia* densities in the water column, and other environmental factors.

ii. Assuming that gradients in the taxonomic composition of *Daphnia* are regularly restored in these reservoirs (as reported previously), we also questioned whether the composition of resting egg banks tracks the water column community, therefore showing a non-random heterogeneous pattern.

V. What is the fate of ephippia after natural disaster and role of egg bank for *Daphnia* community in reservoir?

An answer to this question was enabled by two major floods that hit one of the previously studied reservoirs. We therefore exploited this unique opportunity and investigated the effects of *Daphnia* community recovery with a focus on ephippia. The principal question was: which of the following hypothesis may play a role in a community affected by floods?

i.   The prior egg bank in the sediment was reset by newly imported allochtonous material brought by floods and therefore the dormant egg bank was effectively blocked from making a contribution to the recovery of the *Daphnia* population

ii.   Sediment was resuspended by the flood and the ephippia within were redistributed throughout the reservoir. As a result, dormant eggs (including those previously buried in the sediment) may have been exposed to hatching cues after the flood.

Studied habitat and organisms

Studied *Daphnia* populations originated from man made reservoirs located in the Czech Republic, Central Europe. Built by damming rivers in steep valleys, reservoirs are a special case of anthropogenic lake-like habitats. Their specific hydrologic characteristics and morphology, with a considerable longitudinal depth gradient, result in a large variation of environmental conditions along the main reservoir axis (Straškraba 1998). Although most canyon-shaped reservoirs studied have a relatively short retention time, their pelagic communities show spatial gradients in composition of all important functional groups of biota (e.g., bacteria: (Šimek *et al.* 2008); heterotrophic protists: (Jezbera *et al.* 2003); phytoplankton: (Hejzlar & Vyhnálek 1998); zooplankton: (Seda & Devetter 2000; Seda *et al.* 2007b); fish: (Prchalová *et al.* 2008; Vašek *et al.* 2004).

Daphnia communities in reservoirs may hold several taxa, but frequently found are those of the *Daphnia longispina* species complex (*D. galeata*, *D. longispina* and *D. cucullata,* (Brandl et al. 1989). They may co-exist (Petrusek *et al.* 2008b; Seda *et al.* 2007b) or a particular reservoir may be dominated by a single species only (Seda *et al.* 2007a).

The intensity of sexual reproduction was assessed within the *D. longispina* species complex by examination of the dormant egg bank as well as the link between the dormant and active community and its destiny after a major flood disturbance. More detailed studies regarding sexual reproduction were made in a single species reservoir. The selection of this reservoir excluded the unwanted interspecific interaction of sexual stages leading to hybridization. The investigated species was *Daphnia galeata*, a common species in lakes that have a year-round population and that engage in sexual reproduction mainly in early spring (Jankowski & Straile 2004; Keller & Spaak 2004; Spaak 1995; Spaak & Boersma 2001). *Daphnia galeata* in the studied lake exhibits a seasonal dynamics pattern typical for temperate zone reservoirs (Sommer *et al.* 1986) with a main population peak in spring (Seda 1989).

The determination of taxa and clones of *Daphnia*

To understand the individual roles in sex of facultative asexuals, a precise methodology is necessary to identify key participants on the level of clones and a less specific, but still reliable method for taxa and interspecific hybrid assignment is needed.

Taxonomic determination

The genus *Daphnia* (Cladocera, Anomopoda) comprises roughly 80 species with confused taxonomy (Forro *et al.* 2008; Korovchinsky 2006). Within *Daphnia*, several species complexes are found (Benzie 2005). One frequently found and investigated species complex is *Daphnia* gr. *longispina*, frequently inhabiting lakes including reservoirs in Central Europe (Brandl et al. 1989). Species of this complex are *D. galeata*, *D. longispina* and *D. cucullata* (for nomenclatural issues see (Petrusek *et al.* 2008a). Species of *Daphnia* gr. *longispina* are known to frequently hybridize (Keller *et al.* 2008; Petrusek *et al.* 2008b; Spaak 1997; Spaak & Hoekstra 1995). The determination of hybrids based on classical taxonomy may be misleading, because of the existence of second generation hybrids and backcrosses that may resemble parental taxa (Dlouhá *et al.* 2010).

To reliably distinguish between pure taxa and interspecific hybrids of various extent, methods based on the enzymatic profiles or DNA is necessary. The most used methodology for taxonomic determination is the electrophoresis of allozymes (Hebert & Beaton 1989), methods based on the differentiation of various fragment of DNA lengths such as AFLP (Gili *et al.* 2004) or RFLP (Billiones *et al.*

2004; Skage *et al.* 2007). All give reliable taxonomic data with variable accuracy; and due to their low cost and relatively low equipment demands their use is favored.

Clonal Assignment

For a better understanding of individuals' roles within population interactions, an accurate method is necessary to distinguish structure within a taxon. For this purpose, rough estimates may be provided by allozyme markers (Jankowski & Straile 2004; Mort & Wolf 1986; Spaak 1995; Spaak 1996; Spaak *et al.* 2004). Clonal structure revealed by allozymes has suggested that *Daphnia* populations may contain from a few (in obligate parthenogens, (Hebert & Crease 1980) to several thousand clones (in cyclic parthenogens, (Lynch 1984). Nevertheless, a fine scale clonal composition remained mostly hidden as the method used may refer only to clonal groups (Dlouhá *et al.* 2010; Thielsch *et al.* 2009).

With the use of microsatellites, a much higher genetic diversity in taxa is observed compared to that reported by allozymes (Giessler 1997b; Mort & Wolf 1985; Spaak 1996). True clonal richness, the structure of population and interactions within it, were therefore underestimated. A precise comparison of individuals with identical MLG were indeed shown to belong to clonal lineages (Yin *et al.* 2010) thus supporting the reliability, but low discriminative power of allozymes. Microsatellites are a powerful marker to discriminate clonal lineages, especially if an sufficient number of loci is combined (Koskinen *et al.* 2004; Thielsch *et al.* 2009). An applied high resolution set of microsatellites helped to identify the relationship between the sexually and clonally reproducing parts of the population.

Results and general discussion

Prolonged parthenogenesis and the long term maintenance of clones in reservoirs

Water fleas of the group *Daphnia longispina* are the most common members of temperate zone zooplankton assemblages and are repeatedly reported to dominate the daphniid community in large lakes as well as in special anthropogenic habitats such as reservoirs (Brzezinski *et al.* 2012; Giessler 1997; Hamrová *et al.* 2012; Hart 2004; Hülsmann *et al.* 2012; Petrusek *et al.* 2008; Seda *et al.* 2007b; Spaak & Hoekstra 1995; Weider & Wolf 1991). Both lakes and reservoirs are occasionally hit by natural catastrophes like floods that, however,

affect these water bodies in different ways due to outlet location (Straškraba 1998; Chapter V). Additional environment disturbance occurs in the man-made reservoirs due to management interventions in order to serve their function. Water masses from reservoirs can be unpredictably let out from particular layers, along with a part of the *Daphnia* community. Bearing all this in mind, it is fascinating that we may repeatedly identify the identical clones within *Daphnia* populations (up to 16 % in the inter-annual comparison; Chapter I, II) or similar spatial structuring of the *Daphnia* community (Petrusek *et al.* 2008; Seda *et al.* 2007b).

The long-term persistence of some clones in the lake may result in the local adaptation of clonal lineages (Taylor *et al.* 1999) and the monopolization of the habitat (De Meester *et al.* 2002). The overwintering ability of clones suggests that certain clones are clearly more successful in dealing with natural selection than others. The overwinterers may be strong competitors in spring as they have a larger source pool. Nevertheless, successful survival from one season to another was not correlated with high relative abundance in the population: for instance, the most dominant clone identified in 2009 in the Římov reservoir was of only minor importance in the next season (Chapter II).

Long term clonal propagation enables the maintenance of hybrid lineages whose origin is connected with the hatching out of the sexual ephippia (Chapter IV). Although the hybrid eggs were found in the sediments of all reservoirs where closely related taxa of *Daphnia* gr. *longispina* co-occurred (Chapter IV: Tab. 3), the actual proportions of hybrid eggs were mostly lower than the proportions of hybrids in the water column. This shows that at least some of the hybrid genotypes that hatch are highly competitive and able to increase their relative abundance over the parental taxa (Spaak & Hoekstra 1995; Repka et al. 1999).

The onset of sexual reproduction and the production of males

Shifts from sexual to asexual reproduction, and vice versa, have an evident effect on population structure (Barrés *et al.* 2012) as prolonged asexuality may decrease the effective population size available for sexual reproduction (Frankham 1995). The population of *Daphnia galeata*, studied in detail in Chapters I-III, involves sexual reproduction mainly in early spring time (Jankowski & Straile 2004; Keller & Spaak 2004; Spaak 1995; Spaak & Boersma 2001). After winter, the *Daphnia* population dropped to its yearly minimum (Chapter II; Carvalho & Crisp 1987; Seda 1989), and then an

exponential growth followed. This clonally reproducing population showed strongly reduced clonal richness (MLG/N=0.51 in 2009 and 0.64 in 2010; Chapter II), an excess of heterozygotes and a deviation from Hardy-Weinberg equilibrium (HWE) on most of the investigated loci. At that time, the switch to sexual reproduction in the until now all-female population was triggered. Although later in May the clonal richness seemed to rise again (to approx. 0.8, Chapter II), this has no effect on the already produced sexual *Daphnia* individuals, especially on the males whose production is strictly delimited in time (Chapter I).

The males are produced with surprisingly remarkable regularity in the timing of the production peak and strict spatial delimitation to epilimnion (Chapter I). The first clutches with male individuals were usually detected at the end of April, with the peak of male production at the very beginning of May; this phase was over within the first two weeks of May (Chapter I: Fig. 3). This pattern in the dynamics of male production was quite clear and similar in all the five years studied. Additionally, it was found that there was no bias in a genotype comparison of adult male and previously produced male clutches if the whole male population was included into the comparison (Chapter I). The male group composition was not significantly modified by differences in survival and by various other selective forces acting during the postembryonic development of males. Thus once produced, males have a reasonable chance of passing their genes on to the dormant generation stored in ephippia and therefore reach higher evolutionary impact (Winsor & Innes 2002).

Clonal and spatial structure of the sexual part of the population and its relation to the sexual reproduction and the production dormant offspring

Regarding the production of sexual individuals, the overwintering clones seemed to produce males less often (6-8 %) compared to ephippial females (12-21 %; Chapter II). Rare clones, i.e. recorded only once within 780 individuals genotyped, formed a not negligible part of genotype diversity in the sexual individuals (56-71 %). The males and ephippial females genotype structure was significantly different from the source parthenogenetic population (Chapter I, II) and some of the clones were predominantly active in the production of sexual individuals. For instance, the most frequent clone in 2009, produced a considerable number of male offspring, while the most frequent clone in 2010, formed a substantial part of the ephippial females pool (Chapter II: Fig. 2). This engagement of

only particular clones in the massive production of the sexual individuals was reflected in the lowest recorded clonal richness for these groups (MLG/N = 0.36 for females producing males in 2009, and 0.37 for epilimnetic ephippial females; Chapter II) and both sexual groups were deviating from HWE on a large number of loci. Generally, the investment in sex varies among clones within taxa (De Meester & Vanoverbeke 1999; Spaak 1995; Spaak et al. 2004); differential involvement in sex supports the theory of a strong influence on genotype $\times$ environment interactions at the onset of sexual reproduction (Innes 1997; Innes & Singleton 2000).

The genotypes of both sexes significantly differed among themselves. Diversification at the population level with respect to involvement in sexual reproduction is in agreement with the previous suggestions based on allozyme data (Innes & Dunbrack 1993; Innes & Singleton 2000; Keller & Spaak 2004; Winsor & Innes 2002). Nevertheless, up to 7 % of genotypes were identical in males and ephippial females in both investigated seasons (Chapter II). The simultaneous production of both sexes by an identical clone was also observed in some of the most frequent clones. The low genetic diversity of parents and existence of both sexes produced by the identical clone implies the increased risk of inbreeding that is generally considered as detrimental to the general fitness (Charlesworth & Willis 2009).

Males and ephippial females represented only a minor part of the total *Daphnia* population (Chapter II) but they were distributed in the whole water column although *D. galeata* is traditionally considered a typical epilimnetic taxon (Stich & Lampert 1984). While in the surface strata sexual individuals formed a few per cent of the total *Daphnia* population, in the deep strata their proportion on occasion reached 80 % (Chapter II). Males have previously been reported to be hypolimnetic (Brewer 1998; Spaak & Boersma 2001; Spaak *et al.* 2004). Their occurrence in deeper strata is usually being explained as a reduction of the predation risk (Brewer 1998) and a mechanism of the possible lifespan increase (Pietrzak *et al.* 2010). Ephippial *Daphnia* are vulnerable to visual predation (Mellors 1975) and therefore tend to avoid well-light water layers, but the reason behind the preference for deep layers (below 30 m) remains unclear (Chapter II).

Within the clonal structure of sexual *Daphnia,* we observed the population markedly stratified. Particularly, we found a profound difference between subpopulations in layers separated by larger depth (metalimnion vs. hypolimnion) and non-bordering layers (epilimnion vs. hypolimnion; Chapter II). Spatial structuring of clones

and scarce co-occurrence of ephippial females and males with the same MLG likely lowered risk of inbreeding: the calculated probability of selfing was minor (<1 %). Reproductive isolation via ecological separation might work as a mechanism reducing the origin of hybrids within a taxa scale (Keller *et al.* 2007). The coupling of the production of both sexes might be considered a mechanism to maximize the contribution of such clones to the offspring generation stored in ephippia.

Although the resting eggs were derived from the parental population with reduced diversity, all ephippia genotypes were unique and deviated on fewer loci from HWE compared to parents (Chapter II). The effect of the low genetic diversity of the parents on their dormant progeny might be further altered by the later destiny of ephippia in the sediment. Annual input of the ephippia into the sediment can be mixed by invertebrates (Kearns *et al.* 1996) and fish (Ritvo *et al.* 2004, Matsuzaki *et al.* 2007), or by the impact of turbulent storms (Kerfoot *et al.* 2004). As long as the resting eggs stay viable for years to decades (Cousyn & De Meester 1998), with the maximum up to a century (Cáceres 1998a), the overlap of dormant generations of various age leads to higher genetic variability and thus seasonal impacts become less important.

The course of ephippia production within and over years: the variability in the number of ephippia and the content of healthy eggs

Although we investigated sexual individuals of *Daphnia galeata* in detail during their maximal occurrence in spring, they are also present in the reservoir in other parts of the year. Their proportion in the population is low (Chapter I; Spaak 1995), nevertheless, the ephippia are still produced (Chapter III). During the four years study, the observed total annual production of ephippia (i.e. input into the sediment) was highly and significantly variable among years (Chapter III). It ranged from a minimum 34,000 ephippia.m^{-2} up to 132,000 ephippia.m^{-2}. Such production is in a range of published data (Brendonck & De Meester, 2003) and shows that in large and stable habitats the total production of dormant stages is comparable to that in temporal habitats (regardless of the size of the habitat; Altermatt & Ebert, 2008).

There was a striking difference in the presence of well-preserved eggs, which were found more often in the spring-produced ephippia than in autumn- or winter-produced ephippia. This pattern was consistent in all years investigated. The proportion of empty ephippia

recorded in spring was on average 37 %, in autumn 77 % and in winter 83 %. A certain proportion of ephippia is always laid empty when not fertilized (Zaffagnini 1987). This proportion varies substantially among localities and species: within a single sexual taxon, proportions of empty ephippia in two different studies were 10 and 70 %, respectively (Cáceres, 1998a, Rossi *et al.* 1998). Within hybridizing assemblage of *D. longispina* and *D. galeata,* Keller et al. (2007) reported annual empty ephippia proportions of 32 % and 50 %. In obligate parthenogens, egg abortion has been reported to be as high as 50-100 % (Conde-Porcuna *et al.* 2011).

In total, the spring peak of ephippia production is not only the highest in the year, but also means the highest increment in the resting egg bank because the spring ephippia contain the highest proportion of vital eggs (Chapter III). In contrast to this, the autumn and winter ephippia were observed to display an increased proportion of degraded eggs or empty cases. This may bear witness to the differing conditions that are less suitable for healthy dormant egg production. As the hatching of resting eggs out of ephippia seems unlikely in autumn or winter, the reasons for empty ephippia are likely to be either lower fertilization success and/or increased abortion rate (Conde-Porcuna *et al.* 2011). Both factors are of particular importance for ephippial production, as such empty ephippia represent a zero increment to the dormant community. The variance in egg content significantly alters the importance of particular periods of ephippia production although the ephippia are produced throughout the whole year.

The annual ephippia production consists not only of ephippia that settle down to the sediment but also ephippia captured at the water surface (Slusarczyk & Pietrzak, 2008) or ingested by predators (Mellors, 1975). However, these do not become a part of the resting egg bank and are difficult to quantify; but still may play a role for the *Daphnia* community in some cases, such floods (Chapter V).

The distribution of ephippia in reservoirs and the correspondence between the dormant and active community

The ephippial pool in the sediment of reservoirs may hold up to 10^5 ephippia.m^{-2} (Chapter IV, V; Brendonck & De Meester 2003). There is the variation in the annual production of ephippia and, thereafter, a significant variation in the order of magnitude of annual input into the sediment bank (Chapter III, V). It reflects many factors: the size of the *Daphnia* population (Chapter III), the incidence of sexual reproduction (Chapter I; Cáceres, 1998a,b; Cáceres & Tessier

2004) and others, such as the proportion of floating and sedimentary ephippia (Slusarczyk & Pietrzak, 2008). The seasons with higher ephippia production are interspersed with "ordinary" production (Chapter III, V) and the total number of ephippia in the resting egg bank is likely averaged. Mixing of the sediment may further unify the ephippia density differences among seasons (Kearns *et al.* 1996). This is in the agreement with the surprisingly similar numbers of ephippia in the upper 6-cm layer of sediment in dam part of five different reservoirs, where in four out of five water bodies the sediment hold 160,000 ephippia.m^{-2} (Chapter IV). Contrary to this, the number of ephippia in the tributary zone of studied reservoirs was found more variable (between 6,000 and 67,000 ephippia.m^{-2}). The consistent pattern of ephippia numbers increasing in a downstream direction was observed in all reservoirs (Chapter IV). The difference between the upstream and dam densities was in one or even two orders of magnitude, and this difference was significant. It reflects the differences in *Daphnia* population size in the water column, sedimentation regime, and habitat stability over the year (Chapter IV).

The content of eggs inside ephippia was similar at the reservoir dam regions; both eggs were missing from 32-42 % ephippia; a pair of well-preserved eggs was found in 19-34 % ephippia. Contrary, the ephippia from the upstream regions were less consistent in the egg quality distribution. In particular, substantial variation was found in the proportion of ephippia containing well-preserved eggs: this was very low in the upstream regions of Seč and Vranov but exceeded the values from dam regions in the other three reservoirs (Brno, Vír, Žlutice). It is likely that conditions in the sediment of deep reservoir parts (in particular at the dam) are much more stable, and ephippia deposited in such conditions experience similar seasonal changes of temperature and oxygen depletion. On the other hand, high variation in the environmental conditions of upstream regions may be reflected in variable proportions of ephippial eggs removed from the egg bank due to hatching or degradation.

The longitudinal gradient in taxonomic composition was present in resting eggs (Chapter IV) and was similar to the previously reported water column *Daphnia* (Chapter IV; Petrusek *et al.* 2008b; Seda *et al.* 2007b). A significant agreement between the composition of ephippial eggs and the *Daphnia* community was confirmed. Although not all taxa had similar distributions within the water column and sediment, the summer composition of *Daphnia* in the water column explained 81 % of the variation in ephippial egg composition. The autochthonous production of ephippia at least partly reflects the pelagic community (Jankowski & Straile 2003), and therefore, *Daphnia* egg banks may contribute to the significant

differentiation of pelagic *Daphnia* along local environmental gradients.

The role of ephippia for *Daphnia* community after natural disaster in a reservoir

The fate of ephippia and their significance for *Daphnia* community in large reservoirs was investigated in the effects of two extreme floods affecting the Vranov reservoir, one of the previously studied reservoirs (in Chapter IV). The floods obviously effected *Daphnia* in the water column (Chapter V). After the floods, firstly, abundances were lower at all sampling sites but during the spring the usual *Daphnia* summer abundance were reached at the dam station. In that time, the population abundance was still low at the inflow and middle station. Even though the reservoir was affected by the second major flood in late June, *Daphnia* abundances were comparable to previous years two weeks after this event (Chapter V). Although the *Daphnia* population in the water column reached high abundance soon after the second flood, it is reasonable to assume that the population had suffered due to the magnitude of the floods in 2006. In early spring the growth rate of the population is low and cannot compensate for losses due to washout (Rellstab *et al.* 2007). The water column was not thermally stratified in the time of first flood, therefore, the flood pulse influenced the whole reservoir volume. Contrary, the impact of the second flood in summer on the *Daphnia* population in Vranov was most likely mitigated by the location of the outlet that released only particular water strata. and the epilimnion could served as a refuge. Usually, *Daphnia* in downstream parts of the reservoirs are relatively less affected by floods (Seda & Macháček 1998).

Despite the two extraordinary floods, ephippia were abundantly found at all sampled sites in 2007, and in all sampled sediment layers from the surface down to 6 cm (Chapter V). The general pattern of ephippia distribution in the sediment observed before the flood was maintained in subsequent years, i.e. ephippia numbers increasing in the downstream direction. In the upstream part of the reservoir, ephippia densities significantly increased after the floods (from 6.6 to 43.2×10^3 ephippia m^{-2}). At the dam region, on the contrary, we found a lower number of ephippia stored in the sediment in 2007 than in 2004; however, this trend was not significant.

The floods also resulted in significant changes of the preservation of eggs within each ephippial capsule. One year after the

flood, a significant decrease in the proportion of ephippia containing well-preserved eggs in both upstream and dam regions was noticed. These proportions dropped to less than a half of those recorded before the flood (from 6 % to 2 % at the upstream, and from 23 % to 10 % at the dam); this decrease was not accompanied by an increase in the proportion of aborted eggs. Instead, a nearly two times higher proportion of empty ephippia in both upstream and dam sites were found (increasing from 48 % to 78 %, and from 34 % to 59 %, respectively). In the following years, a slow increase in the proportion of ephippia with well-preserved eggs at all sampled sites along the reservoir longitudinal axis was observed, practically reaching the original pre-flood values in 2009.

Changes in ephippia density and quality, such as the increase of ephippia abundances in the upper part of the reservoir in 2007 raised the question of their origin. Floods may have released the ephippia previously attached to the shoreline while floating or from redistributed reservoir sediments due to sudden and turbulent flood inflows. The mixing of the top surface layers of the sediment might have transport the ephippia contained within and deposit them after the flood to shallower parts of the reservoir bottom, where are more favorable conditions for hatching (Rother *et al*. 2010) due to the access of hatching cues, such as light (Schwartz & Hebert 1987). The ephippia redistribution thus might have facilitated the activation of *Daphnia* genotypes stored in the ephippial egg bank, which would otherwise have remained lost and eggs in long-term dormancy may have contributed to *Daphnia* recovery in the otherwise stable habitat.

Conclusions

In conclusion, large habitats enable the long term maintenance of multiplied clonal lineages that may overwinter in the amictic active phase. Although this erodes genotype diversity before the production of sexual individuals, the genetically variant offspring still may be produced due to mechanisms such as the spatial segregation of closely related sexual females and males (Chapter I, II). The ephippial community might be produced by only part of the population (Chapter III), but the admixture of the sediment dormant generations of various age may reduce the seasonal impacts of low diversity ephippia. Benthic-pelagic coupling showed the correspondence of the active *Daphnia* community and dormant community on a large scale (tributary-dam). The spatial heterogeneity of *Daphnia* taxonomic taxa may be substantially strengthened by the

presence of spatially heterogeneous egg banks (Chapter IV). Additionally, if the community undergoes severe disturbance, such as two extraordinary floods during a couple of months, which may flush out an unknown proportion of the *Daphnia* community, the contribution of individuals hatching from ephippial eggs to the rapid recovery of the *Daphnia* community might also be important (Chapter V). A sexually produced resting egg bank further stabilizes the spatial heterogeneity and local variability of *Daphnia* communities, and may secure the renovation of the community even in large reservoirs.

Further research prospects

During this thesis several topics emerged that would require further studies to better our understanding of them.

The origin and formation of the bathypelagic *Daphnia* community
Daphnia galeata is a species known to have epilimnion preference. They overwinter in deep refuges and after the frost-out of ice cover aggregate in the epilimnion. But later, after the establishment of thermal stratification, a significant part of the population migrate down into dark and cold water and seem to preferably stay there over the summer season. An investigation to possibly answer the following questions is necessary: Are *Daphnia* found in hypolimnion just senescent from upper layers, or is this their location of priority? Are some clones better adapted to hypolimnion conditions and thus to be found frequently in cold temperatures? Are there any markers in which these *Daphnia* differ from epilimnetic ones? Do parasites have any role in hypolimnetic *Daphnia* occurrence?

Unfertilized autumn ephippia and the origin of late summer males
A detailed examination of the autumn course of ephippia production revealed a higher proportion of unfertilized ephippia. Maternal *Daphnia* obviously did not mate with males. Male clutches are scarcely found in autumn but adult males are still present in the water column, albeit in very low abundance. The question is whether these males simply do not co-occur with ephippial females, are too scarce to be able to effectively meet with sexually receptive females, or are too old. Due to migration to the hypolimnion, spring males may reach a behaviorally extended lifespan and therefore those males registered in autumn samples might be those produced in spring and possibly too old to impregnate ephippial females.

The overwintering and long term maintenance of clones in reservoirs

The occurrence of *Daphnia* in winter is not necessarily tied to their maintenance and dominance in the system. Tracking of particular clones should reveal whether the capacity to overwinter is or is not of a stochastic nature and we might be able to track a clone over years in a reservoir. Additionally, an investigation of whether overwintering clones have any competitive advantage during spring development is required.

The hybrid zones of reservoirs as zones of higher sexual rates

During the whole reservoir study we monitored seemingly established hybrid zones in *Daphnia*, usually as transitional zones between habitats, namely the tributary–lacustrine part or the epilimnion–hypolimnion. The question is whether the transitional zones are more favorable for intermediate hybrid progeny compared to the parental species, or whether the hybrids are more frequently produced there and therefore have a larger pool to compete with parental species. If the latter is valid, hybrid zones might be areas of higher sexual reproduction revealed by a higher proportion of hybrid resting eggs and the clonal richness of hybrid clones in the water column.

The increment of hatchlings to the water column *Daphnia* in permanent reservoirs

Daphnia cucullata is frequently found in the tributary zones of reservoirs where its smaller size favors it in size selective predation. It is not known to overwinter as an active stage in the water column, but it is dominant there during the growing season. Its population in tributary zones must come either from upstream migration or from the hatching of ephippia produced there. By investigating *D. cucullata* genotypes, the question of their origin might be answered

Another question of bentic-pelagic coupling arose from the ephippia production study. In *D. galeata* the main peak of ephippia production occurs in spring and these ephippia also contained a significantly higher proportion of healthy resting eggs. As the egg bank assists in establishing clone dominance in the water column, we might observe a gradually rising proportion of alleles of spring clones and their dominance in the system in newly established water bodies.

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Research articles

CHAPTER I

The sexual reproduction of *Daphnia* in a deep temperate reservoir: the phenology and genetics of male formation

Sexual reproduction of *Daphnia* in a deep temperate reservoir: the phenology and genetics of male formation

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Abstract Long-term persistence of a *Daphnia* population is allowed by the production of dormant stages, produced mostly through sexual reproduction. We investigated the spatio-temporal dynamics of male production in a spring population of *Daphnia galeata* in the reservoir, and compared the genetic structure of three groups within this population: male-producing females, female-producing females, and adult males. With a fine resolution sampling design and the use of highly variable microsatellite markers we revealed that: 1) the spring period of male offspring production was delimited in time with minimum interannual variation to about three weeks, and in space to the upper 5m water layer; 2) there were no remarkable changes in the clonal composition of male-producing females within the period of male production; 3) overall certain clones exhibited a higher tendency to produce male offspring and

therefore the clonal structure of male-producing lineages was significantly different from that of female-producing lineages; and 4) the clonal structure of male-producing females was not significantly different from that of adult males occurring later in the reservoir. This suggests that males were not subjected to any significant selective forces till maturity and the male-producing females confer a long-term fitness advantage over female-producing females.

CHAPTER II

II

The sexual reproduction of *Daphnia* in a deep temperate reservoir: avoidance of inbreeding through spatial segregation

Manuscript (first draft)

Sexual reproduction of *Daphnia* in a deep temperate reservoir: avoidance of inbreeding through spatial segregation

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Abstract In species alternating between asexuality and sexuality, the process of forming mictic individuals after prolonged asexuality is essential to maintain genetic variability. In two subsequent years, we focused on the cyclical parthenogen water flea *Daphnia galeata* in a permanent population that sexually reproduces in spring. We aimed at estimating the clonal diversity and spatial distribution of the sexual part of the population (males and ephippial females), compared to these of asexual females. Furthermore, we analyzed the parentage of sexual offspring, the dormant eggs enclosed in molted ephippia. While sexual individuals represented only a minor part of the population in the surface strata, their proportion could reach 80 % in the deep strata. The clonal richness of the population producing sexual individuals was strongly reduced. The genotypes of the both sexes were not a random subset of the population, and also belonged to two genetically distinct groups. Nevertheless, there were in total up to 7 % of genotypes identical for males and ephippial females in both investigated seasons. The observed spatial distribution, showing the presence of different clonal lines at different depth, likely lowers the possibility of siblings mating to only 1 % probability. Even though the clonal diversity of parents was reduced, new unique ephippial genotypes enter the egg bank in the sediment and would mix with other generations.

The following content (20 pages) is included only in the original PhD thesis archived by Faculty of Science, University of South Bohemia.

CHAPTER III

The sexual reproduction of *Daphnia* in a deep temperate reservoir: seasonal patterns in the production and quality of resting eggs

III

Sexual reproduction of *Daphnia* in a deep temperate reservoir: Seasonal patterns in the production and quality of resting eggs

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Abstract Sexual reproduction in cyclical parthenogens produces recombinant offspring, which may refresh the genetic richness of parental populations especially if reproducing clonally for longer periods. We investigated the production of sexual resting eggs, encapsulated in ephippium, of the small planktonic crustacean *Daphnia galeata* in a permanent natural population. Using sediment traps, we monitored the input of ephippia into the resting egg bank in sediments during four successive years. The investigation was performed during three separate seasons (spring Apr – Jul, autumn July – Nov, and winter Nov – Apr) and the content of eggs was evaluated as a proxy of fertilization success. The input of ephippia was the highest in the spring period over all studied years, though varied significantly among years in correlation with *Daphnia* population size. Additionally, ephippia with well-preserved eggs were more frequent in spring compared to autumn or winter ephippia. We attribute this bias to differences in fertilization success, as hatching is likely negligible in the deepest sediment. We conclude that although ephippia and sexual eggs are produced throughout the whole year, the differences in the fertilization success significantly alter the importance of particular periods and highlight the significance of the spring period of sexual reproduction for the annual input to resting egg banks.

The following content (12 pages) is included only in the original PhD thesis archived by Faculty of Science, University of South Bohemia.

CHAPTER IV

The stabilizing effect of resting egg banks of the *Daphnia longispina* species complex for longitudinal taxon heterogeneity in long and narrow reservoirs

IV

The stabilizing effect of resting egg banks of the Daphnia longispina species complex for longitudinal taxon heterogeneity in long and narrow reservoirs

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Abstract We compared the spatial distribution of taxa from the *Daphnia longispina* complex (*D. longispina, D. galeata, D. cucullata*, and their hybrids) in the active water column community and in resting egg banks in five long narrow reservoirs in the Czech Republic (Central Europe). In each reservoir, we sampled at both ends of the longitudinal gradient: in the inflow region and at the dam. Ephippia abundance in the sediments significantly increased in the downstream direction, reflecting differences in the sedimentation regime and *Daphnia* population size. Similarly to the active zooplankton community, in which *D. cucullata* and *D. longispina* tended to occur at opposite ends of the reservoirs, *Daphnia* species and interspecific hybrids in resting eggs revealed a spatially diversified pattern; however, we observed some differences in taxon distributions between sediments and water columns. High relative abundances of hybrid genotypes (up to 16% of resting eggs, and 74% of *Daphnia* in the water column) confirm that interspecific hybridization is frequent in these reservoirs, and some hybrids are successful in competition with the parental taxa. We assume that the spatial heterogeneity of *Daphnia*

taxonomic composition in reservoirs, being affected by the seasonal selection of taxa within the mixed reservoir species pool, is substantially strengthened by the presence of spatially heterogeneous egg banks.

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CHAPTER V

The effects of extreme floods on the *Daphnia* ephippial egg bank in a long narrow reservoir

V

Effects of extreme floods on the *Daphnia* ephippial egg bank in a long narrow reservoir

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Abstract Ephippial egg banks are important reservoirs of dormant stages that allow the recovery of *Daphnia* populations after unfavourable periods. Although the contribution of hatchlings from ephippia to a population in the water column is probably of minor importance in permanent water bodies with a year-round *Daphnia* persistence, this may differ after major disturbances. In 2006, two 500-year floods hit the long and narrow Vranov reservoir (Czech Republic), in which we had investigated zooplankton densities and ephippia distribution in the sediment in preceding years. In this study, we evaluated the impact of those extraordinary floods on the population of the *Daphnia longispina* complex, and particularly on the local dormant egg bank. We considered two alternative hypotheses related to the egg bank: that either the substantial input of new material with the flood completely buried and therefore reset the existing egg bank, or that the sediment including ephippia was redistributed in the reservoir after the flood and dormant eggs could be exposed to hatching stimuli. A year after the floods, we did not observe any sediment layer that would be devoid of ephippia. However, we observed a significant increase in the proportion of empty ephippia and a decrease in the proportion of those containing eggs. We attribute these changes to disturbance of the sediment due to the floods,

which caused redistribution of ephippia stored in the sediment and those detached from the reservoir shoreline. Dormant eggs inside ephippia deposited to shallow parts of reservoir after the floods could therefore receive and respond to hatching stimuli. Hatching from ephippia may have contributed to *Daphnia* recovery after the spring flood; however, a significant proportion of the *Daphnia* population probably survived the summer flood protected in the epilimnetic refuge of the thermally stratified environment.

The dynamics of sexual reproduction and ephippia production of *Daphnia* in reservoirs
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