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Biodiversity and structure of invertebrate communities of
temporary pools along a hydroperiod gradient

Biodiversiteit en structuur van invertebratengemeenschappen in
tijdelijke poelen overheen een hydroperiodegradiënt

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Door:

Liesbet Boven

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INTRODUCTION AND THESIS OUTLINE

WETLANDS, PONDS AND TEMPORARY POOLS: DEFINITION AND CONSERVATION STATUS

Wetlands are defined as “areas where saturation with water constitutes the dominant factor determining the nature of soil development and the types of plant and animal communities living in the soil and on its surface (Cowardin et al. 1979)”. Different wetland types exist according to regional conditions of the soil, topography, climate, hydrology, water chemistry, vegetation and other factors, including human disturbance. Particular wetland types are **ponds and temporary pools**: relatively small water bodies, ranging from 1 m² to 2 hectares, of man-made or natural origin (Biggs et al. 2005). The definition of temporary pools is further narrowed to small waterbodies “that occasionally contain water and once inundated, hold water long enough to allow the development of the aquatic phase of the life cycle of some species (Blaustein & Schwartz 2001)”.

Ecosystem services and biodiversity

Currently, the significance of natural ecosystems to human society is expressed in economic terms (e.g. Costanza et al. 1997, Balmford et al. 2002). The economic value of ecosystems is measured by their ‘ecosystem services’, key functions or processes of ecosystems and species that support human activity and sustain human life (Chapin et al. 2000, Costanza 2008, Costanza et al. 2008). Wetlands, as such, are extremely valuable ecosystems (Dudgeon et al. 2006). Not only do they play a major role in water quality improvement and flood control, but recently also their importance to carbon storage has been acknowledged (Zedler & Kercher 2005). Although biodiversity *in se* can be considered as an ecosystem service provided by wetlands (Dudgeon et al. 2006), conservation efforts are often especially valued when they are linked to the preservation of specific goods or services of immediate economic value (Ehrlich & Ehrlich 1992, Chapin et al. 2000).

Temporary pools are wetlands of particular concern to biodiversity. They are especially valued as sources of regional diversity (γ -diversity), both for plant and invertebrate species (Williams 1997, Forró et al. 2003, Williams et al. 2003, Nicolet et al. 2004). Ponds and pools in general considerably contribute to regional biodiversity through their high community heterogeneity (β -diversity), while especially temporary pools often contain rare or unique species (Collinson et al. 1995, Oertli et al. 2002, Williams et al. 2003, Nicolet et al. 2004). Moreover, temporary pools constitute feeding and breeding sites for waterfowl (Leydi

& White 1998) and are main habitats of species with particular conservation concern, such as large branchiopods (Dumont and Negrea 2002) and amphibians (Semlitsch 2000, Babbitt et al. 2003, Beja & Alcazar 2003, Baber et al. 2004). Functioning as stepping-stones, they are furthermore likely to improve connectivity among aquatic habitats and as such enhance the persistence of amphibian (Gibbs 1993), bird (Naugle et al. 2001) or invertebrate populations through metacommunity dynamics (Holyoak & Lawler 1996, Holyoak et al. 2005).

A changing landscape

Fresh water supports approximately 6% of all described species (see *Hydrobiologia* Vol. 595 (2008) for a recent update on aquatic animal diversity) and its conservation is the over-riding priority during the International Decade for Action – ‘Water for Life’ – 2005 to 2015 (Dudgeon et al. 2006). On a global scale, freshwater wetlands cover less than 3% of the surface area, while they contribute to 40% of annual renewable ecosystem services (Zedler & Kercher 2005). Despite their conservation priority and disproportional function in relation to the area they cover, profound reductions in wetland density and connectivity are associated with the increasing human population and the resulting global landscape changes (Gibbs 2000, Dudgeon et al. 2006). According to some estimates, throughout history half of the global wetland area has been lost, mainly in northern countries during the first half of the twentieth century (OECD/IUCN 1996). One of the main causes of wetland loss is drainage in agricultural regions, while remaining wetlands are often degraded through eutrophication or invasion of exotic species (Zedler & Kercher 2005).

The loss and degradation of temporary water bodies during the last decades is well-documented worldwide (King 1998, Williams et al. 2001, Boothby 2003, Jenkins et al. 2003, Wood et al. 2003), with regional estimates of habitat loss ranging from 50 to 85% (King 1998, Jenkins et al. 2003). Also in Western Europe, drainage and intensive cultivation have had a negative impact on these habitats. In Eastern Europe, agriculture is usually less intensive and large areas of farmland still support a high habitat and species diversity, often including species of conservation concern (European Environment Agency 2004). While environmental pressures are expected to decrease in Western Europe, many areas in Eastern Europe will experience increasing pressure from agriculture. In countries such as Poland and Hungary, which have recently joined the European Union, the conservation of temporary pools and their inhabitants consequently is of high priority (Biggs et al. 2004, Oertli et al. 2005).

Models and long-term data provide evidence of a strong link between the frequency and duration of inundation of temporary aquatic habitats and temperature and precipitation patterns (Brooks 2004, Bauder 2005, Pyke 2005a, Hulsmans et al. 2008). Therefore, also concern is rising with regards to the alteration of these ecosystems’ hydrological conditions

or persistence as a result of climate change and the shift this might entail in reproductive suitability for their inhabiting species (Roshier et al. 2001). Some studies moreover indicate that the ecological implications of climate change may be reinforced by interactions with habitat loss or land use changes and the other way around (Hartig et al. 1997, Pyke 2005b, Pyke & Marty 2005, Piha et al. 2007).

WHY STUDY TEMPORARY POOLS?

Pool ecosystems in general have been suggested as convenient model systems in addressing ecological research questions. Pools often are locally abundant and cover a broad range of ecological gradients (Blaustein & Schwarz 2001, De Meester et al. 2005). Their small size allows quantitative and representative sampling, while their relative simplicity allows them to be easily mimicked or manipulated. Finally, they constitute versatile but biologically realistic natural microcosms (Srivastava et al. 2004).

The use of temporary pools as study systems additionally allows the study of time constraints on population and community processes. Such information is not only relevant to temporary pools but can be applied to diverse ecosystems where time for development is variable and often limiting (e.g. fruits, plants, carrion; Blaustein & Schwarz 2001). One of the main objectives of ecological studies is the protection of ecosystems as proper understanding is needed for designing effective management practices. Given the particular conservation concern of temporary aquatic habitats, the results obtained from ecological studies on temporary pools are highly relevant and directly applicable to the protection of their functioning and diversity (Blaustein & Schwarz 2001).

GENERAL OBJECTIVES OF THE STUDY

Our study aims at characterizing ecological processes that structure invertebrate communities in temporary pools through space and time, by means of an elaborate field study complemented with controlled laboratory experiments. As our study mainly focuses on the ecology of two groups of temporary pool inhabitants that perform a particular function in their habitats, large branchiopods and Cladocera (Branchiopoda – Crustacea; Fig. 1), our results may reach beyond fundamental scientific research and could be applied for conservation purposes.

TEMPORARY POOL INHABITANTS

The recurrent drying and resulting time stress and fluctuations in physical and chemical parameters in temporary pools require different strategies of physiological tolerance, life history adaptations and/or migration from their animal inhabitants (Wiggins et al. 1980).

According to their strategy of tolerating or avoiding drought, “permanent” and “temporary” inhabitants can be distinguished. Permanent inhabitants survive the dry period in the habitat as dormant eggs (e.g. Cladocera and large branchiopods; Brendonck 1996, Brock et al. 2003) (Fig. 1) or other drought-resistant life stages (e.g. Mollusca, Chironomidae, some Coleoptera and Odonata), while temporary inhabitants migrate to permanent waters or terrestrial habitat when the temporary habitats dry (e.g. Odonata, Hemiptera and Coleoptera) (Wiggins et al. 1980; Velasco & Millan 1998).

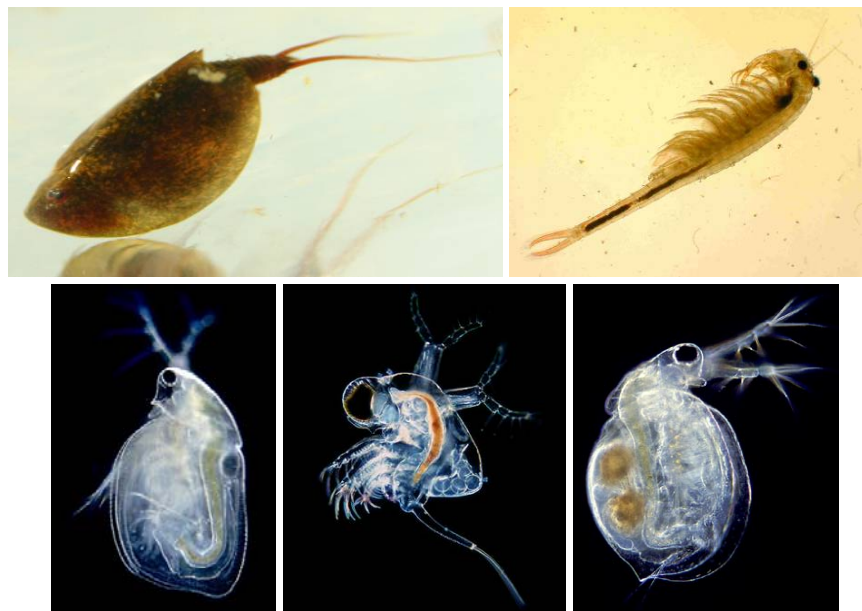


Figure 1. The study organisms: large branchiopods and Cladocera as permanent inhabitants of temporary aquatic habitats. From left to right; upper row: *Lepidurus apus*, *Branchipus schaefferi*, lower row: *Simocephalus vetulus*, *Polyphemus pediculus* and *Ceriodaphnia reticulata*. Pictures by Elly Vaes, László Forró and Wim van Egmond.

Both large branchiopods and cladocerans play a key role in ecosystem functioning. They are highly efficient grazers (Sommer et al. 1986, Lampert 1988, Brendonck 1993, Sommer et al. 2001) and in turn, constitute major prey for invertebrate and other predators. Their conservation, therefore, may be especially important in preserving the ecological quality and species diversity of the temporary pool habitat. Downing & Leibold (2002) showed that changes in species diversity and composition at different trophic levels in ponds affected ecosystem functioning through indirect effects and trophic interactions among species.

Being restricted to temporary waters, large branchiopods face serious threats to persist in many areas, sometimes even extinction (Brendonck 1989, Eng et al. 1990, Mura 1993, Brendonck & Riddoch 1997, Petrov & Petrov 1997, Williams 1997, Damgaard & Olesen 1998, Maier 1998, Eder & Hödl 2002, De Roeck et al. 2007, Brendonck et al. 2008).

They are internationally recognized as a taxon in need of conservation (IUCN 2000). Through their role as 'flagship species' (Belk 1998), moreover, they represent the entire temporary pool ecosystem that is in need of considerable protection. The increasing literature on their alarming status, however, contrasts with the limited number of management and conservation measures that are based on thorough ecological studies (e.g. Angeler et al. 2008a).

TEMPORARY POOL COMMUNITY ECOLOGY

Local communities of temporary pool invertebrates may be strongly influenced by physical and chemical variables, either directly or through trophic interactions, but the most important factor steering species richness and composition is probably the duration of inundation (Williams 2006). Besides local factors, interactions with the landscape and the regional species pool (dispersal) may explain species' distributions and local abundance patterns (Ricklefs 1987, Cornell & Lawton 1992). Recently, temporary pools proved to be valuable ecosystems in assessing the relative importance of local and regional processes in structuring local communities linked through dispersal (i.e. metacommunities). In these temporally variable and often unpredictable habitats, an important fraction of the community remains dormant in the sediment, but may contribute to the active species pool through temporal dispersal dynamics.

Hydroperiod

The importance of hydroperiod (i.e. the duration of inundation) as a structuring factor of temporary pool communities mainly results from two constraints on species' life cycles: predation risk and development time (Schneider & Frost 1996, Wellborn et al. 1996). The changes in species distribution along the hydroperiod gradient are shaped by trade-offs in individual life history traits that determine the success of each species along the gradient (Wellborn et al. 1996, Stoks & McPeck 2003). Fish, only with some exceptions, are excluded from seasonally drying pools as they cannot tolerate or avoid the recurrent droughts. For many invertebrate predators (e.g. Odonata, Coleoptera, Hemiptera) or amphibians, temporary pools constitute suitable feeding and/or breeding sites from which they can escape before drying (Wiggins et al. 1980). However, the time required for their development and the active dispersal strategy of most of these predators theoretically limits their distribution along the hydroperiod gradient. With decreasing pool duration, predator abundance and/or species richness generally diminishes (Corti et al. 1997, Bilton et al. 2001, Wilcox 2001, Rundle et al. 2002), while the importance of a high development rate of the inhabitants increases (Schneider & Frost 1996, Rowe & Dunson 1995). There is, however, conclusive evidence that temporary pools are not 'enemy-free' habitats and that invertebrate

predation may be a biotic stressor regulating inhabiting communities (Arner et al. 1998, Boix et al. 2006), even in ephemeral pools (Blaustein et al. 1995, Brendonck et al. 2002).

With increasing pool duration, more time is available for colonization and community development. Ebert & Balko (1987) proposed an extension of the equilibrium theory of island biogeography to temporary pools as islands in time, in which the duration and frequency of flooding are included, positively correlated to colonization rate and negatively to extinction risk (Fig. 2). Long hydroperiod pools hence may support more species with different life cycle phenologies or colonization strategies (Schneider & Frost 1996, Spencer et al. 1999, Brooks 2000). This may eventually result in nested patterns in which species-poor assemblages of temporary wetlands are not distinct, but rather subsets of species-rich assemblages present in more permanent pools (e.g. Baber et al. 2004). As the hydroperiod shortens, in turn, seasonal succession stages, species turnover and species additions may be truncated (Boix et al. 2004, Jocqué et al. 2007a). Jocqué et al. (2007a) proposed an alternative definition of ephemeral pools as temporary pools in which the limited amount of time available for community development does not allow the replacement of species and species are merely added to the community.

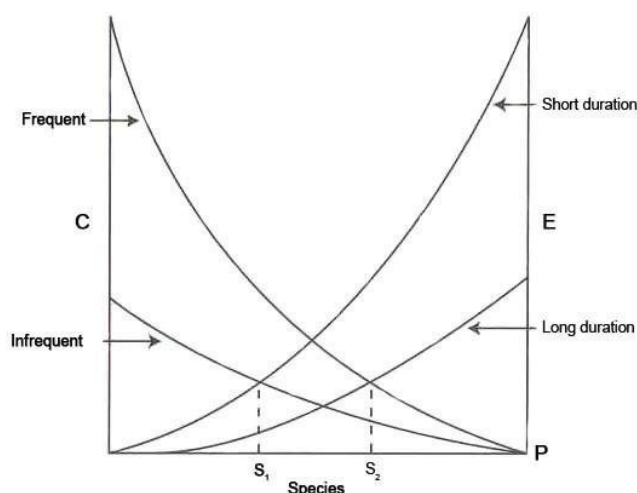


Figure 2. Diagram of colonization (C) and extinction (E) curves for temporary pools as islands in time, analogous to islands in space. P represents local species richness: S_1 is the number of species at equilibrium for a pool that occurs infrequently and has a short duration; S_2 is the number of species at equilibrium for a pool that occurs frequently and has a long duration (from Ebert & Balko 1987).

Pool morphometry and physical and chemical variables

The positive relation between species richness and surface area, as established in the theory of island biogeography (MacArthur & Wilson 1967), has also been demonstrated in temporary pools (Mahoney et al. 1990, March & Bass 1995, King et al. 1996, Brose 2001, Mura & Brecciaroli 2003, Eitam et al. 2004b, Frisch et al. 2006). Larger habitats usually have higher habitat heterogeneity (Williams 1943, Douglas & Lake 1994), are better buffered

against physical and chemical fluctuations and may support larger populations (Spencer et al., 1999).

Larger habitats are possibly also more attractive to actively dispersing insects (Wiggins et al. 1980, Wilcox 2001). Additionally, the relation between pool size and local species richness is often established through a positive correlation between pool size and hydroperiod (Spencer et al. 1999, Brooks 2000, Baber et al. 2004, Tarr et al. 2005, Frisch et al. 2006, De Roeck 2008).

It is generally assumed that physical or chemical pool characteristics imply stronger constraints on the biota in temporary pools than in permanent waters, especially through their wide temporal and spatial variation (Keeley & Zedler 1998, Williams 2006, Magnusson & Williams 2006). The repeated cycles of pool filling, drying and possibly freezing result in significant variation in temperature, oxygen concentration, conductivity and turbidity throughout the pool's inundation cycle (Daborn & Clifford 1974, Lake et al. 1989, Meintjes et al. 1994, Bonner et al. 1997, Lahr et al. 1999, Hancock & Timms 2002, Williams 2006). Moreover, as temporary pools are relatively shallow and the water often has a low buffering capacity, diurnal fluctuations in temperature, oxygen levels and pH in response to changes in air temperature and photosynthesis, respectively, may be especially pronounced (Hamer & Appleton 1991, Meintjes et al. 1994, Scholnick 1994, Keeley & Zedler 1998, Podrabsky et al. 1998, Chan et al. 2005). According to Batzer et al. (2004), invertebrates that successfully exploit seasonal habitats became tolerant to a broad range of environmental conditions, such as water chemistry and hydroperiod length, and therefore might not be negatively affected by the degree of environmental variation in their habitats. According to these authors, such relatively high tolerance level may result in weak or insignificant relations between invertebrate community patterns and habitat characteristics.

Seasonal community dynamics

Seasonal community patterns generally result both from the autogenic development of assemblages through time (e.g. due to facilitation or resource exploitation) as well as from responses of the community to allogenic environmental variability (such as temperature changes) (Reynolds 1988). Given the highly variable nature of the temporary pool environment (e.g. depth and volume, temperature and conductivity, algae and vegetation growth), the seasonal development of their inhabiting communities throughout a single hydrocycle is highly dynamic as well. The gradual recolonization of a newly flooded habitat reflects species' differences in life history strategies. First, permanent residents emerge from the dormant propagule bank and thereafter, actively dispersing insects arrive (Williams 2006). According to the type of food resources that become available, a succession of detritivore, herbivore and predacious macroinvertebrates generally takes place (Meintjes

1996, Moorhead et al. 1998, Lahr et al. 1999, Culioli et al. 2006). Seasonal dynamics often result in an addition of species to the community and an increase in taxon richness over time (Bazzanti et al. 1996, Moorhead et al. 1998, Boix et al. 2004, Culioli et al. 2006, Jocqué et al. 2007a). However, when a continuous turnover or replacement of taxa occurs, taxon richness may reach a plateau (Lake et al. 1989). A drop in taxon richness may occur before desiccation as various taxa leave the habitat due to harsh abiotic conditions or intensified predation pressure (Bazzanti et al. 1996, Lahr et al. 1999, Mura & Breccioliari 2003).

Seasonal dynamics in pool invertebrate assemblages may considerably vary from one year to another according to hydrological and other habitat conditions or prevailing weather conditions (e.g. Jeffries 1994, 2003, 2005, Rettig et al. 2006, Tavernini 2008). Moreover, hydrological conditions and thus the reproductive suitability of pools to various taxa strongly depend on temperature and precipitation patterns (Brooks 2004, Bauder 2005, Pyke 2005a, Hulsmans et al. 2008). Therefore, temporary pool communities might be considerably affected by long-term climatic variation – possibly as a result of climate change.

Regional processes and metacommunities

Aquatic invertebrates have developed strategies to migrate from one habitat to another. While adult insect stages and amphibians are able to disperse between habitats on their own force and actively select habitats to colonise (Pajunen & Pajunen 2003, Rieger et al. 2004, Resetarits 2001, Arav & Blaustein 2006), zooplankton taxa rely mainly on passive dispersal to colonize new habitats. On a local scale, freshwater zooplankton and/or their dormant eggs are readily dispersed by wind (Vanschoenwinkel et al. 2008a, b), water connections (Michels et al. 2001, Hulsmans et al. 2007, Vanschoenwinkel et al. 2008b), amphibians (Bohonak & Whiteman 1999) and mammals (Vanschoenwinkel et al. 2008c), while probably the most effective long-distance dispersal agents are water birds (Figuerola & Green 2002).

As a consequence, depending on the degree at which species move between habitats, freshwater pool communities should not be considered as static, isolated entities in the landscape, but may constitute a dynamic complex of interconnected patches. The notion that spatial dynamics between local communities have to be taken into account in explaining species distribution patterns is the basis of modern metacommunity ecology (Leibold et al. 2004; Holyoak et al. 2005). Moreover, as their biota are often strongly bounded by terrestrial habitat, pool communities are suitable to empirically test existing metacommunity paradigms (Leibold et al. 2004). A species sorting view, in which species distributions are closely linked to local conditions and largely independent of spatial effects such as dispersal limitation, may be best applicable to metacommunities of pool invertebrates, as supported by empirical evidence in highly interconnected ponds (Cottenie et al. 2003) as well as in temporary rock pools (Jocqué 2007, Vanschoenwinkel et al. 2007). However, various metacommunity

processes are likely to interact in real metacommunities in pools (Leibold & Miller 2004). For example, for passive dispersers in temporary rock pools, at high levels of dispersal mass effects probably came into play, facilitating community homogenization, whereas in isolated pools priority effects possibly became more important (Vanschoenwinkel et al. 2007). Additionally, metacommunity dynamics of passive dispersers may be more affected by spatial factors (connectivity) when compared to active dispersers (Jocqué 2007, Van de Meutter et al. 2007).

Ecological role of dormant egg banks

In temporally variable aquatic environments, freshwater zooplankton organisms produce dormant eggs to bridge periods of unfavourable conditions for survival and reproduction (Sars 1885, Fryer 1996, Cáceres 1997a, Hairston and Kearns 2002; Fig. 3). In seasonally drying pools, permanent inhabitants rely on dormant stages to survive recurrent droughts (Brendonck 1996, Brock et al. 2003). During the entire growing period (large branchiopods) or before the onset of unsuitable conditions (cladocerans, rotifers, copepods), dormant stages are produced and deposited in the sediment where they accumulate as a dormant egg bank. The dormant eggs are generally very resistant to harsh conditions, such as freezing and drought, and may remain viable in the sediment for long periods (Hairston et al. 1995, Cousyn & De Meester 1998). When conditions turn favourable again, for example after the filling of a temporary pool by rain or snow melt, species hatch from the dormant egg bank and initiate new populations. By such diapause strategies, zooplankton organisms are able to disperse through time, in analogy with dispersal through space (Templeton & Levin 1979).



Figure 3. Female of *Daphnia atkinsoni* carrying ephippium with dormant eggs (left; picture by Joachim Mergeay), ephippium with dormant eggs of *Simocephalus* (middle; picture by Jochen Vandekerckhove) and *Macrothrix* (right; picture by Tom De Bie). An ephippium is the protective case in which one or two dormant eggs, depending on the species, are encapsulated.

Prolonged diapause beyond the next growing season and partial hatching of zooplankton dormant eggs may act as an adaptive strategy in response to the variability and unpredictability of their natural environment (Cohen 1966, Brown & Venable 1986, Philippi &

Seger 1989). Indeed, in environments where there is a risk of hatching without successful production of the next generation of dormant stages, theoretically each growing season only a fraction of the dormant eggs should hatch, while the non-hatched fraction constitutes a buffer against unsuccessful growing seasons (i.e. diversified bet-hedging; Philippi & Seger 1989, De Meester & De Jager 1993, Van Dooren & Brendonck 1998). In temporary pools, low hatching fractions may serve as a bet-hedging adaptation to the unpredictability in duration and frequency of filling events (Simovich & Hathaway 1997, Brendonck et al. 1998, Brendonck & Riddoch 2002, Philippi et al. 2001).

The dormant zooplankton egg bank furthermore constitutes an important mechanism maintaining species coexistence (Hairston 1996, Cáceres 1997b, Bonis et al. 1995) through the 'storage effect' (Warner & Chesson 1985). Stable coexistence necessarily requires important ecological differences between species in resources, predators, and the use of space and time, four main axes defining niche space (Chesson 2000). However, the storage effect refers to a model in which species are not distinguished by the resources *in se*, but by when they are using them (Chesson 2000). The storage effect implies that in environments where variable environmental conditions result in fluctuations in the recruitment of competing species over time, competitive exclusion can be prevented when an overlap of generations - in this case through the dormant egg bank - allows species to recover from periods of low recruitment (Warner & Chesson 1985).

The timing of diapause termination, triggered by environmental cues, may affect annual or seasonal turnover of zooplankton populations and communities. For example, in a temporary pond in Canada, erratic occurrence patterns and annual changes in anostracan species composition depended on species-specific hatching requirements related to amounts of precipitation at different times of the year (Donald 1982). In another example, changes in the zooplankton community composition in Swan Lake (Canada) from one year to another were attributed to environmental changes in water level, temperature and light conditions that differentially affected the emergence of species (Arnott & Yan 2002). The contribution of emergence to seasonal dynamics of zooplankton assemblages can be assessed by means of emergence traps (Wolf and Carvalho 1989, De Stasio 1990, Cáceres 1998, Hairston et al. 2000, Gyllström 2004). Based on such observations from permanent lakes it was shown that many cladocerans as well as rotifers and copepods hatch often synchronously early in the growing season. Therefore, it was generally assumed that emergence only contributes to the initiation of active populations, while changes in the composition of communities and populations later in the season are driven by biotic processes and abiotic conditions in the water column (e.g. predation, food availability). Field observations, however, sometimes suggested a more important link between hatching processes and seasonal changes in active population size (De Stasio 1990).

In experimental studies it has been shown that dormant stages are able to adjust emergence fractions in response to signals of environmental quality such as conductivity or temperature (Brendonck 1996, Spencer & Blaustein 2001), temperature and photoperiod (Cáceres & Schwalbach 2001) or even predation (Hansson 1996, Rengefors et al. 1998, Spencer & Blaustein 2001, De Roeck et al. 2005). This ability to detect signals related to the probability of future reproductive success and to adjust hatching fractions accordingly may entail an ecological advantage of delaying the hatching response beyond the onset of the growing season. A strategy by which clones or species are able to adjust the timing of hatching within the growing season depending on environmental conditions indicative of a high expected relative fitness is defined as 'temporal habitat selection' (cf. Vanoverbeke 2000) and could result in a more important link between hatching and seasonal population or community dynamics than generally assumed.

In permanent lakes, recruitment from the dormant egg bank and its contribution to the development of spring populations may vary considerably from year to year depending on the size of overwintering populations (Cáceres 1998). In temporary pools, on the other hand, the initiation of active populations solely depends on emergence, due to the lack of populations bridging the dry period. Assessing the contribution of emergence to active community dynamics could shed more light on the mechanisms driving coexistence and succession of competing species (Gyllström & Hansson 2004).

Dormant egg banks as tools for diversity studies

The presence of dormant egg banks as a biotic reservoir both of genetic and species diversity (Hairston 1996) not only has important implications for the ecological and evolutionary dynamics of the zooplankton (Brendonck & De Meester 2003). The integration of annual and seasonal variation in species composition and population genetic structure of zooplankton communities in the sediment entails some methodological applications as well. Detailed morphological or genetic analysis of dormant stages deposited in undisturbed historically stratified aquatic sediments, as such, allows the historical reconstruction of species composition and diversity against the known history of a pool, for example in the context of anthropogenic impacts (e.g. pollution, eutrophication, fish predation), and to reliably estimate its pristine state (Brendonck & De Meester 2003, Jeppesen et al. 2001).

Through the integration of temporal and spatial variation in community structure in the most recently deposited eggs, the analysis of the 'active egg bank' (i.e. upper 2-3 cm; Cáceres & Hairston 1998) may be a more reliable method to assess species richness in contemporary zooplankton communities than sampling the active community, even at repeated occasions (Vandekerkhove et al. 2005a). Positive results obtained from exploratory work done on this topic (May 1986, Havel et al. 2000, Crispim & Watanabe 2001, Duggan et

al. 2002) were reinforced by elaborate research by Vandekerckhove et al. (2005b). In a comparative study of 88 European lakes, these authors demonstrated that the identification of hatchlings obtained from egg bank samples enabled the detection of about twice as many species than identification of six subsequent active community samples.

THE KISKUNSAĞ NATIONAL PARK: A PRISTINE STUDY AREA

The Kiskunság National Park was established in 1975 and is located 85 km southwest of Budapest (Hungary), on the plain between the Danube and the Tisza River (Fig. 4a). It has an area of almost 500 km². The highly diverse landscape of Kiskunság makes part of the Natura 2000 Network and its wetlands are considered of international importance by the Ramsar Convention. Due to the high local abundance of temporary freshwater pools with variable hydroperiods and local habitat characteristics and the limited impact of traditional and rather extensive agricultural activities in most parts of the area, Kiskunság constitutes a unique study system for addressing ecological research questions, especially related to biodiversity issues.

The Hungarian continental climate with cold winters and warm dry summers favors the formation of vernal pools filling in early spring with snowmelt. As air and water temperature increase during springtime, evaporation also increases. Depending on the morphometry of pools and local weather conditions, the pools usually are dry again in spring or summer.

Within the west part of the Kiskunság area, a total of 82 pools were selected for this study (Fig. 4b), belonging to five habitat types: meadow pools, clay pits, pools in arable land, orchard pools and wheel tracks (Fig. 5). Meadow pools, pools in arable land and pools in orchards occur as natural depressions. Depending on the soil type, their transparency and degree of vegetation cover varies. Orchard pools are rare, whereas meadow pools and pools in arable land occur throughout the study area. Clay pits are man-made pools in the vicinity of the village of Fülöpszállás and originate from the excavation of clay for building houses. They are rather turbid and deep. Wheel tracks are man-made tracks and typically occur on clayey soils. These habitats are usually small and shallow, extremely turbid and with little or no vegetation. The different pool types are distributed along a typical environmental gradient that characterizes the study area. Eastwards, reed marshes and meadows on peat soils constitute the main biotopes, whereas more to the west, meadows on alkaline or clayey soils and salt lakes typically characterize the study area. The main land use types in the study area consist of agriculture, cattle grazing and mowing (Fig. 6). Although within the protected areas of the national park agricultural activities are rather extensive, some parts are also intensively cultivated, especially westwards towards the Danube valley. In the most southern

part of the study area the landscape gradually gets drier and the more sandy soils are used for orchards. In the northeast, forests predominantly occur.

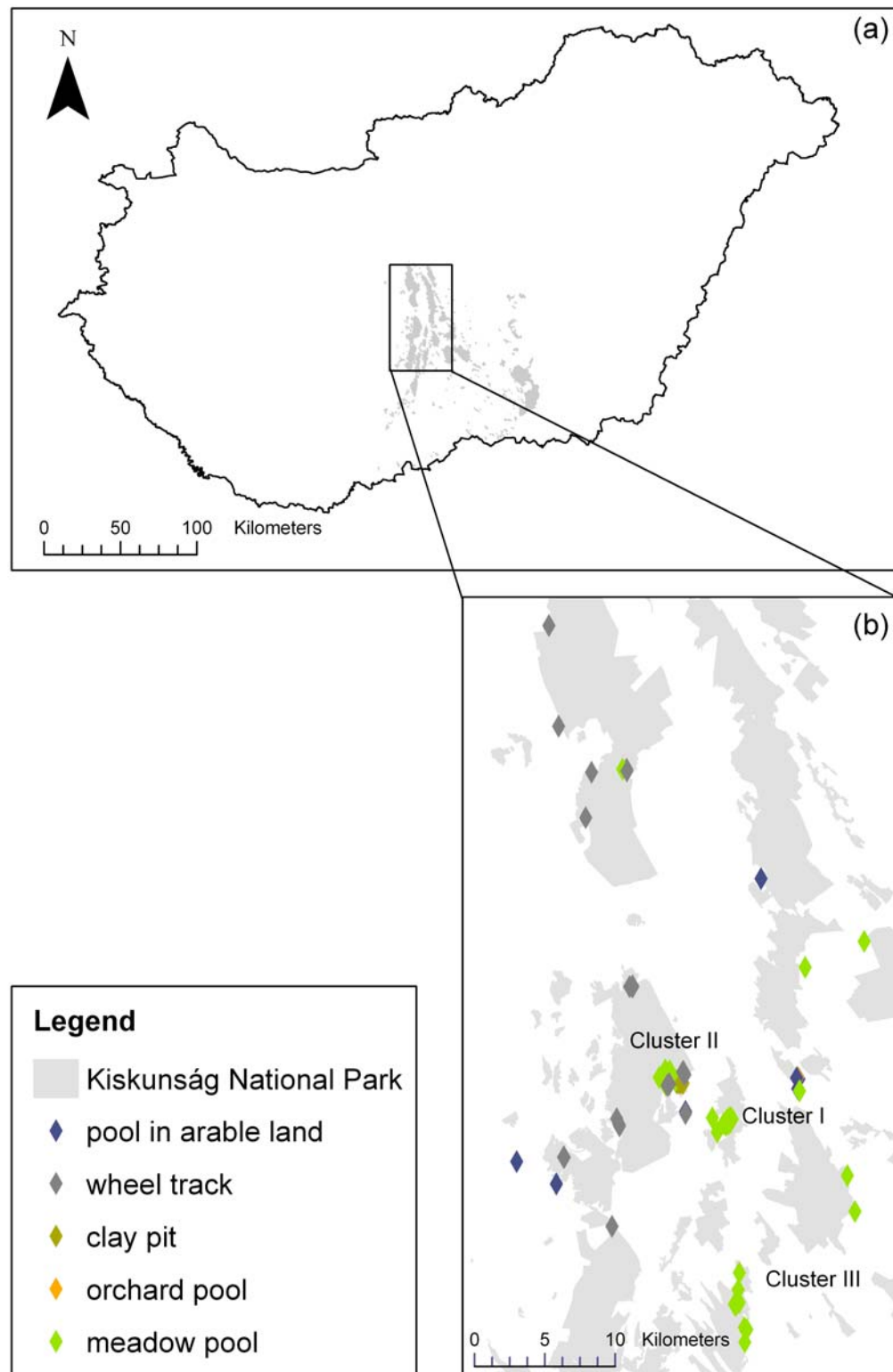


Figure 4. Location of the study area (a) and study pools (b) in the Kiskunság area in Hungary.



Figure 5. The five types of temporary study pools that were sampled in Kiskunság: meadow pools (upper row), clay pits (second row), pools in arable land (third row), orchard pools (lower row, left) and wheel tracks (lower row, middle and right).



Figure 6. The main land use types characterizing the landscape of the study area: agriculture (upper row, left), grazing (upper row, right) and mowing (lower row).

From all 82 pools, large branchiopods were sampled in the spring of 2005 and/or 2006 in order to evaluate their regional species richness and occurrence frequency and explain the distribution of the most common species (Chapter 1; Table 1). For a subset of 36 pools, mainly meadow pools and clay pits, limnological characteristics were monitored through an intensive sampling survey carried out in the spring of 2005 (Chapter 3; Table 1). These pools were equally distributed over three pool clusters: cluster I and cluster II are located around the villages Fülöpszállás and Soltszentimre; cluster III is part of a nature reserve close to the small town of Kiskörös (Fig. 4b). During the same spring, for a subset of 18 of these pools, the active cladoceran and macroinvertebrate communities were characterized (Chapters 2 and 4; Table 1). Due to dry weather conditions in the preceding winter and spring, only four of these pools could be sampled again in 2007 (Chapter 6; Table 1). The dormant branchiopod communities were collected from a subset of 12 out of 18 pools (spring 2007) and their species richness (Chapters 1, 2 and 7) and hatching dynamics (Chapter 5) were characterized in the laboratory (Table 1).

OUTLINE OF THE THESIS

A schematic overview of the thesis outline is presented in figure 7 and table 1.

In the **first chapter**, the nature and conservation value of the Kiskunság pools is assessed through an intensive monitoring of the regional species richness and occurrence frequency of large branchiopods. Additionally, we compare the relative importance of local environmental characteristics as well as spatial factors in explaining the distribution of large branchiopods. As such, we aim at increasing the ecological background on the basis of which effective conservation measures for this flagship group of temporary aquatic habitats should be formulated.

In the **second chapter**, we assess the importance of the temporary study pools for regional diversity of Cladocera in Kiskunság. By means of a comparative literature study of 36 studies on temporary wetlands throughout the world, we further aim at uncovering general processes underlying patterns of regional cladoceran species richness.

In **chapter 3**, we assess the water quality and vegetation cover and their seasonal changes in pools in relation to their duration of inundation. The documented changes in habitat characteristics will be useful in explaining the seasonal dynamics in the local zooplankton communities.

In **chapter 4**, we investigate to what extent the seasonal changes in species richness and composition of cladoceran communities are associated with changing habitat characteristics and/or interactions with the macroinvertebrate community. The second main aim of this chapter is to investigate the impact of hydroperiod length on species richness and community development in the cladoceran communities throughout the growing season. While the general pattern of invertebrate seasonal changes has been well examined in other studies, we want to especially supply more information on the dynamics within one of the respective taxonomic groups.

Next, in **chapter 5** we compare the cladoceran community composition of four study pools between two years with contrasting weather conditions. We analyse to what extent the observed differences might be related to changes in pool dimensions, water quality and vegetation cover or biotic interactions resulting from the different inundation pattern of the pools from one year to another. Such studies may help to increase our insights in processes underlying interannual variation in community structure.

Temporary pools are especially suitable to study the link between recruitment from dormant egg banks and active community dynamics as the initiation of active populations strongly depends on the dormant egg banks. In the following chapter (**Chapter 6**), we therefore investigate to what extent hatching preferences of cladoceran species were associated with abundance patterns during the growing period, as expected under the hypothesis of temporal habitat selection. The hatching response of several cladoceran

species to temperature and photoperiod cues mimicking different moments during the growing season is investigated in controlled experiments and compared to data on their abundance in the field.

In **chapter 7**, for a total of 12 pools, the cladoceran species list as assessed through the identification of hatchlings retrieved from the dormant egg bank is compared with the species richness estimates on the basis of active community samples at repeated occasions throughout the season. The efficiency of dormant egg bank analysis for cladoceran species richness assessment in our temporary study pools is compared to results obtained in permanent lakes, where it proved to be an efficient alternative for active community sampling.

Finally, the results of each chapter are integrated in a general discussion where we also discuss their implications for conservation.

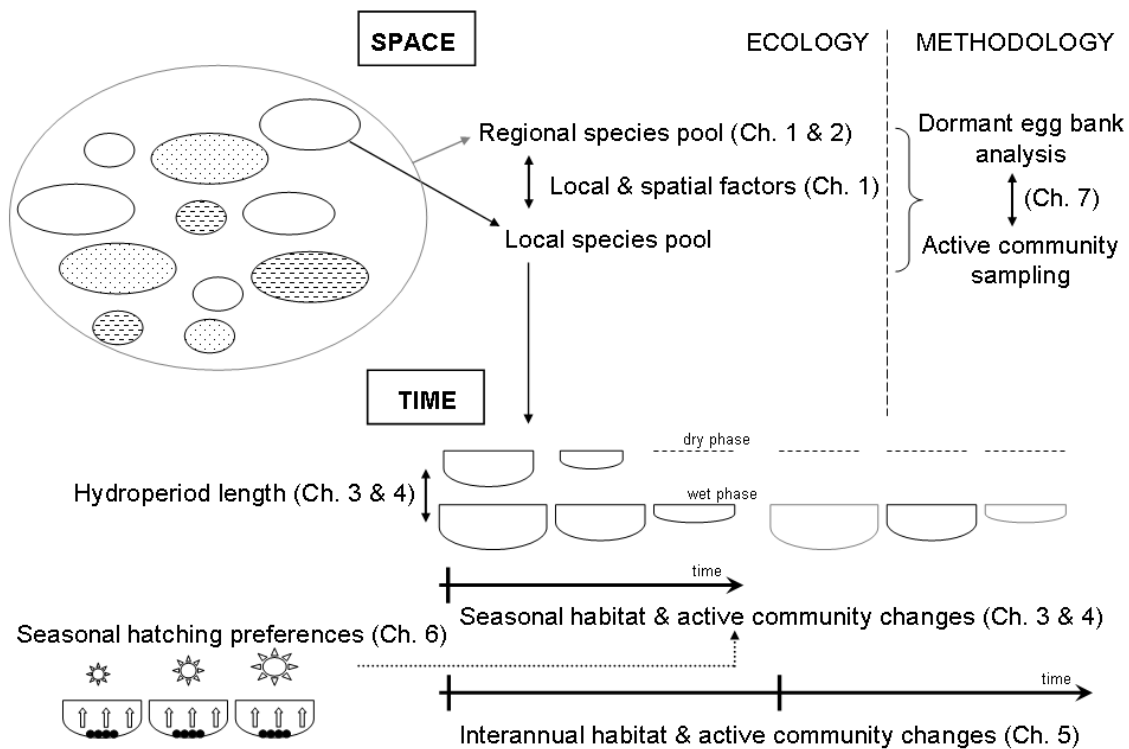


Figure 7. Schematic overview of the ecological processes and methodological application covered in the indicated chapters of this study. Regional species diversity and ecological processes structuring branchiopod communities in space were studied in chapters 1 and 2. Temporal variation in the temporary pool environment and branchiopod communities was studied in chapters 3, 4 and 5. In chapter 6, we discuss the results of a laboratory experiment in which hatching requirements of species were assessed and related to active community dynamics. In chapter 7, we assessed the efficiency of dormant egg bank analysis for species richness assessment.

INTRODUCTION AND THESIS OUTLINE

Table 1. Schematic overview of the ecological processes and methodological application covered in this study. We studied ecological processes structuring communities of large branchiopods and Cladocera in space and time, temporal variation in water quality variables and vegetation cover and a methodological application for cladoceran species richness assessment. For each aspect related to community ecology, we specify which fraction of the community was studied: the active community (AC) or dormant egg bank (DEB). For each aspect, we further outline the year of the study, the habitat types considered (MP: meadow pool; CP: clay pit; PA: pool in arable land; OP: orchard pool; WT: wheel track), the number of pools (n) monitored and in which chapter the respective results are presented. In chapter 6, we discuss the results of a laboratory experiment in which hatching requirements of cladocerans were assessed and related to active community dynamics.

ECOLOGY	Sampling		Habitats		Chapter
	Community	Spring	Habitat type	n	
Large branchiopods					
Regional diversity and species distribution	AC (+ DEB)	2005, 2006	MP, CP, PA, OP, WT	82 (12)	1
Cladocera					
Regional diversity	AC (+ DEB)	2005	MP, CP	18 (12)	2
Seasonal variation	AC	2005	MP, CP	18	4
Interannual variation	AC	2005, 2007	MP, CP	4	5
Recruitment from dormant egg banks		experimental	MP, CP	12	6
Water quality and vegetation cover					
Seasonal variation		2005	MP, CP	36	3
Interannual variation		2005, 2007	MP, CP	4	5
METHODOLOGY					
Cladoceran species richness assessment	AC vs. DEB	2005	MP, CP	12	7

CHAPTER 1

DIVERSITY AND DISTRIBUTION OF LARGE BRANCHIOPODS IN KISKUNSAÁG (HUNGARY) IN RELATION TO LOCAL HABITAT AND SPATIAL FACTORS: IMPLICATIONS FOR THEIR CONSERVATION

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Abstract Large branchiopods are threatened worldwide by the loss and degradation of their temporary aquatic habitats due to drainage and intensive agriculture. Sound ecological knowledge of their diversity and distribution is a prerequisite to formulate effective conservation measures. We collected large branchiopods in 82 temporary freshwater pools in Kiskunság (Hungary) belonging to five habitat types and complemented our field survey with dormant propagule bank analysis. Eleven species were found, with large branchiopods occurring in more than half of the study systems. The significant independent effect of local habitat characteristics on the presence of the most common large branchiopod species indicates that the local environment may have acted as a selective force, resulting in efficient species sorting. Colonization success of different species was possibly differentially affected by invertebrate predation risk and hydroperiod. The extent to which the spatial structure in species distribution was linked to dispersal limitation or hidden environmental gradients remains unknown. Meadow pools and wheel tracks contributed most to regional species richness through the presence of rare and exclusive species. The high regional species richness and occurrence frequency of large branchiopods make Kiskunság a true “hot spot” of large branchiopod diversity. To conserve branchiopod diversity, we stress the importance of high habitat diversity in the landscape and the need to conserve neglected habitats such as wheel tracks.

Keywords Anostraca, habitat size, Notostraca, Laevicaudata, Spinicaudata, transparency, vegetation cover

INTRODUCTION

Large branchiopods are a flagship group of temporary pools (Belk 1998). The relatively rapid growth and maturation and the production of dormant stages make them highly adapted to the recurrent filling and drying of temporary pools, producing both aquatic and terrestrial phases (Brendonck 1996). Most large branchiopod species rely on temporary aquatic systems (Dumont & Negrea 2002) as their relatively large body size does not allow them to coexist with fish predators in permanent ponds and lakes (Kerfoot & Lynch 1987).

Temporary aquatic habitats house a wide array of specialized life-forms often with a high level of endemism and thus make an important contribution to regional biodiversity (Williams et al. 2003, De Meester et al. 2005). Regardless of their particular conservation concern, they have been progressively lost from the European landscape or have been degraded over the past decades (Williams et al. 2001). Besides the deterioration of habitats, also the increasing distance among remaining populations in a metapopulation configuration may ultimately lead to loss of local populations and eventually of the species from a region (Hanski & Gaggiotti 2004). Especially in Western Europe, drainage and intensive cultivation have had a negative impact on these habitats, thereby threatening the persistence of large branchiopod populations (Mura 1993, Williams 1997, Eder & Hödl 2002). In countries such as Belgium (Brendonck 1989), Great Britain (Williams 1997), Germany (Maier 1998) and Austria (Eder & Hödl 2002), large branchiopods became rare or even close to extinction. In Eastern Europe, on the other hand, agriculture is usually less intensive and large areas of farmland still support a high habitat and species diversity, often including species of conservation concern (European Environment Agency 2004). The Banat province in Serbia, for example, is a true “hot spot” of large branchiopod diversity where a total of ten frequently coexisting species were found in an area of only 0.3 ha (Petrov & Cvetković 1997). While environmental pressures are expected to decrease in Western Europe, many areas in Eastern Europe will experience increasing pressure from agriculture. In countries such as Poland and Hungary, which have recently joined the European Union, the conservation of large branchiopods and their temporary habitats consequently is of high priority (Oertli et al. 2005).

Potential factors explaining large branchiopod diversity patterns at a local and regional scale include: (1) local environmental characteristics: physical habitat characteristics (e.g. pond size, hydroperiod) and abiotic and biotic conditions (e.g. water quality, biotic interactions); (2) landscape features and dispersal possibilities (e.g. connections); and (3) historical factors. Local species richness generally increases with habitat size (MacArthur & Wilson 1967), possibly due to its correlation with habitat heterogeneity (Williams 1943, Douglas & Lake 1994, Brose 2001).

A positive relation between pool depth and large branchiopod species richness was for example observed by Gallagher (1996) and Thiery (1991). Hamer & Appleton (1991) showed that large branchiopods coexisting in one pool occupied spatially distinct habitat patches strongly differing in vegetation structure and predation risk. In order to avoid competition, co-occurring anostracan species often show different life cycle strategies (Mura 1991, Maeda-Martinez et al. 1997, Moscatello et al. 2002, Beladjal et al. 2003). The coexistence of species that develop at various times throughout the season is consequently advantaged in long-lasting pools as suggested by Petrov & Cvetković (1997). On the other hand, due to low predation pressure in small pools that regularly dry out, some species may prefer or are restricted to short-lived habitats such as rain pools, puddles and wheel tracks (Hössler et al. 1995). Differences in length of wet phase as well as in local conditions such as turbidity, salinity or presence of vegetation may result in distinct temporary habitat types housing different large branchiopod assemblages (Eng et al. 1990, Eder & Hödl 1996, Timms & Sanders 2002). As such, the presence of diverse habitat types in the landscape may support a high regional species richness.

Spatial dynamics may also affect species distributions, for example through colonization-extinction dynamics or dispersal limitation (Cornell & Lawton 1992, Shurin 2000). The notion that the spatial structure of natural populations has to be taken into account is the basis of modern metapopulation ecology (Hanski & Gaggiotti 2004). On a local scale, freshwater zooplankton and/or their dormant eggs are readily dispersed by wind (Vanschoenwinkel et al. 2008a, b), amphibians (Bohonak & Whiteman 1999) and water connections (Michels et al. 2001, Hulsmans et al. 2007), while probably the most effective long-distance dispersal agents are water birds (Figuerola & Green 2002). The effective importance of dispersal limitation for large branchiopods probably varies strongly between geographic areas, depending on the proximity of source populations and the availability of dispersal vectors. Demeter (2005) found that large branchiopod habitats in the Ciuc basin (Romania) were located in areas with high habitat density and thus higher chance for dispersal. He attributed the lack of Anostraca from apparently suitable habitats in a high mountain area to the absence of suitable dispersal vectors (Demeter 2004). As species may be absent from otherwise suitable habitat patches due to dispersal limitation, studies that do not take the spatial configuration of populations (isolation, connectivity) into account, may miss out on important information.

The increasing literature on the alarming status of large branchiopods and their habitats contrasts with the limited number of management and conservation measures. Ideally, such measures are based on thorough ecological studies investigating the relative importance of local

habitat characteristics and spatial metapopulation structures in explaining diversity patterns and species distribution.

In this paper, we present the species richness and distribution patterns of large branchiopods in different pool types in the Kiskunság area (Hungary), a relatively pristine area where temporary pools are still relatively abundant. We compare the relative importance of local environmental characteristics as well as spatial factors in explaining the distribution of the most common large branchiopod species. Based on our results, we suggest some conservation measures for large branchiopods and their habitats.

MATERIALS AND METHODS

Study area

The Kiskunság National Park in Hungary (Fig. 1a), covering an area of almost 500 km², is located 85 km southwest of Budapest in the Kiskunság area. In the west part of this area, we sampled 82 temporary freshwater pools (Fig. 1b). Five different pool types were distinguished: wheel tracks (n = 29), clay pits (n = 6), meadow pools (n = 38), pools in arable land (n = 8) and one orchard pool. The geographical distribution of pool types reflects the typical environmental gradient characterizing the study area. Eastwards, reed marshes and bog meadows constitute the main biotopes and meadow pools on peat soils typically occur. More westwards, the study area is characterized by salt lakes on alkaline soils and the main pool types are clay pits, wheel tracks and meadow pools on clayey soils. Pools in arable land occur throughout the gradient, while orchard pools are rarely observed.

All pools are initially inundated after snow melt in early spring (February - March) and gradually dry by summer. Throughout the year, they can be replenished with rain fall. The duration and frequency of inundation of the pools strongly depends on pool morphology (depth and surface area) and local weather conditions.

Field sampling

Pools were sampled between mid-April and mid-May in 2005 (n = 45) and/or 2006 (n = 44) (Table 1). The shape of each pool was compared to known geometric figures of which the dimensions were measured to calculate its surface area (m²). Maximum depth (cm) was assessed using a calibrated stick. The measurement of water transparency using a Snell's tube was practically impossible in the shallowest habitats (10 – 20 cm) on clayey soil as clay particles would be stirred up and influence our measurements. For further analyses, bottom visibility (0/1) was therefore used as a measure of water transparency. Depth of pools did not interfere with

transparency measurements and pools without bottom visibility were on average shallower. In each pool, an integrated water sample of about 9 L was taken from the surface of three randomly chosen locations. From this sample, temperature ($^{\circ}\text{C}$), conductivity ($\mu\text{S cm}^{-1}$), pH and dissolved oxygen concentration (% saturation) were measured in the field with a multimeter (WTW 340i). When the water was too shallow to collect an integrated water sample, measurements were made directly in the pool. Percentage cover with macrophytes (floating, submersed, and emerged vegetation) was visually estimated (10% accuracy). Table 2 provides an overview of the measured values for the different local habitat variables per pool type. The spatial configuration of the pools was reconstructed in a GIS environment and the distance between each pair of pools was measured using the distance tool in ArcGIS 8.0.

Large branchiopods were sampled qualitatively by searching all subhabitats with a sweep net (250- μm mesh). In clay pits and meadow pools sampled in 2005, quantitative sampling was carried out using quadrats. Four open quadrats were placed on the bottom of the pool and trapped animals were removed with a sweep net (250- μm mesh). Qualitative samples were simultaneously collected and additionally screened. Collected animals were preserved in 70% ethanol and identified to species level using the identification key in Eder & Hödl (1996). For further analysis, presence/absence data were used.

Table 1. Overview of the number of study pools (n) and the total number of species found (S_{tot}) per pool type during the spring of 2005 and 2006, from dormant egg bank analysis (DEB) and during the whole study. For each pool type, the number of exclusive species (S_{excl}) is also given.

	2005		2006		DEB		Whole study		
	n	S_{tot}	n	S_{tot}	n	S_{tot}	n	S_{tot}	S_{excl}
Clay pits	5	0	3	0	4	3	6	3	0
Meadow pools	32	5	10	4	9	7	38	9	4
Pools in arable land	/	/	8	3	/	/	8	3	0
Pool in orchard	/	/	1	1	/	/	1	1	0
Wheel tracks	8	2	22	5	/	/	29	5	2
Total:	45	7	44	8	13	7	82	11	

Pools that were not sampled are indicated with an '/'.

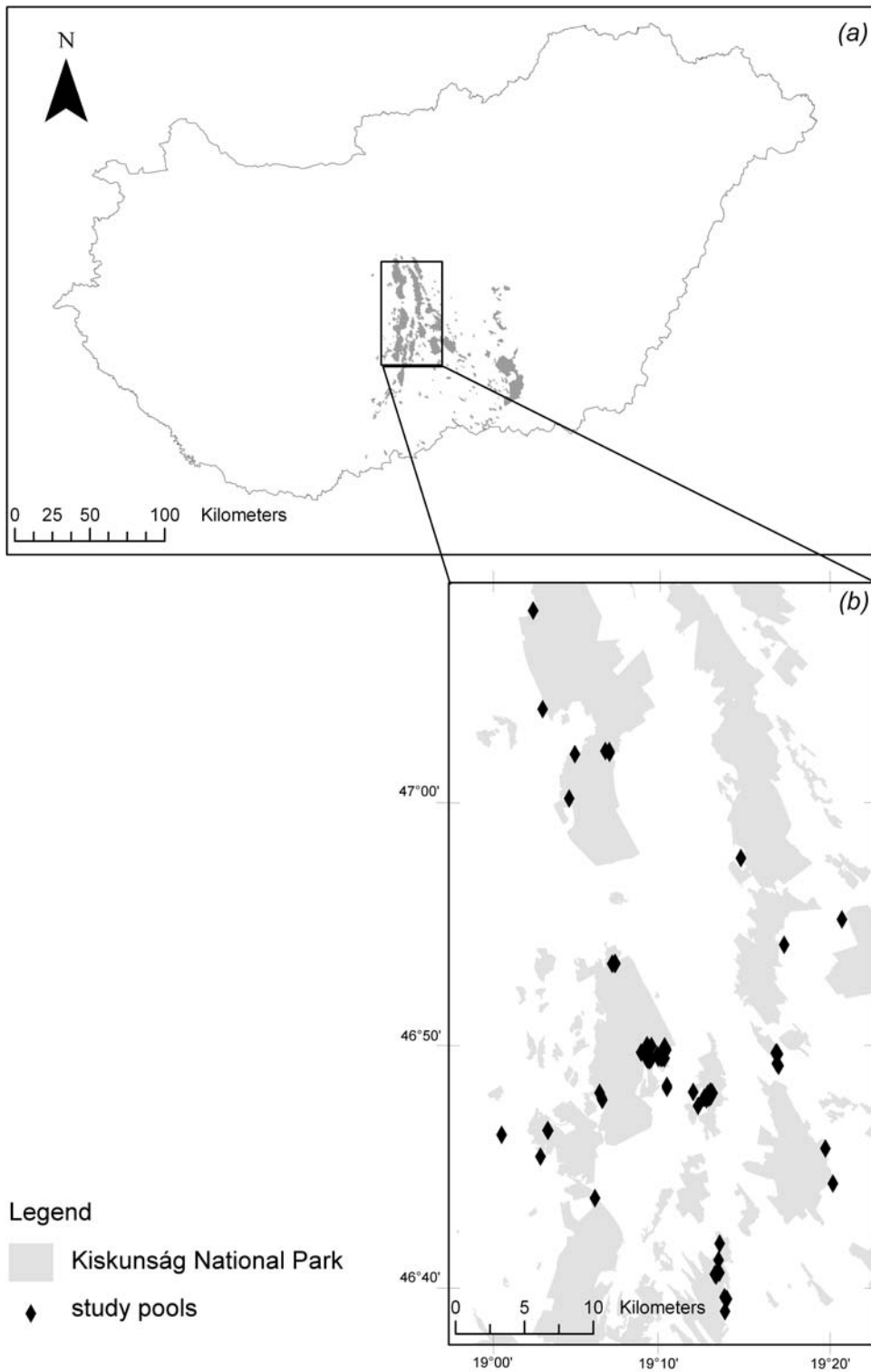


Figure 1. Location of the Kiskunság National Park in Hungary (a) and of the study pools within the study area (b).

For a subset of 13 pools (Table 1), the species list was complemented by hatching and identifying viable dormant eggs from the sediment. We selected eight meadow pools and four clay pits as these were generally larger and deeper and the chance that we missed some species in the field was probably higher. The upper 3 cm of sediment was collected in each pool from random locations ($n = 15$ to 20 ; ± 20 cm²) representative of all existing microhabitats (depth, vegetation type). Sediment with dormant eggs was dried and stored for three months in the dark at room temperature. Thereafter, we inundated for each pool (a) dry sediment and (b) dormant eggs isolated from sediment (using the sugar flotation method: Onbé 1978; Marcus 1990). For each pool about 150g of sediment was inundated in 2-l aquaria filled with EPA medium (Anonymous 1985) at 10 and 20°C. To increase species detection, dormant eggs were next isolated from about 4kg of sediment for each pool. They were inundated at 12, 18 and 24°C (EPA medium). Using different temperature treatments, we aimed at optimizing hatching conditions for a maximum number of species. During a period of about one month, hatchlings were isolated twice a week, grown to adult stage and identified to species level using Eder & Hödl (1996). Finally, isolated dormant eggs that did not hatch were identified to species level using Thiéry & Gasc (1991). We acknowledge that, as we inundated the sediment and dormant eggs only once, the local species pool might still be underestimated as species might delay hatching over different inundations.

Table 2. Overview of local environmental characteristics (mean $\pm$ standard error and range) for each of the habitat types.

	Clay pits (n = 6)	Meadow pools (n = 38)	Pools in arable land (n = 8)	Pool in orchard (n = 1)	Wheel tracks (n = 29)
Conductivity ($\mu\text{S cm}^{-1}$)	1775 $\pm$ 307 563 – 2510	941 $\pm$ 52 453 – 2090	1110 $\pm$ 268 607 – 2820	593	1552 $\pm$ 247 415 – 6370
Temperature (°C)	14.2 $\pm$ 0.3 13.1 – 15.1	17.8 $\pm$ 0.7 10.7 – 27.1	19.7 $\pm$ 1.3 15.1 – 26.7	19.5	20.3 $\pm$ 0.9 11.9 – 29.3
pH	8.7 $\pm$ 0.1 8.2 – 9.0	8.4 $\pm$ 0.1 7.2 – 9.7	7.7 $\pm$ 0.1 7.3 – 8.2	7.5	8.7 $\pm$ 0.1 7.3 – 10.0
Oxygen saturation (%)	82 $\pm$ 4 71 – 96	100 $\pm$ 5 42 – 185	83 $\pm$ 7 49 – 112	86	82 $\pm$ 5 18 – 155
Transparency (Snell's depth in cm)	27.2 $\pm$ 2.5 20.0 – 37.5	34.0 $\pm$ 1.2 19.5 – 50.0	16.6 $\pm$ 3.9 0 – 29.0	19.0	* *
Vegetation cover (%)	66 $\pm$ 11 30 – 100	90 $\pm$ 3 40 – 100	21 $\pm$ 8 0 – 50	70	1 $\pm$ 0.12 0 – 2
Surface area (m ²)	1460 $\pm$ 659 188 – 3758	11183 $\pm$ 3298 11 – 77616	1199 $\pm$ 448 216 – 3360	1180	9 $\pm$ 2 0.15 – 33
Maximum depth (cm)	71.1 $\pm$ 13.3 43.0 – 112.5	37.5 $\pm$ 2.6 13.0 – 90.0	24.3 $\pm$ 2.4 15.0 – 37.0	29.0	14.2 $\pm$ 0.7 10.0 – 20.0

Data analysis

The presence/absence of the most common large branchiopod species (found in more than three pools throughout the study; Table 3) as observed in the field was investigated in relation to local environmental and spatial characteristics using Canonical Correspondence Analysis (CCA). We first applied forward selection on the entire set of measured variables to identify those variables that best explained the branchiopods' distribution. Next, the selected variables were incorporated into variation partitioning models (Borcard et al. 1992) to separate the contribution of local and spatial factors in explaining the presence of species. The significance of the models was tested using Monte-Carlo permutations ($n = 999$). Analyses were carried out in Canoco for Windows 4.5 (Lepš & Šmilauer 2003).

Local environmental characteristics included morphometry (maximum depth, surface area), water quality (conductivity, dissolved oxygen, pH, temperature and transparency) and percentage vegetation cover. The spatial factors we used were pool coordinates extracted from the distance matrix of pair wise distances between pools, using Principle Coordinate Analysis (PrCoord 1.0; Canoco for Windows 4.5). A total of 41 axes with positive eigenvalues were extracted and included in CCA with forward selection. Additionally, the average distance of a pool to all other study pools was included in the forward selection procedure as a general measure of isolation (Isol). Distance measures only included our study pools, but not other pools in the area, and were representative for both years.

The Fager's affinity index was calculated for each pair of species as a relative measure of species co-occurrence, analogous to the approach in Maeda-Martinez et al. (1997): $I_F = 2n_{1+2}/(n_1+n_2)$ where n_{1+2} = number of joint occurrences of species 1 and 2, n_1 = total number of occurrences of species 1 and n_2 = total number of occurrences of species 2. We compared the index calculated from simultaneous co-occurrences in the field and from occurrences in the dormant egg bank and/or during subsequent sampling seasons.

RESULTS

Species richness and co-occurrence

In more than half of the study systems (48 out of 82), large branchiopods were found in the field and/or in the dormant egg bank. A total of eleven species was found: six Anostraca; two Notostraca and three Conchostraca (Table 3). *Branchipus schaefferi* and *Tanymastix stagnalis* were the most commonly observed species throughout the study (in 25 and 13 pools, respectively). In the field, usually only one species occurred per pool (82.5% of all species observations). Less frequently, species co-occurred simultaneously with two (five pools, 12.5%)

or three (two pools, 5%). Dormant egg bank analysis revealed six additional large branchiopod habitats and increased the number of co-occurrences. In two more pools, two species co-occurred and in four more habitats, three species occurred together. Four and five species were found together each time in the sediment of a single pool.

Except for *Eubbranchipus grubii* and *Chirocephalus shadini*, all species were found together simultaneously with one or more species relatively frequent (Table 3). *Triops cancriformis* never occurred alone and was found together with *Leptasteria dahalacensis* (Fager's index of 0.50) and/or *B. schaefferi* (0.27). *Chirocephalus carnuntanus* was found alone, with *T. stagnalis* (0.33) or with *Lynceus brachyurus* (0.31). *Lepidurus apus* and *L. brachyurus* were also found together in the field (0.28). The subsequent monitoring of pools during two springs, as well as dormant egg bank analysis, revealed a higher frequency of co-occurrence for some species (Table 3). In one pool that was monitored during both years we found two different species each year (*C. shadini* and *C. carnuntanus*, respectively). In two other pools, we did not find any large branchiopods in the field, but we found *B. schaefferi*, *T. stagnalis* and *T. cancriformis* in the dormant egg bank. Species most often found together in the egg bank of the same habitat were: *T. cancriformis* and *T. stagnalis* (0.55; n = 6); *T. cancriformis* and *B. schaefferi* (0.53; n = 9) and *B. schaefferi* and *T. stagnalis* (0.32; n = 6). As for species that were less frequently observed, there was a relatively high affinity between *Imnadia yeyetta* on the one hand and *C. shadini* (0.40), *T. cancriformis* (0.36) and *T. stagnalis* (0.27) on the other hand and between *C. carnuntanus* and *C. shadini* (0.25).

Local environmental and spatial factors

Local environmental as well as spatial factors explained a significant amount of variation in the presence/absence of the most common large branchiopod species (Table 4). The independent effect of both local and spatial variables was highly significant, but they also exerted a considerable shared effect. Local factors included maximum depth, surface area, oxygen saturation percentage, pH, transparency and percentage vegetation cover. Spatial factors that significantly correlated to large branchiopod community composition included pool coordinates along axes 1, 4, 6, 21, 22 and 27. About 20% of the variation in the presence/absence of species remained unexplained by the selected explanatory variables.

Table 3. Large branchiopod species found in Kiskunság with their number of records, frequency of co-occurrence and European distribution (after Brtek & Thiéry 1995). The number of records includes records from field collections in 2005 and 2006 (Field) and additional records from dormant egg banks (DEB). The frequency of co-occurrence of a species is expressed as the proportion of its habitats in which it was found together with one or more species simultaneously (Time) or throughout different years and/or in the dormant egg bank (Habitat).

	# Records		% Co-occurrence		Distribution in Europe
	Field	DEB	Time	Habitat	
ANOSTRACA					
<i>Branchipus schaefferi</i> Fischer, 1834	19	6	21	40	Continental Europe and Mediterranean islands
<i>Tanymastix stagnalis</i> (Linnaeus, 1758)	6	7	33	62	widespread
<i>Chirocephalus carnuntanus</i> (Brauer, 1877)	5	0	40	60	Eastern Europe (Pannonian Lowland)
<i>Chirocephalus shadini</i> (Smimov, 1928)	2	1	0	66	Eastern Europe and Russia
<i>Branchinecta ferox</i> (Milne Edwards, 1840)	1	0	100	100	Eastern Europe and Spain
<i>Eubranchipus grubii</i> (Dybowski, 1860)	1	0	0	0	Central Europe
NOTOSTRACA					
<i>Triops cancriformis</i> (Bosc, 1801)	3	6	100	100	widespread
<i>Lepidurus apus</i> (Linnaeus, 1758)	8	0	25	25	Continental Europe
LAEVICAUDATA					
<i>Lynceus brachyurus</i> Müller, 1776	6	0	50	50	Eastern Europe, Northern Europe and Russia
SPINICAUDATA					
<i>Imnadia yeyetta</i> Hertzog, 1935	0	2	/	100	Eastern Europe (Pannonian Lowland) and Camargue
<i>Lepthesteria dahalacensis</i> (Rüppell, 1837)	1	0	100	100	Eastern Europe, Mediterranean Islands and Russia

Table 4. Results of variance partitioning (percentage of explained variation, F - and p -value) of CCA model relating the presence of large branchiopod species to local environmental and spatial variables. Local environmental variables ([E]) include maximum depth, oxygen saturation percentage, percentage vegetation cover, pH, surface area and transparency. Coordinates along six axes (1, 4, 6, 21, 22 and 27) were used as spatial variables ([S]). Symbols according to Cottenie *et al.* (2003): [E+S] = total explained variation by environmental and spatial variables; [E] = variation explained by environmental variables; [S] = variation explained by spatial variables; [E|S] = pure environmental variation; [S|E] = pure spatial variation; [E∩S] = variation shared by environmental and spatial variables; 1 - [E+S] = unexplained variation.

	% expl. var.	F	p
[E+S]	80.3	5.45	0.001
[E]	48.3	3.43	0.001
[S]	54.4	4.38	0.001
[E S]	25.9	3.52	0.001
[S E]	32.0	4.34	0.001
[E∩S]	22.4		
1-[E+S]	19.7		

Branchipus schaefferi (Fig. 2a) and *T. cancriformis* (Fig. 2b) were positively associated with turbid, sparsely-vegetated pools with a small depth and surface area. In the field, these species were almost exclusively found in wheel tracks. The other species were generally associated with deeper and/or larger pools (Fig. 2). *Tanymastix stagnalis* was found in diverse habitat types in the field and was associated with relatively low pH values (Fig. 2c). *Lepidurus apus* (Fig. 2d) and *L. brachyurus* (Fig. 2e) were associated with transparent pools with a high vegetation cover and *L. apus* was positively associated with high oxygen concentrations. Both species were observed in meadow pools on peat soils. *C. carnuntanus* occurred in different pool types at various local conditions and its presence was not especially associated with any of the selected environmental variables (Fig. 2f). *Branchipus schaefferi*, *T. stagnalis* and *T. cancriformis* also hatched from the sediment of clay pits and meadow pools on clay soils (Fig. 2a, b and c). For *L. apus*, *Lynceus brachyurus* and *Chirocephalus carnuntanus* no new records were added from the dormant egg banks. As for the rare species, the anostracans *Chirocephalus shadini* and *Eubranchipus grubii* were found in transparent, vegetated meadow pools on peat soils, but *C. shadini* also hatched from a meadow pool on clayey substrate. *Branchinecta ferox* and the conchostracan *L. dahalencis* were found in small, turbid wheel tracks. *Imnadia yeyetta* hatched from two meadow pools on clayey substrate.

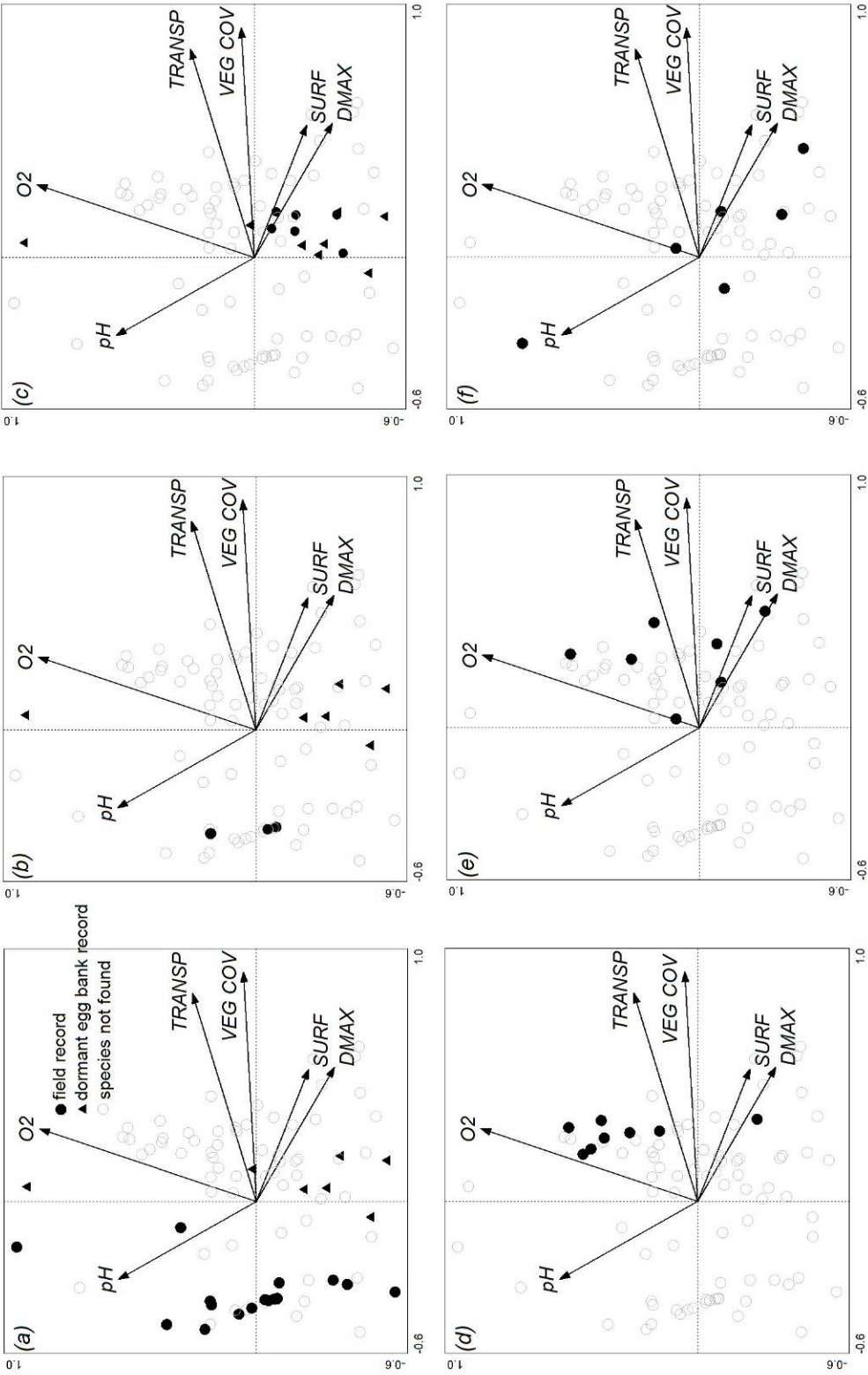


Figure 2. Ordination plots illustrating the distribution of the most common large branchiopod species in Kiskunság in relation to local environmental characteristics (PCA of selected local environmental characteristics and samples; first two axes explain 52.1% of total variation in the data): (a) *B. schaefferi*; (b) *T. cancriformis*; (c) *L. apus*; (d) *L. stagnalis*; (e) *L. brachyurus* and; (f) *C. carnuntanus*. Legend to abbreviations: DMAX = maximum depth; O2 = oxygen saturation percentage; pH; SURF = surface area; TRANSP = transparency; VEG COV = percentage vegetation cover).

Branchipus schaefferi and *T. cancriformis* were typically found in pools in the western part of the study area (Fig. 3a and b), whereas *L. apus* and *L. brachyurus* were largely restricted to the south-eastern part (Fig. 3d and e). *Chirocephalus carnuntanus* and *T. stagnalis* occurred in the east and northwest (Fig. 3c and f).

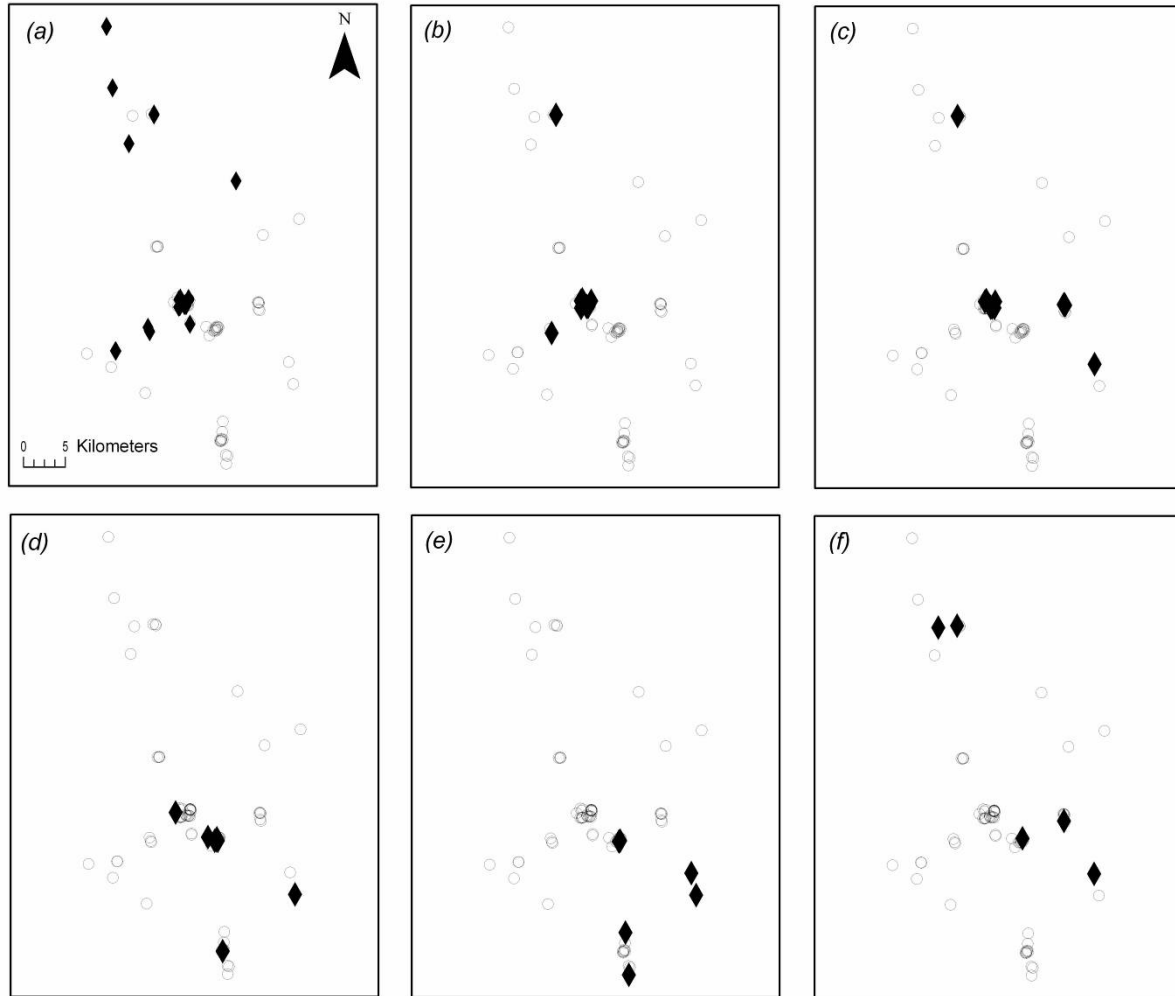


Figure 3. The spatial distribution of the most common large branchiopod species in Kiskunság as observed during our study: (a) *B. schaefferi*; (b) *T. cancriformis*; (c) *T. stagnalis*; (d) *L. apus*; (e) *L. brachyurus* and; (f) *C. carnuntanus*. Empty circles represent pools where the respective species was not found, black diamonds represent pools where the species was effectively observed in the field or in the dormant egg bank.

Habitat type

Meadow pools and wheel tracks were characterized by the highest total species richness (nine and five species, respectively) and were the only habitat types with exclusive species (i.e. species not found in any other habitat type) (Table 1). *Chirocephalus shadini*, *E. grubii*, *L.*

brachyurus and *L. apus* were only observed in meadow pools, while *B. ferox* and *L. dahalacensis* were exclusive species of wheel tracks.

DISCUSSION

Species richness

We encountered eleven large branchiopod species during our study of 82 temporary freshwater pools in and around the Kiskunság National Park in 2005 and 2006. This is more than half of the described Hungarian large branchiopod fauna consisting of 19 species (Forró 2000). The anostracans *B. schaefferi* and *T. stagnalis* were the most common species in our survey. Both species are also widely distributed in Europe (Brtek & Thiéry 1995), which may be indicative of the eurytopic nature or particular dispersal and colonization abilities of these species. In our study area, species with a more limited geographical distribution also occurred. *Chirocephalus shadini*, *B. ferox* and the conchostracan species (*L. brachyurus*, *I. yeyetta* and *L. dahalacensis*), for example, more typically occur in Eastern Europe, while *C. carnuntanus* is restricted to the Pannonian Lowland (Brtek & Thiéry 1995). Compared to other regions in Europe (Belgium: six species with one observation since 1959 (Brendonck 1989, Loneux 2002); UK: two species from about 20 locations (Williams 1997); Germany: ten species (Maier 1998); Southwest Portugal: five species in 20% of all pools (Machado et al. 1999); Eastern Poland: three species in 32% of all pools (Biggs et al. 2004); Ciuc Basin Romania: six species in 18% of all pools (Demeter 2005)), many large branchiopod species were found in a relatively high number of pools in the Kiskunság area.

Both Petrov & Cvetković (1997) and Timms & Sanders (2002) defined large branchiopod “hot spots” (the Banat province in Serbia and the middle Paroo catchment in Australia, respectively) as relatively small areas (0.30 ha and 2000 km², respectively), where a high proportion of the regional (national) species pool occurs and species co-occurrence is frequent. In Banat, 53% of the national large branchiopod species pool was represented and more than one species was found in 67% of all habitats (Petrov & Cvetković 1997). In the Paroo, 50% of the known conchostracan fauna and 57% of the national anostracan species pool was observed and in 21%, respectively 55% of all collections, species co-occurred (Timms & Richter 2002, Timms & Sanders 2002). According to this definition, the Kiskunság area, where 58% of all Hungarian species were found in an area of about 1800 km² (~2% of Hungary) and species and anostracan co-occurrence was common (31 and 21% of all large branchiopod habitats, respectively), should also be considered as a “hot spot” of large branchiopod diversity. The presence of such a species-rich area contrasts with the general trend of declining large

branchiopod diversity throughout Europe concurrent with an unprecedented rate of habitat destruction or change by agricultural and urban development (Brendonck 1989, Maier 1998, Williams et al. 2001, Eder & Hödl 2002). Dormant propagule banks may increase the resilience of large branchiopods to anthropogenic stress (Angeler et al. 2008b). However, as for some species only a few or small populations were found, and no information on dormant egg banks sizes is available, it is difficult to make unequivocal conclusions on the conservation status of the large branchiopod species in the area.

Local environmental variables

There was a distinct segregation of species among habitats with contrasting local environments (Fig. 2). Small, turbid and sparsely-vegetated wheel tracks were typically inhabited by *B. schaefferi*, *T. cancriformis* and *L. dahalacensis*. *Branchipus schaefferi* and *T. cancriformis* also occurred in larger clay pits and meadow pools with more abundant vegetation, often together with *T. stagnalis*. *Lepidurus apus*, *L. brachyurus*, *E. grubii* and *C. shadini* were representative species of meadow pools with very abundant vegetation. In several studies, species were found in similar habitats as in our survey. *Branchipus schaefferi*, *T. cancriformis* and *L. dahalacensis* were found in muddy rain pools in Germany, Austria and Yugoslavia (Hössler et al. 1995, Hödl & Eder 1996, Petrov & Petrov 1997). *Eubranchipus grubii* and *C. shadini* occurred in meadow pools in Austria, which were considered as the preferred habitats of the latter species (Hödl & Eder 1996). *Lepidurus apus* and *L. brachyurus* were observed in densely-vegetated waters in Spain and Yugoslavia (Alonso 1985, Petrov & Petrov 1997) and in pools on peat marshes in France (Rabet et al. 2005). Only our observation of the anostracan *B. ferox* in a wheel track was rather unusual as this species is considered characteristic for alkaline lakes (Löffler 1991, Eder & Hödl 2003).

The significant independent effect of local habitat characteristics on the presence of the most common large branchiopod species indicates that the local environment may have acted as a selective force, resulting in efficient species sorting (Leibold et al. 2004, Holyoak et al. 2005, Vanschoenwinkel et al. 2007) and thereby explaining the observed association of species with contrasting habitats. For example, the presence of abundant vegetation most likely implicates a high abundance of invertebrate predators such as Odonata and beetle larvae (Lombardo 1997, Burks et al. 2002, Van de Meutter et al. 2004). The low frequency and small size of anostracan populations in the densely-vegetated meadow pools may be indicative of such an elevated predation risk. In only 13% of all meadow pools we found anostracans in the field and a maximum of only 15 adult specimens was collected during one five-minute search

period (L. Boven, personal observations). Anostracans associated with densely-vegetated habitats may be locally adapted to high predation pressure by specific hatching or life cycle strategies. *Chirocephalus shadini* and *E. grubii*, for example, are coldwater species typically occurring early in spring (Eder et al. 1997, Petrov & Cvetković 1997). By hatching early after snow melt, they probably mature before the predator community is well developed. A similar strategy was observed for anostracans in temporary pools in South Africa that developed before actively dispersing predators colonized their habitats (Hamer & Appleton 1991).

The morphology of pools also affected species distribution, possibly through its association with the duration of inundation. Although hydroperiod is not solely dependent on morphological measurements (Marcus & Weeks 1997, Skidds & Golet 2005), the maximum depth or surface-to-volume ratio of pools are often used as indicators of the length of the aquatic phase (King et al. 1996, Marcus & Weeks 1997, Jocqué et al. 2007b). Small and shallow habitats, in our study typically wheel tracks, are most likely inundated for shorter periods than the other pool types, which had variable dimensions, but were generally larger and deeper (Table 2). *Branchipus schaefferi* and *T. cancriformis* are characterized by very fast growth and maturation, especially at high temperatures (Flößner 1972, Defaye et al. 1998, Beladjal et al. 2003) and are therefore presumably well adapted to survive in such short-lived habitats. In their larger but relatively shallow habitats, *T. stagnalis* is likewise advantaged by hatching at relatively low temperature and rapidly growing to maturation (Al-Tikiry & Grainger 1990, Grainger 1991, Mura 1991), avoiding extinction through early desiccation. Although not observed in the field, *B. schaefferi*, *T. cancriformis* and *T. stagnalis* also hatched from the sediment of meadow pools and claypits. In these relatively turbid habitats (Fig. 2a and c), *B. schaefferi* and *T. stagnalis* may be relatively safe from invertebrate predators (Woodward & Kiesecker 1994, Hössler et al. 1995), while in transparent meadow pools they are possibly more susceptible to visual predation. Another hypothesis that might explain the success of *B. schaefferi* and *T. stagnalis* in these meadow pools and clay pits implies that they are possibly restricted to shallow, littoral zones with high desiccation and low predation risk. In temporary pools in South Africa, fast-growing species occupied the peripheral region of a pool, while the pelagic area was inhabited by large branchiopods with a slower development (Hamer & Appleton 1991). Finally, the slow growth and maturation of *L. brachyurus* (Rabet et al. 2005) and *L. apus* (Kuller & Gasith 1996) may limit these species to habitats with a long duration. In temporary pools in France, *L. brachyurus* matured only after three months and was found in rather deep systems as well (0.70 - 2.50 m) (Rabet et al. 2005). Although both notostracans were observed together in rain pools

in Israel, larval development of *L. apus* was obviously slower than that of *T. cancriformis* (Kuller & Gasith 1996).

Chirocephalus carnuntanus was found in various habitats. Although *C. carnuntanus* possibly is a habitat generalist, the low encounter rate makes it difficult to make any statements on the species' distribution.

Spatial factors

The shared effect of local and spatial factors on large branchiopod community composition is most likely explained by spatial-environmental covariation. The spatial distribution of species indeed partly reflects the distribution of suitable habitat types along the characteristic environmental gradient of the study area. *Branchipus schaefferi* and *T. cancriformis* typically occurred in pools in the western part (Fig. 3) where main pool types are clay pits, wheel tracks and meadow pools on clayey soils. *Lepidurus apus* and *L. brachyurus* mainly occurred more to the south-eastern part of the study area where meadow pools on peat soils more typically occur. However, the significant independent spatial effect indicates that this pattern probably linked with environmental differences can only partly explain species distribution. The observed spatial effects might to some extent also be due to dispersal limitation or, alternatively, to regional variation in local habitat variables that we did not take into account (e.g. anthropogenic disturbance). The extent to which dispersal limitation may contribute to the spatial distribution of species needs to be investigated in more detail. For example, *L. apus* and *L. brachyurus* apparently were restricted to the more southern pools, whereas meadow pools present in different other parts are also expected to be suitable habitats for these species. As both species were also found in more isolated pools, dispersal limitation seems an unlikely explanation. Furthermore, given the protected status of our study area with high habitat density and abundant dispersal vectors, communities may be highly interconnected. Ample temporary connections between pools are established after snowmelt and by a network of irrigation channels. Amphibian and bird populations, which constitute known dispersal vectors (Bohonak & Whiteman 1999, Figuerola & Green 2002), are abundant in the area. Large branchiopod dormant eggs may also be easily dispersed by wind in our study area, as pools were separated by distances of a few meters up to 50 km in a flat landscape. Vanschoenwinkel et al. (2008) measured considerable wind dispersal of large branchiopod propagules on an isolated rock pool outcrop, though on a very local scale (few hundred m). Dormant egg transport over longer distances is, however, not unlikely over the long term. In order to more reliably interpret the observed patterns, more intensive long-term monitoring is required. Such study would especially

be relevant when contrasted to patterns observed in highly fragmented areas where dispersal limitation is effectively expected (e.g. Flanders, different parts of Western Europe). The degree of connectivity between habitats might indeed be related to anthropogenic disturbance through habitat loss and fragmentation (Hanski 1999, Lehtinen et al. 1999, Cushman 2006).

Finally, in areas with either natural or artificial physical barriers to dispersal, the creation of new habitats for conservation purposes may not be effective. In the Ciuc basin in Romania, for example, large branchiopods were especially found in areas with high habitat density (Demeter 2005). In a high mountain area, the absence of anostracans was related to the absence of suitable dispersal vectors (Demeter 2004). Therefore, we strongly recommend the integration of spatial factors in any future studies investigating large branchiopod distribution patterns and in the development of conservation schemes.

Species co-occurrence

Species co-occurred in 31% of all large branchiopod habitats in our study. This percentage was most likely underestimated as field surveys were limited in time and dormant egg banks were only searched for a subset of pools. In temporary ponds in Mexico, Arizona (Maeda-Martínez et al. 1997), Serbia (Petrov & Cvetković 1997), the Camargue (France; Waterkeyn et al. submitted) and Morocco (Thiéry 1991), co-occurrence was more frequently observed (43, 47, 67, 79 and 90%, respectively). In 21% of all large branchiopod habitats, we found co-occurring anostracans. In Northeast Australia, 55% of all collections included more than one anostracan species (Timms & Sanders 2002) and 21% more than one conchostracan (Timms & Richter 2002), but repeated collections were made for each pool. In Morocco, anostracans co-occurred in 50% of all habitats (Thiéry 1991). As in the mentioned studies, large branchiopod species sometimes co-occurred simultaneously in our study pools (17%), but we only rarely found more than one anostracan at the same time.

The coexistence of branchiopods in temporary wetlands in general is ascribed to a combination of abundant food resources and low intensity of competitive interactions due to the annual truncation of community development (King et al. 1996, Simovich 1998). However, the common co-occurrence of anostracans, notostracans and conchostracans, as also observed during our study, is probably established through resource partitioning as each group uses different food types (Dumont & Negrea 2002). Especially when filter-feeding anostracans co-occur, mechanisms of competition avoidance come into play. Frequently co-occurring anostracans, but also conchostracans in the Paroo catchment, for example, avoided competition through differences in body size and thus in the size of filtered prey or food particles (Timms &

Sanders 2002, Timms & Richter 2002). A reduction in temporal overlap of competitive life stages through different life cycle strategies (growth rate, size) also explained the occurrence of two-species assemblages of anostracans (Hathaway & Simovich 1996, Mura 1991, Beladjal et al. 2003), as well as the coexistence of six anostracan and up to ten large branchiopod species in a single pond in Morocco (Thiery 1991). In our study pools, resource overlap may be too high to allow anostracans to occur simultaneously, even at different life stages. However, other factors such as predation level and food type availability may differ between our study pools and for example the Moroccan pools and contribute to the low level of co-occurrence we observed.

Furthermore, species may co-occur in the same habitat but occupy different temporal niches (different times throughout one growing season or different growing seasons). Differences in the seasonal appearance of species explained the co-occurrence of an *Artemia* and *Branchinella* species in a saline pond in Italy (Moscatello et al. 2002), of the notostracans *L. apus* and *T. cancriformis* in Austrian pools (Gottwald & Eder 1999) and enabled the persistence of up to seven large branchiopod species in a single habitat in the Banat province (Serbia; Petrov & Cvetković 1997). In one temporary pond in North America, five anostracans were able to coexist as the species-specific hatching requirements allowed different species to recolonize the habitat from the dormant egg bank from year to year (Donald 1982). Although during subsequent samplings of meadow pools and clay pits during the spring of 2005 (end of March to July) no additional species were found (*L. Boven*, personal observations), dormant egg bank analysis revealed new large branchiopod records for a subset of these pools. Therefore, temporal niche segregation may still be an effective mechanism enabling species co-occurrence in our study pools.

Conclusions and conservation implications

Our observation of a high regional species richness (γ -diversity) and occurrence frequency of large branchiopods in Kiskunság underlines the considerable natural and conservation value of the studied habitats. 'Between habitat diversity' (β -diversity) of large branchiopod communities resulted from the distinct species composition of habitats with contrasting local environments. This probably resulted from efficient species sorting of large branchiopods between different habitat types. The considerable contribution of meadow pools and wheel tracks to the regional species pool due to the presence of rare and exclusive species furthermore supports the importance of maintaining high habitat diversity (size, transparency, vegetation cover) in the landscape. We also have shown that habitats such as wheel tracks and puddles, which are often

overlooked and especially vulnerable to urbanization (e.g. road hardening), can be of particular conservation interest.

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CHAPTER 2

TEMPORARY FRESHWATER POOLS IN KISKUNSÁG (HUNGARY) ARE HOT SPOTS OF CLADOCERAN DIVERSITY

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Abstract We assessed regional cladoceran species richness of 18 temporary freshwater pools in a rather pristine area in the south of Hungary by sampling the active community at repeated occasions throughout one growing season and through identification of hatchlings retrieved from the dormant egg bank. We encountered a total of 33 cladoceran species, which constitutes 68% of the currently described regional species pool of the Kiskunság area. As such, we also demonstrated the importance of small freshwater pools as sources of regional biodiversity. Our study pools are furthermore amongst the most species rich temporary freshwater habitats with respect to Cladocera as revealed by a comparative literature study of 36 studies carried out worldwide. The size of the regional cladoceran species pool of temporary wetlands in these studies turned out to be significantly related to the maximum surface area, depth and/or hydroperiod length of the pools and/or to the degree of variation in these hydromorphological characteristics. We argue that in Kiskunság, Cladocera may thrive in the temporary pools mainly due to their relatively long hydroperiod, which probably relaxed constraints on development time and did not limit colonization success of species. Furthermore, in the well-preserved landscape of Kiskunság, regional coexistence of species is likely enhanced as habitat diversity is preserved and negative impacts such as drainage, pollution or habitat fragmentation are still limited. Given the recent joining of Hungary to the European Union, an increasing pressure from agriculture on the landscape can however be expected and the conservation of its habitat and species diversity may not be evident.

Keywords active community, Cladocera, conservation, species richness

INTRODUCTION

In temporary waters, the recurrent drying and resulting time stress and fluctuations in physical and chemical parameters require different strategies of physiological tolerance, life history adaptations and/or migration from their inhabitants (Wiggins et al. 1980). The relatively low local diversity of temporary pools, especially when compared to permanent ponds or lakes (Collinson et al. 1995, Nicolet 2001, Della Bella et al. 2005, Davies et al. 2008, De Bie et al. 2008), may result from these various stress factors associated with their temporary nature. With increasing pool duration, constraints on development time diminish (Schneider & Frost 1996) and pools may support more species with different life cycles or hatching phenologies. With more time available, chances of colonisation are also higher and extinction rate decreases (Ebert & Balko 1987, Schneider & Frost 1996, Spencer et al. 1999). However, the lower species richness of temporary pools when compared to permanent lakes may as well be due to their smaller habitat size. Larger habitats usually have higher habitat heterogeneity (Williams 1943, Douglas & Lake 1994), are buffered against physicochemical fluctuations and may support larger populations (Spencer et al. 1999). Additionally, larger habitats are possibly more attractive to actively dispersing insects (Wiggins et al. 1980, Wilcox 2001) and local stress events may have a smaller impact (De Bie et al. 2008).

Although the temporary pool environment does not always support a high local diversity, their fauna and flora usually contribute considerably to regional diversity (Williams 1997, Forró et al. 2003, Williams et al. 2003, Nicolet et al. 2004, De Bie et al. 2008). Small pools or ponds in general strongly differ in species composition amongst each other, while temporary pools often contain rare or unique species that are not found elsewhere (De Meester et al. 2005). The high community heterogeneity (β -diversity) of pools and ponds probably results from their physical heterogeneity, related to the relatively small catchment area, and from their isolation degree (Williams et al. 2003).

The importance of temporary pools in maintaining regional biodiversity and the ecological quality of the landscape in general has been acknowledged only recently. Although their relatively small size provides 'easy-win' opportunities for enhancing landscape biodiversity (Williams et al. 2003), the loss and degradation of temporary waterbodies constituted a persistent threat worldwide during the last decades (Brendonck & Williams 2000, Williams et al. 2001, Boothby 2003, Grillas et al. 2004, Jenkins et al. 2003). Also in Western Europe, drainage and intensive cultivation have had a negative impact on these habitats, thereby threatening the persistence of their inhabitants. In Eastern Europe, on the other hand, agriculture is usually less intensive and large areas of farmland still support a high habitat and species diversity, often

including species of conservation concern (European Environment Agency 2004). While environmental pressures are expected to decrease in Western Europe, many areas in Eastern Europe will experience increasing pressure from agriculture. In countries such as Poland and Hungary, which have recently joined the European Union, the conservation of temporary habitats consequently is of high priority (Biggs et al. 2004). Additionally, due to the direct link between temporary pool hydrology (frequency and duration of inundation) and temperature and precipitation patterns, also climate change may have a considerable impact on temporary pools and their communities (Bauder 2005, Pyke 2005a, b, Hulsmans et al. 2008).

Cladocerans (Crustacea - Branchiopoda) are amongst the best represented taxa inhabiting temporary freshwater pools (Williams 2006). Their adaptations to persist in these habitats consist of the production of dormant eggs that are resistant to the recurrent droughts, and of a fast growth and reproduction allowing them to quickly recolonize the habitats after inundation (Wiggins et al. 1980). Although their species numbers in temporary waters are highly variable, high levels of cladoceran species richness have been observed in several studies (Mahoney et al. 1990, King et al. 1996, Simovich 1998, Frisch et al. 2006, Serrano & Fahd 2006, Medley & Havel 2007). Mechanisms promoting the coexistence of cladocerans in temporary pools may include resource partitioning through a diversification of food type or spatial niche segregation (e.g. benthic-littoral versus pelagic species). Furthermore, species may co-occur in the same habitat but occupy different temporal niches (different moments throughout one growing season or different growing seasons) (Mahoney et al. 1990, Boven et al. in press (Chapter 3)). By allowing species to recover from periods of low recruitment, dormant egg banks may prevent competitive exclusion and as such promote the coexistence of species (i.e. the storage effect; Warner & Chesson 1985, Cáceres 1997b). As spatial and temporal niche availability increase with increasing pool size and duration, local species richness is generally positively correlated to surface area, depth and/or hydroperiod (Mahoney et al. 1990, King et al. 1996, Mura & Brecciaroli 2003, Eitam et al. 2004a). King et al. (1996) and Simovich (1998) further argued that high levels of branchiopod coexistence were due to a combination of abundant food resources and low intensity of competitive interactions due to the annual truncation of community development. Finally, on a regional scale, cladoceran species richness may be favored by local variation in habitat characteristics (habitat diversity) and connectivity between pools. Differences in pool size and length of wet phase as well as in local conditions such as salinity or presence of vegetation may result in distinct temporary habitat types housing different branchiopod assemblages (Simovich 1998, Frisch et al. 2006). Furthermore, although high dispersal rates may result in homogenization of communities and a decrease in regional

diversity (Mouquet & Loreau 2003), in interconnected communities (i.e. metacommunities), dispersal may facilitate habitat recolonization after local extinctions, thereby promoting the regional persistence of species (Holyoak & Lawler 1996).

In the current study, we assess regional cladoceran species richness in 18 temporary freshwater pools in Kiskunság, a rather pristine area in the south of Hungary. The measured cladoceran species richness is discussed in relation to the known regional species pool of Kiskunság. By means of a comparative literature study of 36 studies on temporary wetlands throughout the world, we further aim at uncovering general processes underlying regional cladoceran species richness patterns.

MATERIALS AND METHODS

Study Area

The Kiskunság National Park, covering an area of almost 500 km², is located 85 km southwest of Budapest (Hungary), on the plain between the Danube and the Tisza River. The area between the villages of Szabadszállás and Kiskörös was selected as the study area. It is located on the border of alkali lands of the Danube valley, situated mainly in the so-called Turján lands with reed marshes and bog meadows as main biotopes. Within this area, three pool clusters were chosen. Cluster I (46° 47' N 19° 14' E), mainly containing wet meadows, is located outside the national park along the road between Fülöpszállás and Soltszentimre. Cluster II (46° 50' N 19° 10' E) is located in the national park in the Felső Kiskunsági tavak, typically characterized by alkaline lakes, and around the village of Fülöpszállás. Cluster III (46° 40' N 19° 14' E) is part of the nature reserve Kiskörösi Turjános and consists of wet meadows surrounded by fen and upland forests. Clusters were separated by a distance of five (I and II), ten (I and III), and 15 km (II and III). For a detailed description of these clusters, we refer to Chapter 3; Boven et al. 2008a).

The Hungarian continental climate favours the formation of vernal pools. In early spring (February - March), pools fill with snowmelt. As air and water temperature increase during springtime, evaporation also increases. Depending on the morphology of the pools and local weather conditions, the pools usually become dry again in spring or summer.

Sampling of active communities

A total of 18 pools, equally distributed over the three pool clusters, were selected. The length of inundation of the pools as observed in the spring of 2005 varied from less than ten to more than 16 weeks. Sampling of the active communities started at the end of March 2005, two weeks

after inundation by snow melt. Pools were initially sampled every two weeks (March – May) and thereafter monthly (June – July) in a random order. In July, when most pools were dry, sampling stopped. The most long-lived pools were consequently sampled seven times (T_1 : end of March; T_2 : early April; T_3 : end of April; T_4 : early May; T_5 : end of May; T_6 : June; T_7 : July).

At each sampling occasion, cladocerans were sampled quantitatively by collecting an integrated volume of pool water (50 L at the first and second sampling occasion; 100 L at all other occasions). Water was collected with a measuring cup from four peripheral and four central locations and poured over a plankton net (mesh size: 64 μm). Qualitative samples were collected by searching all microhabitats with a 64- μm plankton net. Collected animals were preserved in 70% ethanol and identified to species level using Flößner (2000). From each quantitative sample, subsamples were processed until 300 specimens were counted. For species richness estimations, quantitative samples containing less than 300 specimens were supplemented with corresponding qualitative samples. From these we identified an additional number of specimens either up to 300 or until all collected animals were checked (when less than 300 specimens were present).

Dormant egg bank analysis

Recently, the identification of hatchlings obtained from dormant egg banks has proved to be a valuable method for the estimation of cladoceran species richness in permanent aquatic systems as dormant propagule banks integrated temporal and spatial variation in the cladoceran community structure (Vandekerkhove et al. 2005a, b). To complete the species list obtained with active samples, we additionally performed an analysis of the dormant egg bank for a subset of twelve study pools from which the active cladoceran community was characterized. The dormant egg banks were sampled when the pools were dry (spring 2007) by collecting the upper 3 cm of sediment from 18 random locations (10 x 10 cm^2) representative of all microhabitats (depth, vegetation type) in each pool. Sediment with ephippia (i.e. the protective case in which one or two eggs, depending on the species, are contained) was dried and stored for three months in the dark at room temperature. Next, ephippia were isolated from the sediment (2.4 - 6.0 kg; average 4.3 kg per pool) using the sugar flotation method (Onbé 1978, Marcus 1990). For each pool, the harvested ephippia were next divided over three subsamples. Each subsample was exposed to one of three conditions of temperature with covarying photoperiod (12°C and 12h light/12h dark; 18°C and 14h light/10h dark; 24°C and 17h light/7h dark), mimicking different seasonal conditions (early and late spring and summer, respectively). The ephippia were inundated with 1.5 L of EPA medium (Anonymous 1985; initial conductivity of 800

$\mu\text{S cm}^{-1}$ and continuously aerated) in 2-L aquaria that were placed in incubators with the respective temperature and photoperiod settings. During a period of about one month, newly emerged hatchlings were isolated from the aquaria at repeated occasions (resp. 2, 6, 12, 18, 21 and 26 days after inundation). They were grown in 100-mL vials filled with the medium from which they hatched and were fed a few drops of *Scenedesmus obliquus* algae every other day. Once they reached maturity, they were identified to species level using Flöbner (2000). We acknowledge that, as we inundated the dormant eggs only once, the local species pool might still be underestimated as species might delay hatching over different inundations.

Comparative literature study

To perform a comparative literature study of regional cladoceran species richness in temporary pool ecosystems, we gathered data from a total of 36 studies carried out in 25 different areas worldwide, including our own study in Kiskunság. The following data were collected from each study as far as they were available (Table 1): (a) total number of cladoceran species encountered (regional species richness; S_y); (b) sampling regime: number of sampled pools (N), number of sampling occasions (n) and period covered by the sampling (number of years and/or months); (c) hydromorphological features of the study pools: range of hydroperiod length (number of months; $HP_{\min}$ to $HP_{\max}$), pool depth ($D_{\min}$ to $D_{\max}$) and surface area ($A_{\min}$ to $A_{\max}$); (d) presence of fish (yes: present in some pools; no: absent from all pools); (e) conservation status (P: pristine; D: disturbance; V: variable); and (f) geographical range (scale) of the study area.

Next, we investigated whether the observed levels of regional cladoceran species richness could be explained by any of the mentioned study area characteristics or varied according to the sampling effort (sampling regime or scale of the study areas). Using a General Linear Model, we investigated whether regional species richness was related to the number of sampled pools, number of sampling occasions, variation in depth ($D_{\text{var}} = D_{\max} - D_{\min}$) or surface area ($A_{\text{var}} = A_{\max} - A_{\min}$) of the pools, the scale of the study area (continuous predictor variables) or the presence of fish or conservation status (categorical predictor variables). Variation in pool depth was strongly correlated to maximum pool depth (Spearman Rank correlation: $r = 0.92$, $p < 0.001$) and variation in surface area to maximum surface area ($r = 0.98$, $p < 0.001$). Therefore, we were not able to completely separate both effects. As variation in depth was strongly correlated with hydroperiod variation ($r = 0.86$, $p < 0.001$), we only used depth in our analyses. However, we acknowledge that the effects of both variables are difficult to disentangle. As the categorical predictor variables did not cause any significant interaction or main effects, we finally integrated the remaining significant continuous predictor variables in a multiple regression model. For all

analyses, the number of sampled pools and sampling occasions, variation in pool surface area and scale of the study area were logarithmically transformed to meet assumptions of linearity and equal variance of the data. We did not take those studies into account with only one study pool ($N = 1$) as there was no variation in pool morphometry. For the remaining 30 studies, missing data for certain variables were replaced by the average of the respective variable over all studies. When the conservation status was unspecified, it was classified as 'variable (V)'. We believe that our results are not biased by these actions as they were consistent when the analyses were repeated excluding studies with missing data for any of the explanatory variables. GLMs and multiple regression were performed in Statistica 8.0 (StatSoft Inc. 2007).

Table 1. Overview of temporary pools throughout the world, in order of the regional species richness (Sy) of their cladoceran communities. For each study, we provided data on the sampling regime (N = number of sampled pools; n = number of sampling occasions; period = number of months (M) or years (Y) covered by the sampling), hydromorphological features of the study pools (range of hydroperiod length (HP; number of months), depth and surface area) and the presence of fish (yes: present in some pools; no: absent from all pools), the conservation status (P: pristine; D: disturbance; V: variable) and geographical range (scale) of the study area. Where the respective information was not available, cases are empty.

	Sy	Sampling regime			Study pools				Study area		References
		N	n	period	HP (mths)	Depth (m)	Surface area	Fish	Conservation status	Scale	
Carolina Bay (Carolina, N America)	44	23	3	3 M	1 - 12	0.19 - 1.30	0.4 - 50.2 ha	no	variable (V)	650 km ²	Mahoney et al. 1990
Doñana (SW Spain)	40	~65			<3 - 12	0.05 - 3.00	20 m ² - 30000 ha	yes	national park (P)	1000 km ²	Serrano & Fahd 2005, Fahd et al. 2000 & 2007, Frisch et al. 2006
Missouri floodplain ponds (N America)	37	29	1	1 M	3 - 12	0.10 - 3.20	60 - 3510 m ²	yes	cattle reservoirs (D)	125 km ²	Medley & Havel 2007
Doñana dune lagoons (SW Spain)	33	~40			<3 - >7	0.20 - 3.00	<100 m ² - 32.7 ha	yes	national park (P)	25 km ²	Serrano & Fahd 2005, Fahd et al. 2000 & 2007
	27	19	5	1 - 2 M; 3 Y	<3 - >7	0.20 - 3.00	<100 m ² - 32.7 ha	yes	national park (P)	25 km ²	Serrano & Fahd 2005
	17	18	2	2 M	<3 - >7	0.20 - 3.00	<100 m ² - 32.7 ha	yes	national park (P)	25 km ²	Fahd et al. 2000
	24	36	1	1 M	<3 - >7	0.20 - 3.00	<100 m ² - 32.7 ha	yes	national park (P)	25 km ²	Fahd et al. 2007
	20	9	4	2 M	<3 - >7	0.20 - 3.00	<100 m ² - 32.7 ha	yes	national park (P)	25 km ²	Fahd et al. 2007
Kiskunság (Hungary)	33	18	7	4 M	1.5 - >4	0.18 - 1.15	11 m ² - 6.8 ha	no	national park (P)	200 km ²	This study
Flanders (Belgium)	29	38	1	3M	<1 - 12		<25 m ² - 2 ha	no	variable (V)	13000 km ²	De Bie et al. 2008
California vernal pools (N America)	25	128	3	4 M	<1 - 6	<0.10 - >1.00	<300 m ² - >2.5 ha	no	variable (V)	300 km transect + 93 km ²	Simovich 1998
Australian gnammas (W Australia)	24	36	1	2 M		0.02 - >1.00	0.16 - 300 m ²	no			Bayly 1997
Victoria (Australia)	22	53	1	8 M		<0.50	1 - 440 m ²	no			Morton & Bayly 1977
Cordillera del Tunari peat pools (Bolivia)	21	61	1	1 M	3 - 6	0.03 - 0.50	1.5 - 532 m ²	no	pristine (P)	140 km ²	Coronel et al. 2007
Doñana marshes (SW Spain)	21	25	1	3 M	<3 - 12	0.05 - 1.50	20 m ² - 30000 ha	yes	national park (P)	1000 km ²	Frisch et al. 2006
Eastern Numidia (Algeria)	20	27	1	1 M	5 - 12	0.05 - 1.20	<1 ha	yes		1200 km ²	Samraoui 2002
Carolina Bay (Carolina, N America)	19	1	21	2 - 9 M; 2 Y	2 - 9	1.00	1 ha	no			Taylor & Mahoney 1990

Table 1. continued.

	Sy	Sampling regime			Study pools				Study area		References
		N	n	period	HP (mths)	Depth (m)	Surface area	Fish	Conservation status	Scale	
California vernal pools (N America)	18	58	3	4 M	1 - 6	<0.10 - >1.00	<300 m ² - >2.5 ha	no	variable (V)	300 km transect	King et al. 1996
Camargue (France)	17	30	3	3 M	5 - 9	0.15 - 0.87	564 m ² - 14 ha	yes	national park (P)	26 km ²	Waterkeyn et al. 2008
Western Cape (South Africa)	16	62	1	3 M	1 - 11	0.03 - 1.50	5 m ² - 8.6 ha	no	variable (V)	30000 km ²	E. De Roeck unpubl. data
Bluff Spring Ponds (Illinois, N America)	16	13	~64	4 - 8 M; 3 Y	4 - 8	<0.95		no	drainage (D)		Jenkins et al. 2003
Roadside pond Victoria (Australia)	14	1	12	7 M	7	0.86	370 m ²	no	roadside (D)		Lake et al. 1989
Castelporziano Nature Reserve (Latium, Italy)	12	2	24	12 M	11 - 12	0.69 - 0.75	500 - 699 m ²	no	nature reserve (P)	60 km ²	Seminara et al. 2008
Oasis of Palo (Latium, Italy)	11	9	24	12 M	3 - 12	0.28 - 1.30	9 - 800 m ²	no	protected area (P)	1.2 km ²	Mura & Brecciaroli 2003
S Germany	10	8	12	4 - 8 M; 2 Y	5 - 8	0.20 - 1.05	3 - 500 m ²	no	military area (D)		Maier et al. 1998
Flanders (Belgium)	7	30	1	8 M			<0.1 ha	no	variable (V)	13000 km ²	Forró et al. 2003
Australian rock pools (W Australia)	7	52	1	1 M		0.03 - 0.23	1 - 60 m ²	no	pristine (P)		Jocqué 2007
California vernal pools (N America)	6	54	4	3 M	<1 - 3	0.06 - 0.38	12 - 648 m ²	no	variable (V)	32 km ²	Ebert & Balko 1987
Israeli rock pools (Israel)	6	52	2	2 M	1.5 - 6	<0.80	0.01 - 166 m ²	no	overgrazing (D)	2 km ²	Eitam et al. 2004
Bluff Spring Ponds (Illinois, N America)	5	15	8	2 M	1.5 - 2.5	0.16 - 0.56	200 m ² - 0.4 ha	no		16 ha	Steven & Jenkins 2000
N Apennines mountain pools (Italy)	5	9	22	6 M; 2 Y	3 - 6	0.30 - 1.20	36 - 396 m ²	no	pristine (P)	4 km ²	Tavernini 2008, Tavernini et al. 2005
Korannaberg rock pools (South Africa)	5	36	4	4 M	<1 - 3.5	0.05 - 0.38	0.6 - 50 m ²	no	pristine (P)	<1 ha	B. Vanschoenwinkel unpubl. data
Sahelian pond (Senegal)	4	1	~50	8 M	8	1.20	<1 ha	no	extensive agriculture (D)		Lahr et al. 1999
Zingaro Nature Reserve (Sicily, Italy)	3	2	14	7 M	7	0.30 - 0.60	175 m ² - 0.1 ha	no	cattle reservoirs (D)	1.7 km ²	Castelli et al. 2006
Israeli rock pools (Israel)	3	25	5	5 M	1 - 5		0.15 - 13.0 m ²	no		800 m ²	Spencer et al. 1999
Australian gnamma (SW Australia)	1	1	10	2 M	<3	0.74	97 m ²	no			Bayly 2001
Botswana rock pools	1	18	~15	<1 M	<1	<0.20	1.3 - 14 m ²	no	pristine (P)		Jocqué et al. 2006
Utah rock pools (N America)	1	27	~6	<1 M	<1	0.01 - 0.20	0.3 - 1.1 m ²	no	pristine (P)		Jocqué et al. 2007b

RESULTS

We encountered a total of 32 cladoceran species in the active community of 18 temporary freshwater pools throughout one growing season. Using dormant egg bank analysis for the subset of twelve pools, one more species could be added to the regional species pool of the study pools (Table 2).

Table 2. Overview of the described regional cladoceran fauna of Kiskunság (Hungary) including temporary freshwater pools and sodic lakes. From our samples, *Bosmina* could not be identified to species level due to the bad condition of the limited number of specimens.

		Temporary freshwater pools		Sodic lakes
		This study	Forró 2003	Forró 2003
	Fam. Sididae			
1	<i>Diaphanosoma brachyurum</i> (Liévin, 1848)	+		+
2	<i>Diaphanosoma mongolianum</i> (Uéno, 1938)			+
	Fam. Macrothricidae			
3	<i>Macrothrix hirsuticornis</i> Norman et Brady, 1867	+	+	+
4	<i>Macrothrix laticornis</i> (Jurine, 1820)			+
5	<i>Macrothrix rosea</i> (Jurine, 1820)	+		+
6	<i>Bunops serricaudatus</i> (Daday, 1884)	+		
7	<i>Wlassicsia pannonica</i> Daday, 1904		+	+
	Fam. Daphniidae			
8	<i>Moina brachiata</i> (Jurine, 1820)	+	+	+
9	<i>Moina macrocopa</i> (Straus 1820)		+	+
10	<i>Moina micrura</i> Kurz, 1875	+		+
11	<i>Megafenestra aurita</i> (Fischer, 1849)	+	+	+
12	<i>Scapholeberis mucronata</i> (O.F. Müller, 1776)	+		+
13	<i>Scapholeberis rammneri</i> Dumont et Pensaert, 1983	+	+	+
14	<i>Simocephalus exspinosus</i> (Koch, 1841)	+		+
15	<i>Simocephalus vetulus</i> (O.F. Müller, 1776)	+		+
16	<i>Daphnia atkinsoni</i> Baird, 1859	+	+	+
17	<i>Daphnia magna</i> Straus, 1820	+	+	+
18	<i>Daphnia curvirostris</i> Eylmann, 1887	+		+
19	<i>Daphnia pulex</i> Leydig, 1860			+
20	<i>Daphnia galeata</i> Sars, 1863			+
21	<i>Daphnia longispina</i> (O.F. Müller, 1776)	+		+
22	<i>Daphnia cucullata</i> Sars, 1862			+
23	<i>Ceriodaphnia affinis</i> Lilljeborg, 1901	+		
24	<i>Ceriodaphnia laticaudata</i> P.E. Müller, 1876	+		
25	<i>Ceriodaphnia megops</i> Sars, 1862			+
26	<i>Ceriodaphnia quadrangula</i> (O.F. Müller, 1785)			+
27	<i>Ceriodaphnia reticulata</i> (Jurine, 1820)	+	+	+
28	<i>Ceriodaphnia rotunda</i> Sars, 1862			+
	Fam. Chydoridae			
29	<i>Eurycercus lamellatus</i> (O.F. Müller, 1776)	+		
30	<i>Chydorus latus</i> Sars, 1862			+
31	<i>Chydorus ovalis</i> Kurz, 1875			+

Table 2. continued.

		Temporary freshwater pools		Sodic lakes
		This study	Forró 2003	Forró 2003
32	<i>Chydorus sphaericus</i> (O.F. Müller, 1776)	+	+	+
33	<i>Pleuroxus aduncus</i> (Jurine, 1820)	+		+
34	<i>Pleuroxus laevis</i> Sars, 1861	+		
35	<i>Alonella excisa</i> (Fischer, 1854)	+		+
36	<i>Alonella nana</i> (Baird, 1843)			+
37	<i>Disparalona rostrata</i> (Koch, 1841)			+
38	<i>Dunhevedia crassa</i> King, 1853	+	+	+
39	<i>Alona affinis</i> (Leydig, 1860)			+
40	<i>Alona costata</i> Sars, 1862			+
41	<i>Alona guttata</i> Sars, 1862	+		+
42	<i>Alona quadrangularis</i> (O.F. Müller, 1776)			+
43	<i>Alona rectangula</i> Sars, 1861	+	+	+
44	<i>Kurzia latissima</i> (Kurz, 1875)	+		
45	<i>Acroperus harpae</i> (Baird, 1835)	+		+
46	<i>Graptoleberis testudinaria</i> (Fischer, 1848)	+		
47	<i>Oxyurella tenuicaudis</i> (Sars, 1862)	+		+
48	<i>Leydigia leydigi</i> (Schoedler, 1863)			+
49	<i>Tretocephala ambigua</i> (Lilljeborg, 1901)	+		
	Fam. Polyphemidae			
50	<i>Polyphemus pediculus</i> (Linné, 1761)	+		+
	Fam. Bosminidae			
51	<i>Bosmina</i> sp. Baird, 1846	+		+
		33	12	43
Total species richness:		35		

The regional cladoceran species richness of temporary pool ecosystems worldwide was not significantly related to the sampling regime of the respective studies, neither to the presence of fish or conservation status of the study area (GLM: $p > 0.05$). We did find a significant effect both of the degree of variation in depth ($MS_{(1, 21)} = 319.54$, $F = 5.78$, $p = 0.03$) and surface area of the study pools ($MS_{(1, 21)} = 709.08$, $F = 12.83$, $p = 0.002$) (Whole model: $MS_{(8)} = 402.31$, $F = 7.28$, $p < 0.001$). Regional species richness significantly increased with increasing variation in depth (multiple regression: $\beta = 0.41$, $t = 3.05$, $df = 27$, $p = 0.005$) (Fig. 1a) and surface area of the temporary study pools ($\beta = 0.52$, $t = 3.91$, $df = 27$, $p < 0.001$) (Fig. 1b). Together, these variables explained 66.9% of total variation in species richness ($F_{(2,27)} = 27.34$; $p < 0.001$).

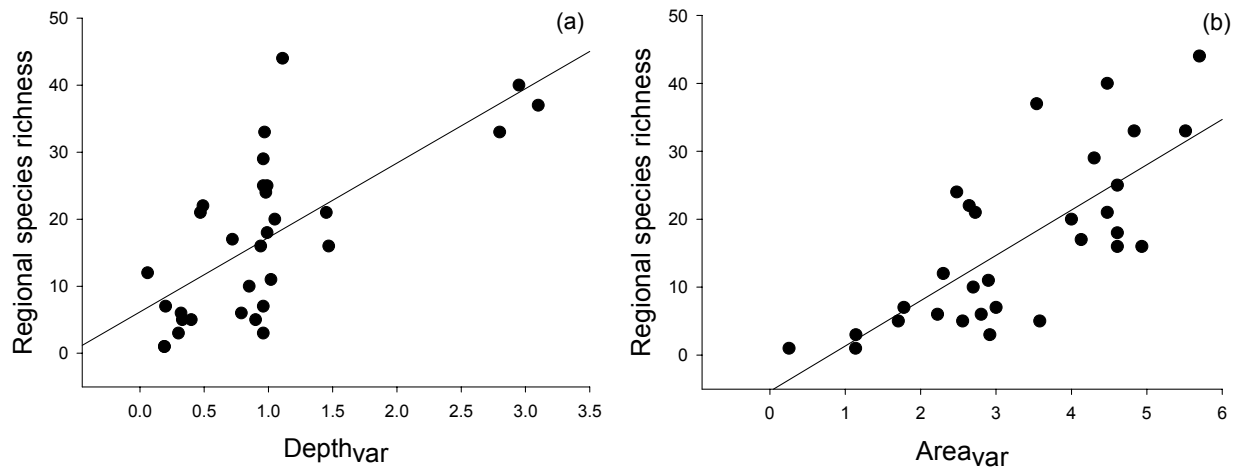


Figure 1. Scatter plots illustrating the relationship between regional cladoceran species richness of various temporary pool ecosystems throughout the world and the degree of variation in (a) depth (Depth_{var}; m) and (b) surface area (Area_{var}; m²; logarithmically transformed) of the respective study pools.

DISCUSSION

During our study of 18 temporary freshwater pools in Kiskunság (Hungary), we encountered a total of 33 cladoceran species. In general, the use of dormant egg bank analysis was highly inefficient for regional species richness assessment as only one species could be added to the regional species list based on active community samples. In the Kiskunság area, 43 cladoceran species have already been observed since 1894 (Forró 2003 and references therein; Table 2) of which 25 were also found throughout our study. In addition, we found eight new species for the area: *Bunops serricaudatus*; *Ceriodaphnia affinis*; *Ceriodaphnia laticaudata*; *Eurycercus lamellatus*; *Graptoleberis testudinaria*; *Kurzia latissima*; *Pleuroxus laevis*; and *Tretocephala ambigua*. Only twelve cladoceran species were recorded from small temporary pools in Kiskunság before (Forró 2003). The total number of species found in temporary freshwater pools, also including our study (35 species), consequently constitutes 68% of the currently described regional species pool of Kiskunság (51 species; Table 2). The cladoceran fauna of the large sodic lakes in Kiskunság (43 species), which were the main focus of previous studies in the area, comprises 84% of the regional species richness (Table 2). Considering the relatively small surface area covered by the temporary study pools (cumulative surface area: 8 ha) when compared to the size of the lakes (cumulative surface area: more than 630 ha), our findings underline the importance of temporary freshwater pools as sources of regional (γ) biodiversity also in Kiskunság. The considerable contribution to regional biodiversity of small waterbodies such as ponds and particularly temporary pools (Williams 1997, Forró et al. 2003, Williams et al.

2003, Nicolet et al. 2004, Davies et al. 2008, De Bie et al. 2008) is generally attributed to their highly variable species composition (Oertli et al. 2002, Williams et al. 2003, De Meester et al. 2005).

Regional species richness of the Kiskunság pools was most comparable with the number of species found in temporary freshwater pools in the dune lagoons in Doñana National Park in Spain (Serrano & Fahd 2005, Fahd et al. 2000, 2007). When also considering the temporary pools in the marshes (Frisch et al. 2006), Doñana presumably is a richer area. However, given the relatively small sampling effort of our study (18 pools; one growing season) when compared to the studies that have been carried out in Doñana (about 65 pools; some were monitored during three subsequent years), our findings are rather exceptional. Furthermore, to our knowledge, only Carolina Bay ponds (44 species; Mahoney et al. 1990) and Missouri floodplain ponds (37 species; Medley & Havel 2007) in North America are characterized by a richer regional species pool. A rather high number of species was also found in a single temporary pond in Carolina Bay (19 species; Taylor & Mahoney 1990) and Victoria, Australia (14 species; Lake et al. 1989). The lack of a significant effect of sampling regime and scale of the study area in our comparative analysis of literature data suggests that differences in the total number of species encountered in each area were not due to a variable sampling effort among the studies considered. We can therefore reliably conclude that Kiskunság pools house rich cladoceran communities.

As revealed by our comparative literature study, temporary wetland systems are generally characterized by a richer cladoceran species pool as variation in depth and surface area of the pools increases. However, as the degree of variation was strongly correlated with maximum values, levels of regional cladoceran species richness might as well relate to maximum pool depth and surface area. Additionally, the positive effect of pool depth probably partly results from an increase in pool hydroperiod. We therefore argue that prolonged hydroperiods, related to the relatively large depth of pools, may be the main underlying cause for the elevated species richness in our study area. Indeed, our study pools may be considered as relatively long-lived on the basis of the worldwide comparison (Table 1). The shortest observed hydroperiod during spring (seven weeks) still exceeds the time needed both for clonal and sexual reproduction of cladocerans and the production of dormant eggs and constraints on development time as such are probably weakened, especially when compared for example to ephemeral rock pools (Spencer et al. 1999, Bayly 2001, Eitam et al. 2004a, Jocqué 2007, Jocqué et al. 2006, 2007b). With increasing pool duration, more time is available for colonization and succession of species with a variable life history or hatching phenology (Schneider & Frost

1996). Indeed, especially in long hydroperiod pools, seasonal community dynamics resulted in the accumulation of species throughout the growing season (Chapter 4). Moreover, the continuous growth of aquatic macrophytes and algae throughout spring (Boven et al. 2008a; Chapter 3) probably enhanced the development of species rich communities. Macrophytes generally provide structural heterogeneity (van den Brink et al. 1994, Taniguchi et al. 2003), refuges for predation (Van de Meutter et al. 2005a) and substrate for periphyton as an important food source for cladocerans (Balayla & Moss 2003). The high species richness of branchiopods in Californian vernal pools was likely attributed to abundant food resources originating both from the input of autochthonous and allochthonous organic material (King et al. 1996, Simovich 1998). Additionally, the relatively large depth and surface area of most of our study pools probably also favoured species coexistence by providing diverse microhabitats. In general, however, the measured positive relation between variation in pool depth (hydroperiod) and surface area and the size of the regional species pool indicates the overall importance of high habitat diversity in promoting regional coexistence of species. In Kiskunság, as well as in other of the most species rich areas (Missouri, Carolina Bay, Doñana), not only large long-lived pools are typically present, but also wide gradients of pool depth, surface area and duration are characteristic features (Table 1). Small habitats with a short duration may be locally species poor, but are of considerable importance in maintaining metacommunity dynamics and stability and the regional persistence of species and populations (Semlitsch 2000, Holyak et al. 2005, Tarr et al. 2005). Therefore, in order to conserve global species diversity, the maintenance of habitat diversity rather than the exclusive protection of large long-lived pools should be promoted.

There was a striking absence of a relation between regional species diversity and conservation status in our comparative analysis. Although natural landscape features are more likely to be preserved both in areas where national or international guidelines for habitat or species conservation are implemented, as well as in remote pristine areas, this does therefore not necessarily result in a high regional diversity. Indeed, in our comparative analysis, pristine areas were often rock pool systems on remote mountaintops, which partly due to the small size and ephemeral duration as well as the low productivity of their habitats generally support lower species diversity. In national parks, pools were typically situated along wide gradients of pool depth, surface area and duration and therefore probably supported a rich cladoceran species pool. The Kiskunság area, nevertheless, is relatively well-preserved (Natura 2000 Network and Ramsar Convention), supporting high habitat diversity and hence a rich regional species pool as any negative impacts of agricultural activities and urbanization are still limited (e.g. drainage or

pollution). Unlike pools in highly fragmented landscapes, the temporary pools in Kiskunság furthermore may constitute interconnected communities between which dispersal is enabled through various vectors such as wind, temporary water connections and animals (amphibians, water birds, deer and cattle). If in such metacommunities dispersal rates are not too high to homogenize the communities through mass effects, they may contribute to high levels of regional diversity (Mouquet & Loreau 2003), as observed in Kiskunság. The presence of an area like Kiskunság contrasts with the general trend of habitat destruction, change or fragmentation by agricultural and urban development throughout Europe. However, the recent joining of Hungary to the European Union may result in an increasing pressure from agriculture on the landscape and its temporary habitats.

Finally, regional cladoceran species richness may also vary according to other factors such as type of vegetation, soil type and productivity levels of pools as well as climatic, geographical or historical factors. However, such factors could not be included in our study as for most study areas only limited information was available.

Conclusion

The results of this study in combination with a previous study on the large branchiopod communities (Boven et al. 2008b; Chapter 1) indicate that temporary freshwater pools in Kiskunság are a hot spot of branchiopod diversity. Given the importance of branchiopods in the food chain of temporary aquatic waters, we argue that their conservation will be important in preserving the ecological quality and functioning of these ecosystems. This is especially important in the European context of ongoing habitat destruction and degradation of the landscape for agriculture and urban development. Any human impact resulting in a strong decrease in maximum pool size and duration could adversely affect the regional cladoceran species pool and therefore should be restricted. However, in order to protect regional species diversity, we strongly promote the maintenance of a high diversity of habitat types along a gradient of pool depth, surface area and hydroperiod length rather than the exclusive protection of large, deep pools.

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CHAPTER 3

SEASONAL DYNAMICS IN WATER QUALITY AND VEGETATION COVER IN TEMPORARY POOLS WITH VARIABLE HYDROPERIODS IN KISKUNSÁG (HUNGARY)

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Abstract The seasonal dynamics in habitat characteristics of temporary freshwater pools were studied in relation to hydroperiod and geographical location for a set of 36 pristine pools in and around the Kiskunság National Park (Hungary). The pools were geographically distributed over three clusters and their hydroperiod varied from seven to more than 16 weeks. Biweekly to monthly monitoring started two weeks after inundation (March) and lasted until most pools were dry again (July). Throughout the season, nutrient concentrations and conductivity increased in all study pools. Algal biomass increased after short hydroperiod pools were already dry, resulting in an increase in the amount of suspended solids and turbidity in pools with a relatively long hydroperiod. Both the longer inundation period and conditions later in the season may have contributed to the stronger algal growth in these pools. Oxygen was not depleted towards the end of the season, potentially due to growing algae and vegetation. The high buffering capacity of the water may have contributed to the near constant pH levels through time. Pools in one of the clusters were typically more turbid, less vegetated and had higher amounts of suspended matter when compared to pools in the two other clusters. Temporary pool characteristics were related to local environmental conditions and seasonal dynamics differed according to the duration of inundation.

Keywords inundation period, macrophyte cover, physicochemical pool characteristics, seasonal changes, temporary aquatic habitats

INTRODUCTION

Temporary freshwater pools are habitats of particular conservation concern. Although temporary pools generally have a lower species richness compared to permanent systems (Collinson et al. 1995, Nicolet 2001, Della Bella et al. 2005, Davies et al. 2008, De Bie et al. 2008), they often contain highly specialized and unique species and strongly contribute to regional biodiversity (De Meester et al. 2005). Yet, over the past decades, these habitats have been progressively lost from the European landscape or have been degraded (Mura 1993, Williams et al. 2001, Boothby 2003, Grillas et al. 2004, Jenkins et al. 2003). In Western Europe, particularly drainage and intensive cultivation have a negative impact on these systems. In Eastern Europe, on the other hand, agriculture is usually less intensive and large areas of farmland still support a high habitat and species diversity, often including species of conservation concern (European Environment Agency 2004). While environmental pressures are expected to decrease in Western Europe, many areas in Eastern Europe will experience increasing pressure from agriculture (EEA 2004). The consequent change from traditional to intensive cultivation methods may affect species and habitat diversity in an undesirable way (Bernaldez 1991, EEA 2004). In order to better understand the vulnerability of temporary freshwater systems and the expected impact of intensified agriculture on their diversity, more information on their habitat characteristics is needed.

Temporary aquatic habitats exhibit recurrent filling and drying events. Once inundated, they may hold water long enough to allow the completion of the aquatic phase of the life cycle of some plant and animal species (Blaustein & Schwartz 2001). These hydroperiod dynamics drive seasonal variation in physical and chemical conditions as documented for many systems (Daborn & Clifford 1974, Freimuth & Bass 1994, Bonner et al. 1997, Lahr et al. 1999, Hancock & Timms 2002). The degree of seasonal variation in physical and chemical characteristics varies according to climate zone. For example, in temperate regions the surface water of very shallow ponds may approach 40°C in summer (Williams 2006) and in alpine and arctic regions water temperatures may drop below 0°C (Daborn & Clifford 1974). Also, the pool type (size and soil type) affects fluctuations in physical and chemical variables. Fluctuations in pH are usually more pronounced in diluted ephemeral rock pools (see Scholnick 1994, Chan et al. 2005) than in pools with clay substrate (Hancock & Timms 2002), as they depend not only on the intensity of photosynthesis, but also on buffering capacity associated with pool substrate (Williams 2005). Besides temporal variation, habitat characteristics of pools often show considerable spatial variability. For example, Podrabsky et al. (1998) found a high degree of spatial variation in dissolved oxygen and pH values among different ephemeral rain pools in Venezuela. Spatial

variation may even occur at a small scale. In South African pans, for example, large differences in temperature, oxygen, pH and conductivity were observed even among neighboring pools (Meintjes et al. 1994). While temporal and spatial variation in habitat characteristics of temporary pools is well documented, little is known about variation in pool characteristics in relation to the length of inundation. Additionally, it is not known whether temporal changes in pool characteristics differ according to the length of the hydroperiod of pools.

Stress caused by variability in environmental characteristics and especially the occasional drying of the habitats, may explain both why temporary pools generally have a lower species richness compared to permanent systems and why they often contain highly specialized species that do not occur in other habitats. The seasonal changes of physical and chemical characteristics could also be important in explaining seasonal changes in biological communities.

The aim of this study is to document the temporal (seasonal) dynamics in water quality and vegetation cover in vernal pools with variable duration of inundation in the Kiskunság National Park in Hungary. In this rather well-preserved area, temporary aquatic habitats are still relatively abundant, covering a wide gradient in inundation period.

MATERIALS AND METHODS

Study area

The Kiskunság National Park was established in 1975 and is located 85 km southwest of Budapest (Hungary), on the plain between the Danube and the Tisza River. It has an area of almost 500 km². The area between the villages of Szabadszállás and Kiskörös was selected as the study area. It is located on the border of alkali lands of the Danube valley, situated mainly in the so-called Turján lands with reed marshes and bog meadows as main biotopes. Within this area, three pool clusters were chosen. Cluster I (46° 48' 14.3" N 19° 16' 17.9" E) with mainly wet meadows is located outside the national park along the road between Fülöpszállás and Soltszentimre. Cluster II (46° 49' 42.1" N 19° 13' 35.3" E) is located in the national park in the Felső Kiskunsági tavak with alkaline lakes and around the village of Fülöpszállás. Cluster III (46° 41' 11.7" N 19° 16' 16.5" E) is part of the nature reserve Kiskörösi Turjános and consists of wet meadows surrounded by fen and upland forests. Clusters were separated by a distance of five (I and II), ten (I and III) and 15 km (II and III). A general description of each cluster is included in Table 1.

Table 1. Overview of the three pool clusters with geographical location (GPS coordinates), conservation status, main land use type, main soil type and main habitat type. For each cluster, we also provided the range and average value of size and depth of pools, as well as the number of pools that was sampled for each hydroperiod class (HP_S: short hydroperiod; HP_M: medium long hydroperiod; HP_L: long hydroperiod).

	Cluster I	Cluster II	Cluster III
Location	46° 49' 42.1" N 19° 13' 35.3" E	46° 48' 14.3" N 19° 16' 17.9" E	46° 41' 11.7" N 19° 16' 16.5" E
Conservation status	unprotected	national park	nature reserve
Land use			
Mowing	extensive; uncontrolled	no	extensive; controlled by national park
Grazing	extensive	extensive	no
Arable farming	no	extensive to intensive	no
Soil type	sand	clay; sand	sand
Habitat type	meadow pools	clay pits; meadow pools	meadow pools
Pool size			
Range	11 m ² – 1.38 ha	18 m ² – 64.08 ha	290 m ² – 67.89 ha
Average	0.54 ha	1.55 ha	1.25 ha
Pool depth			
Range	10.0 – 65.0 cm	22.0 – 108.0 cm	17.0 – 96.0 cm
Average	36.6 cm	49.0 cm	43.5 cm
Number of pools by hydroperiod			
HP _S (< 10 weeks)	2	1	4
HP _M (10 – 18 weeks)	7	6	6
HP _L (> 18 weeks)	3	5	2

The Hungarian continental climate with cold winters and warm dry summers favors the formation of temporary pools filling in early spring (February – March) with snowmelt. Water loss due to infiltration is negligible at that time since the soil is saturated or still frozen, while evaporation is limited due to the low temperatures. As air and water temperature increase during springtime, evaporation also increases. Depending on the morphology of the pools and local weather conditions, the pools usually are dry again in spring (April – June) or summer (July – August). According to a recently suggested classification framework of temporary waters (see Williams 2005), our study pools are classified as intermittent (i.e. having a more or less predictable hydrocycle), freshwater meso- and macrohabitats from the temperate grasslands. Here, they will be further referred to as temporary freshwater ‘pools’.

Field sampling

A total of 36 pools equally distributed over the three clusters were selected. Within each cluster, study systems were selected that differed in size (surface area) and hydroperiod (depth). In an attempt to have a well balanced distribution over hydroperiods (short, medium and long) without prior knowledge, pools were selected over three depth classes on the basis of maximal depth: < 30 cm, 31 to 45 cm and > 45 cm. The effective hydroperiod of the pools as later measured in the field varied from seven to more than 16 weeks. For subsequent analysis, habitats were divided into three hydroperiod classes based upon their absolute hydroperiod (number of weeks): short (< 10 weeks; HP_S), medium long (10 – 13 weeks; HP_M) and long hydroperiod (> 16 weeks; HP_L). Depth classes did not always correspond with hydroperiod classes as more than half of the pools turned out to have a medium long hydroperiod. In addition, most of the short hydroperiod pools were located in cluster III, whereas cluster II contained a greater proportion of the long hydroperiod habitats (Table 1). This discrepancy is probably related to soil conditions of the study clusters. Cluster II, located in the vicinity of the village Füllöpszállás, is characterized by a less permeable clayey soil than the sandy soils in the other clusters. The vicinity of forest in cluster III may also affect the hydrology through increased evapotranspiration and soil infiltration, lowering the water table in this area. Pool surface when fully inundated ranged from 11 m² to almost 68 ha and was not correlated with either maximal depth or hydroperiod. Within each cluster, pools were generally close to each other (i.e. within a few hundred meters). A general description of the pools in each cluster is included in Table 1.

Sampling started two weeks after inundation by snow melt (March 2005), when all pools were in a similar and early succession stage. Even when some pools did not dry before winter, they all froze to the bottom, a condition that is physiologically similar to drought for the biota. Sampling was initially conducted biweekly (March – May) and thereafter monthly (June – July) until most pools dried. In July, we stopped sampling. The remaining pools ($n = 7$) did not dry until August and some very probably did not dry at all in 2005 because of relatively low temperatures and high rainfall. The longest hydroperiod pools were sampled seven times (end of March up to July). Pools were sampled in a random order during each sampling round.

At each sampling, habitat characteristics were assessed. Maximum depth was measured using a calibrated stick. Water transparency was measured using a Snell's tube at a central location. Snell's depth is equivalent to Secchi depth, but is measured in a tube (70 cm long, 6 cm diameter) that is impermeable to light. This device is useful in shallow, clear water to develop a relative measure of transparency. An integrated water sample of about nine liters was taken from the surface at three randomly chosen locations. From this sample, conductivity ($\mu\text{S cm}^{-1}$),

pH and dissolved oxygen concentration (% saturation) were measured in the field with a WTW 340i multimeter. (Oxygen concentration was not measured in the earliest sample.) From the same sample, chlorophyll *a* concentration (mg L^{-1}) was determined in the lab by filtering 1.0 – 1.5 L of water and using the methanol extraction method (Talling & Driver 1963). The amount of suspended solids (mg L^{-1}) was also determined by filtering 1.0 – 1.5 L of water and by measuring the dry weight of the residue on a Whatman GF/C filter dried for 24h at 105°C. Total phosphorus (TP, mg L^{-1}) was determined with the acid persulphate digestion method using a portable spectrophotometer (Hach DR/700; detection range: 0 to 1.17 mg L^{-1}). Alkalinity of each sample was determined using sulphuric acid titration. Percentage cover with macrophytes (floating, submersed and emerged vegetation) was estimated at each pool ($\pm 10\%$).

Data analysis

Repeated measures ANOVA was used to analyze seasonal dynamics in habitat characteristics (Statistica 6.0; Statsoft Inc. 2005). Time (repeated factor), hydroperiod class and cluster were used as independent variables. Each pool was treated as a replicate. Interactions that could not be tested due to limited replication were excluded from the model (cluster x hydroperiod, time x cluster x hydroperiod). Given the unbalanced design we used type III sum of squares as advised by Quinn & Keough (2002). A relative time scale was applied to enable comparisons of temporal dynamics of habitat characteristics among hydroperiod classes, despite early drying of short hydroperiod pools. This is an intrinsic problem when comparing pools with variable hydroperiods. Therefore, for each pool, the inundation period was divided into an early, middle and late succession stage. The early (T_{early}) and late (T_{late}) stages were respectively the first and the last time the pool was sampled. The middle stage (T_{middle}) was chosen halfway between the early and late stages. As such, in pools that dried up quickly (HP_S), the middle and late succession stages refer to earlier moments in spring than in pools that dried up later (HP_M and HP_L). Interpretation of relations between time and hydroperiod, must therefore be done cautiously. Interaction might be due to differences in time elapsed between succession stages and/or to seasonal effects and hence not only to hydroperiod. In order to obtain a more reliable interpretation for variables with a significant time x hydroperiod interaction, we also applied repeated measures ANOVA using an absolute time scale for each hydroperiod class individually. We used data from three, five or seven successive sampling occasions for pools belonging to HP_S , HP_M and HP_L , respectively (T_1 : end of March; T_2 : early April; T_3 : end of April; T_4 : early May; T_5 : end of May; T_6 : June; T_7 : July).

Assumptions of normality and equal variance of data were checked prior to analysis (Shapiro-Wilks and Levene's test; Statistica 6.0). When necessary, data were transformed logarithmically or exponentially. Total phosphorus data at certain stages were missing for ten pools due to meter malfunctioning. Missing data occurred equally in all clusters and hydroperiod classes and consequently these samples were deleted from analysis of nutrient data. For vegetation cover, analyses using the absolute time scale could not be performed for the short hydroperiod class as no variance occurred between two sampling times. Throughout the manuscript, means are reported with standard error.

RESULTS

Nutrients, alkalinity and conductivity

Over the season, total phosphorus concentration ranged from undetectable concentrations to 0.73 mg L^{-1} (cluster III, HP_L , T_{late}). Alkalinity ranged from $124 \text{ mg CaCO}_3 \text{ L}^{-1}$ (cluster II, HP_L , T_{early}) to $929 \text{ mg CaCO}_3 \text{ L}^{-1}$ (cluster III, HP_M , T_{late}), with an average of $390 \text{ mg CaCO}_3 \text{ L}^{-1}$. The lowest conductivity measured was $348 \mu\text{S cm}^{-1}$ (cluster II, HP_L , T_{early}) and the highest was $2310 \mu\text{S cm}^{-1}$ (cluster III, HP_M , T_{late}). Alkalinity and conductivity were tightly correlated throughout the season ($r = 0.84$). Therefore, only conductivity values were further analyzed. There were significant differences in phosphorus concentration and conductivity over time (Table 2). TP concentration was significantly higher at the late succession stage ($0.073 \pm 0.0032 \text{ mg L}^{-1}$) when compared to the early and middle stages ($0.0067 \pm 0.0038 \text{ mg L}^{-1}$ and $0.032 \pm 0.0201 \text{ mg L}^{-1}$) (Tukey HSD: $p = 0.0026$ and 0.029) (Fig. 1a). Conductivity values were significantly lower in the early succession stage ($758 \pm 56 \mu\text{S cm}^{-1}$) in comparison with the middle and late ones ($894 \pm 66 \mu\text{S cm}^{-1}$ and $958 \pm 76 \mu\text{S cm}^{-1}$) (Tukey HSD: $p < 0.001$) (Fig. 1b). Both patterns were observed throughout the different hydroperiod classes.

Table 2. Results of Repeated Measures ANOVA with time (repeated factor), hydroperiod class (HP) and pool cluster as factors on various habitat characteristics (*df*: degrees of freedom; *F*-value and *p*-value). Values in bold are significant ($p < 0.05$). Chlorophyll *a* concentration, amount of suspended solids and conductivity were logarithmically transformed. Percentage cover with vegetation was exponentially transformed.

	Total phosphorus			Conductivity			Transparency			Suspended solids		
	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>
CLUSTER	2	1.77	0.20	2	1.51	0.24	2	10.13	< 0.001	2	5.03	0.013
HP	2	0.13	0.88	2	1.20	0.31	2	0.74	0.49	2	1.26	0.30
Error	21			31			31			31		
TIME	2	6.88	0.0026	2	19.79	< 0.001	2	18.48	< 0.001	2	24.47	< 0.001
TIME x CLUSTER	4	1.88	0.13	4	1.21	0.31	4	1.49	0.22	4	1.48	0.22
TIME x HP	4	0.90	0.47	4	0.14	0.97	4	3.18	0.019	4	3.46	0.013
Error	42			62			62			62		

	Oxygen			pH			Chlorophyll <i>a</i>			Vegetation cover		
	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>
CLUSTER	2	1.19	0.32	2	2.37	0.11	2	2.79	0.077	2	13.02	< 0.001
HP	2	2.51	0.10	2	2.13	0.14	2	1.20	0.31	2	2.14	0.14
Error	26			31			31			31		
TIME	1	1.09	0.31	2	2.48	0.092	2	36.68	< 0.001	2	0.45	0.64
TIME x CLUSTER	2	2.00	0.16	4	1.23	0.31	4	1.61	0.18	4	3.35	0.015
TIME x HP	2	0.55	0.58	4	3.45	0.013	4	4.81	0.0019	4	2.70	0.038
Error	26			62			62			62		

Transparency and suspended solids

The lowest and the highest transparency observed were Snell's depth measurements of 6 (cluster II, HP_M, T_{late}) and 49 cm (cluster I, HP_L, T_{middle}), respectively. Pools in cluster II were less transparent than pools in other clusters (Table 3) (Tukey HSD: $p < 0.05$). The amount of suspended solids ranged from 0.6 mg L⁻¹ (cluster I, HP_L, T_{early}) to 137.2 mg L⁻¹ (cluster II, HP_M, T_{late}), with pools in cluster II having higher concentrations of suspended solids than other pools (Table 3) (Tukey HSD: $p < 0.05$). For both variables there was a significant interaction between time and hydroperiod class (Table 2). In pools with long or medium long hydroperiod, transparency was lower and suspended solids measurements were higher at the late succession stage than at the early or middle succession stage (Tukey HSD: $p < 0.05$), while this was not the case for short hydroperiod pools (Fig. 1c and d).

Repeated measures ANOVA on data from the absolute time scale revealed that in all hydroperiod classes, transparency and the amount of suspended solids did not change significantly from March through the end of April (T₁ to T₃). However, from the beginning of May or June onwards, transparency values declined in pools with a medium long or long hydroperiod (T₄ to T₅ in HP_M, $F_{(4,60)} = 21.78$, $p < 0.001$; T₆ to T₇ in HP_L, $F_{(6,36)} = 8.85$, $p < 0.001$; Tukey HSD: $p < 0.05$). Suspended solid levels increased from the end of May onwards (at T₅ in HP_M, $F_{(4,60)} = 15.89$, $p < 0.001$; from T₅ to T₇ in HP_L, $F_{(6,36)} = 9.78$, $p < 0.001$; Tukey HSD: $p < 0.05$).

Table 3. Mean values and standard errors of pool characteristics per cluster. For each pool, three observations were made (at the early, middle and late succession stage respectively), resulting in 36 observations per cluster. For oxygen saturation, data are missing from the earliest stage.

	Cluster I	Cluster II	Cluster III
Total phosphorus (mg L ⁻¹)	0.030 ± 0.010	0.021 ± 0.0086	0.012 ± 0.0091
Conductivity (μS cm ⁻¹)	837 ± 58	902 ± 58	1015 ± 58
Transparency (Snell's depth in cm)	34 ± 1	25 ± 1	33 ± 1
Suspended Solids (mg L ⁻¹)	8.1 ± 3.1	20.8 ± 3.1	12.4 ± 3.1
Oxygen (%)	97 ± 8	109 ± 9	110 ± 10
pH	8.4 ± 0.1	8.5 ± 0.1	8.3 ± 0.1
Chlorophyll a (mg L ⁻¹)	10.4 ± 9.8	15.8 ± 9.8	30.7 ± 9.8
Vegetation cover (%)	99 ± 3	68 ± 3	97 ± 3

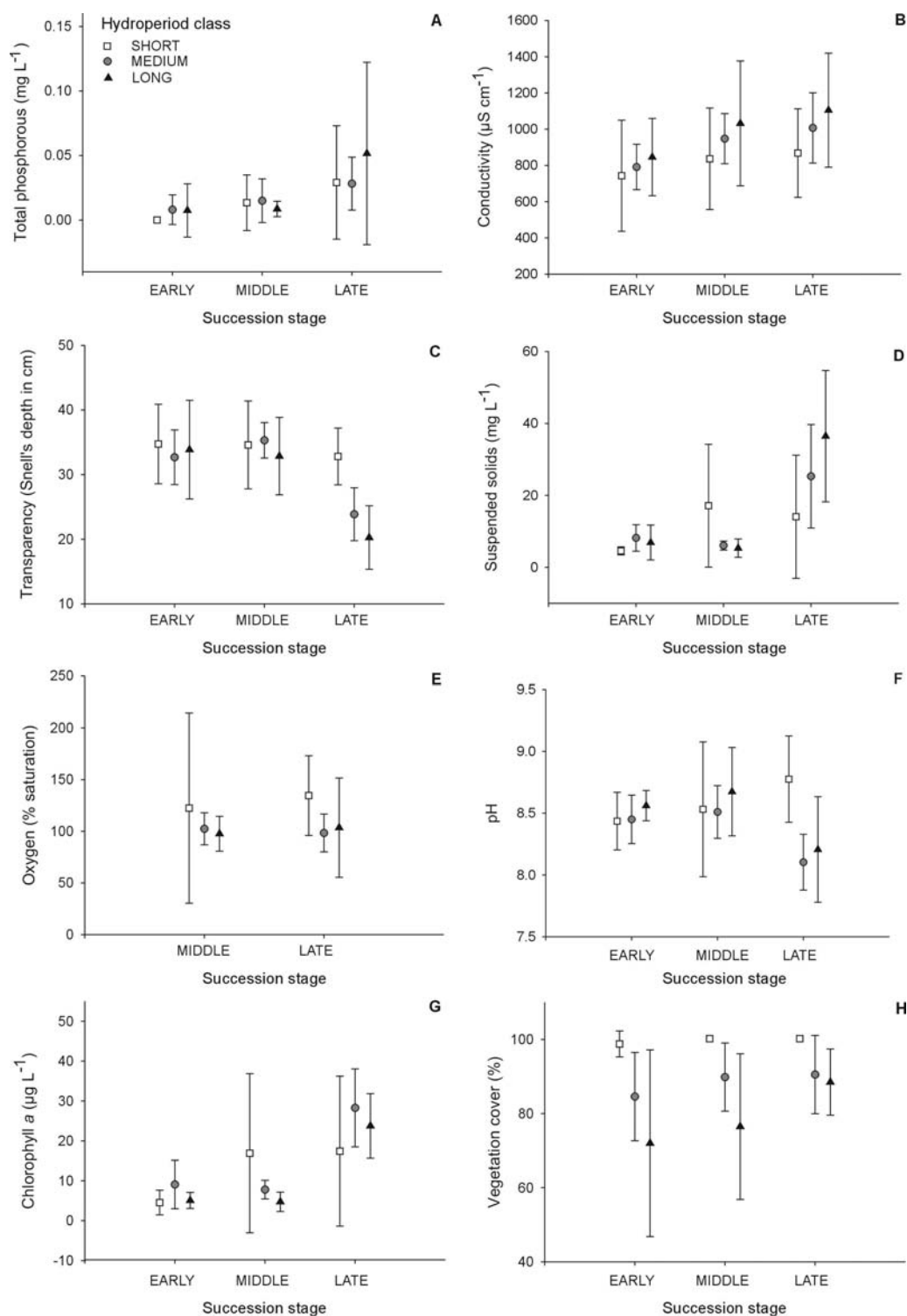


Figure 1. (a) Total phosphorus concentration (mg L^{-1}), (b) conductivity ($\mu\text{S cm}^{-1}$), (c) transparency (Snell's depth in cm), (d) amount of suspended solids (mg L^{-1}), (e) oxygen saturation level (%), (f) pH, (g) Chlorophyll a concentration (mg L^{-1}) and (h) vegetation cover percentage at early, middle and late succession stages (data omitted for the early stage for oxygen due to missing measurements) of temporary pools with variable hydroperiods (short, medium and long). Means are given with 95% confidence intervals.

Oxygen and pH

Oxygen saturation levels typically varied between 50 and 150%. However, extreme saturation percentages ranging from 31 (cluster III, HP_L, T_{late}) to 236% (cluster III, HP_L, T_{late}) occurred occasionally. Oxygen concentration did not vary significantly through time or among hydroperiod classes and clusters (Fig. 1e, Table 2).

The pH of the water averaged 8.4 with a range of 7.3 (cluster I, HP_L, T_{late}) to 9.7 (cluster II, HP_M, T_{middle}) and was relatively stable over time (Fig. 1f). There was a significant interaction between time and hydroperiod class (Table 2). For pools with medium long hydroperiod only, pH measurements at the late successional stage were significantly lower than at the middle stage (Tukey HSD: $p = 0.014$).

Algal biomass and vegetation cover

Chlorophyll *a* concentrations ranged from 1.4 mg L⁻¹ (cluster I, HP_S, T_{early}) to 604.0 mg L⁻¹ (cluster III, HP_L, T_{late}). Chlorophyll concentrations in pools of cluster III were generally, although not significantly, higher when compared to other clusters (Table 3) (Tukey HSD: $p > 0.2$). There was a significant interaction between time and hydroperiod class (Table 2). In pools with long or medium long hydroperiod, chlorophyll *a* measurements at the late succession stage were significantly higher than at the early and middle succession stage (Tukey HSD: $p < 0.05$), while this was not the case for short hydroperiod pools (Fig. 1g). Repeated measures ANOVA on absolute dates showed that chlorophyll concentrations did not change in pools with a short hydroperiod, or in the early months (T₁ to T₄) in pools with longer hydroperiods (HP_M and HP_L). From the end of May onwards, values in medium or long hydroperiod pools increased significantly (at T₅ in HP_M, $F_{(4,60)} = 19.78$, $p < 0.001$; from T₅ to T₆ in HP_L, $F_{(6,36)} = 12.57$, $p < 0.001$; Tukey HSD: $p < 0.01$).

Over the season, vegetation cover varied from 10 to 100%. Vegetation consisted mainly of emergent grasses such as *Juncus*, *Carex* and *Phragmites* as well as emergent water plants or wet meadow species with submersed parts (such as *Mentha aquatica*, *Ranunculus aquatilis*, *Caltha palustris*, *Iris pseudacorus*, *Euphorbia* sp. and *Symphytum* sp.). Floating species were rarely observed. There was a significant interaction between time and hydroperiod class (Table 2), indicating that vegetation cover increased through time in pools with a long hydroperiod, but not in pools with short or medium hydroperiods (Fig. 1h). The increase in vegetation cover in the long hydroperiod class was not significant when using the absolute time scale. Pools in cluster II had lower vegetation cover (Table 2) (Tukey HSD: $p < 0.001$) and were characterized by a stronger increase in vegetation cover with time (from 67 ± 8 to $84 \pm 6\%$) than pools in cluster I

(99 ± 7 to $100 \pm 5\%$) or cluster III (96 ± 7 to $98 \pm 5\%$). The interaction effect between time and cluster was significant (Table 2).

DISCUSSION

Temporary pools offer unique opportunities for addressing ecological research questions (Srivastava et al. 2004, De Meester et al. 2005). However, due to periodic drying, they are challenging systems to study. Analyses of seasonal changes in water quality or biological communities in pools with different durations of inundation are potentially confounded by the intrinsic problem of missing data from pools that dry quickly. As illustrated in our study, different statistical approaches can be used to tackle this problem. However, relating hydroperiod length to observed patterns remains complex. Besides having more chances for habitat changes to occur, a longer duration of inundation will encompass seasonality effects that might contribute directly or indirectly to any observed abiotic or biotic habitat variation.

Nutrients and conductivity

In all pool types, phosphorus concentration and conductivity increased towards the late succession stages (Fig. 1a and b). Typically, mineralization of dead plant material accumulated during the dry phase causes an initial nutrient release upon flooding (Williams 2006). Throughout the season, nutrients and ions are released from decomposing organic matter and by excretion of living organisms, causing a rise both in nutrient concentration and conductivity. As water from pools evaporates, ions are concentrated and conductivity further increases, a phenomenon also observed in a semi-permanent pond in western Canada (Dabron & Clifford 1974), Australian clay pans (Hancock & Timms 2002) and desert potholes (Chan et al. 2005). Although cluster II was located inside the national park, anthropogenic activities, especially agriculture, were well developed in this area; however, enrichment of these pools was not evident.

Transparency and suspended solids

Turbidity and the amount of suspended solids increased from May, when short hydroperiod pools were already dry. Consequently, at the late succession stage of pools with a medium long or long hydroperiod, turbidity and suspended solids were relatively high (Fig. 1c and d). Increased algal biomass (see following) probably contributed to the observed temporal differences. Seasonal increases in turbidity in a pond in Oklahoma were attributed to an increase of the phytoplankton community, local rainfall and an input of organic matter in the habitat

(Freimuth & Bass 1994). A temporal increase in turbidity was also observed in small temporary pans in South Africa (Meintjes et al. 1994) and Australian clay pans (Hancock & Timms 2002). Increases there were due to resuspension of sediment by wind as the pans evaporated. In our study, the extensive vegetation in most pools probably stabilized bottom materials and resuspension through evaporation and wind was less important. Suspended clay particles from the underlying substrate in cluster II probably contributed to the high turbidity and amounts of suspended material in pools of this cluster. Relatively high algae concentrations also contributed to high turbidity in these systems.

Oxygen and pH

We expected oxygen concentrations to decrease with time due to concentration of organisms, bacterial decomposition and rising water temperatures, but oxygen concentrations remained fairly constant (Fig. 1e). Production of oxygen by macrophytes and algae and mixing of water by rainfall may have compensated for any losses. In forested pools in Mississippi, oxygen levels decreased with time (Bonner et al. 1997), but these pools had little to no vegetation, low chlorophyll a levels and a meager phytoplankton community. In ephemeral desert pools, the rise in oxygen levels was also linked to an increase in algal biomass (Scholnick 1994).

In our study, pH only varied minimally (Fig. 1f). In Australian clay pans, pH was also fairly constant (Hancock & Timms 2002). In desert potholes and pools, on the other hand, pronounced pH fluctuations were observed (Scholnick 1994, Chan et al. 2005). Fluctuations in pH are usually caused by the removal of CO₂ from photosynthetic activity (Brönmark & Hansson 2005). In our study systems, pH fluctuations were probably adequately buffered. Indeed, the alkalinity of our study systems was high (average measurements of 390 mg CaCO₃ L⁻¹), presumably resulting from surrounding alkaline soils. Modest seasonal fluctuations in pH in a semi-permanent pond in Canada (8.5 to 9.0) were likewise attributed to the buffering capacity of the water (Daborn & Clifford 1974).

Algal biomass and vegetation cover

Initial nutrient releases and the progressive rise of nutrient concentrations throughout the season provided ideal growth conditions for algae. Algal concentrations were highest in the late succession stage of medium long and long hydroperiod pools (Fig. 1g). In pools with a longer inundation period, algae had more time to develop and benefited from higher summer temperatures, thus both hydroperiod length and seasonal effects probably contributed to this pattern.

Cluster II had pools where a littoral zone with macrophytes and an open water area could be distinguished. As the season proceeded, vegetation invaded the basin from the littoral zone, while pools in the other clusters were already completely vegetated immediately after inundation. The more limited vegetation cover of pools in cluster II could be related to the higher turbidity. Turbidity adversely affects the growth of macrophytes through light limitation (Scheffer et al. 1993). In turn, vegetation tends to enhance water clarity through the stabilization of bottom material and a reduction of nutrient availability for algae.

Conclusions

All temporary pools in this study were characterized by a rise in nutrient concentration and conductivity with time. Algal biomass increased relatively late in the season in pools that retained water. Enhanced algal growth in pools with a longer hydroperiod could be due to either a longer inundation period or more favorable late-season conditions. Temporary pool characteristics such as turbidity, amount of suspended solids and vegetation cover differed among pool clusters, probably due to local environmental conditions such as soil type.

The documented changes in habitat characteristics likely affected the local biological communities. The increase in phytoplankton biomass, for example, created more food for zooplankton and some macroinvertebrates. Especially in pools with a long hydroperiod this may have altered community composition. Oxygen and pH conditions were favorable for growth and reproduction of many biota. Variation in zooplankton and macroinvertebrate communities among clusters might be expected because turbidity and vegetation cover are important factors explaining species distribution (De Szalay & Resh 2000, Cottenie et al. 2001, Van de Meutter et al. 2005b).

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CHAPTER 4

IMPACT OF HYDROPERIOD ON SEASONAL DYNAMICS IN TEMPORARY POOL CLADOCERAN COMMUNITIES

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Abstract We studied seasonal dynamics in cladoceran communities in relation to changing habitat characteristics and biotic interactions in 18 temporary freshwater pools in Kiskunság (Hungary), with an inundation period varying from less than ten weeks (short) to ten to 13 weeks (medium long) and more than 16 weeks (long). Pools were sampled for cladocerans and macroinvertebrates and habitat characteristics were monitored starting two weeks after snow melt (end of March 2005) until July. Analysis of early-season dynamics (March – April), when all three hydroperiod classes were represented, did not reveal differences in cladoceran communities recolonizing pools with a different hydroperiod. Species turnover later in the season (May - July) resulted in the association of some species with the medium and long hydroperiod class. Cumulative species richness increased with hydroperiod length from an average of ten species in short hydroperiod to 15 species in long hydroperiod pools. The longer time available most likely allowed more species to gradually occupy available niches in pools with a longer hydroperiod. With time, feeding and growth conditions became more favorable for predominantly small and/or plant-associated species, while the absence of large *Daphnia* may be due to predation risk. A decline of pools with a long inundation cycle, through drainage and/or climate change, may adversely affect cladoceran communities and needs particular attention in the conservation of temporary pools and their inhabitants.

Keywords community composition, habitat characteristics, predation, species richness, temporary waters, truncation, zooplankton

INTRODUCTION

The length of the inundation period (i.e. hydroperiod) is generally recognized as an important structuring factor of invertebrate communities in temporary pools (Schneider & Frost 1996, Wellborn et al. 1996), and it may also affect their seasonal dynamics. In pools with a relatively long hydroperiod more time is available for community development and a gradual occupation of niches, resulting in a higher chance for successful colonization and recruitment by a more diverse set of taxa (Boix et al. 2000, Brooks 2000, Fischer et al. 2000, Baber et al. 2004, Tavernini et al. 2005). In pools with a longer filling cycle, additional feeding and spatial niches may occur later in the season, allowing more species to occur in the habitat. For example, different types of food resources that become available throughout the season enable the succession of detritivore, herbivore and predacious macroinvertebrates (Meintjes 1996, Moorhead et al. 1998, Lahr et al. 1999, Culioli et al. 2006), while the developing aquatic vegetation creates suitable microhabitats for plant-associated zooplankton species which gradually replace planktonic species later in spring or summer (Hann & Zrum 1997). Species that need more time to develop from dormant stages or need to disperse into the habitat are – at least theoretically – excluded from very short-lived habitats, typically resulting in more simplified communities (Grillas et al. 2004, Tavernini et al. 2005). As the hydroperiod shortens, some succession stages may furthermore be truncated (Boix et al. 2004). In ephemeral rock pools in Botswana, the short hydroperiod did not allow replacement of species through time (Jocqué et al. 2007a).

During the recurrent filling events of temporary pools, gradual changes occur in the invertebrate communities. In a newly flooded habitat, permanent residents typically appear first. They survive the dry period as dormant eggs (e.g. cladocerans, large branchiopods) or other drought-resistant life stages (e.g. snails, chironomids, some beetles and odonates) (Wiggins et al. 1980). A fast growth and reproduction allow them to swiftly recolonize the habitat after inundation. Next, actively dispersing insects (odonates, bugs and beetles) arrive from permanent or other temporary habitats (Wiggins et al. 1980). The recolonization after filling generally results in an increase in taxon richness over time (Lake et al. 1989, Bazzanti et al. 1996, Moorhead et al. 1998, Boix et al. 2004, Culioli et al. 2006, Jocqué et al. 2007a). After some time, taxon richness may reach a plateau when a continuous turnover or replacement of taxa occurs (Lake et al. 1989) and niche saturation is possibly accomplished. Both changing abiotic conditions (conductivity, water level, temperature) and biotic interactions (competition, predation and facilitation) are important and possibly interacting factors shaping seasonal community dynamics. In Spanish temporary ponds, community changes at early, middle and late inundation phases were related to availability of food sources, biotic interactions and temperature increases, respectively (Boix et al. 2004). Invertebrate succession in a Carolina Bay pond (U.S.A.; Taylor & Mahoney 1990) as well as

in South African temporary waters (Meintjes 1996) was explained by changes in the nature of available food resources and predation. A drop in taxon richness often occurs before desiccation as various taxa leave the habitat due to harsh abiotic conditions or intensified predation pressure (Bazzanti et al. 1996, Lahr et al. 1999, Mura & Breccioliari 2003). Higgins & Merritt (1999) suggested that both physical constraints and biotic interactions play a role, irrespective of the succession phase. For example, early detritivores are cold-adapted, profit from favorable food conditions in a microbiologically enriched environment, but also take advantage of the relatively low predator pressure.

While the general pattern of invertebrate seasonal changes has been elaborately examined, there is a lack of information on the dynamics within each of the succeeding taxonomic groups. The aim of our study is therefore to investigate into more detail temporal changes in species richness and composition of cladoceran communities, in pools with a different hydroperiod. We expect that hydroperiod length may act as a limiting factor to species richness and community development also at lower taxonomic levels. To contribute further to the clarification of processes underlying seasonal community changes, we will also investigate whether any of the observed patterns were associated with changing habitat characteristics and/or interactions with the macroinvertebrate community. Temporary freshwater habitats in and around the Kiskunság National Park (Hungary) were selected as the study systems for our research.

MATERIAL AND METHODS

Study area

We selected three pool clusters in the Kiskunság National Park in Hungary, located 85 km southwest of Budapest in the Kiskunság area. Cluster I (46° 48' 14.3" N 19° 16' 17.9" E) is located outside the national park, cluster II (46° 49' 42.1" N 19° 13' 35.3" E) is located partly in the national park and cluster III (46° 41' 11.7" N 19° 16' 16.5" E) is part of a nature reserve within the park. Clusters are separated by a distance of 5 (I and II), 10 (I and III), and 15 km (II and III). A detailed description of the pool clusters is presented in Boven et al. (2008a; Chapter 3).

The Hungarian continental climate favors the formation of vernal pools. In early spring (February - March), these pools fill with snow melt. During springtime, their water level gradually drops as air and water temperature, and consequently evaporation, increase. Depending on pool morphology and local weather conditions, pools usually become dry again in spring (April - June) or summer (July - August). Because of relatively low temperatures and high rainfall in the spring of 2005, some pools exceptionally did not dry before August.

Field sampling

A total of 18 pools, equally distributed over the three clusters and with a variable length of inundation were selected. Pools from spatially distinct replicates (clusters) were selected to improve the power of our analysis. To correct for spatial variation in the communities, we checked the hydroperiod x cluster interaction or included cluster as a covariable in our statistical analyses. Cluster effects will not further be discussed in the results and discussion as they were not the main focus of this study. Pools were divided into three classes based on their absolute hydroperiod (number of weeks as observed in the spring of 2005): short (<10 weeks; HP_S; n = 6), medium long (10 - 13 weeks; HP_M; n = 7) and long hydroperiod class (>16 weeks; HP_L; n = 5). Hydroperiod and maximum surface area of pools were not correlated. However, due to the correlation between hydroperiod and maximum depth of pools, a feature inherent to temporary aquatic systems, we were not able to completely separate the effects of both variables.

Sampling started at the end of March 2005, two weeks after snow melt when all pools were in a similar succession stage. Even when some pools remained flooded during winter, they all froze to the bottom, a condition that is physiologically similar to drought for the biota. Pools were initially sampled every two weeks (March – May) and thereafter monthly (June – July) in a random order. In July, when most pools were dry, sampling stopped. Pools with the longest hydroperiod were consequently sampled seven times (T₁: end of March; T₂: early April; T₃: end of April; T₄: early May; T₅: end of May; T₆: June; T₇: July).

At each sampling occasion, cladocerans were sampled quantitatively by collecting an integrated volume of pool water (50 L at the first and second sampling occasion; 100 L at all other occasions). Water was collected with a measuring cup from four peripheral and four central locations and poured over a plankton net (mesh size: 64 μ m). Qualitative samples were collected by searching all microhabitats with a 64- μ m plankton net. Collected animals were preserved in 70% ethanol and identified to species level using Flöbner (2000). *Bosmina* could not be identified to species level due to the bad condition of the limited number of specimens. *Simocephalus vetulus* and *Simocephalus exspinosus* were not always distinguishable and therefore grouped together. From each quantitative sample, subsamples were processed until 300 specimens were counted. For species richness estimations, quantitative samples containing less than 300 specimens were supplemented with corresponding qualitative samples. From these we identified an additional number of specimens either up to 300 or until all collected animals were checked (when less than 300 specimens were present). Absolute species abundances (# specimens L⁻¹) were calculated from quantitative samples.

Simultaneously, at each sampling occasion, macroinvertebrates were sampled quantitatively. Four open quadrants (height: 50 cm; diameter: 65 cm) were placed on the

bottom of the pool, two at a central and two at a peripheral location. Trapped animals were removed with a sweeping net (mesh size: 250 μm) and preserved in 70% ethanol. Collected animals were identified to different taxonomic levels using De Pauw & Vannevel 1993 (general), Savage (1989) and Nieser (1982) (Hemiptera), Drost et al. (1992) and Klausnitzer (1996) (Coleoptera) and Eder & Hödl (1996) (large branchiopods). The volume of water enclosed by the quadrant was calculated and absolute abundances of taxa were calculated (# specimens l^{-1}). Furthermore, at each sampling, the maximum water depth (cm) was measured using a calibrated stick. Water transparency was measured using a Snell's tube at a central location. An integrated water sample of about 9 L was taken from the surface at three randomly chosen locations. From this sample, temperature ($^{\circ}\text{C}$), conductivity ($\mu\text{S cm}^{-1}$), pH and dissolved oxygen concentration (% saturation) were measured in the field with a WTW 340i multimeter. (Oxygen concentration was not measured in the two earliest samples.) From the same sample, chlorophyll a concentration (mg L^{-1}) was determined in the lab by filtering 1.0 – 1.5 L of water and using the methanol extraction method (Talling and Driver 1963). The amount of suspended solids (mg L^{-1}) was also determined by filtering 1.0 – 1.5 L of water and by measuring the dry weight of the residue on a Whatman GF/C filter dried for 24h at 105°C . Percentage cover with macrophytes (floating, submersed, and emerged vegetation) was estimated at each pool ($\pm 10\%$).

Data analysis

We first analyzed changes in cladoceran communities from March until April (T_1 to T_3 ; early-season dynamics), when pools from all three hydroperiod classes were represented and a comparison of communities between hydroperiod classes was not confounded by seasonality effects. Repeated measures ANOVA was used to analyze changes in species richness (number of species) (Statistica 8.0; StatSoft Inc. 2007). Time (repeated factor), hydroperiod class and cluster were used as independent variables. Each pool was treated as a replicate. Cluster x hydroperiod and time x cluster x hydroperiod interactions were not significant and excluded from the model. Assumptions of normality and equal variance of the data were checked prior to analysis (Shapiro-Wilks and Levene's test). Redundancy Analysis (RDA, Canoco for Windows 4.5; Lepš & Šmilauer 2003) was used to investigate changes in species composition using time, hydroperiod class and time x hydroperiod class as explanatory variables. Species data were absolute abundances (logarithmically transformed) of cladoceran species that occurred in more than two pools. The significance of the models was tested using Monte Carlo permutations ($n=999$), performed by a split-plot design. Table 1 gives an overview of the explanatory variables, covariables and level of permutations used. For samples taken after April, changes in species richness were investigated for each of the remaining hydroperiod classes separately (HP_M : T_1 to T_4 ; and HP_L : T_1 to T_7) using Repeated

Measures ANOVA with time (repeated factor) and cluster as independent variables (Statistica 8.0).

Table 1. Explanatory variables, covariables and permutation levels of RDA to test for early-season dynamics in cladoceran communities. Species data were logarithmically transformed absolute abundances of common species from March to April (T_1 to T_3).

Effect	Environmental variable(s)	Covariable(s)	Level of permutations
Time	Time (T_1 , T_2 , T_3)	Pool n°	Split-plot (time)
Hydroperiod	Hydroperiod class (HP_S ; HP_M ; HP_L)	Time, cluster	Whole-plot (pool)
Time x Hydroperiod	$T_1 \times HP_S$; $T_1 \times HP_M$; $T_1 \times HP_L$; $T_2 \times HP_S$; $T_2 \times HP_M$; $T_2 \times HP_L$; $T_3 \times HP_S$; $T_3 \times HP_M$; $T_3 \times HP_L$	Time, pool n°	Split-plot and whole-plot

In a second series of analyses, we performed an integrated analysis of the cladoceran communities over the whole inundation cycle of all pools (whole-inundation dynamics). For each pool, the inundation period was therefore divided into an early, middle and late succession stage. The early (T_{early}) and late (T_{late}) stages were respectively the first and the last time the pool was sampled. The middle stage (T_{middle}) was chosen halfway between T_{early} and T_{late} . As such, we corrected for any variation in sampling effort between hydroperiod classes (i.e. number of samples taken). A cumulative species list was created based on the selected succession stages. Full-factorial ANOVA was used to test for significant differences in cumulative species richness between hydroperiod classes and clusters and the interaction between both (Statistica 8.0). Canonical Correspondence Analysis (CCA, Canoco for Windows 4.5) was performed to investigate differences in the composition of the cumulative species lists (presence/absence data) using hydroperiod class as explanatory variable. The significance of the model was tested using unrestricted Monte Carlo permutations ($n = 999$). Cluster was included as a covariable. Species that occurred in less than two pools were omitted.

Finally, for each hydroperiod class separately, we investigated the association between seasonal variation in the cladoceran community composition on the one hand, and habitat characteristics and the macroinvertebrate community on the other. Therefore, we used constrained ordination (RDA) of the absolute species abundances (log transformed) at the three representative succession stages. We only considered species found in more than one habitat and at more than one stage. First, forward manual selection was used to identify explanatory variables significantly related to the cladoceran community composition. Besides

time, explanatory variables included maximum depth, temperature, conductivity, amount of suspended solids, chlorophyll a concentration, turbidity and percentage vegetation cover (habitat characteristics) and the abundance of macroinvertebrate detritivores and predators (competition and predation interactions). Except for maximum depth, values of all habitat variables generally increased with time (Boven et al. 2008a; chapter 3) and were therefore a priori selected as explanatory variables. As for the macroinvertebrate functional groups, only herbivore abundance did not change considerably in time (L. Boven, unpub. data) and was hence not selected as an explanatory variable. Next, the selected variables were included in variance partitioning models (Borcard et al. 1992) to separate the effects of the different variable groups (time, habitat characteristics and macroinvertebrates). When testing for unique effects of time, we used habitat characteristics and macroinvertebrates as covariables and vice versa. We were primarily interested in the relation between seasonal variation in cladoceran abundances and habitat or macroinvertebrate variables. As all analyses where time is used as a covariable correct for such variation, their results are only used to calculate shared effects. As for each pool we dealt with repeated observations in time, the significance of the models was tested by using Monte-Carlo permutations at the split-plot level (i.e. permuting succession stages; $n = 999$). All RDA analyses were carried out in Canoco for Windows 4.5, with pool number included as covariable.

RESULTS

Early-season dynamics (March - April)

Within one month after snow melt (T_1 - T_2), 25 cladoceran species (81% of all 31 species found during the study) occurred in the pools (Table 2). Two weeks later (T_3), 28 species (90% of total) were found. On average, seven (T_1) to eight cladoceran species (T_2 and T_3) were found per pool.

The increase in cladoceran species richness over time (except in HP_M ; Fig. 1) was not significant (Repeated Measures ANOVA: $p = 0.05$). There were no significant differences in species richness between pools from different hydroperiod classes.

Significant temporal changes occurred in the cladoceran community composition (RDA: $F = 7.09$; $p = 0.001$; 12.0% explained variance). Especially six weeks after snow melt (T_3), cladoceran species abundances changed, explaining 10.6% of total variance in the species data ($F = 12.31$; $p = 0.001$). Species generally increased in abundance, except for *Daphnia atkinsoni*, which was associated with the earliest sampling times (Fig. 2). Also *Macrothrix hirsuticornis* and the rare *Daphnia magna* and *Bosmina* disappeared after six weeks (T_3 ; Table 2). There were no significant differences in the community composition of pools from different hydroperiod classes ($F = 2.99$; $p = 0.17$). Also the time x hydroperiod interaction was not significant ($F = 0.97$; $p = 0.53$).

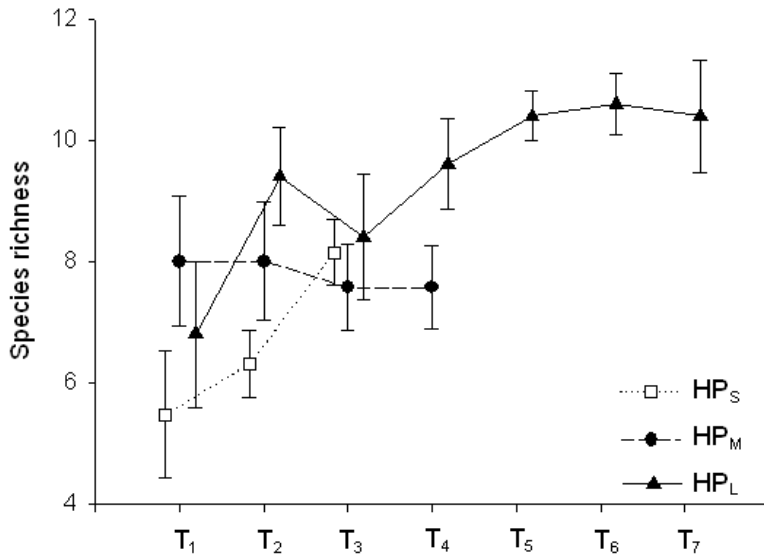


Figure 1. Species richness (number of species; mean and standard error) as a function of time (T_1 : end of March; T_2 : early April; T_3 : end of April; T_4 : early May; T_5 : end of May; T_6 : June; T_7 : July) for the three hydroperiod classes (HP_S : short; HP_M : medium long; HP_L : long hydroperiod class).

Table 2. Species list of all cladocerans found in the study, ordered according to the timing of their maximum frequency of occurrence. The frequency of a species' occurrence (i.e. the number of pools in which the species is recorded) at each sampling occasion (T_1 : end of March; T_2 : early April; T_3 : end of April; T_4 : early May; T_5 : end of May; T_6 : June; T_7 : July) is expressed relative to its maximal frequency ($F_{\max}$) observed throughout the season (white: species absent; light grey: <25% of $F_{\max}$; dark grey: 25-75% of $F_{\max}$; and black: 75-100% of $F_{\max}$). Rare species that were omitted from multivariate analyses are indicated with an asterisk.

		T_1 March	T_2 April	T_3	T_4 May	T_5	T_6 June	T_7 July
1	<i>Eurycercus lamellatus</i> (O.F. Müller, 1776)	Black	Dark grey	Dark grey	Dark grey			
2	<i>Bosmina</i> sp. Baird, 1846*	Black						
3	<i>Daphnia atkinsoni</i> Baird, 1859	Black	Dark grey	Light grey				
4	<i>Macrothrix hirsuticornis</i> Norman et Brady, 1867	Black						
5	<i>Chydorus sphaericus</i> (O.F. Müller, 1776)	Black	Dark grey	Dark grey	Dark grey	Dark grey	Dark grey	Light grey
6	<i>Daphnia longispina</i> (O.F. Müller, 1776)	Black	Dark grey	Dark grey	Dark grey	Dark grey	Dark grey	Dark grey
7	<i>Alonella excisa</i> (Fischer, 1854)	Black	Black	Black	Dark grey	Dark grey	Dark grey	Dark grey
8	<i>Ceriodaphnia laticaudata</i> P.E. Müller, 1876	Black	Dark grey	Dark grey				Black
9	<i>Daphnia curvirostris</i> Eylmann, 1887	Light grey	Black	Dark grey	Dark grey			
10	<i>Daphnia magna</i> Straus, 1820*		Black					
11	<i>Moina brachiata</i> (Jurine, 1820)	Dark grey	Black	Dark grey	Light grey	Dark grey		
12	<i>Megafenestra aurita</i> (Fischer, 1849)		Black	Black				
13	<i>Polyphemus pediculus</i> (Linné, 1761)	Dark grey	Black	Black	Dark grey	Dark grey	Dark grey	Dark grey
14	<i>Simocephalus vetulus</i> (O.F. Müller, 1776)/ <i>Simocephalus exspinosus</i> (Koch, 1841)	Dark grey	Black	Black	Dark grey	Dark grey	Dark grey	Dark grey
15	<i>Ceriodaphnia reticulata</i> (Jurine, 1820)	Dark grey	Black	Black	Dark grey	Dark grey	Dark grey	Dark grey
16	<i>Ceriodaphnia affinis</i> Lilljeborg, 1901*		Black	Black				
17	<i>Scapholeberis rammneri</i> Dumont et Pensaert, 1983	Dark grey	Black	Black	Dark grey	Dark grey	Dark grey	Dark grey
18	<i>Alona rectangula</i> Sars, 1861	Dark grey	Black	Black	Dark grey	Dark grey	Dark grey	Dark grey
19	<i>Dunhevedia crassa</i> King, 1853	Dark grey	Black	Black	Dark grey	Dark grey	Dark grey	Dark grey
20	<i>Pleuroxus aduncus</i> (Jurine, 1820)	Dark grey	Black	Black	Dark grey	Dark grey	Dark grey	Dark grey
21	<i>Pleuroxus laevis</i> Sars, 1861*		Black	Black	Dark grey	Dark grey		
22	<i>Graptoleberis testudinaria</i> (Fischer, 1848)	Dark grey	Black	Black	Dark grey	Dark grey	Dark grey	Dark grey
23	<i>Diaphanosoma brachyurum</i> (Liévin, 1848)	Light grey	Dark grey	Dark grey	Dark grey	Dark grey	Dark grey	Dark grey
24	<i>Macrothrix rosea</i> (Jurine, 1820)	Light grey	Dark grey	Dark grey	Dark grey	Dark grey	Dark grey	Dark grey
25	<i>Acroperus harpae</i> (Baird, 1835)*	Dark grey		Dark grey	Dark grey	Dark grey		
26	<i>Scapholeberis mucronata</i> (O.F. Müller, 1776)		Dark grey	Dark grey		Dark grey	Dark grey	
27	<i>Oxyurella tenuicaudis</i> (Sars, 1862)*		Dark grey	Dark grey		Dark grey		
28	<i>Tretocephala ambigua</i> (Lilljeborg, 1901)		Dark grey	Dark grey		Dark grey	Dark grey	Dark grey
29	<i>Kurzia latissima</i> (Kurz, 1875)*					Dark grey	Dark grey	Dark grey
30	<i>Alona guttata</i> Sars, 1862*				Dark grey	Dark grey	Dark grey	Dark grey
31	<i>Moina micrura</i> Kurz, 1875*				Dark grey	Dark grey	Dark grey	Dark grey

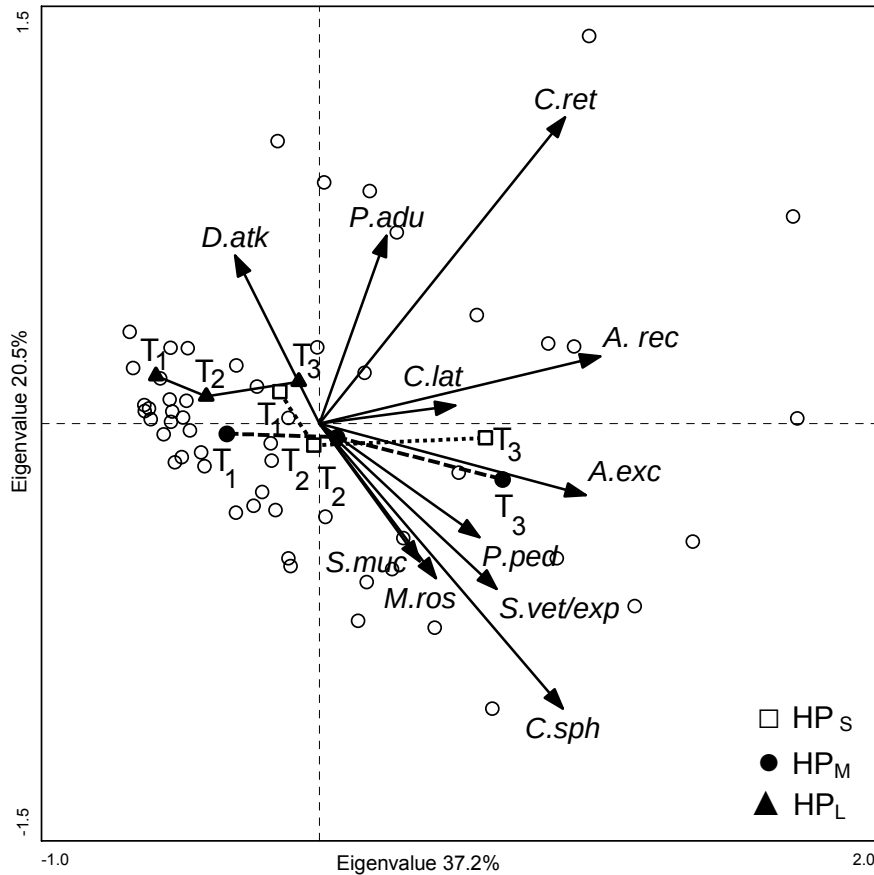


Figure 2. Biplot of samples (open circles) and species (arrows) (PCA), with time as supplementary variable for each hydroperiod class separately (time x hydroperiod interaction) (HP_S: short; HP_M: medium long; and HP_L: long hydroperiod class) (T₁: end of March; T₂: early April; and T₃: end of April). 11 species that best fitted the model are included. Species legend: *D.atk*: *Daphnia atkinsoni*; *P.adu*: *Pleuroxus aduncus*; *C.ret*: *Ceriodaphnia reticulata*; *A.rec*: *Alona rectangularis*; *C.lat*: *Ceriodaphnia laticaudata*; *A.exc*: *Alonella excisa*; *P.ped*: *Polyphemus pediculus*; *Simo*: *Simocephalus exspinosus/vetulus*; *C.sph*: *Chydorus sphaericus*; *S.muc*: *Scapholeberis mucronata*; *M.ros*: *Macrothrix rosea*. Species data were logarithmically transformed absolute abundances.

Whole-inundation dynamics (March - July)

Two months after snow melt (T₄), when pools with a short hydroperiod were already dry, six species had disappeared from the pools (Table 2: 2 to 4; 10; 12; 16), but thereafter three new ones appeared (Table 2: 29 to 31). In pools with a long hydroperiod species richness increased after two months and on average ten (T₄, T₅ and T₇) to 11 species (T₆) were recorded per pool (Fig. 1). This increase was not significant (Repeated Measures ANOVA: $p > 0.05$). In pools with a medium long hydroperiod the average species richness ($n = 8$) remained constant ($p > 0.05$).

Over the whole inundation cycle, there was a significant difference in species richness between hydroperiod classes (Full-factorial ANOVA: $F_{(2, 9)} = 8.55$; $p = 0.0083$). Significantly more species occurred in pools with a longer hydroperiod (Mean $\pm$ SE: $14.60 \pm$

1.21) than in pools with a short or medium long hydroperiod (respectively 9.83 ± 0.70 and 11.57 ± 0.65) (Tukey HSD: $p < 0.01$). The cluster x hydroperiod interaction was not significant.

There were significant differences in community composition among pools with variable hydroperiods (CCA: $F = 2.00$; $p = 0.001$; 17.66 % explained variance). Except for the common cladocerans *Alonella excisa*, *Ceriodaphnia reticulata*, *Chydorus sphaericus*, *Daphnia longispina* and *Simocephalus vetulus* or *Simocephalus exspinosus*, some species were associated with one or more hydroperiod classes (Fig. 3). *Megafenestra aurita* was restricted to pools with a short hydroperiod and *Eurycercus lamellatus* to pools with a medium long hydroperiod. *Daphnia curvirostris* was found more often in pools with a short hydroperiod. *Graptoleberis testudinaria*, *Alona guttata* and *Macrothrix rosea* were more often observed in the medium long and long hydroperiod classes than in short hydroperiod pools. *Scapholeberis mucronata*, *Moina brachiata* and *D. atkinsoni* occurred only in pools with a relatively long hydroperiod (HP_M and HP_L), and more often in the medium long hydroperiod class. *Diaphanosoma brachyurum* and *Moina micrura* only occurred in long hydroperiod pools. All species associated with the medium long and/or long hydroperiod class, except for *E. lamellatus*, *D. atkinsoni* and *M. brachiata*, were typically most frequent relatively late in the season (May – July) (Table 2).

Impact of habitat characteristics and interactions with macroinvertebrates

Time (succession stage) in combination with selected habitat characteristics and macroinvertebrate variables explained an increasing amount of variation in the cladoceran communities with an increasing hydroperiod (Table 3). Only in pools with a short hydroperiod, the selected variables were not significantly related to the cladoceran community composition.

For all hydroperiod classes, the changing habitat quality throughout the inundation cycle (i.e. shared effect of time and habitat characteristics) (Table 4e) and the unique effect of time (Table 4b) showed a stronger association with the changing cladoceran communities than the increase in macroinvertebrate predator abundance (i.e. shared effect of time and predator abundance) (Table 4g) (Fig. 4). When compared to these effects, also the shared effect of time, habitat characteristics and predation (Table 4j) explained a considerable amount of variation especially in pools from the medium and long hydroperiod class.

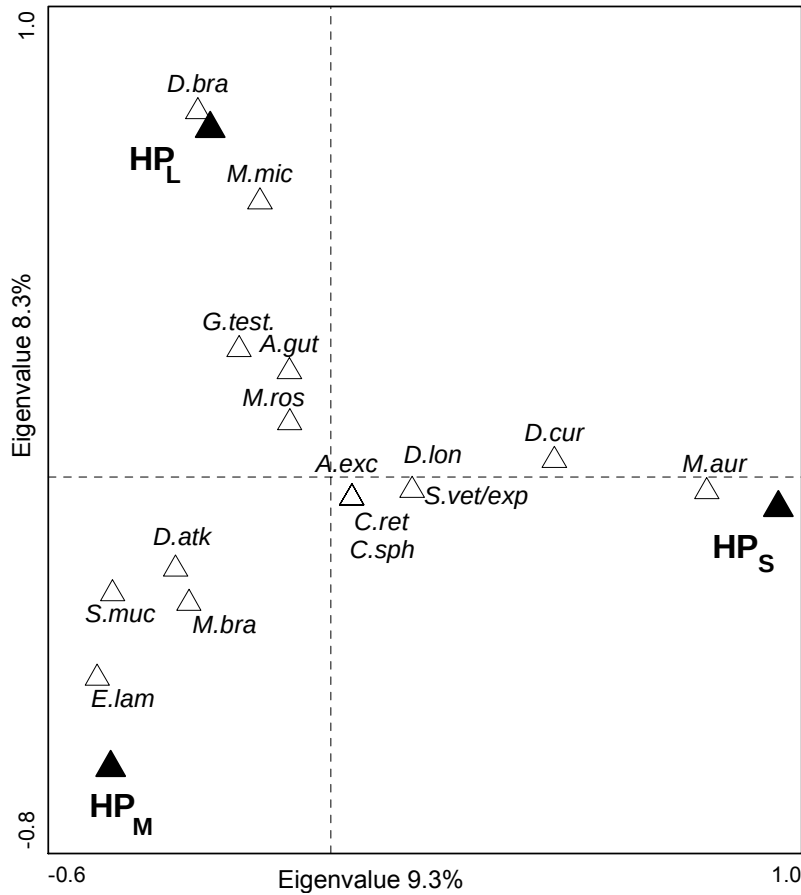


Figure 3. Biplot of species (white triangles) and hydroperiod classes (black triangles; HP_S: short; HP_M: medium long; and HP_L: long hydroperiod class) (CCA). 16 species that best fitted the model are included. Species legend: *D.bra*: *Diaphanosoma brachyurum*; *M.mic*: *Moina micrura*; *G.test*: *Graptoleberis testudinaria*; *A.gut*: *Alona guttata*; *M.ros*: *M. rosea*; *C.sph*: *C. sphaericus*; *A.exc*: *A. excisa*; *C.ret*: *C. reticulata*; *Simo*: *S. exspinosus/vetullus*; *D.lon*: *Daphnia longispina*; *D.cur*: *Daphnia curvirostris*; *M.aur*: *Megafenestra aurita*; *D.atk*: *Daphnia atkinsoni*; *S.muc*: *Scapholeberis mucronata*; *M.bra*: *Moina brachiata*; *E.lam*: *Eurycercus lamellatus*. The distance of a species to a hydroperiod class is proportional to its relative frequency in the respective hydroperiod class.

Table 3. Results of RDA (percentage of variance explained, *F*- and *p*-value) on absolute abundances of common species at three representative succession stages, per hydroperiod class (HP_S: short; HP_M: medium long; and HP_L: long hydroperiod class). Explanatory variables include time as well as habitat characteristics (chl a: chlorophyll a concentration; temp: temperature; cond: conductivity; veg cov: vegetation cover) and macroinvertebrate variables (pred: predator abundance) selected using forward manual selection. As for the short hydroperiod class, time and predation did not contribute significantly to variation in the cladoceran communities, but were included in the model to improve comparability of the results. Significant values are indicated in *italic*.

	Time + Habitat + Macroinv.	% expl. var.	<i>F</i>	<i>p</i>
HP _S	Time + chl a + pred	29.7	2.76	0.058
HP _M	Time + chl a, temp, cond + pred	46.0	3.80	0.001
HP _L	Time + chl a, temp, veg cov + pred	71.1	4.96	0.002

Table 4. Variation partitioning results (RDA; percentage of variance explained, F - and p -value; /: unique effects, +: common effects, $\cap$: shared effects), per hydroperiod class (HP_S: short; HP_M: medium long; and HP_L: long hydroperiod class). Species data were absolute abundances of common species at three representative succession stages. Explanatory variables include time (T), habitat characteristics (Hab; see table 3) and macroinvertebrate predator abundance (Pred).

		HP _S			HP _M			HP _L		
		% expl.			% expl.			% expl.		
		var.	F	p	var.	F	p	var.	F	p
a	Hab / T + Pred	8.5	3.18	0.049	12.0	1.98	0.021	15.8	2.21	0.098
b	T / Hab + Pred	3.7	0.69	0.77	7.8	1.93	0.022	12.1	2.53	0.067
c	Pred / T + Hab	4.3	1.59	0.44	2.1	1.034	0.30	6.3	2.66	0.091
d	T + Hab / Pred	17.9	2.22	0.070	24.4	2.42	0.001	39.2	3.28	0.004
e	T $\cap$ Hab / Pred	5.7			4.6			11.3		
f	T + Pred / Hab	9.4	1.17	0.55	11.1	1.83	0.023	23.9	3.33	0.03
g	T $\cap$ Pred / Hab	1.4			1.2			5.5		
h	Hab + Pred / T	14.5	2.70	0.082	13.9	1.71	0.04	27.4	2.86	0.043
i	Hab $\cap$ Pred / T	1.7			-0.2			5.3		
j	Hab $\cap$ T $\cap$ Pred	4.4			18.5			14.8		

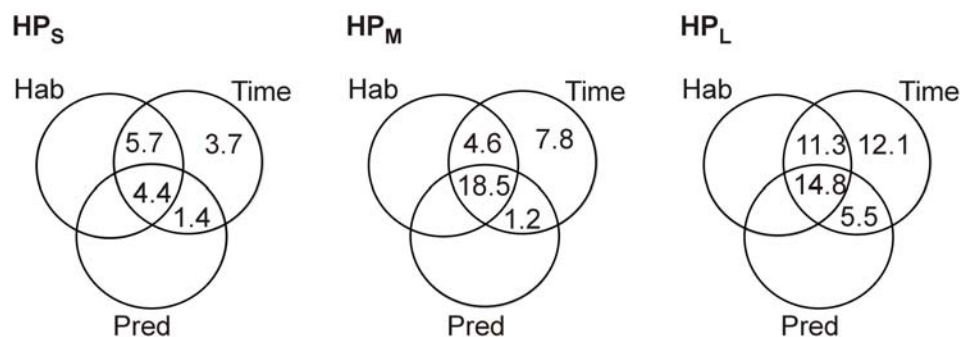


Figure 4. Overview of the percentage of variance explained by the shared effect of time and habitat (T $\cap$ Hab / Pred), the shared effect of time and predation (T $\cap$ Pred / Hab), the shared effect of time, habitat and predation (T $\cap$ Hab $\cap$ Pred) and the unique effect of time (T / Hab+Pred) for each hydroperiod class (HP_S: short; HP_M: medium long; and HP_L: long hydroperiod class). Habitat characteristics are different for each hydroperiod class (see table 3). The unique and shared effects of habitat and predation are not illustrated here (see material and methods).

DISCUSSION

Recolonization of pools by cladocerans started within two weeks after snow melt. At that time, an average of seven cladoceran species was found per pool. Species that were not observed at that time either hatched later or were too low in abundance to be detected early in spring. In permanent lakes and ponds, hatching from diapausing eggs by many cladocerans as well as by rotifers and copepods is mainly observed synchronously early in

the growing season (reviewed in Brendonck & De Meester 2003, see also Wolf & Carvalho 1989, De Stasio 1990, Hairston et al. 2000). Therefore, it is generally assumed that changes in the abundance of species later in the season are driven by seasonal processes and conditions (e.g. food availability, predation) rather than by emergence (Hairston et al. 2000). On the other hand, the timing of hatching of *Daphnia magna* clones in permanent lakes and ponds depended on environmental conditions indicative of a high relative fitness (Vanoverbeke 2000). The applicability of such temporal habitat selection for temporary pools cladocerans requires further research on selective hatching behavior.

The early-season species composition of cladoceran communities did not differ significantly between pools with a different hydroperiod and any community changes were comparable in all hydroperiod classes. Species turnover relatively late in the season in pools with a longer hydroperiod (HP_M and HP_L) contributed to the overall difference in cladoceran community composition between hydroperiod classes. Species associated with the medium long and/or long hydroperiod classes indeed were more frequently found from May onwards, when short hydroperiod pools were already dry. Moreover, the longer time span allowed more species to gradually occupy available niches and resulted in the higher cumulative number of species in pools with a long hydroperiod than in pools with a short to medium long hydroperiod. Likewise, in temporary ponds in Spain and Italy, cumulative zooplankton taxon richness differed among hydroperiod classes while momentary richness did not (Serrano & Fahd 2005, Tavernini et al. 2005). Such truncation of community development by a decreasing hydroperiod, here illustrated for the cladoceran community, was until now only presented for succeeding functional groups of temporary pool invertebrates (Boix et al. 2004, Jocqué et al. 2007a). Other mechanisms that are generally accepted to shape community structure across a hydroperiod gradient, such as constraints of development time in short hydroperiod pools and a higher predation risk in pools with a long inundation period (Schneider & Frost 1996, Wellborn et al. 1996), cannot account for the observed species distribution in our study. For example, the development time of *D. atkinsoni*, which is assumed to be similar to that of *D. magna* (± 10 days at 20°C ; Ebert 2005), is faster than the shortest observed inundation period (seven weeks) and therefore can not explain its absence from short hydroperiod habitats. Further, we have no indications of a higher predation risk in pools with a longer inundation (L. Boven unpubl. data) limiting the distribution of species as *M. aurita* and *E. lamellatus* to habitats with a shorter hydroperiod.

Although seasonal variation in the cladoceran communities was related more to the changing habitat quality than to the increase in invertebrate predation, a considerable amount of variation was explained by their shared effect. As predator abundance was correlated both with chlorophyll concentration and temperature (Pearson correlation: $r = 0.61$

and 0.46; $p < 0.05$), it remains difficult to relate changes in the cladoceran communities to any of both effects.

In temporary pools, the invertebrate predator community usually increases throughout the season (Lake 1989, Moorhead et al. 1998, Lahr et al. 1999, L. Boven unpubl. data), and may therefore imply an increasing predation pressure on the cladoceran communities. In our pools, however, there was an increase in abundance of a growing number of cladoceran species throughout the season. The predominantly small cladocerans observed in the study pools (Chydoridae and *Ceriodaphnia*) are possibly no major prey organisms of the main invertebrate predators present (*Chaoborus*, Dytiscidae, Hydrophilidae, *Notonecta* and Odonata). On the other hand, the early disappearance of *D. atkinsoni* and the overall small proportion of relatively large *Daphnia* species in the cladoceran communities may be due to predation, for example by the size-selective *Notonecta* (Murdoch et al. 1984, Arner et al. 1998, Steiner & Roy 2003).

The development of planktonic algae was a main environmental change during the inundation cycle of the studied pools (Boven et al. 2008a; chapter 3). This may be indicative of gradually improving feeding conditions for planktonic filter feeders as reflected by the increase in population size of some species (e.g. *Ceriodaphnia* spp.). However, the continuous increase in algal biomass also indicates that planktonic algae were not efficiently grazed and suggests that substrate-associated algae (periphyton) may be a more important food source for cladocerans and possibly also for other herbivores inhabiting the pools. This is reflected by the association between cladoceran community changes and the increase in biomass of aquatic vegetation, as a main periphyton substrate. The developing vegetation probably created additional feeding niches and suitable microhabitats for macrophyte associated species such as Chydoridae and *Simocephalus* sp. (Flößner 2000, Balayla & Moss 2003). Likewise, Chydoridae and *Simocephalus* replaced planktonic *Daphnia* in a pond in Carolina Bay (Taylor & Mahoney 1990) and in Canadian wetlands, cladoceran communities were gradually dominated by phytophilous species towards summer (Hann & Zrum 1997). The increase in temperature gradually improved growth conditions for cladocerans as well as for their algae food resources and as such may have contributed to the increase in population size of most species. *Daphnia atkinsoni*, on the other hand, may have suffered from the rising water temperatures either directly or through concomitant changes in algal quality (e.g. algae type or nutritional value), as generally observed for large-bodied cladocerans in lakes or ponds (Moore et al. 1996, Lynch 1978, Steiner 2003). Finally, the unique effect of time on cladoceran community composition may integrate unmeasured environmental variables and biological characteristics of the species. In pools with a relatively long hydroperiod, a higher amount of seasonal variation in the cladoceran communities could be related to habitat quality changes and the unique time effect.

Especially in pools with a relatively long hydroperiod more algal food resources became available and more (complex) vegetation structures developed later in the season (Boven et al. 2008a; chapter 3) generating additional feeding niches and microhabitats. Moreover, in pools with a longer hydroperiod there was indeed more time for species to grow and develop and possibly for additional hatching, resulting in the observed community changes.

Conclusions and implications for conservation

Pools with a different hydroperiod were recolonized by similar cladoceran communities early in the season, but community turnover relatively late in the season resulted in the overall difference in cladoceran community composition between hydroperiod classes. The longer time that was available for the occupation of available niches probably contributed to the higher cladoceran diversity in pools with a relatively long hydroperiod, while in short hydroperiod pools community development was truncated. The observed community changes towards predominantly smaller and/or plant-associated cladocerans throughout the inundation could be related to improving feeding and growth conditions for these species, while the absence of large *Daphnia* is possibly due to predation.

We argue that in developing adequate conservation measures in temporary aquatic habitats, knowledge of community assembly and temporal dynamics are of key importance. For example, in order to reliably estimate diversity in these habitats, simple community snapshots are not recommended, as a lot of temporal variation is missed. Furthermore, we argue that a reduction of the length of the aquatic phase of pools may lead to severe negative effects on the cladoceran communities, which through indirect effects or direct trophic interactions may ultimately interfere with the ecological quality and functioning of these ecosystems (Downing & Leibold 2002). Such reduction could result from drainage for agricultural purposes or from climate change (e.g. decreasing winter precipitation, increasing drought events and spring temperatures).

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CHAPTER 5

INTERANNUAL VARIATION IN SPECIES RICHNESS AND COMPOSITION OF TEMPORARY POOL CLADOCERAN COMMUNITIES

Liesbet Boven & Luc Brendonck

Unpublished manuscript

Abstract Interannual changes in temporary pools and their inhabiting communities are expected to result from climate-induced variation in local hydrological conditions. Studies investigating changes at the community level during years with different weather conditions therefore could provide valuable information on community structuring processes, especially in relation to hydrological variation. We compared species richness and composition of cladoceran communities in four temporary pools in Kiskunság (Hungary) between a wet year (2005) versus a dry one (2007). In May 2007, a subset of cladoceran species in lower abundance occurred in the pools when compared to a comparable moment in the wetter year. This was probably due to a decrease in microhabitat availability in the smaller and shallower pools in the dry year where cladocerans likely experienced more intense biotic interactions with a predator-dominated macroinvertebrate community. The currently ongoing trends of decreasing total annual and winter precipitation as well as the increasing frequency of droughts in Hungary may considerably alter hydrological conditions of temporary pools. Although temporary pool species are characterized by a relatively high tolerance to environmental variation, they might not be able to adapt fast enough to these ongoing changes. Based on our results, it can be expected that species richness of local cladoceran communities will be adversely affected. Given the importance of cladocerans in the food web of temporary waters, this may ultimately interfere with the ecological quality and diversity at different trophic levels of these vulnerable ecosystems.

Keywords drought, hydromorphology, macroinvertebrate predators, temporary aquatic habitat, zooplankton

INTRODUCTION

Climate change is one of the main threats to biodiversity and functioning of natural ecosystems (Chapin et al. 2000, Sala et al. 2000). Forecasting ecosystem responses to climate change is difficult and in many habitat types hampered by the lack of ecological studies assessing ecosystem functioning and relevant processes structuring their biological communities. Small and temporary water bodies that rely on local climatic conditions may be early warning systems. Their usually simple communities may allow quick signaling of changes in community structure and functioning due to climate change.

Current evidence indicates that not only for small rock pools with a granite basin (Hulsmans et al. 2008), but also for other types of temporary pools on a more permeable soil (Brooks 2004, Bauder 2005, Pyke 2005a), a direct link exists between the hydroregime of the pools and temperature and precipitation patterns. As the hydroregime is crucial for various processes in temporary pools, variation in climatic features - possibly as a result of climate change - is expected to have a considerable impact on temporary pools and their communities. Especially a shortening of the hydroperiod may decrease reproductive chances of many species (Schneider & Frost 1996, Pyke 2005a, b, Hulsmans et al. 2008) or interfere with the seasonal succession of species. In temporary mountain pools, yearly variation in seasonal zooplankton dynamics and the presence/absence of taxa was related to variation in weather conditions and its effect on pool duration (Tavernini 2008). Not only local but also regional processes may vary from year to year according to weather conditions, and significantly affect the diversity and composition of pool communities. In an agricultural landscape, connectivity established through the presence of ponds and ditch networks probably buffered negative effects of agriculture on amphibian population abundance during normal weather conditions (Piha et al. 2007). However, after a severe drought, their effect was insufficient to support amphibian populations. Alternatively, in a long-term (24 years) study, Altermatt et al. (2008) demonstrated a significant impact of climate change on the dynamics of a *Daphnia* metacommunity in a set of rock pools. Owing to the increasing frequency of warmer and drier weather, resting stages were increasingly exposed to animal and wind dispersal in the desiccated pools resulting in increased colonization rates.

Cladocerans are a major component of the communities inhabiting temporary freshwater pools (Williams 2006). They are able to persist in these habitats by the production of dormant eggs that are resistant to recurrent droughts, and by a fast growth and reproduction allowing them to quickly recolonize the habitats after inundation (Wiggins et al. 1980). Cladocerans generally play a key role in the functioning of freshwater aquatic ecosystems. They are efficient primary consumers controlling algae growth in ponds and lakes (Sommer et al. 1986, Lampert 1988, Sommer et al. 2001), while they also constitute an important food source for various predators (zooplankton, insect larvae and adults and

amphibian larvae). Given the importance of cladocerans in the food web, changes in their species diversity and composition may affect the ecological quality of these habitats or species diversity at various trophic levels associated with the temporary pool habitat.

Information on factors steering cladoceran community structure and determining cladoceran species richness in temporary waterbodies is mounting. Especially the duration of the aquatic phase (hydroperiod) and - often herewith correlated - habitat size are globally recognized as significant for maintaining cladoceran rich communities. With some exceptions, most studies on community structure are based on single snap-shots of the communities or observations throughout one growing season. Given the dynamic nature of these temporary pool communities both within and between growing seasons (Donald 1982, Rettig et al. 2006, Tavernini 2008, Chapter 4), such studies are likely to miss a considerable amount of variation. The global lack of long-term studies is not surprising since the importance of temporary pools for regional biodiversity has been acknowledged only recently (Williams 1997, Forró et al. 2003, Williams et al. 2003, Nicolet et al. 2004, De Bie et al. 2008). Studies investigating changes at the community level between years with different weather conditions, therefore, could considerably increase our insights in community development and structuring processes, especially in relation to hydrological variation.

In this study, we compare the species richness and composition of cladoceran communities in four temporary pools in Kiskunság (Hungary) between two years with different climate conditions. The difference in pool phenology during both years was due to differences in winter and spring precipitation and spring temperatures. We analyse to what extent the observed differences in the cladoceran communities from one year to another might be related to changes in pool dimensions, water quality and vegetation cover or biotic interactions (competition and predation).

MATERIALS AND METHODS

Study area

The Kiskunság National Park in Hungary is located 85 km southwest of Budapest in the Kiskunság area. Within this area, temporary pools are generally very abundant in spring. The Hungarian continental climate favors the formation of vernal pools. In early spring (February - March), these pools fill with snowmelt. During springtime, they gradually dry as air and water temperature, and consequently evaporation, increase. Depending on pool morphology and local weather conditions, pools usually are dry again in spring or summer. We studied the pools in the spring of both 2005 and 2007. Although much different, temperature and precipitation patterns in these periods were not exceptional in comparison with longer term climatic variation in the region (Fig. 1). Low winter and spring precipitation in combination

with relatively high spring temperatures in 2007 when compared to 2005 (Fig. 2) resulted in the early drying of many pools in 2007.

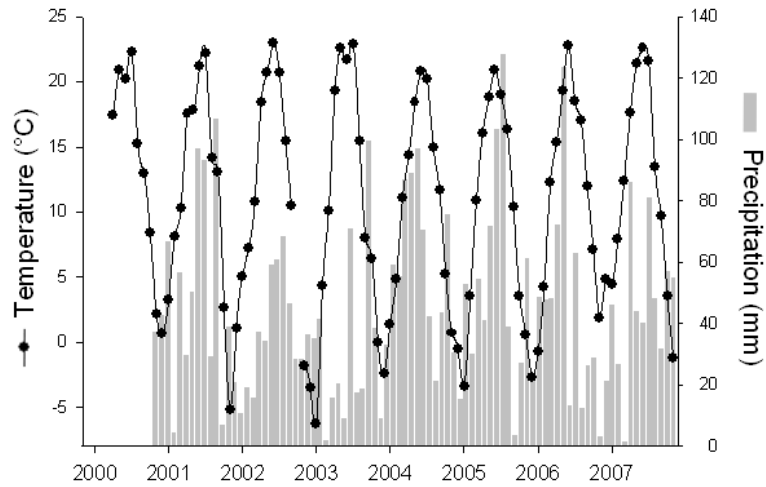


Figure 1. Monthly meteorological data for Kiskunság from May 2000 until December 2007: average air temperature (°C) and amount of precipitation (mm) (data from the Ecological and Botanical Research Institute).

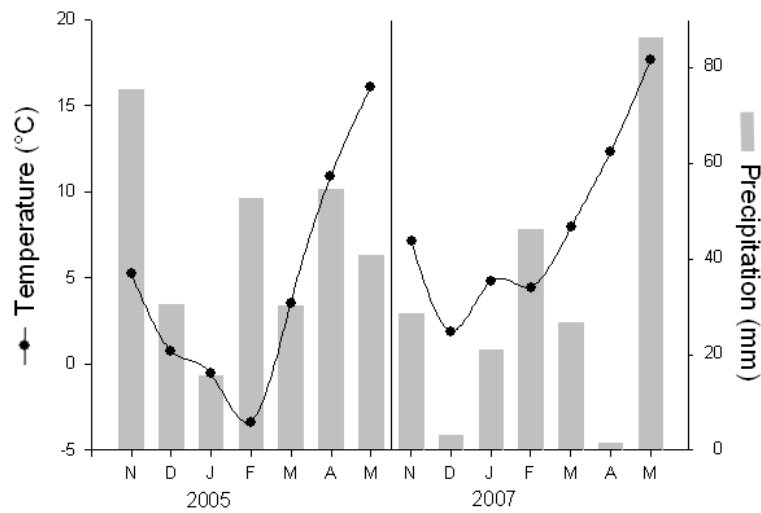


Figure 2. Monthly meteorological data for Kiskunság for the preceding winter and spring of 2005 and 2007 (November – May): average air temperature (°C) and amount of precipitation (mm) (data from the Ecological and Botanical Research Institute).

Field sampling

In 2005, 36 pools with a variable length of inundation were selected (Chapter 3). These pools were monitored subsequently throughout the growing season, starting two weeks after snowmelt (end of March) until most pools were dry (July). Early in May 2007, only four of the selected pools contained water and could be sampled. Based on precipitation and temperature patterns (Fig. 2), we assume that at that time pools were already inundated for three to four months. The four pools were sampled once early May 2007 (T_{07}), whereas in

2005 the same pools were sampled at seven occasions ($T_{05,1}$: March; $T_{05,2}$: early April; $T_{05,3}$: late April; $T_{05,4}$: early May; $T_{05,5}$: late May; $T_{05,6}$: June; $T_{05,7}$: July).

At each occasion, cladocerans were sampled quantitatively by collecting an integrated volume of pool water (50 L at $T_{05,1}$ - $T_{05,2}$; 100 L at $T_{05,3}$ - $T_{05,7}$; 5 - 80 L at T_{07}). Water was collected with a measuring cup from four peripheral and four central locations and poured over a plankton net (mesh size: 64 μm). Qualitative samples were collected by searching all microhabitats with a 64- μm plankton net. Collected animals were preserved in 70% ethanol and identified to species level using Flößner (2000). From each quantitative sample, subsamples were processed until 300 specimens were counted. For species richness estimations, samples containing less than 300 specimens were supplemented with corresponding qualitative samples. From these we identified an additional number of specimens either up to 300 or until all collected animals were checked (when less than 300 specimens were present). Absolute species abundances (# specimens L^{-1}) were calculated from quantitative samples.

At each occasion, the maximum water depth (cm) was measured using a calibrated stick. Surface area (m^2) of the pools was estimated only in March and June - July 2005 and in May 2007 by comparing the shape of each pool to known geometric figures of which the dimensions were measured. At each sampling occasion, water transparency was measured using a Snell's tube at a central location. An integrated water sample of about 9 L was taken from the surface at three randomly chosen locations. From this sample, conductivity ($\mu\text{S cm}^{-1}$) and pH were measured in the field with a WTW 340i multimeter. Total alkalinity ($\text{mg CaCO}_3 \text{L}^{-1}$) was determined from a subsample (250 mL) using sulphuric acid titration. Given the strong correlation between conductivity and alkalinity ($r = 0.93$; $p < 0.001$), and missing conductivity measurements for some pools, only alkalinity was used in further analyses. From the same sample, chlorophyll *a* concentration (mg L^{-1}) was determined in the lab by filtering 1.0 – 1.5 L of water and using the methanol extraction method (Talling and Driver 1963). The amount of suspended solids (mg L^{-1}) was also determined by filtering 1.0 – 1.5 L of water and by measuring the dry weight of the residue on a Whatman GF/C filter dried for 24h at 105°C. Finally, at each occasion, percentage cover with macrophytes (floating, submersed and emerged vegetation) was estimated ($\pm 10\%$).

To assess quantity and quality of potential competitors and predators in the study pools, also macroinvertebrates were sampled. At three occasions in 2005, representative of an early, middle and late succession stage ($T_{05,1}$: March; $T_{05,3}$: late April; and $T_{05,7}$: July), pools were sampled in a quantitative way. Four open quadrants (height: 50 cm; diameter: 65 cm) were placed on the bottom of each pool, two at a central and two at a peripheral location. Trapped animals were removed with a sweep net (mesh size: 250 μm). In 2007, we only took qualitative samples by searching all microhabitats with a sweep net (mesh size:

250 μm). Collected animals were preserved in 70% ethanol and identified to different taxonomic levels using De Pauw & Vannevel (1993) (general), Savage (1989) and Nieser (1982) (Hemiptera), Drost et al. (1992) and Klausnitzer (1996) (Coleoptera) and Eder & Hödl (1996) (large branchiopods). They were classified as herbivores, detritivores, predators or omnivores according to their main food type as described by De Pauw & Vannevel (1993). Due to the different sampling methods, we only used relative abundances of the macroinvertebrate taxa in further analyses.

Data analysis

We wanted to investigate whether characteristics of the study pools were different in May 2007 when compared to their status at about the same period (late April or early May) in 2005 or whether they were more comparable with other succession stages (March – early April or late May – July) in 2005. Variables considered were pool morphometry, water quality, vegetation cover, macroinvertebrate community composition (proportion of macroinvertebrate herbivores and predators and taxa composition) and cladoceran community structure (total abundance, species richness and composition). We first used Repeated Measures ANOVA and post-hoc Tukey HSD tests (Statistica 8.0; Statsoft Inc. 2007) to analyze differences between sampling occasions in 2005 and 2007 (repeated factor) in maximum depth and surface area of the pools, in the proportion of macroinvertebrate herbivores and predators as well as in total cladoceran abundance (number of cladocerans L^{-1}) and species richness. Surface area and the proportion of herbivores and predators were only available for two and three occasions in 2005, respectively. Prior to analysis, surface area, total cladoceran abundance and the proportion of macroinvertebrate predators were logarithmically transformed to meet assumptions of normality and equal variance. The proportion of detritivores could not be compared statistically as they were absent from all samples in May 2007.

Next, we used Redundancy Analysis (RDA) to investigate differences between the sampling occasions in 2005 and May 2007 in (a) water quality and vegetation cover, (b) macroinvertebrate community composition and (c) cladoceran community composition, using time (sampling occasion in 2005 or 2007) as single explanatory variable. Using forward selection, we identified those sampling occasions that significantly contributed to variation in the respective habitat characteristics. Species data (i.e. dependent variables) were: (a) water quality variables (alkalinity, chlorophyll a concentration, pH, suspended solids concentration, temperature and transparency) and percentage vegetation cover; (b) relative abundances of common macroinvertebrate taxa; (c) absolute and relative abundances of common cladoceran species. When investigating relative abundances, input data were absolute abundances standardized by sample norm. Rare taxa or species that were present only in

one pool were excluded from analysis as they may disproportionately influence the results. Macroinvertebrate and cladoceran abundance were logarithmically transformed prior to analysis. To investigate differences between sampling occasions in 2005 and May 2007 in the cladoceran species composition, we also used Canonical Correspondence Analysis (CCA) with forward selection on the presence/absence of common cladoceran species using time (sampling occasion in 2005 or 2007) as single explanatory variable. The significance of the models was tested using Monte Carlo permutations ($n = 999$), performed by a split-plot design (permutations at split-plot level i.e. sampling occasion in 2005 or 2007) and with pool as covariable. RDA and CCA were performed in Canoco for Windows 4.5 (Lepš & Šmilauer 2003).

RESULTS

There were significant differences in maximum depth (Repeated Measures ANOVA: $MS_{(7,21)} = 1930.5$, $F = 20.35$, $p < 0.001$) and surface area of the four study pools ($MS_{(2, 6)} = 4.86$, $F = 18.23$, $p = 0.003$) among sampling occasions in both years. Maximum depth was significantly lower in May 2007 when compared to all other occasions in 2005 (Tukey HSD: $p < 0.001$) (Fig. 3a). The flooded surface area was significantly smaller in May 2007 when compared to March 2005 (Tukey HSD: $p = 0.002$), but did not differ significantly from the area measured in June or July 2005 (Fig. 3b).

There were significant overall differences in water quality and vegetation cover of the study pools among sampling occasions (RDA: 35.5% explained variance; $F = 2.57$; $p = 0.001$). Variation in habitat characteristics was especially due to differences between the late samplings in 2005 (late May - July) and May 2007 on the one hand and the earlier stages in 2005 (March – early May) on the other hand (forward selection: $T_{05,5}$, $T_{05,6}$, $T_{05,7}$ and T_{07} ; 32.6%, $F = 4.57$, $p = 0.001$) (Fig. 4). In late stages in 2005 as well as in May 2007, pools were relatively turbid, with high concentrations of chlorophyll *a* and suspend solids as well as high temperature and alkalinity values in most pools (Fig. 5).

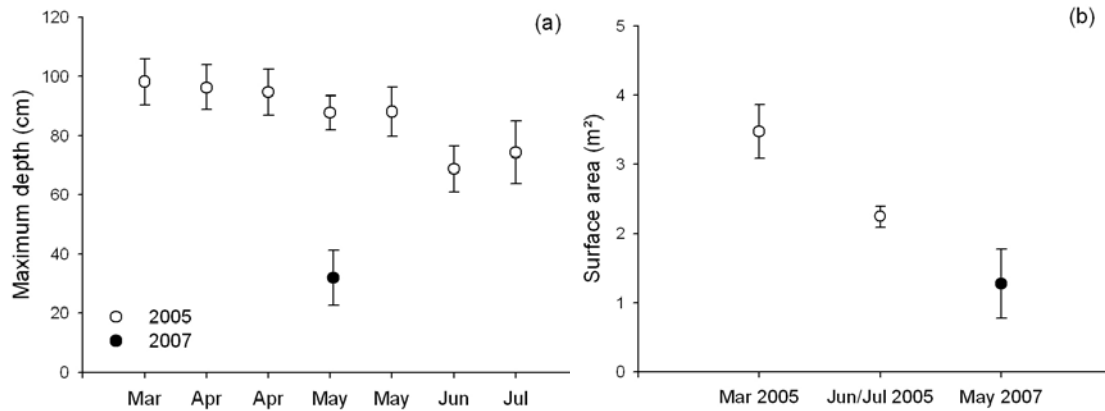


Figure 3. (a) Maximum depth (mean $\pm$ S.E.; cm) and (b) surface area (logarithmically transformed) (mean $\pm$ S.E.; m²) of the four study pools in function of time (March, early April, late April, early May, late May, June and July).

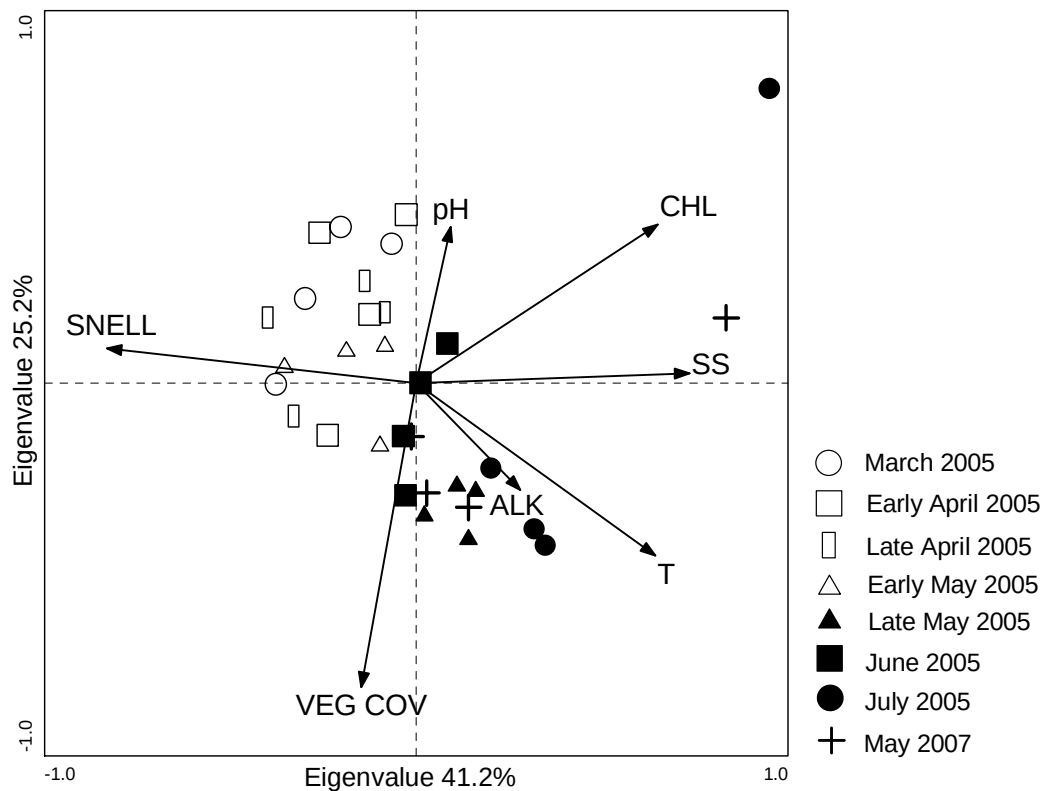


Figure 4. Biplot of water quality variables and vegetation cover (arrows) and pools (symbols) at the different sampling occasions in 2005 and 2007 (PCA with pool as covariable). Legend: ALK: alkalinity; CHL: chlorophyll a concentration; pH; SS: suspended solids concentration; T: temperature; TRANSP: transparency; VEG COV: percentage vegetation cover. For 2005, white symbols represent pools relatively early in spring, whereas black symbols represent late spring and summer. Crosses represent pools in May 2007.

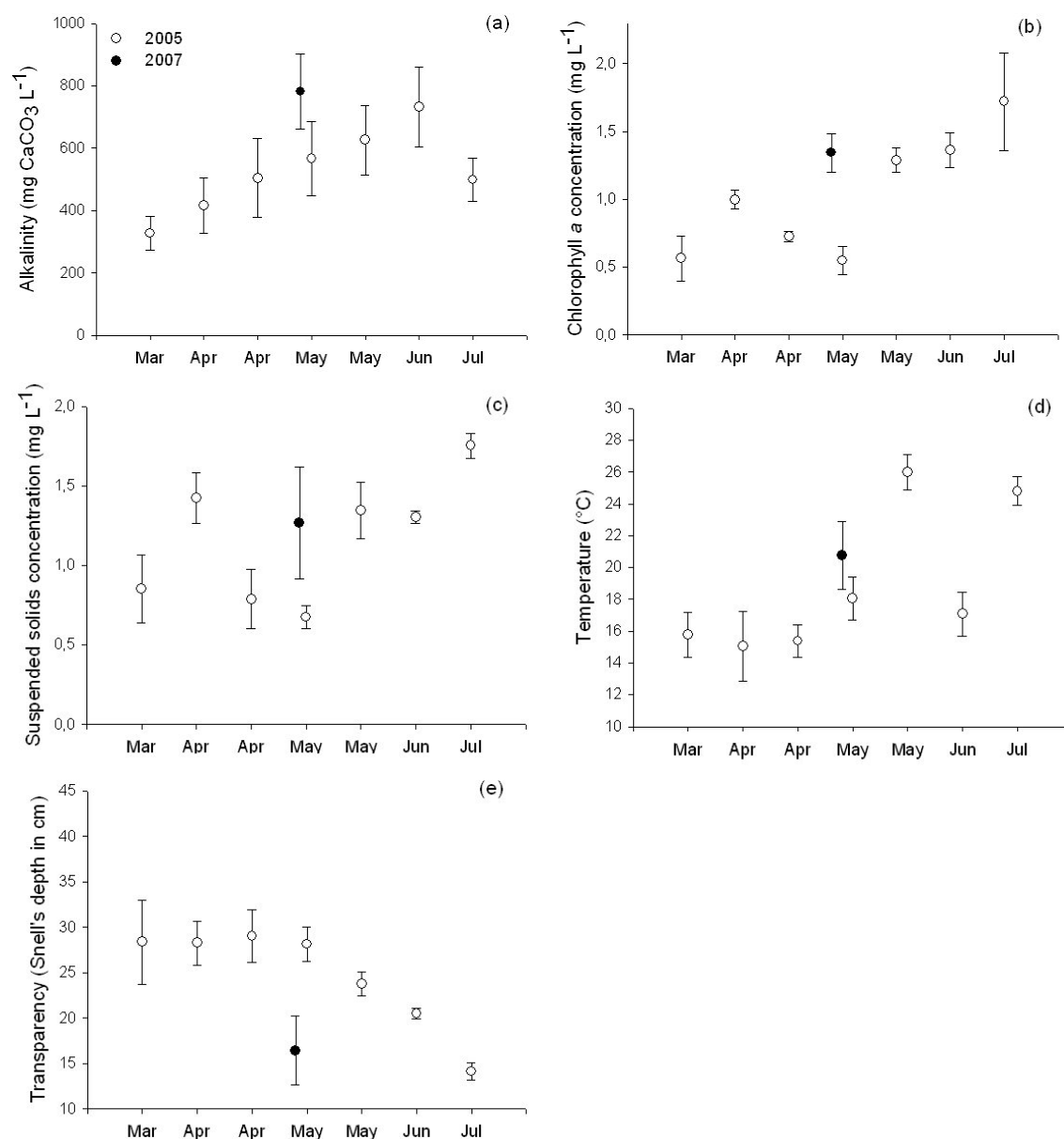


Figure 5. Water quality variables of the four study pools (mean $\pm$ S.E.) in function of time (March, early April, late April, early May, late May, June and July): (a) alkalinity ($\text{mg CaCO}_3 \text{ L}^{-1}$); (b) chlorophyll a concentration (mg L^{-1} ; log transformed); (c) suspended solids concentration (mg L^{-1} ; log transformed); (d) temperature ($^{\circ}\text{C}$); (e) transparency (Snell's depth in cm).

The composition of the macroinvertebrate community differed significantly between all sampling occasions in both years ($T_{05,1}$, $T_{05,3}$, $T_{05,7}$ and T_{07}) (RDA: 46.7%; $F = 3.80$; $p = 0.001$). Samples taken during the spring of 2007 were especially positively associated with high relative abundances of Coleoptera and Hemiptera (Fig. 6). Overall, predators constituted the largest proportion of the macroinvertebrate communities in May 2007 (Table 1; Fig. 7), but their proportion was only significantly higher when compared to the earliest sampling in 2005 (Repeated Measures ANOVA: $MS_{(3,9)} = 0.84$; $F = 20.06$; $p < 0.001$) (Tukey HSD: $p < 0.001$). The proportion of herbivores in May 2007 did not differ significantly from any sampling in 2005 ($p > 0.05$) (Fig. 7). The proportion of detritivores was comparable with values found in July 2005 (Fig. 7), but the difference could not be tested statistically.

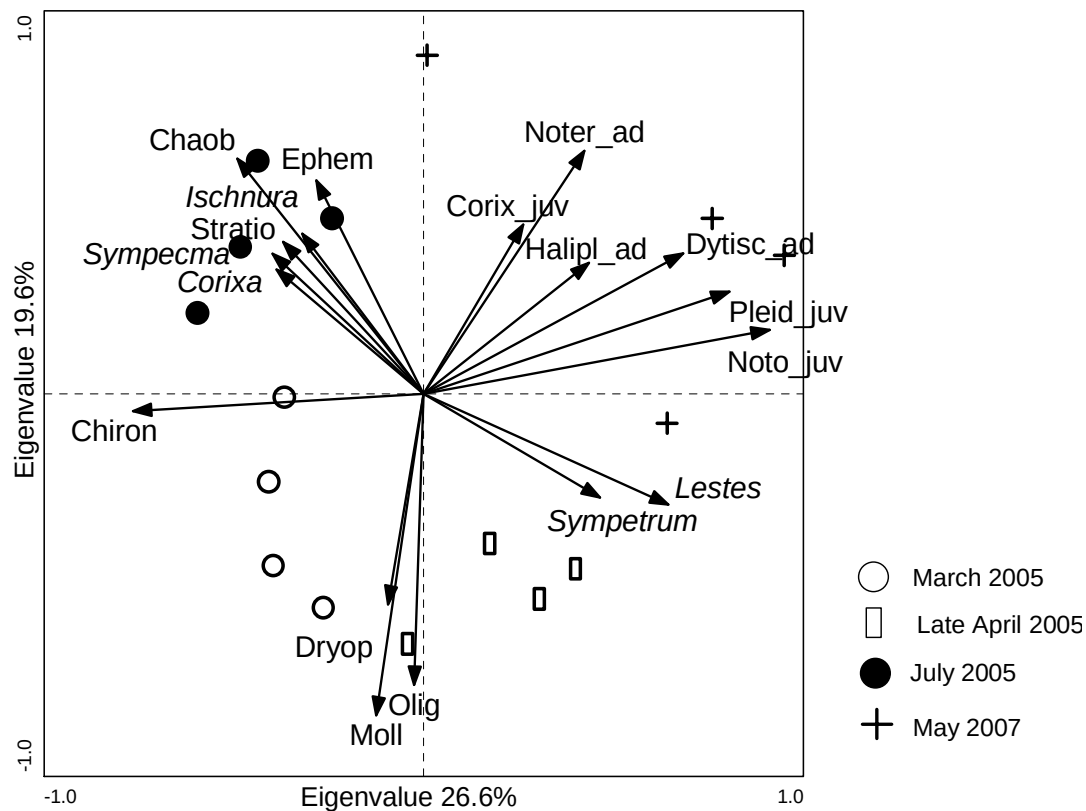


Figure 6. Biplot of macroinvertebrate taxa (arrows) and pools (symbols) at the different sampling occasions in 2005 and 2007 (PCA). 18 taxa that best fitted the model are included. Legend: Chaob: Chaoboridae; Chiron: Chironomidae; Corixa: adult *Corixa*; Corix_juv: juvenile *Corixidae*; Dryop: Dryopidae; Dytisc_ad: adult *Dytiscidae*; Ephem: Ephemeroptera; Halipl_ad: adult *Haliplidae*; Ischnura; Lestes; Moll: Mollusca; Noter_ad: adult *Noteridae*; Noto_juv: juvenile *Notonectidae*; Olig: Oligochaetae; Pleid_juv: juvenile *Pleidae*; Stratio: *Stratiomyidae*; Sympecma; Sympetrum. Species data were logarithmically transformed relative proportions. For 2005, white symbols represent the early and middle succession stage of pools in spring, whereas black symbols represent the late succession stage in summer. Crosses represent pools in May 2007.

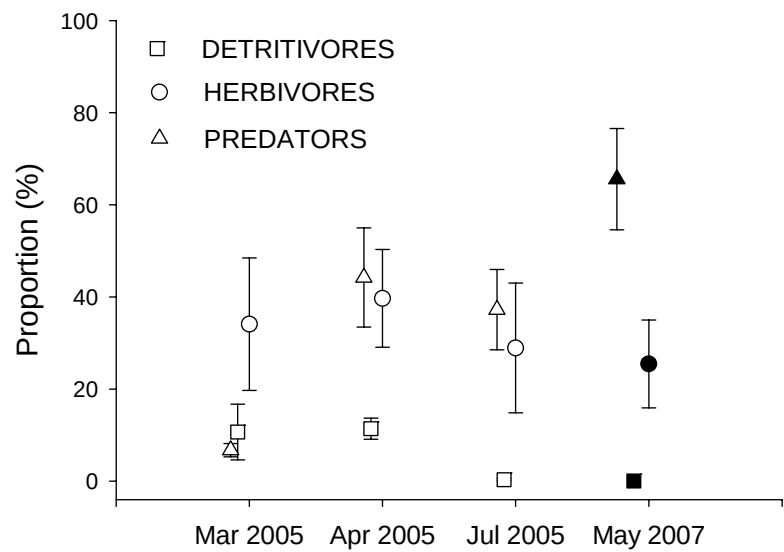


Figure 7. Proportion of the macroinvertebrate community composed of detritivores, herbivores and predators (mean $\pm$ S.E.; %) in the study pools in function of time (white symbols: early March, late April and July 2005; black symbols: May 2007).

Table 1. Macroinvertebrate taxa encountered in the study pools during the dry year (2007) and the functional group to which they were assigned.

		Functional group
Acari		predators
Gastropoda		herbivores
Crustacea	<i>Asellus aquaticus</i>	herbivores
Ephemeroptera	Baetidae	herbivores
Odonata	<i>Lestes viridis</i>	predators
	<i>Lestes dryas</i>	predators
	<i>Aeschna</i>	predators
	<i>Sympetrum</i>	predators
Diptera	Chaoboridae	predators
	Chironomidae	omnivores
	unidentified	omnivores
Hemiptera	Corixidae	omnivores
	Pleidae	predators
	<i>Notonecta</i>	predators
Coleoptera	<i>Halipus</i>	predators
	<i>Noterus clavicornis</i>	predators
	<i>Noterus classicornis</i>	predators
	Dytiscidae	predators
		<i>Bidessus</i>
		<i>Coelambus</i>
		<i>Hydroporus</i>
		unidentified
	Hydraenidae	herbivores
	Hydrochidae	herbivores
	Hydrophilidae	omnivores

In the spring of 2005, we found a total of 25 cladoceran species in the study pools at seven repeated sampling occasions. At the single sampling occasion in early May, 12 species were collected in 2005 and eight in 2007. The following species were present in May 2007: *Alona rectangula*, *Alonella excisa*, *Ceriodaphnia reticulata*, *Chydorus sphaericus*, *Daphnia longispina*, *Pleuroxus aduncus*, *Scapholeberis rammneri* and *Simocephalus* sp.

There were significant differences in total cladoceran abundance (Repeated Measures ANOVA: $MS_{(7, 21)} = 4.14$; $F = 20.78$; $p < 0.001$) and species richness ($MS_{(7, 21)} = 17.50$; $F = 5.29$; $p = 0.001$) among sampling occasions in both study years. Total cladoceran abundance in May 2007 was significantly lower when compared to all but the two earliest sampling occasions in 2005 (Tukey HSD: $p < 0.05$) (Fig. 8a). The average species richness per pool was significantly lower in May 2007 when compared to all sampling occasions in 2005, except March and late April (Fig. 8b).

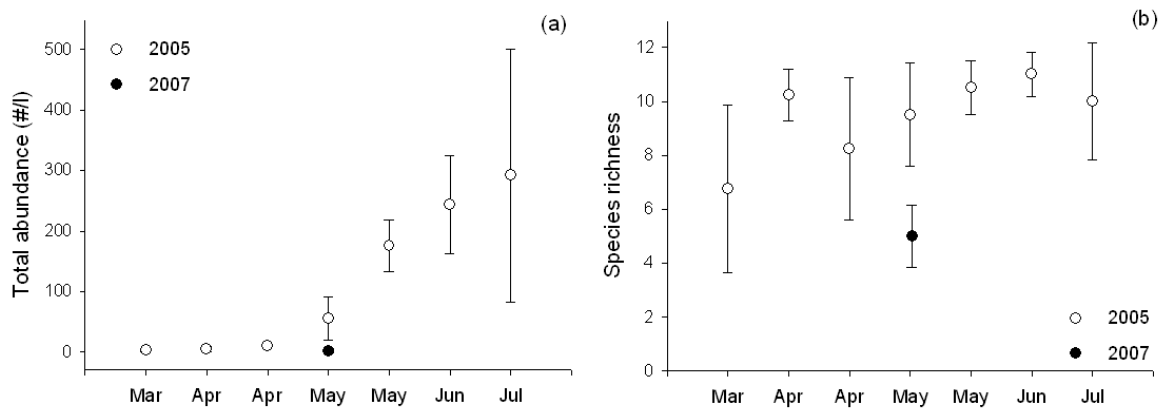


Figure 8. (a) Total cladoceran abundance (number of specimens L^{-1}) and (b) species richness (mean $\pm$ S.E.) of the cladoceran communities in the study pools in function of time (late March, early April, late April, early May, late May, June and July 2005 and May 2007).

There were significant differences in cladoceran community composition among the sampling occasions in both years with regards to presence/absence (CCA: 39.3%; $F = 2.55$; $p = 0.001$), as well as relative proportions (RDA: 36.2%; $F = 2.73$; $p = 0.001$) and absolute abundances of species (58.2%; $F = 5.47$; $p = 0.001$). Variation in presence/absence patterns was especially due to differences between various stages in 2005 on the one hand versus early May 2007 and late April 2005 on the other hand (forward selection: $T_{05,1}$, $T_{05,2}$, $T_{05,4}$, $T_{05,5}$, $T_{05,6}$ and $T_{05,7}$; 37.3%, $F = 2.83$, $p = 0.001$). Absolute abundances of species were generally different in later stages in 2005 (May – July) in comparison with May 2007 and the three earliest stages in 2005 (March – April) (forward selection: $T_{05,4}$, $T_{05,5}$, $T_{05,6}$, $T_{05,7}$; 56.6%, $F = 10.17$, $p = 0.001$) (Fig. 9). A significant amount of variation in the relative proportions of species was due to differences between the first and the late samplings in 2005 (March and late May - July) and May 2007 on the one hand and the middle stages in 2005 (early April – early May) on the other hand (forward selection: $T_{05,1}$, $T_{05,5}$, $T_{05,6}$, $T_{05,7}$ and T_{07} ; 32.1%, $F =$

2.97, $p = 0.001$) (Fig. 10). Together, these analyses indicate that in May 2007, similar species were present as at a comparable moment in 2005 (end of April), but in low abundances comparable to early in the growing season in 2005 (Fig. 9). Species were also represented in different proportions of the total community when compared to various stages in 2005 (Fig. 10). In May 2007, communities were mainly composed of *Ceriodaphnia reticulata* and *Chydorus sphaericus* (Fig. 10).

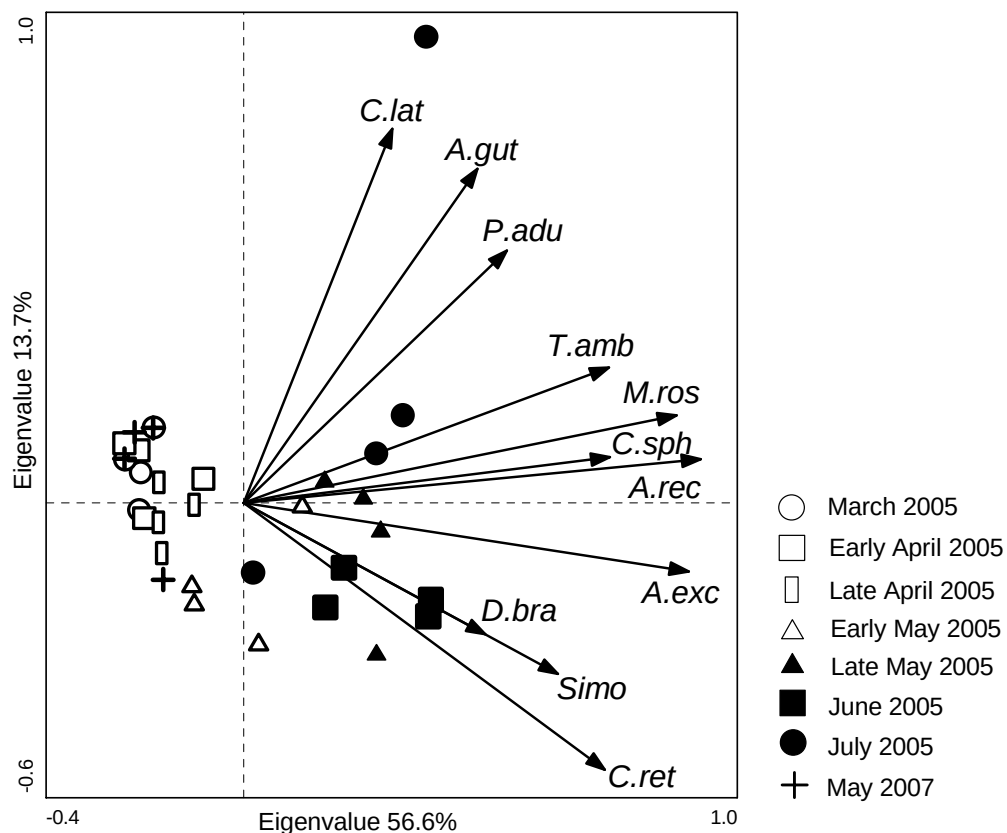


Figure 9. Biplot of cladoceran species (arrows; logarithmically transformed absolute abundances) and pools (symbols) at the different sampling occasions in 2005 and 2007 (PCA with pool as covariable). 11 species that best fitted the model are included. Legend: *A.exc*: *Alonella excisa*; *A.gut*: *Alona guttata*; *A.rec*: *Alona rectangulara*; *C.lat*: *Ceriodaphnia laticaudata*; *C.ret*: *Ceriodaphnia reticulata*; *C.sph*: *Chydorus sphaericus*; *D.bra*: *Diaphanosoma brachyurum*; *M.ros*: *Macrothrix rosea*; *P.adu*: *Pleuroxus aduncus*; *Simo*: *Simocephalus expinosus/vetullus*; *T.amb*: *Tretoccephala ambigua*. Species data were. For 2005, white symbols represent pools relatively early in spring, whereas black symbols represent late spring and summer. Crosses represent pools in May 2007.

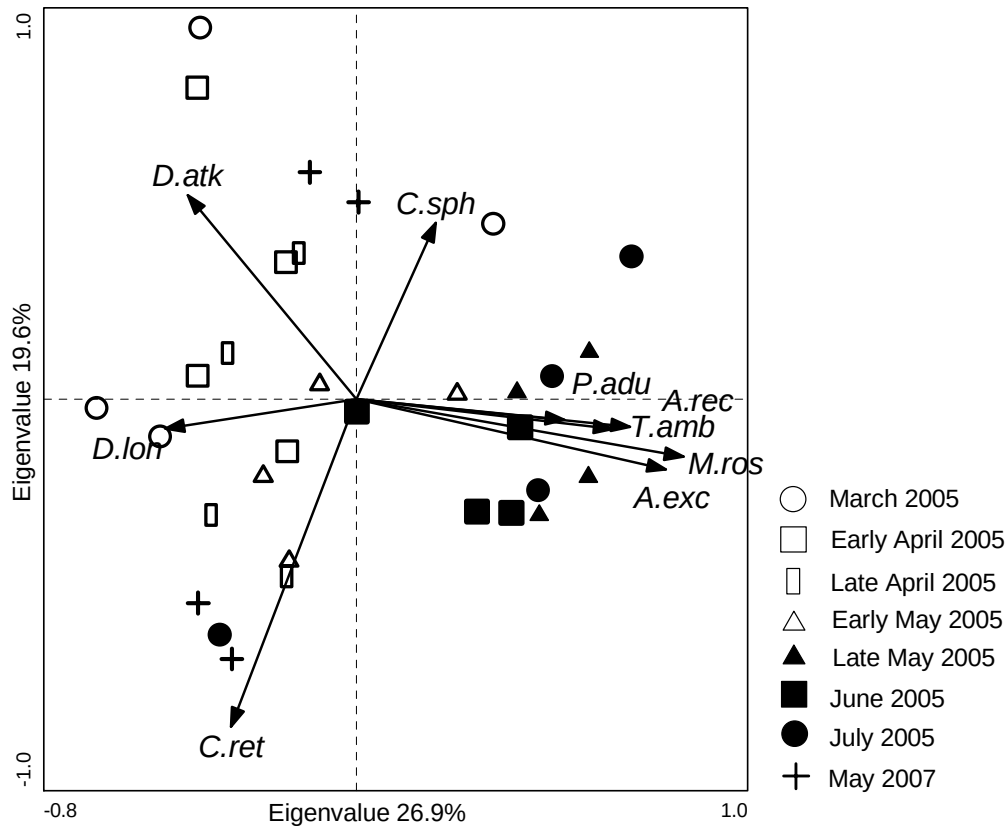


Figure 10. Biplot of cladoceran species (arrows; logarithmically transformed absolute abundances, standardized by sample norm) and pools (symbols) at the different sampling occasions in 2005 and 2007 (PCA with pool as covariable). 9 species that best fitted the model are included. Legend: *A.exc*: *Alonella excisa*; *A.rec*: *Alona rectangulara*; *C.ret*: *Ceriodaphnia reticulata*; *C.sph*: *Chydorus sphaericus*; *D.atk*: *Daphnia atkinsoni*; *D.lon*: *Daphnia longispina*; *M.ros*: *Macrothrix rosea*; *P.adu*: *Pleuroxus aduncus*; *T.amb*: *Tretocephala ambigua*. For 2005, white symbols represent pools relatively early in spring, whereas black symbols represent late spring and summer. Crosses represent pools in May 2007.

DISCUSSION

Studies investigating community changes in temporary pools during years with different weather conditions may be relevant to increase our knowledge of the ecological processes that structure the invertebrate communities, particularly in relation to hydrological variation. Such information may ultimately allow predicting the effects of climatic variation - possibly as a result of climate change.

In early May 2007, which was the drier year, study pools contained a subset of species when compared to a comparable moment during the spring of the wetter 2005 (end of April). Unlike in 2005, however, communities were mainly composed of *Ceriodaphnia reticulata* and *Chydorus sphaericus* whereas other species only contributed to a small extent to the total community. Together, these findings indicate that in May 2007, pools were in a

similar stage of seasonal species assembly when compared to the same moment in 2005, but communities were far less diverse and dominated by only a few species. Additionally, in May 2007, species were only present at low abundances comparable to the situation in early stages of 2005. These temporal differences in cladoceran community structure and diversity were associated with interannual variation in local pool characteristics. In the spring of 2007, pools were characterized by a considerably smaller flooded surface and shallower depth when compared to the spring of 2005, probably due to lower winter and spring precipitation and higher evaporation going along with higher spring temperatures (Fig. 2). High water temperatures in the spring of 2007 may have stimulated algae growth, resulting in relatively high suspended solids concentrations and elevated turbidity. High conductivity values at that time were probably related to the concentration of ions due to evaporation. Especially the decrease in microhabitat availability in smaller and shallower pools probably explains the low cladoceran diversity and overall abundance. Local species richness generally increases with habitat size (MacArthur & Wilson 1967), partly due to its correlation with habitat heterogeneity (Williams 1943, Douglas & Lake 1994, Brose 2001). In the smaller pool basin (Fig. 3b), a smaller proportion of the dormant egg bank was inundated with less eggs being exposed to appropriate hatching cues, whereas the lack of spatial niches probably did not allow a strong population growth of the species that effectively hatched.

Cladocerans probably also experienced more intense predation in the small pool volumes in the spring of 2007 as the macroinvertebrate community at that time was mainly composed of predators. The low availability of spatial refuges associated with the small surface area and limited depth of the pools therefore possibly constituted an additional stress factor to cladoceran prey in the spring of 2007. Likewise, Boulton et al. (1992) found a strong decrease in invertebrate densities after a severe drought in a desert stream, due to altered habitat availability and concentration of predators as stream volume shrank. The high relative abundance of predatory taxa in early May 2007 partly reflects natural seasonal succession. As the nature of available food resources in temporary pools changes with time of inundation, a shift in dominance usually occurs from detritivores to herbivores and finally predators (Meintjes 1996, Moorhead et al. 1998, Lahr et al. 1999). Additionally, predatory taxa usually need more time to complete their life cycles and therefore dominate later stages. On the other hand, while most predators recolonize the habitats from overwintering grounds, many herbivores and detritivores rely on the activation of dormant life stages (e.g. Mollusca, Crustacea, Ephemeroptera, Oligochaeta; Wiggins et al. 1980). As for cladocerans, most probably a smaller proportion of such dormant stages was inundated in the smaller pool basin, and herbivores and detritivores were therefore possibly less well represented during the dry year.

Regional processes may also vary from year to year as a response to climatic variation. Under dry weather conditions, an increased distance among habitats and a decrease in habitat density is expected due to smaller pool surfaces and increased numbers of dry pools. In a metapopulation configuration, such changes may adversely affect colonization-extinction dynamics (Hanski & Gaggiotti 2004). In an agricultural landscape, amphibian abundance was positively related to the amount of ditches and ponds established under normal weather conditions (100 ha; Piha et al. 2007). After a severe drought, however, it was the proportion of forests (positive association) and cultivated fields (negative association) within a radius of 1 km that explained amphibian population abundance. These authors therefore concluded that the positive effects of connectivity in buffering adverse impacts from agriculture on population persistence may not be effective during extremely dry years. The low species richness and cladoceran abundance observed in our study in the dry year is unlikely to be due to dispersal limitation, as cladocerans rely on emergence from long-lived dormant egg banks after flooding. Additionally, cladocerans mainly rely on passive dispersal of dormant stages, which may even be positively affected under dry weather conditions. This was observed by Altermatt et al. (2008) in a *Daphnia* metacommunity in rock pools: as a consequence of warmer and drier weather, resting stages of *Daphnia* were increasingly exposed to animal and wind dispersal in desiccated pools. During a 24-year period, this resulted in increased colonization rates and a more dynamic and diverse metacommunity. In their study, however, the number of potential wet habitats for *Daphnia* was not limiting. Decreases in habitat availability owing to dry weather conditions on the long term may still adversely affect colonization success and the regional coexistence of species.

During the last decades, total annual and winter precipitation in Hungary significantly decreased, possibly as a result of climate change (Domonkos 2003). Although drought events are a characteristic feature of the Hungarian climate, they were more frequent during the past 50 years (Szinell et al. 1998) and are predicted to increase in frequency in the next century (Gálos et al. 2007). If these trends persist, hydrological conditions of temporary pools in Kiskunság will be altered, eventually resulting in smaller pools with on average shorter hydroperiods. It is not clear what the outcome of such climatic changes will be on the communities inhabiting the temporary pools. Temporary pool invertebrates are naturally tolerant to a relatively high degree of environmental variation, even in hydroperiod length (Batzler et al. 2004). Additionally, through their dormant egg banks (Hairston 1996) and adaptive hatching behavior (i.e. bet-hedging; Ellner et al. 1998, De Meester & De Jager 1993), zooplankton communities in temporary pools may be substantially buffered against unsuccessful growing seasons and the unpredictable nature of their habitat. It remains, however, unsure whether the invertebrate inhabitants will be able to adapt fast enough to such long-term and possibly drastic changes. If this is not the case, our results suggest that

species richness and composition of the cladoceran communities in these pools and eventually of the regional species pool may be negatively affected. Given the importance of cladocerans in the food web of temporary waters, through indirect effects and trophic interactions this may ultimately interfere with the functioning and diversity at different trophic levels of these ecosystems. However, predicting species and community responses to long-term climatic variation should be done with caution. Such long-term effects depend on the evolutionary potential of species to respond to environmental changes or, in the absence of a strong adaptive response, of the buffering capacity of the dormant egg bank against repeated emergence in an unsuitable environment. Especially in temporary pool ecosystems, there is still a considerable need of long-term monitoring and sound ecological research on ecosystem responses to future environmental change including global change.

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CHAPTER 6

RECRUITMENT FROM DORMANT EGG BANKS IN TEMPORARY POOLS: DO CLADOCERANS CHOOSE THEIR MOMENTS OF GLORY?

Liesbet Boven, Joost Vanoverbeke & Luc Brendonck

Unpublished manuscript

Abstract In temporally variable aquatic systems, zooplankton organisms often rely on the production of dormant eggs to survive periods of adverse conditions, such as recurrent droughts. It is generally assumed that hatching of different species from the dormant egg bank is synchronous and only contributes to the initiation of active populations at the onset of a period suitable for growth and reproduction, but not to changes in abundance or species composition later on in the inundation period. Theoretically, however, species may adjust the timing of hatching to environmental conditions where their relative fitness is expected to be highest. Such temporal habitat selection may allow temporal niche differentiation of competing species and should result in a positive correlation between hatching percentages and the relative abundance of the species in the water column over the course of a growing period. In Hungarian temporary pools, temporal patterns of abundance throughout the inundation period were observed to be different for cladoceran species co-occurring in the same pools. To test whether species-specific abundance patterns could be correlated to species-specific hatching preferences, we performed controlled laboratory experiments in which we compared the hatching response of species to experimental conditions of temperature and photoperiod mimicking different moments of time in the growing season. We observed differences among species in their hatching response to the different experimental conditions, with a positive correlation between hatching percentages of the species and their abundance in the field at the corresponding seasonal time. This differential hatching response to temperature and photoperiod cues might thus be an adaptive response allowing species to initiate populations from the resting egg bank mainly when their relative fitness is expected to be high. Such response is in agreement with temporal habitat selection and may result in a more important contribution of dormant egg bank dynamics to active community patterns than generally assumed.

Key words Branchiopoda, temperature, temporal habitat selection, timing of hatching, photoperiod

INTRODUCTION

In temporally variable aquatic environments, freshwater zooplankton organisms produce dormant eggs to bridge periods of unfavourable conditions for survival and reproduction (Fryer 1996, Cáceres 1997a, Hairston & Kearns 2002, Brendonck & De Meester 2003). The permanent inhabitants of seasonally drying pools, for example, rely on the production of resting stages to survive recurrent droughts (Brendonck 1996, Brock et al. 2003). During the entire growing period (large branchiopods) or just before the onset of unsuitable conditions (cladocerans, rotifers, copepods) these resting stages are produced and deposited in the sediment where they accumulate as a dormant egg bank. The dormant eggs are generally very resistant to harsh conditions, such as freezing and drought, and remain viable in the sediment for long periods (Hairston et al. 1995, Cousyn & De Meester 1998). Therefore, dormant egg banks can be considered as a biotic reservoir both of species richness and genetic diversity (Hairston 1996, Brendonck & De Meester 2003). When conditions turn favourable again, for example after the filling of a temporary pool by rain or snow melt, species hatch from the dormant egg bank and initiate new populations.

Seasonal population and community dynamics could be considerably affected by emergence from the dormant egg banks. Based on observations from permanent lakes, where hatching by many cladocerans as well as rotifers and copepods is often observed synchronously early in the growing season (Wolf & Carvalho 1989, Cáceres 1998, Hairston et al. 2000, Gyllström 2004), it is generally assumed that emergence only contributes to the initiation of active populations, while changes in the composition of communities and populations later in the season are driven by biotic processes and abiotic conditions in the water column (e.g. predation, food availability). However, for *Ceriodaphnia* two subsequent hatching peaks were observed in spring and summer (De Stasio 1990, Arnott & Yan 2002) and probably accounted for the subsequent increase in population size in a permanent pond (De Stasio 1990). In addition, Vanoverbeke (unpubl. data) observed no preferential hatching of *Daphnia magna* resting eggs under early spring conditions in experiments involving different seasonal treatments. Rather high hatching percentages were observed under all seasonal conditions.

Hatching as soon as conditions become favourable for growth and survival may indeed not always be the most successful strategy. If resting eggs are able to detect signals relating environmental quality to the probability of future reproductive success (e.g. temperature and predation; Hansson 1996, Rengefors et al. 1998, Spencer & Blaustein 2001, De Roeck et al. 2005), delaying the hatching response beyond the onset of the growing season may in some cases be more advantageous. Indeed, temporal habitat selection implies that species are able to adjust the timing of hatching within the growing season depending on environmental conditions indicative of a high expected relative fitness. Such process may facilitate temporal

niche dynamics, where competing species do not differentiate in the resources they use, but when they are most active using them (Chesson 2000). Temporal niche dynamics generally result in stable coexistence if variable environmental conditions result in fluctuations in the recruitment of competing species over time and when species are able to recover from unsuccessful periods through 'storage' of reproductive capacity (Warner & Chesson 1985). Dormant egg banks effectively promote the coexistence of species through the storage effect (Hairston 1996, Cáceres 1997b, Bonis et al. 1995). Moreover, Hedrick (1995) theoretically showed that, especially when temporal habitat selection is involved, prolonged diapause enhances the occurrence of stable genetic polymorphism in temporally varying environments. Analogously to promoting genetic diversity within populations, temporal habitat selection may thus be an important mechanism of temporal niche dynamics promoting the coexistence of species and maintaining species diversity in communities.

Most studies on the ecological dynamics of dormant egg banks, including the few studies investigating the link between dormant and active populations, have been focusing on permanent ponds and lakes. In temporary pools, the recurrent droughts inevitably imply a strong dependence on the resting egg banks. Indeed, the initiation of active populations solely depends on emergence, due to the lack of active populations bridging the dry period. Therefore, temporary pools may be especially suitable to study the link between hatching dynamics and the dynamics of the active community during the growing period. In the current study, we want to investigate to what extent cladoceran species from temporary pools exhibit differential hatching preferences toward seasonal hatching cues representing early-spring, late-spring and summer in the region of sampling. In addition, we want to study whether hatching preferences are associated with abundance patterns of these species during the growing period, as is expected under the hypothesis of temporal habitat selection. In controlled laboratory experiments, we therefore investigate the hatching response of seven cladoceran species from Hungarian temporary pools to temperature and photoperiod cues mimicking different moments during the growing period and compare the hatching response to data on the abundance of these species in the field.

MATERIALS AND METHODS

Study area

The pools selected for our study are located in the Kiskunság area in Hungary, situated approximately 85 km southwest of Budapest. The Hungarian continental climate favors the formation of vernal pools. In early spring (February-March), these pools fill with snowmelt. During springtime, they gradually dry as air and water temperature and consequently evaporation increase. Depending on pool morphology and local weather conditions, pools usually are dry again in late spring or summer. The study pools are distributed over three

pool clusters and their hydroperiod as observed in the spring of 2005 varied from less than ten weeks (short) to more than four months (long). A more detailed description of the pools is presented in Boven et al. (2008a; Chapter 3).

In a total of 18 pools, equally distributed over the three pool clusters and with a variable length of inundation, we sampled the cladoceran communities at subsequent occasions in the spring of 2005. For these pools, a clear seasonal succession in the cladoceran community was observed throughout spring (Chapter 4). *Daphnia atkinsoni* appeared as an early species and disappeared by the end of April, while most other species increased in abundance from April onwards (Fig. 1). *Daphnia curvirostris* and *Moina brachiata* already disappeared in May, whereas *Alona rectangula*, *Ceriodaphnia reticulata* and *Macrothrix rosea* continuously increased until summer. *Simocephalus* was present throughout spring and summer and peaked in May. For a detailed description of the seasonal dynamics in the cladoceran communities, we refer to chapter 4.

Experimental design

For a subset of twelve of the 18 pools from which the active cladoceran community was characterized, the dormant egg banks were sampled when the pools were dry. For each pool, three sediment subsamples were collected by removing each time the upper 3 cm of sediment from six random locations (10x10 cm²) representative of all microhabitats (depth, vegetation type) present in the pool. Dormant eggs of Cladocera are usually encapsulated in a protective case (ephippium) containing one or two eggs depending on the species. Sediment with ephippia was dried and stored for three months in the dark at room temperature. Next, from each sediment subsample, ephippia were isolated (0.8-2.0 kg of sediment; average 1.4 kg) using the sugar flotation method (Onbé 1978, Marcus 1990). After the isolation procedure, ephippia were stored in the dark at 4°C in order to avoid exposing them to hatching cues as much as possible.

Prior to the experiment, for each pool, three egg samples were composed each with one-third of the ephippia of each sediment subsample. Each egg sample was exposed to one of three treatments of covarying temperature and light conditions, mimicking different moments in the growing season (12°C and 12 h light/12 h dark: early spring; 18°C and 14 h light/10 h dark: late spring; 24°C and 17 h light/7 h dark: summer). The ephippia were inundated with about 1.5 l of EPA medium (Anonymous 1985) in 2-l aquaria that were placed in incubators with the respective temperature and photoperiod settings. We imposed the same initial conductivity (i.e. 800 μ S/cm at inundation) to all treatments. In preliminary experiments this proved to be a suitable condition to assess hatching characteristics of different cladoceran species at different temperatures for our study systems. As

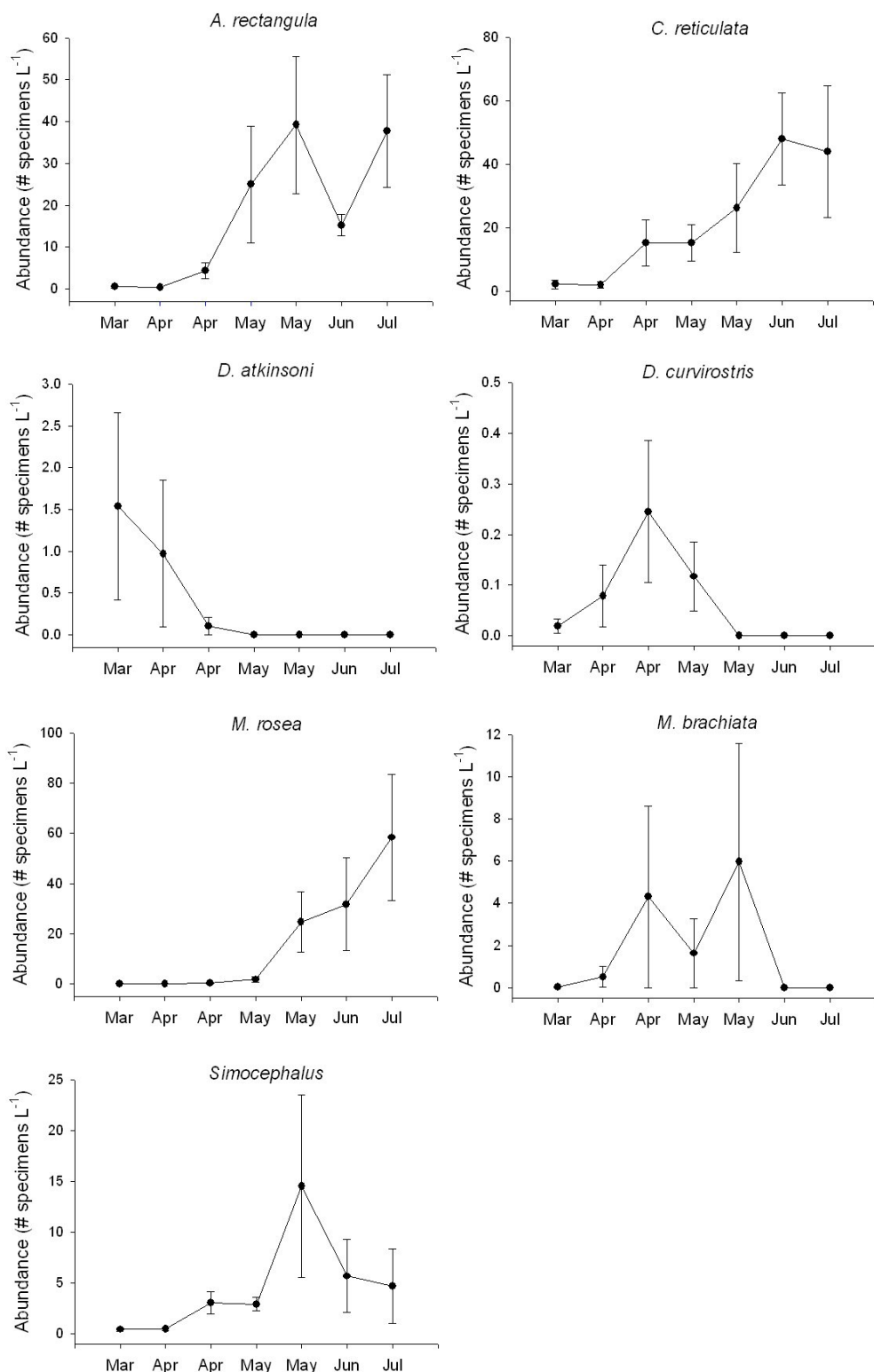


Figure 1. Absolute abundance of cladoceran species (number of specimens per liter; mean $\pm$ S.E.) as a function of time during one growing season (end of March, early April, end of April, early May, end of May, June and July 2005) in 18 temporary freshwater pools in Kiskunság (Hungary; new figure on the basis of data in chapter 4).

the medium was not renewed, conductivity slightly (max. 5%) increased during the experiment, especially in the late spring and summer treatments. This pattern was consistent with our observations in the field (Boven et al. 2008a; Chapter 3). In order to ensure a high oxygen supply, the hatching medium was aerated at least a few hours before inundation of the dormant eggs and continuously during the experiment.

During a period of about one month, newly emerged hatchlings were isolated from the aquaria at repeated occasions (resp. 2, 6, 12, 18, 21 and 25 days after inundation). They were grown in the medium from which they hatched and were fed with a few drops of *Scenedesmus obliquus* every other day. Once they reached maturity, they were identified to species level using Flößner (2000).

Finally, viable dormant eggs that did not hatch during the incubation period were identified to genus or species level based on ephippium morphology using Vandekerckhove et al. (2004a) and Mergeay et al. (2005) and counted in order to calculate hatching percentages of the different species. Eggs were counted after decapsulation from the ephippia and were considered viable if a healthy embryo was detected. When a species was not present in the dormant egg bank of a pool sample, the concerning sample was not included in the calculation of average hatching percentages for the respective species.

Data analysis

To detect any species-specific hatching preferences for different conditions of temperature and photoperiod, we compared the chance of successful hatching of resting eggs between species and seasonal treatments and studied the interaction. By means of a Generalized Linear Model with logit link we modeled the hatching response (hatched versus unhatched) of viable dormant eggs with species and seasonal treatment as independent variables. For each species, data from the different pools were pooled per seasonal treatment as zero values for some pools (i.e. no resting eggs) did not allow to use pool as an extra independent variable in the analysis. *Alona rectangula* was excluded from this analysis as estimates of hatching percentages at the summer treatments were probably not reliable due to reproduction. The hatching response of each species to the seasonal treatments was studied into more detail by performing a similar Generalized Linear Model for each species separately with season as independent variable.

To further study the possible occurrence of temporal habitat selection, we also tested for a correlation between average hatching percentages of the different species in the different seasonal treatments and the average abundance of these species in the field. Natural abundances that were used for comparison were determined two weeks after the experimental conditions were realized in the field (early April for early spring, early May for late spring and July for summer). We first calculated the Spearman Rank correlation between

hatching percentages and abundances for all seven species together. To be able to compare among species, these were standardized prior to analysis. For each species, each hatching percentage at one of the treatments or abundance at one of the moments throughout the season was therefore divided by, respectively, the average hatching percentage over the different treatments or abundance over the growing season of that species. Given the limited number of data, we consider the correlation as described above as the most straightforward way to test this relationship. To account for the interdependency of data referring to the same species, however, we additionally calculated the Spearman Rank correlation between average hatching percentages under the different seasonal treatments and average field abundances at corresponding moments throughout the season for each species separately. Next, we used a single sample t-test to test whether on average the correlation coefficients significantly differed from zero. For *Alona rectangula* the correlation coefficient could not be calculated due to the limited number of data. All analyses were performed in Statistica 8.0 (StatSoft 2007).

RESULTS

In general, hatching percentages comprised the whole range from 0 to 100%. Besides a significant difference between species in the overall proportion of hatchers (Wald stat. = 161.40; $df = 5$; $p < 0.001$), we also detected a significant interaction between seasonal treatment and species (Wald stat. = 134.57; $df = 10$; $p < 0.001$). *Alona rectangula* hatched more successfully at the late spring condition when compared to the early spring condition (Wald stat. = 90.77; $df = 1$; $p < 0.001$) (Fig. 2), but for the summer conditions we lacked reliable data. *Ceriodaphnia reticulata* showed a higher hatching percentage at late spring and summer conditions when compared to early spring conditions (Wald stat. = 31.49; $df = 2$; $p < 0.001$) (Fig. 2). At early spring conditions, a larger proportion of *D. atkinsoni* resting eggs hatched when compared to late spring and summer conditions (Wald stat. = 19.28; $df = 2$; $p < 0.001$) (Fig. 2). Hatching success was higher at the summer condition when compared to both other treatments for *M. rosea* (Wald stat. = 118.67; $df = 2$; $p < 0.001$) and at the late spring treatment for *M. brachiata* (Wald stat. = 26.45; $df = 2$; $p < 0.001$) (Fig. 2). *Simocephalus* and *D. curvirostris* did not show a significantly different hatching response to the seasonal treatments ($p > 0.05$) (Fig. 2). The goodness of fit of each regression was considered adequately high as the ratio of the Pearson χ^2 to its degrees of freedom was close to one.

We found a significant correlation between the hatching percentages of the species at each experimental condition and their abundances in the field at corresponding moments throughout the growing season (Spearman Rank correlation: $r = 0.65$; $p = 0.002$) (Fig. 3). The Spearman Rank correlation coefficient calculated for the different species separately on

average differed significantly and positively from zero (mean $\pm$ S.D. = 0.73 ± 0.25 ; Single sample t-test: $df = 5$, $t = 7.01$, $p < 0.001$).

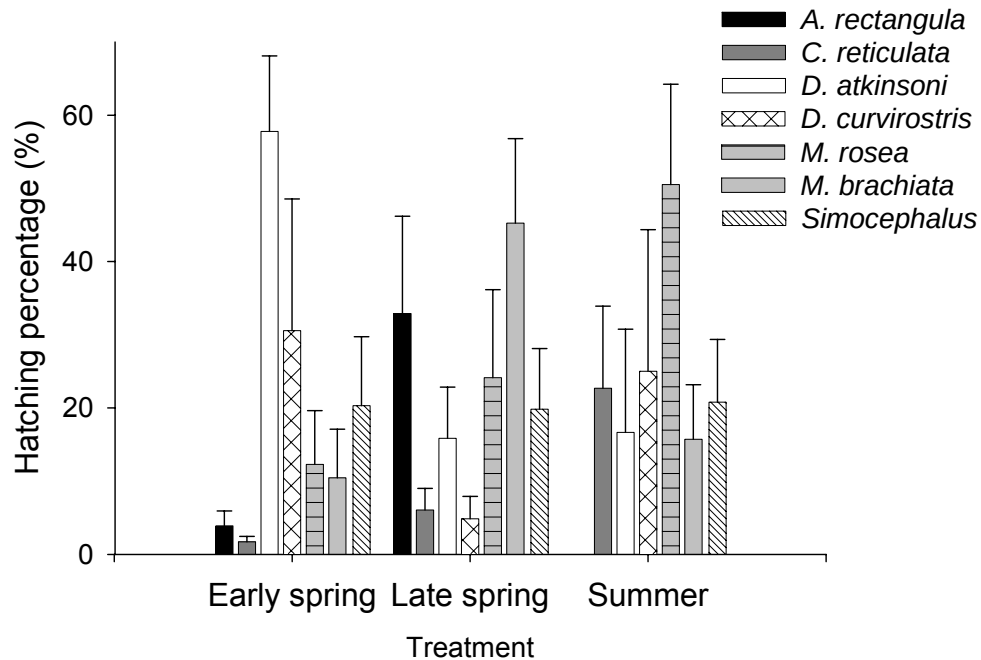


Figure 2. Cumulative hatching percentages (%; mean $\pm$ S.E.) of seven cladoceran species that hatched from the sediment of twelve temporary pools under three different seasonal treatments (early spring: 12°C and 12 h light/12 h dark; late spring: 18°C and 14 h light/10 h dark; summer: 24°C and 17 h light/7 h dark) during a period of 25 days.

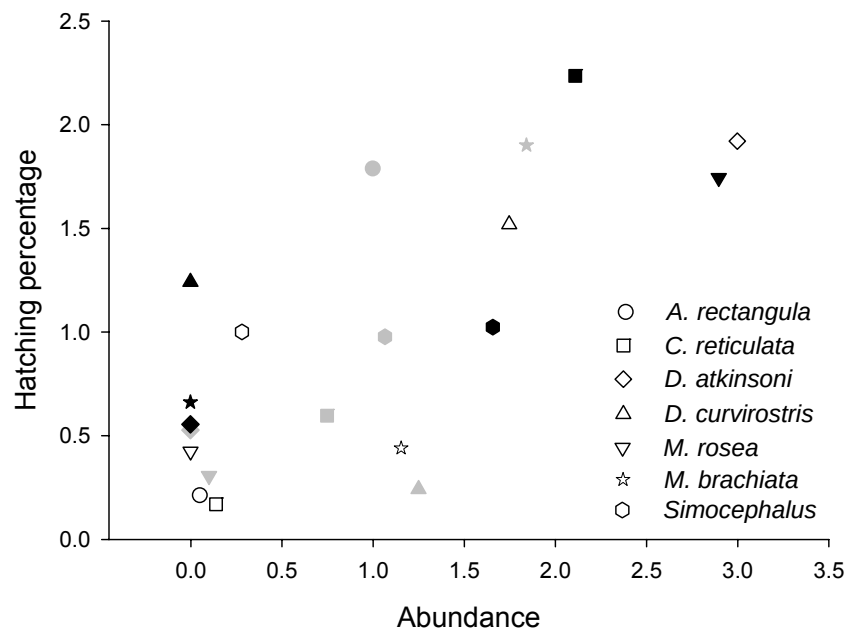


Figure 3. Relationship between standardized hatching percentages of species under different seasonal treatments and their abundances at corresponding moments throughout the growing season in the field (white symbols: early spring; grey symbols: late spring; black symbols: summer). The standardization method is explained in materials and methods.

DISCUSSION

Throughout the hatching experiment, cumulative hatching percentages varied between species and seasonal treatments, with average cumulative percentages per species per treatment ranging from 2% to 60%. Our results showed clear differences between the species in seasonal hatching preferences. Moreover, these hatching preferences were strongly correlated to the abundance patterns of the species observed in the field. *Daphnia atkinsoni*, appearing early in the field (March – April), also hatched more successfully at early spring conditions, while species occurring late in the field, *A. rectangula*, *C. reticulata*, *M. brachiata* and *M. rosea* (April – May or July), showed higher hatching percentages under late spring and/or summer conditions. Only for *D. curvirostris* and *Simocephalus* we did not detect a significantly different hatching response to the seasonal treatments. The differential hatching response of several investigated species, with maximal hatching percentages corresponding to the seasonal timing of their maximal abundance in the field, suggests that species are able to adjust their hatching fractions depending on the quality of the habitat as indicated by temperature and photoperiod. Our observed patterns of adjustment of emergence fractions of cladocerans in response to environmental cues are corroborated by several studies measuring emergence *in situ* as well as in laboratory experiments. In a laboratory experiment where natural conditions of photoperiod and water temperatures of a lake (Oneida Lake, New York) were mimicked during 34 weeks, *Ceriodaphnia* and *Alona* started hatching and reached their hatching peak at late spring and summer conditions, respectively (Cáceres & Schwalbach 2001). In the field, *Daphnia* emerged just before the spring increase in temperature in a lake, while the timing of emergence of *Ceriodaphnia* coincided with the temperature rise (Gyllström 2004). In a pond and a lake, *Ceriodaphnia reticulata* emerged both in late spring and summer (De Stasio 1990). Finally, field emergence rates of *Alona* were positively affected by a rise in temperature above 20°C (Arnott and Yan 2002). Although it is generally not clear to which species these studies exactly refer, the biology and temperature preferences of most species of small-bodied genera like *Alona* and *Ceriodaphnia* might be comparable. An adjustment of emergence fractions in response to temperature, and even to the presence of predators, has also been observed for algae (Hansson 1996, Rengefors et al. 1998) and for large branchiopods (Spencer & Blaustein 2001, De Roeck et al. 2005).

As many cladocerans, however, have been observed to hatch synchronously early in the growing season, changes in their abundance later in the season are assumed to be driven by seasonal processes in the water column rather than to result from seasonal emergence patterns (Wolf & Carvalho 1989, Cáceres 1998, Hairston et al. 2000, Gyllström 2004). Our results suggest that in temporary pools, emergence from dormant egg banks may contribute more to seasonal dynamics of the active populations than is generally assumed. If

preferential hatching behaviour of species to seasonal cues, as observed in our study, results in differential emergence peaks of species throughout the season, this could cause or contribute to the observed seasonal changes in population size and community composition. Some observations from permanent ponds and lakes also suggest a more important link between dormant egg bank and active community dynamics. Two subsequent hatching peaks have already been observed throughout the growing season for *Ceriodaphnia* (De Stasio 1990, Arnott & Yan 2002). The increase in hatching of *Ceriodaphnia reticulata* also contributed to its increase in abundance in the pond (De Stasio 1990). In a field study on zooplankton emergence, it was suggested that changes in the community composition in a lake from one year to another were due to environmental changes in water level, temperature and light conditions that differentially affected the emergence of species (Arnott & Yan 2002).

A species-specific hatching response to environmental cues might be an adaptive response to the species-dependent suitability of conditions for growth and recruitment throughout the season, allowing species to mainly initiate active populations when their relative fitness is expected to be high, as predicted under temporal habitat selection. We observed a significant positive correlation between hatching preferences and abundance patterns of cladoceran species in the field. For the current study systems, we have no other data besides population densities to assess seasonal species performance in the field. However, we cannot unequivocally conclude whether increased population sizes in the field are a direct result of increased population growth rate (i.e. a high relative fitness) of species or of increased numbers of hatchlings. Literature data, though, provide further evidence that our field observations may reflect optimal conditions for population growth. The competitive ability of large-bodied zooplankton species is adversely affected by elevated temperatures (Moore et al. 1996). In experiments, seasonal community shifts from large-bodied zooplankton like *Daphnia* to smaller species such as *Ceriodaphnia* were attributed to changes in their competitive ability with increasing temperatures (Lynch 1978) or concomitant changes in algal quality (C:P ratio; Steiner 2003). In our study systems, the large-bodied *Daphnia atkinsoni* has its highest abundance in early spring (Fig. 1) and disappears almost completely from the water body of the investigated pools later during the growing period. Hatching of *D. atkinsoni* was favored by early-spring conditions, even though a considerable amount of eggs still hatched under late-spring and summer conditions (but see below). Both *Moina brachiata* and *Macrothrix rosea* are reported as thermophilous species (Flößner 2000). In laboratory experiments, *M. brachiata* effectively showed its highest reproductive success at 25 and 30°C when compared to lower temperatures (Maier 1993). From our field samples (Fig. 1), it shows that both species do indeed have a maximal field density under late-spring and summer conditions. Both species preferably hatched at

late spring or summer conditions in our hatching experiment. These observations provide some (albeit circumstantial) evidence that conditions with maximal hatching response are associated with seasonal conditions providing optimal growth conditions for the respective species in the field and support the idea that temporal habitat selection does occur in our study pools.

Although *D. atkinsoni* disappeared almost completely from the water column under late-spring and summer conditions, a proportion of the incubated eggs still hatched under these conditions. In addition, most other species showed preferential hatching behavior dependent on the seasonal conditions, but all of them nevertheless hatched under all seasonal treatments, including the early spring treatment. Likewise, these species recolonized the pools in the field within two to four weeks after snow melt although they mostly remained less abundant early in the growing season (Fig. 1; Chapter 4). These observations indicate that a fraction of the egg bank of each species may hatch at any season, independent of the actual conditions, and suggest that species may also apply some risk-spreading strategy within one growing period as opposed to risk spreading over different growing periods. Indeed, given that seasonal hatching cues are only partly predictive of future reproductive success, spreading the risk by enabling a (small) proportion of the eggs to hatch under all conditions suitable, though possibly not optimal, for growth and reproduction may further increase the chance of a species to persist in a habitat exposed to variable environmental conditions.

Conclusion

For several cladoceran species from temporary pools, maximal hatching was observed under experimental conditions mimicking conditions prevailing in the field when their active population size was also peaking. This pattern is suggestive of an adaptive response to the natural variability in growth and recruitment conditions, allowing species to initiate populations at optimal conditions and may be indicative of temporal habitat selection. Temporal habitat selection, which implies that genotypes or species are able to adjust the timing of hatching depending on environmental conditions indicative of a high relative fitness, may be an important mechanism facilitating temporal niche differentiation and the maintenance of species diversity in communities. Indeed, in variable environments, the role of prolonged diapause in promoting the coexistence of species (Hairston 1996, Cáceres 1997b) may be more considerable when a mechanism of habitat selection is involved. Our results suggest that the contribution of emergence from dormant egg banks to active community dynamics may be more important than often assumed. Our conclusions, however, should be further confirmed using long-term *in situ* monitoring of emergence and/or long-term laboratory hatching experiments.

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CHAPTER 7

DORMANT EGG BANK ANALYSIS FOR CLADOCERAN SPECIES RICHNESS ASSESSMENT IN TEMPORARY POOLS

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Unpublished manuscript

Abstract The use of dormant egg bank analysis has proven to be an efficient method for cladoceran species richness assessment in permanent lakes. In order to evaluate its efficiency in temporary pools, we compared the cladoceran species richness of 12 temporary freshwater pools in a rather pristine area in the south of Hungary assessed through active community sampling as well as dormant egg bank analysis. Active snap-shot samples were collected at repeated occasions throughout one growing season (March – July). To assess species composition in the dormant egg bank, we exposed dormant eggs in the laboratory to three treatments of temperature and covarying light conditions (12°C and 12h light/12h dark; 18°C and 14h light/10h dark; 24°C and 17h light/7h dark) during one month and identified hatchlings collected at repeated occasions. Using both methods, we encountered a total of 33 cladoceran species. Only one species, *Bunops serricaudatus*, exclusively hatched in the laboratory and was never observed in the field. Fourteen species were only observed in the field, but never hatched. For a single pool, the total number of species detected from three active community samples collected at subsequent or random moments throughout the season (11.4 ± 0.8 and 11.6 ± 0.7 , respectively) was on average significantly higher than the species number assessed using dormant egg banks (8.4 ± 0.9). When collecting active community samples at moments maximally spread in time, two samples were already more effective (11.3 ± 0.8) than dormant egg bank analysis. Finally, for a single pool also more species were exclusively found in the field (7.3 ± 0.8 versus 1.3 ± 0.4). The lack of integration of temporal variation in the hatched assemblages and low efficiency of dormant egg bank analysis for species richness assessment when compared to permanent lakes may be related to low densities of heterogeneously distributed ephippia and the relatively low hatching success of temporary pool cladocerans. In order to reliably estimate local cladoceran species richness in shallow temporary pools, biodiversity monitoring schemes should preferably make use of active community samples collected at different moments spread in time (e.g. early, middle and late inundation stages).

Key Words active community, cladocerans, diversity estimation, dormant community, hatching

INTRODUCTION

Single snap-shot samples of the active component of cladoceran communities are usually not sufficient to detect their characteristic temporal and spatial dynamics (Sommer et al. 1986, Burks et al. 2002), which may eventually result in an underestimation of the local species pool. The identification of hatchlings obtained from dormant egg banks has therefore been suggested as a valuable tool for the estimation of cladoceran species richness in permanent aquatic systems (Vandekerkhove et al. 2005a). Dormant propagule banks indeed integrate temporal and spatial variation in community structure and their analysis therefore may be more reliable than sampling the active community, even at repeated occasions (Vandekerkhove et al. 2005a). This was effectively demonstrated in a comparative study of 88 European lakes, where the identification of hatchlings obtained from egg bank samples enabled the detection of about twice as many species than identification of six subsequent active community samples (Vandekerkhove et al. 2005b). Both methods were complementary, however, as some species were only detected by one of the two methods. Also in Missouri floodplain ponds (Havel et al. 2000) and in a Brazilian reservoir (Crispim & Watanabe 2001), more cladoceran species were detected after hatching sediment than in the water column at three or more sampling occasions.

Like in permanent lakes, the use of dormant egg banks may be a cost-efficient alternative method for diversity estimations in temporary freshwater pools as it is less time consuming and less expensive than active sampling (Vandekerkhove et al. 2005b). Additionally, the recurrent drying of temporary pools, either predictable or unpredictable, may often practically impede sampling of the active communities (e.g. Angeler et al. 2008b). Furthermore, seasonal community dynamics may strongly differ depending on the duration of inundation of the pools and interfere with diversity estimates especially when these are based on single community snap-shots (Chapter 4). The efficiency of dormant egg bank analysis in temporary pools has not been assessed so far.

In the current study, the efficiency to determine cladoceran species richness in temporary pools on the basis of dormant egg banks is compared with richness estimations on the basis of repeated active community samplings. For this study, we selected a total of twelve temporary freshwater pools in Kiskunság, a rather pristine area in the south of Hungary.

MATERIALS AND METHODS

Study Area

The Kiskunság National Park, covering an area of almost 500 km², is located 85 km southwest of Budapest (Hungary) in the Kiskunság area. Within this area, 12 temporary pools, equally distributed over three pool clusters and with a variable length of inundation,

were selected for this study. Pools were divided into three classes based on their absolute hydroperiod (number of weeks as observed in the spring of 2005): short (<10 weeks; $n = 4$), medium long (10-13 weeks; $n = 4$) and long hydroperiod class (>16 weeks; $n = 4$). For a detailed description of the pool clusters, we refer to Boven et al. (2008a; Chapter 3).

Sampling of active communities

Sampling of the active communities started at the end of March 2005, two weeks after inundation by snow melt. Pools were initially sampled every two weeks (March – May) and thereafter monthly (June – July) in a random order. In July, when most pools were dry, sampling stopped. The most long-lived pools were consequently sampled seven times (T_1 : end of March; T_2 : early April; T_3 : end of April; T_4 : early May; T_5 : end of May; T_6 : June; T_7 : July).

At each sampling occasion, cladocerans were sampled quantitatively by collecting an integrated volume of pool water (50 l at the first and second sampling occasion; 100 l at all other occasions). Water was collected with a measuring cup from four peripheral and four central locations and poured over a plankton net (mesh size: 64 μm). Qualitative samples were collected by searching all microhabitats with a 64- μm plankton net. Collected animals were preserved in 70% ethanol and identified to species level using Flößner (2000). From each quantitative sample, subsamples were processed until 300 specimens were counted. Quantitative samples containing less than 300 specimens were supplemented with corresponding qualitative samples. From these we identified an additional number of specimens either up to 300 or until all collected animals were checked (when less than 300 specimens were present).

Dormant egg bank analysis

The dormant egg banks were sampled when the pools were dry (spring 2007) by collecting the upper 3 cm of sediment from 18 random locations (10 x 10 cm^2) representative of all microhabitats (depth, vegetation type) present in the pool. Sediment with ephippia (i.e. the protective case in which one or two eggs, depending on the species, are contained) was dried and stored for three months in the dark at room temperature. Next, ephippia were isolated from the sediment (2.4 - 6.0 kg; average 4.3 kg per pool) using the sugar flotation method (Onbé 1978, Marcus 1990). Resting egg isolation has been shown to increase overall hatching success (Vandekerckhove et al. 2004b). For each pool, the harvested ephippia were next divided over three subsamples. Each subsample was exposed to one of three conditions of temperature with covarying photoperiod (12°C and 12h light/12h dark; 18°C and 14h light/10h dark; 24°C and 17h light/7h dark). The ephippia were inundated with 1.5 L of EPA medium (Anonymous 1985; initial conductivity of 800 $\mu\text{S cm}^{-1}$ and continuously

aerated) in 2-L aquaria that were placed in incubators with the respective temperature and photoperiod settings. During a period of about one month, newly emerged hatchlings were isolated from the aquaria at repeated occasions (resp. 2, 6, 12, 18, 21 and 26 days after inundation). Two weeks has been suggested as a minimum incubation period for hatching experiments aimed at species richness assessment (Vandekerckhove et al. 2005a). They were grown in 100-mL vials filled with the medium from which they hatched and were fed with a few drops of *Scenedesmus obliquus* algae every other day. Once they reached maturity, they were identified to species level using Flößner (2000). The total number of hatchlings that was retrieved from the egg sample of a single pool ranged from 11 to 150 with an average of 72 specimens. In this calculation, species that presumably reproduced during the inundation period were not taken into account. We acknowledge that, as we inundated the dormant eggs only once, the local species pool might be underestimated if some species delay their hatching throughout different inundations.

Data analysis

We first assessed whether an incubation period of 26 days was effective in detecting most species. We performed Repeated Measures ANOVA on the cumulative number of detected species per pool with the number of days after the start of inundation as repeated factor. By including incubation condition as an additional repeated factor in this analysis, we were able to compare the total number of species that hatched from the egg bank of the pools between the three experimental treatments and to look for any interactions with time. The first sampling was not included in the analysis, as for the 12°C treatment no species hatched until six days after inundation, resulting in a lack of variance. Repeated Measures ANOVA was performed in Statistica 8.0 (Statsoft Inc. 2007). Assumptions of normality and equal variance of the data were met.

Next, we compared the efficiency of dormant egg bank analysis for species richness estimation with repeated sampling of the active communities. Previous analyses pointed out that there was a significant difference in species richness and composition of the assemblages that hatched under different experimental conditions. Given also the low overall hatchling abundance, we considered the total number of species that hatched at all three experimental treatments together as the dormant community of a single pool. Furthermore, for each pool, we virtually created 'pooled' samples of the active communities by pooling the communities encountered in each of the 'snap-shot' samples. Snap-shot samples from different sampling occasions were first pooled chronologically (Table 1). Next, the order in which samples were pooled was randomized in Excel (Table 2). Finally, snap-shot samples were pooled according to a pre-determined scheme in which the time between subsequently pooled snap-shot samples was maximized (Table 3). According to the duration of inundation

and thus the number of collected snap-shot samples from each pool, the number of chronologically pooled samples and the number and composition of randomly and schematically pooled samples differed between pools. For each snap-shot ($n = 7$) and pooled sample ($n = 3 \times 6$) we calculated the local species richness and compared these with the number of species that hatched from the dormant egg banks, using t-tests for dependent samples (Statistica 8.0). We also used a t-test to compare the average number of species that was exclusively found during the field sampling throughout the whole inundation cycle of a single pool and from dormant egg bank analysis.

Finally, we tested whether the similarity in species composition between the dormant and active communities increased if more than one active sample was considered, as would be expected given that dormant egg banks integrate temporal variation in zooplankton communities (Vandekerkhove et al. 2005a). Therefore, we compared the average similarity in community composition (Bray Curtis similarity index based on the presence/absence of species; Primer 5; Clarke & Warwick 2001) between the dormant egg bank assemblage and, respectively, active snap-shot and chronologically pooled samples, using t-tests for dependent samples. Chronologically pooled samples are expected to be the less efficient method to integrate temporal variation and thus to give a minimum estimate of integration of variation when compared to both other pool schemes. As the similarity in active and dormant community composition did not change significantly throughout the growing season (for snap-shot samples) or as more active samples were pooled (for pooled samples), we averaged all similarity indices of active and pooled samples for a single pool. Assumptions of normality and equal variance of the data were met.

Table 1. Schematic overview of the subsequent samples taken from the active cladoceran community (snap-shot samples) and the way they were combined into chronologically pooled samples. In brackets, we indicated the number of pools that were inundated.

	Snap-shot samples	Pooled samples - chronological					
March	T ₁ (n = 12)						
Early April	T ₂ (n = 12)	AC2					
Late April	T ₃ (n = 12)		AC3				
Early May	T ₄ (n = 11)			AC4			
Late May	T ₅ (n = 7)				AC5		
June	T ₆ (n = 4)					AC6	
July	T ₇ (n = 4)						AC7

Table 2. Schematic overview of the subsequent samples taken from the active cladoceran community (snap-shot samples) and the way they were combined into randomly pooled samples for a pool that did not dry until July (T₇; left) or until early May (T₄; right).

Snap-shot samples	Pooled samples – random – long hydroperiod pool						Snap-shot samples	Pooled samples – random – short hydroperiod pool		
T ₆							T ₄			
T ₄	AC2						T ₂	AC2		
T ₂		AC3					T ₃		AC3	
T ₇			AC4				T ₁			AC4
T ₃				AC5						
T ₅					AC6					
T ₁						AC7				

Table 3. Schematic overview of the subsequent samples taken from the active cladoceran community (snap-shot samples) and the way they were combined into schematically pooled samples for a pool that did not dry until July (T₇; left) or until early May (T₄; right).

Snap-shot samples	Pooled samples – schematic – long hydroperiod pool						Snap-shot samples	Pooled samples – schematic – short hydroperiod pool		
T ₁							T ₁			
T ₇	AC2						T ₄	AC2		
T ₄		AC3					T ₂		AC3	
T ₂			AC4				T ₃			AC4
T ₆				AC5						
T ₃					AC6					
T ₅						AC7				

RESULTS

We encountered a total of 32 cladoceran species in the active community of the 12 temporary study pools throughout one growing season (Table 4). In the laboratory, a total of 19 species hatched from the dormant egg bank of the same pools. Overall, only one species that hatched from the dormant egg bank (*Bunops serricaudatus*) was never observed in the field. Fourteen species that were observed in the field throughout the growing season did not hatch in the laboratory (Table 4).

Table 4. Overview of the cladoceran species encountered in the active communities throughout one growing season (AC) and/or hatched from the dormant egg bank (DEB) of the study pools.

		AC	DEB
1	<i>Diaphanosoma brachyurum</i> (Liévin, 1848)	+	
2	<i>Macrothrix hirsuticornis</i> Norman et Brady, 1867	+	
3	<i>Macrothrix rosea</i> (Jurine, 1820)	+	+
4	<i>Bunops serricaudatus</i> (Daday, 1884)		+
5	<i>Moina brachiata</i> (Jurine, 1820)	+	+
6	<i>Moina micrura</i> Kurz, 1875	+	
7	<i>Megafenestra aurita</i> (Fischer, 1849)	+	
8	<i>Scapholeberis mucronata</i> (O.F. Müller, 1776)	+	+
9	<i>Scapholeberis rammneri</i> Dumont et Pensaert, 1983	+	+
10	<i>Simocephalus exspinosus</i> (Koch, 1841)	+	+
11	<i>Simocephalus vetulus</i> (O.F. Müller, 1776)	+	+
12	<i>Daphnia atkinsoni</i> Baird, 1859	+	+
13	<i>Daphnia magna</i> Straus, 1820	+	+
14	<i>Daphnia curvirostris</i> Eylmann, 1887	+	+
15	<i>Daphnia longispina</i> (O.F. Müller, 1776)	+	
16	<i>Ceriodaphnia affinis</i> Lilljeborg, 1901	+	
17	<i>Ceriodaphnia laticaudata</i> P.E. Müller, 1876	+	+
18	<i>Ceriodaphnia reticulata</i> (Jurine, 1820)	+	+
19	<i>Eurycercus lamellatus</i> (O.F. Müller, 1776)	+	
20	<i>Chydorus sphaericus</i> (O.F. Müller, 1776)	+	+
21	<i>Pleuroxus aduncus</i> (Jurine, 1820)	+	+
22	<i>Pleuroxus laevis</i> Sars, 1861	+	
23	<i>Alonella excisa</i> (Fischer, 1854)	+	+
24	<i>Dunhevedia crassa</i> King, 1853	+	+
25	<i>Alona guttata</i> Sars, 1862	+	
26	<i>Alona rectangula</i> Sars, 1861	+	+
27	<i>Kurzia latissima</i> (Kurz, 1875)	+	
28	<i>Acroperus harpae</i> (Baird, 1835)	+	
29	<i>Graptoleberis testudinaria</i> (Fischer, 1848)	+	
30	<i>Oxyurella tenuicaudis</i> (Sars, 1862)	+	+
31	<i>Tretocephala ambigua</i> (Lilljeborg, 1901)	+	+
32	<i>Polyphemus pediculus</i> (Linné, 1761)	+	
33	<i>Bosmina</i> Baird, 1846	+	
Total species richness:		32	19

The number of species that hatched from the dormant egg bank significantly increased throughout the incubation period ($F_{(4, 44)} = 53.40$, $p < 0.001$) (Fig. 1). There was also a significant difference in the number of species that hatched at the different treatments (Repeated Measures ANOVA: $F_{(2, 22)} = 7.91$; $p = 0.003$) (Fig. 1). On average, a higher species number was retrieved at 18°C (mean $\pm$ S.E.: 6.3 ± 0.7) than at 24°C (5.3 ± 0.6) or 12°C (4.8 ± 0.8), but only the difference between 18°C and 12°C was significant (post-hoc Tukey HSD: $p = 0.002$). Finally, also the interaction effect between incubation condition and time after inundation was significant ($F_{(8, 88)} = 4.74$, $p < 0.001$). The detected species number significantly increased until 12 days after the start of incubation at 18°C and 24°C and until 18 days after the start of incubation at 12°C (post-hoc Tukey HSD: $p > 0.05$).

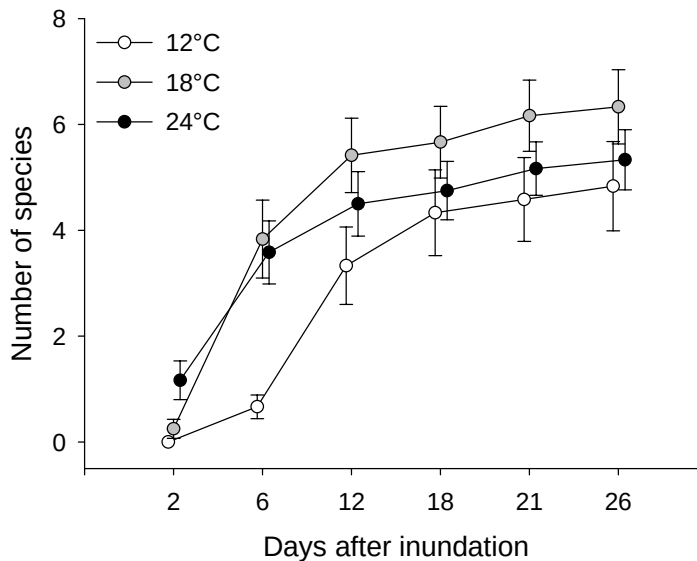


Figure 1. Cumulative number of species (mean $\pm$ S.E.) that hatched from the dormant egg bank in function of time (number of days after inundation) for each incubation treatment.

Local species richness as assessed using dormant egg banks did not differ significantly from the local species richness in any of the active snap-shot samples (Fig. 2a) or from pooled samples derived from two chronologically or randomly pooled samples (Fig. 2b), but was significantly lower than the species number assessed from all other numbers of the different types of pooled samples (Fig. 2b) (Table 5). The number of species exclusively found using one of both methods was on average significantly higher for the cumulative field sampling (7.3 ± 0.8) than for the dormant egg bank analysis (1.3 ± 0.4) ($t = 6.18$; $df = 11$; $p < 0.001$). Species that were most often exclusively found in the field were *A. excisa* (12 pools), *D. longispina* (ten pools), *S. rammneri* (six pools), *S. mucronata*, *A. guttata*, *G. testudinaria* and *P. pediculus* (each in five pools). Species that could most often be added to the species

lists by hatching the dormant community included *M. brachiata* (three pools), *A. rectangula*, *C. laticaudata* and *M. rosea* (each for two pools).

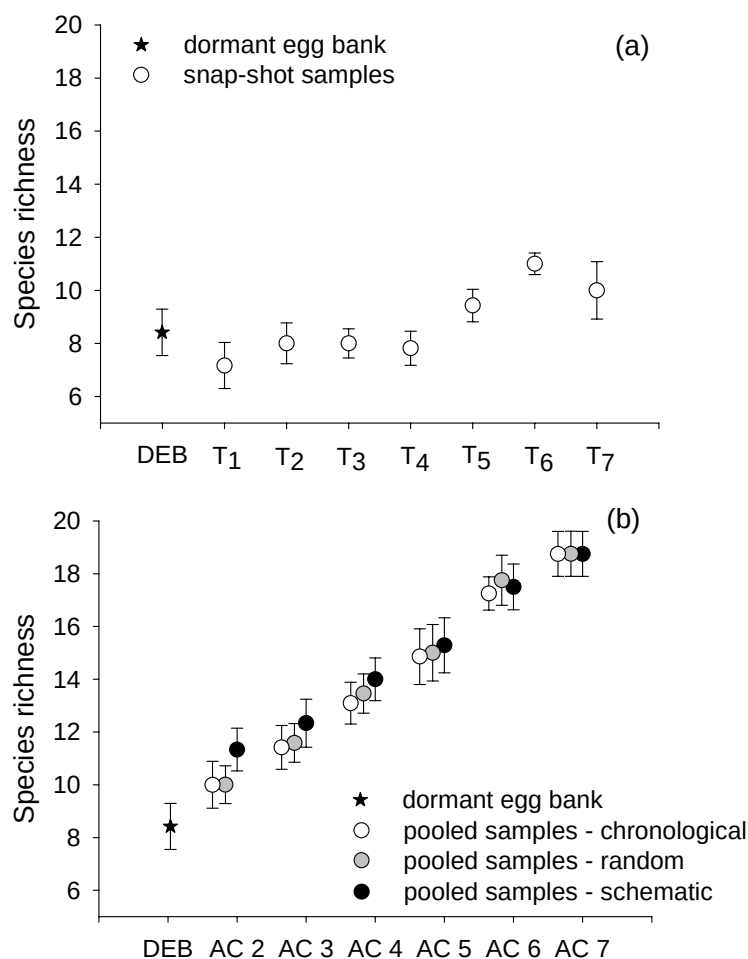


Figure 2. Average local species richness (mean $\pm$ S.E.) of the 12 study pools as assessed using dormant egg bank analysis (DEB) and active community snap-shot samples (a) or pooled samples (b). The composition of pooled sampled is explained in Tables 1, 2 and 3.

Table 5. Results of t-tests for dependent samples (degrees of freedom, t - and p -value) comparing the number of species that hatched from the dormant egg bank of a pool with the local species richness of subsequent active community snap-shot samples and chronologically, randomly or schematically pooled samples.

Snap-shot samples				Pooled samples							
				Chronological				Random		Schematic	
	<i>df</i>	<i>t</i>	<i>p</i>		<i>df</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
T ₁	11	1.20	0.25	AC2	11	-1.91	0.08	-1.97	0.07	-4.16	0.002
T ₂	11	0.55	0.60	AC3	11	-3.85	0.003	-4.03	0.002	-4.77	<0.001
T ₃	11	0.50	0.63	AC4	10	-4.92	<0.001	-4.68	<0.001	-6.42	<0.001
T ₄	10	1.09	0.30	AC5	6	-4.50	0.004	-4.78	0.003	-5.60	0.001
T ₅	6	0.62	0.56	AC6	3	-4.90	0.02	-4.39	0.02	-6.48	0.007
T ₆	3	-0.58	0.60	AC7	3	-5.99	0.009	-5.99	0.009	-5.99	0.009
T ₇	3	1.00	0.39								

The average similarity in species composition with the dormant cladoceran community was not significantly different for active snap-shot (Bray Curtis similarity index: 58.3 ± 2.0) or chronologically pooled community samples (62.6 ± 3.7) ($p > 0.05$).

DISCUSSION

We evaluated the assessment of species richness in the dormant fraction of cladoceran communities in temporary pools of Kiskunság as an alternative method to frequent sampling of the active communities to properly cover temporal and spatial variation. Incubation of the dormant egg bank at a single condition of intermediate temperature and photoperiod (18°C; 14h light/10h dark) allowed the detection of a maximum number of species for a single pool. Based upon similar findings in hatching experiments of cladocerans in various shallow lakes throughout Europe, Vandekerkhove et al. (2005a) likely suggested an intermediate temperature of 15°C for hatching experiments aimed at assessing cladoceran species richness. Though, given the significantly different species assemblages that hatched under different conditions in our study, together with the low overall hatchling abundance, we considered the total cladoceran assemblage that hatched under the three experimental conditions to detect a maximum number of species. Analogously to Vandekerkhove et al. (2005a), we found that an inundation period of almost one month probably allowed the detection of most species.

Yet, the analysis of the dormant egg banks for species richness assessment of cladocerans did not prove an efficient alternative method to active community samples in our temporary study pools. Overall, we encountered a total of 33 cladoceran species in all 12 study pools using both methods, but only one of these species was never found in the field (*Bunops serricaudatus*). The dormant community of a single pool was on average not more similar to active communities from subsequent samples that were pooled than to single community snap-shots, which is indicative of a lack of integration of seasonal variation in the hatched cladoceran assemblage. The total number of species that was detected from three or more subsequent or randomly collected samples of the active community (three samples: 11.4 ± 0.8 and 11.6 ± 0.7 , respectively) was considerably higher than the species number assessed using dormant egg banks (8.4 ± 0.9). When considering samples that were taken at moments spread maximally in time, two samples already detected more species (11.3 ± 0.8) than dormant egg bank analysis. Finally, for a single pool also more species were exclusively found in the field (on average about 7) than detected after incubation of the dormant eggs under three different conditions of temperature and photoperiod (on average about one). Also in South-African temporary wetlands, dormant egg bank analysis was not an effective method for cladoceran species richness estimation and significantly more species were found in one or two active community samples (E. De Roeck, unpublished

data). In a comparative study of 88 European lakes, on the other hand, identification of hatchlings obtained from egg bank samples enabled the detection of about twice as many species than identification of six subsequent active community samples (Vandekerkhove et al., 2005b). Both methods were complementary as some species were only detected by one of the two methods. Our results are also contrary to findings of Havel et al. (2000) and Crispim & Watanabe (2001) who detected more species after incubation of the dormant egg banks than in the water column.

The low efficiency of dormant egg bank analysis as a method for the estimation of species richness in our study pools may have a number of causes. First, egg densities were low and ephippia probably heterogeneously distributed. We measured average densities of merely one ephippium (*Daphnia*) to 30 (*Ceriodaphnia*) or 126 (*Alona*) ephippia per kg dry sediment (equivalent of about 0.1 m²). Resting egg densities in lakes are usually high (e.g. Carvalho & Wolf (1989): 1.1-12.0 x 10⁴ eggs/m²; Cáceres (1998): 2.5-8.0 x 10⁴ eggs/m² for *Daphnia*), but in shallow pools egg production may be lower due to the small size of active zooplankton populations. Furthermore, the horizontal distribution of resting eggs is patchy (Thiéry 1997) and the presence of extensive macrophyte beds may further hamper a homogenous distribution of the deposited ephippia through the limitation of resuspension, for example through wind, as also suggested by Vandekerkhove et al. (2005a). Additionally, ephippia are progressively exposed to wind erosion and organic decomposition during the dry stage, which may result in the loss of a considerable fraction of the ephippia. For the mentioned reasons, a limited number of collected sediment samples from shallow, temporary pools with extensive vegetation growth may contain low amounts of ephippia and the hatching procedure may result in an underestimation of the local species pool.

Low hatching fractions or species-specific hatching requirements may further contribute to an inefficient detection of temporary pool species. Throughout our hatching experiment, maximal hatching fractions of species ranged between 20% and 60% (Chapter 5). The number of hatchlings retrieved from the dormant egg bank was low (1-51 and on average 16 per kg (dry) sediment), especially when compared to the study of Vandekerkhove (2004; 7-2518 per kg (wet) sediment). Low hatching frequencies of permanent inhabitants of temporary pools in general may serve as a bet-hedging adaptation to the unpredictability in duration and frequency of filling events of the habitat (Simovich & Hathaway 1997, Brendonck et al. 1996, Brendonck & Riddoch 2002, Philippi et al. 2001). Furthermore, species-specific hatching requirements related to climatic conditions explained the highly erratic occurrence patterns of temporary pool anostracans throughout different years in Canadian temporary pools (Donald, 1982). In our study pools, for several cladocerans the hatching response to temperature and photoperiod conditions was highly variable and species-specific (chapter 5). We effectively observed a significant difference in

species composition of the assemblages that hatched between the three experimental treatments. We partly corrected for such species-specific hatching response as species richness of the dormant community of the study pools was assessed from the total species pool that hatched at three different conditions.

Concluding, we acknowledge that as we did not standardize the number of individuals identified for each of both methods, the high number of individuals that was identified from the active samples when compared to the low number of hatchlings that was retrieved during the experiment most likely resulted in a bias in species richness towards the active sampling method. This disparity most likely resulted from low resting egg densities and hatching success and can therefore be considered as an inherent shortcoming to the use of dormant egg bank analysis in our study pools. The results of our study indicate that the identification of hatchlings retrieved from dormant egg banks may not be equally effective in all types of temporary pools. Biodiversity monitoring schemes in shallow temporary pools should preferably make use of active community samples in order to reliably estimate local cladoceran species richness. In doing so, active community samples should preferably be collected at different moments spread in time such as in an early, middle and late inundation stage.

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GENERAL DISCUSSION AND CONCLUSIONS

In a European context, there is growing concern that any further extension of drainage practices and more intensive cultivation of the Eastern European landscape will negatively affect the relatively high levels of habitat and species diversity that still characterize many of its farmland areas. The protection of relatively small freshwater wetlands such as ponds and temporary pools could play a major role in the protection of landscape integrity and regional biodiversity in these areas. Until now there is a lack of studies monitoring the levels of biodiversity that currently characterize small freshwater wetlands in Eastern European countries (Biggs et al. 2004, Oertli et al. 2005) or that contribute to a better understanding of their functioning and the factors structuring their inhabiting communities. Fundamental and applied ecological studies are needed to formulate effective management and conservation strategies for these valuable habitats. Our study aimed at filling some of these gaps, and especially focused on ecological processes that structure keystone invertebrate communities through space and time in a set of pristine temporary pools in Kiskunság (Hungary).

Temporary freshwater pools in Kiskunság as a “hot spot” of branchiopod diversity

According to the criteria followed by Petrov & Cvetković (1997) and Timms & Sanders (2002), temporary freshwater pools in Kiskunság can be defined as “hot spots” of large branchiopod diversity (Chapter 1). This conclusion was based on our observations, in comparison with available literature information, that more than half (11 species; 58%) of the known Hungarian large branchiopod fauna was found in an area of about 1800 km² (2% of Hungary’s total surface area) and that co-occurrence was common (31% of all large branchiopod habitats), even among anostracan species (21% of all large branchiopod habitats). Our observed pattern contrasts with the decline in large branchiopod diversity observed throughout Central and Western Europe during the past decades (Brendonck 1989, Maier 1998, Williams 1997, Williams et al. 2001, Eder & Hödl 2002). Similar criteria can be applied to cladoceran diversity. In a subset of merely 18 pools, located in an even smaller area ($\pm$ 200 km²), we encountered 34% of the known Hungarian cladoceran fauna. Local species richness ranged from eight to 19 species, indicating not only frequent, but also high levels of co-occurrence. With a regional species pool of 33 species, our study pools were furthermore amongst the most species rich temporary freshwater habitats with respect to Cladocera when compared to a total of 36 studies carried out worldwide

(Chapter 2). The high levels of diversity of both small and large branchiopods observed throughout our study underline the considerable nature and conservation value of the temporary freshwater pools in Kiskunság and stress their need for protection.

Ecological processes structuring temporary pool communities through space

The high regional species richness of large branchiopods was established by high habitat diversity (Chapter 1). Habitats with contrasting local environments, particularly vegetated meadow pools and small, turbid wheel tracks, were characterized by distinct large branchiopod assemblages. Both habitat types contributed considerably to the regional large branchiopod species pool through the presence of rare and exclusive species. Especially habitats such as wheel tracks and puddles are often overlooked and especially vulnerable to urbanization (e.g. road hardening). We have shown that they house taxa in need of conservation. Cladocerans were only sampled in meadow pools and clay pits. Habitat diversity also contributed to regional cladoceran diversity. A comparative literature study indicated that the high level of regional cladoceran species richness we observed in the pools was likely due to the high degree of variation in habitat size and duration (Chapter 2).

The significant independent effect of local habitat characteristics on the presence of the most common large branchiopod species (Chapter 1) indicated that the local environment may have acted as a selective force, resulting in efficient species sorting (cf. Leibold et al. 2004). Our results hereby confirm findings in previous studies on aquatic invertebrate metacommunities providing empirical evidence for a species sorting view, in which species distributions are closely linked to local conditions: Cottenie et al. (2003; highly interconnected permanent ponds), Vanschoenwinkel et al. (2007) and Jocqué (2007; temporary rock pools) and Waterkeyn et al. (2008; temporary pools).

Species sorting might explain the observed association of large branchiopod species with contrasting habitats (Chapter 1). Predation risk by invertebrates such as *Notonecta*, Odonata and beetle larvae in densely-vegetated pools and the short duration and unpredictable nature of inundation of the smallest and shallowest habitats probably were the main factors affecting colonization success of the different large branchiopod species. Vegetation cover generally is an important characteristic related to the trophic state of shallow water bodies (clear water – turbid state equilibrium; Scheffer 1993) and has previously been shown to affect zooplankton community structure of shallow lakes (Declerck et al. 2005), permanent ponds (Cottenie et al. 2001) and temporary pools (Jocqué 2007, De Roeck 2008). Our study confirms the importance

of macrophytes as a main habitat characteristic structuring local communities of branchiopods in temporary pools.

We also found evidence of spatial variables (geographical position) determining the large branchiopod species distribution. The extent to which the observed spatial pattern was due to dispersal limitation or to regional variation in local habitat variables that were not included in our study, however, should be investigated in more detail. Long-term species monitoring should therefore be combined with a landscape study that more accurately quantifies the degree of habitat connectivity through various potential dispersal pathways (e.g. over-land distance to all other pools in the area, water connections) and characterizes a maximum number of environmental gradients (e.g. land use). Such study would be especially relevant when contrasted to patterns observed in highly fragmented areas where dispersal limitation is effectively expected (e.g. Flanders, different parts of Western Europe). In protected areas such as the Kiskunság National Park, where habitat density is high and dispersal vectors such as water, wind and animals are abundant, communities are expected to be highly interconnected. However, depending on the proximity of source populations and the availability of dispersal vectors, the relative importance of dispersal limitation is expected to change. The degree of connectivity between habitats might therefore be related to anthropogenic disturbance through habitat loss and fragmentation (Hanski 1999, Lehtinen et al. 1999, Cushman 2006). Dispersal limitation may interfere with colonization-extinction dynamics in natural metacommunities, threaten the persistence of populations and eventually result in the loss of species from a region. Therefore, studies investigating species distribution and regional diversity patterns in relation to spatial factors and possible impacts of dispersal limitation in different types of landscapes will be highly relevant for the development of future conservation schemes.

Ecological processes structuring temporary pool communities through time

Intraseasonal variation and the impact of hydroperiod

Studying temporal (seasonal) variation in aquatic systems holding water for a variable duration has many analytical challenges. Besides having more chances for habitat changes to occur, a longer duration of inundation will encompass seasonality effects that might contribute directly or indirectly to any abiotic or biotic habitat variation. Therefore, it remains complex to relate observed patterns of habitat quality and communities dynamics unequivocally to hydroperiod length of the study pools. Even with complex statistical approaches (Chapters 3 and 4), we never succeeded in completely disentangling these confounding effects. When cautiously

interpreting the results, however, our integrated approach may constitute a useful framework also for future studies.

Due to early drying of short hydroperiod pools, we could not compare all inundation phases of pool types at the same time scale. To enable comparisons of pool characteristics we therefore performed ANOVA or ordination analyses according to a relative time scale. For each pool, the inundation period was divided into an equal number of succession stages. The first and the last time a pool was sampled represented an early and late succession stage, respectively. The middle stage was chosen halfway between the early and late stages. In pools that dried up early, the later succession stages therefore corresponded to earlier moments in spring than in pools that dried up later. When for local habitat characteristics (water quality and vegetation cover; Chapter 3) a significant time x hydroperiod interaction was detected for a certain variable, a more reliable interpretation of the pattern was next obtained by performing an absolute time scale analysis for each hydroperiod class individually. For the biological communities (Cladocera; Chapter 4), we tried to minimize confounding effects by considering merely the presence-absence of species and species richness estimations instead of abundances, which are more strongly subjected to seasonality and time effects. Moreover, our relative time scale corrected for any variation in sampling effort between hydroperiod classes (i.e. number of samples taken), which was especially relevant for species richness estimations.

Throughout the growing season, nutrient concentrations and conductivity increased and we observed algae and vegetation growth in the pools (Chapter 3). Oxygen was never depleted and pH changes appeared to be efficiently buffered. In pools with a relatively long hydroperiod, both the longer inundation period and conditions later in the season may have contributed to stronger algal growth and a concomitant increase in amount of suspended solids and turbidity.

As habitat quality changed with time, cladoceran communities became gradually dominated by small and/or plant-associated species on which there was no apparent negative impact of the increasing invertebrate predator community (Chapter 4). In contrast to the dominant pattern of an increase in abundance of a growing number of cladoceran species throughout the season, the large ctenodaphnid *Daphnia atkinsoni* disappeared from the pools early in spring. The presence of large-bodied zooplankton species such as *Daphnia* and anostracans (Chapter 1) exclusively early in the succession of the meadow pools and clay pits, as well as their overall small contribution to the branchiopod communities, suggest that size-selective predation may be effective in these pools, even early in the growing season. It is often assumed that temporary pools are 'enemy-free' due to the lack of fish predators. However, studies have demonstrated severe predation by invertebrates or amphibians in temporary

aquatic systems (Woodward & Kiesecker 1994, Brendonck et al. 2002, De Roeck et al. 2005, Boix et al. 2006), effectively structuring prey communities (Blaustein et al. 1995, 1996, Arner et al. 1998). *Notonecta* has even been demonstrated to act as a size-selective predator (Arner et al. 1998, Steiner & Roy 2003).

In long hydroperiod pools, there was more time for cladoceran community development and the gradual occupation of available niches by species with a variable seasonal phenology (i.e. early versus late species: *Daphnia* versus predominantly small-bodied and/or plant-associated species; Chapter 4). In short hydroperiod pools, the truncation of seasonal succession, in turn, significantly reduced the cumulative local species pool. To our knowledge, our study was the first to provide empirical evidence of a truncation of community development by a decreasing hydroperiod within Cladocera, one of the first invertebrate groups colonizing a temporary pool after inundation.

Both local and regional cladoceran species richness was probably favored by the relatively long hydroperiod of these habitats (Chapters 2 and 4, but see Chapter 5). This relaxed constraints both on species and community development time, while on the other hand inducing enough disturbance to avoid dominance of particular species in a climax type of community. The annual truncation of community development that characterizes temporary pools is considered an important mechanism relaxing competitive interactions in these habitats and thereby allowing high levels of species coexistence (King et al. 1996, Simovich 1998, Brendonck & Williams 2000). Large branchiopod local species richness, on the contrary, was low in meadow pools and clay pits (Chapter 1). Probably, the long hydroperiod of the pools entailed an elevated predation risk, especially for large-bodied planktonic prey.

Interseasonal variation and the impact of climatic variation

In Kiskunság, pools are usually abundant. However, due to low winter and spring precipitation and relatively high spring temperatures in 2007, only four of the 36 pools selected in 2005 contained water (Chapter 5). In May 2007, their cladoceran communities were impoverished and the pools supported only small populations when compared to the previous wet year (end of April 2005). This pattern most likely resulted from a decrease in microhabitat availability in the smaller and shallower pools and possibly more intense biotic interactions in the presence of a macroinvertebrate community dominated by predators. Weather conditions were not exceptional during any of both study years and droughts are a typical feature of the Hungarian climate (Szinell et al. 1998). Therefore, we expect the invertebrate fauna, being adapted to considerable environmental variation and disturbance effects in their temporary habitats, to recover from such

occasional droughts. Especially zooplankton species, through their dormant egg banks (Hairston 1996) and adaptive hatching behaviour (i.e. bet-hedging; Ellner et al. 1998, De Meester & De Jager 1993, Brendonck et al. 1998), are expected to be substantially buffered against such unsuccessful growing seasons.

A direct link between the hydroregime and temperature and precipitation patterns has been demonstrated in different types of temporary pools (Brooks 2004, Bauder 2005, Pyke 2005a, Hulsmans et al. 2008). As the hydroregime is crucial for various processes in temporary pools (e.g. Chapter 4), long-term climatic variation - possibly as a result of climate change - is expected to considerably affect their inhabiting communities. If the currently ongoing trends of decreasing total annual and winter precipitation and increasing frequency of droughts in Hungary (Szinell et al. 1998, Domonkos 2003, Gálos et al. 2007) are going to continue in the future, hydrological conditions of the Kiskunság pools are expected to be altered eventually resulting in smaller pools with on average shorter and more variable hydroperiods. Not only the impoverishment of communities in the small pool volumes during the dry year (Chapter 5), but also the truncation of seasonal community development in early drying pools (Chapter 4) suggests that species richness of cladoceran communities could be adversely affected by such changes. Given the significant preference of some species for seasonal hatching cues (temperature and photoperiod; Chapter 6), a changing pool phenology may interfere with seasonal community development as some species might not receive appropriate hatching cues and fail to initiate active populations. On the other hand, species may hatch under conditions that are no longer guaranteeing sufficient time for maturation and growth. Given the importance of cladocerans in the food web of temporary waters, alterations in their communities may ultimately interfere with the diversity at different trophic levels and the ecological quality of these ecosystems. However, species and community responses to long-term climatic variation are not evident to predict. Such long-term effects depend on the evolutionary potential of species to respond to environmental changes or the buffering capacity of the dormant egg bank against unfavourable growing seasons. Especially in these temporary pool ecosystems, there is still a considerable need of long-term monitoring and sound ecological research on species, community and ecosystem responses to (long-term) environmental changes.

Recruitment from dormant egg banks

Through controlled laboratory experiments we found evidence of a species-specific hatching response of cladocerans to temperature and photoperiod cues simulating different moments throughout the growing period. For most species, maximal hatching fractions occurred at

conditions corresponding with the seasonal timing of maximal abundance in the field (Chapter 6). Hatching of *D. atkinsoni*, an early-spring species in the study pools, was favored by early-spring conditions (low temperatures and relatively few light hours). The low-temperature preference of *D. atkinsoni* is rather surprising, considering the southern distribution of the species within Europe (Flößner 2000). However, in the more northern parts, *D. atkinsoni* tends to disappear when temperature rises in spring or summer (Johnson 1952, Flößner 2000, Louette & De Meester 2004). Johnson (1952) ascribed this observation to a thermal race. However, following Louette & De Meester (2004), we would like to stress that biotic interactions might also contribute to its seasonal occurrence. The competitive ability of large-bodied zooplankton species is adversely affected by elevated temperatures (Moore et al. 1996). Given the overall dominance of small-bodied taxa in the study pools, *D. atkinsoni* might face severe competition especially in late spring and summer and experience optimal growth conditions during the cold-water stage. Additionally, size-selective invertebrate predation may decrease its chances for survival. Species such as *Moina brachiata* and *Macrothrix rosea*, which were especially abundant later in spring or summer, preferably hatched at warmer conditions under more light hours. *Moina brachiata* and *M. rosea* are reported as thermophilous species (Flößner 2000). In laboratory experiments, *M. brachiata* effectively showed its highest reproductive success at 25 and 30°C when compared to lower temperatures (Maier 1993). We conclude that the observed species-specific hatching response to environmental cues is strongly suggestive of temporal habitat selection - an adaptive response allowing species to initiate populations through emergence from the dormant egg bank mainly when their relative fitness is expected to be high.

Temporal habitat selection may be an important mechanism facilitating temporal niche differentiation of competing species. Analogously to promoting genetic diversity within populations (Hedrick 1995), it may also promote the coexistence of species and the maintenance of species diversity in communities in temporally variable environments. Furthermore, the observed response may result in a more important contribution of hatching to active community patterns than merely the initiation of active populations at the onset of the growing season, as is generally assumed (Wolf & Carvalho 1989, Cáceres 1998, Hairston et al. 2000, Gyllström 2004).

In order to verify whether temporal habitat selection effectively occurs in zooplankton species from temporary pools or other habitat types, a complementary approach of controlled experiments and field observations is recommended. For the current study system, for example, it remains unclear whether increased population sizes in the field are a direct result of increased population growth rate (i.e. a high relative fitness) of species rather than of an increased number

of hatchlings. It should be more precisely determined whether seasonal hatching preferences of species effectively correlate to the timing of optimal growth conditions. The seasonal performance of species in the field could be more directly assessed by means of life history experiments rather than from population sizes observed in the field. This relation could be further disentangled by means of long-term *in situ* monitoring of emergence and seasonal abundance patterns, by which the effective contribution of hatching to the active population size of the different species is determined.

Methodological applications: dormant egg bank analysis for species richness assessment

The identification of hatchlings retrieved after incubation of the dormant egg banks was not effective in detecting the relatively high species number that characterized the active cladoceran communities of the study pools throughout one single growing season (Chapter 7; Table 1). An equal species number was detected from dormant egg bank analysis and single 'snap shots' of the active community. However, cladoceran communities were highly dynamic throughout the season (Chapter 4) and this temporal variation was not captured in the assemblages that hatched from the dormant eggs (Chapter 7). The identification of only two active samples, collected at moments maximally spread in time, was already more effective than dormant egg bank analysis in detecting a maximum number of cladoceran species. The identification of three to seven subsequent active community samples throughout the growing season enabled the detection of almost twice as many species than egg bank analysis (Table 1). In a study of 88 European lakes this was the other way around (Vandekerkhove et al. 2005b). The lower efficiency of dormant egg bank analysis in comparison with various other studies (Table 1) is likely explained by low resting egg densities, relatively low hatching fractions and species-specific storage or hatching requirements of the temporary pool cladoceran species. For large branchiopods, dormant egg bank analysis was more efficient for estimating the local species pool (Table 1). However, its success varied between pool types and was partly due to the fact that we identified dormant eggs in addition to hatchlings (Chapter 1). Some species, on the other hand, never hatched (*L. apus* and *L. brachyurus*). Until further testing of the method in various temporary pool ecosystems, the identification of hatchlings retrieved from the sediment for branchiopod species richness assessment should especially be used to complement active community samples.

The results of our study are in agreement with findings in South African temporary pools (E. De Roeck, unpubl. data; Table 1). The reduced efficiency of the method might especially

apply to temporary wetland species. However, the studies by Havel et al. (2000), Crispim & Watanabe (2001) and Vandekerkhove et al. (2005b) also considered temporary water bodies and found that the method was efficient, just like for permanent lakes. Their temporary study systems were generally deeper lakes (Table 1). These findings suggest that, depending on wetland dimensions, the use of dormant egg bank analysis for branchiopod species richness assessment might not be effective in temporary aquatic ecosystems.

GENERAL DISCUSSION

Table 1. Overview of studies that evaluate the efficiency of identification of hatchlings from the dormant egg banks (DEB) when compared to active community samplings at repeated occasions (AC) for species richness assessment of various zooplankton groups. For each method, we provide the average number of species that was detected for a single waterbody (S_{cum} ; mean $\pm$ S.E.) as well as the average number of species that was exclusively detected by the respective method (S_{excl} ; mean $\pm$ S.E.). Values in brackets indicate the number of species exclusively found over the whole study. Information that was unavailable is indicated with an asterisk.

		May 1986	Havel et al. 2000	Crispim & Watanabe 2001	Duggan et al. 2002	Vandekerkhove et al. 2005a	Vandekerkhove et al. 2005b	De Roeck unpubl.	This study	
Taxonomic group		Rotifera	Micro- crustaceans	Cladocera	Rotifera	Cladocera	Cladocera	Cladocera	Cladocera	large branchiopods
Habitat	Habitat type	lake	floodplain ponds	reservoir	lakes	shallow lake	shallow lakes	pools	pools	pools
	Permanence	permanent	temporary / permanent	temporary	permanent	permanent	temporary / permanent	temporary	temporary	temporary
	n	1	12	1	2	1	88	64	12	12
	Area	13.3km ²	4 - 323ha			2.9ha	0.25 - 200ha	5m ² - 8.6ha	95m ² - 2ha	95m ² - 2ha
	Depth (m)	25.5	<1 - 5.5	5	12 - 25	1	<5	0.03 – 1.50	0.20 – 1.08	0.20 – 1.08
AC	# samplings	>156	2-72	3	>17	6	6	1-2	3-7	3-7
	S_{cum}	15	9.9 $\pm$ 3.8	3	*	22	5.1 $\pm$ 0.6	3.5 $\pm$ 1.7	14.3 $\pm$ 1.1	0.4 $\pm$ 0.2
	S_{excl}	0	*	0	min. 2	6	* (6)	* (8)	7.3 $\pm$ 0.8 (14)	0.0 $\pm$ 0.0 (0)
DEB	S_{cum}	15	14.25 $\pm$ 10.8	7	13 - 25	22	7.9 $\pm$ 0.8	1.7 $\pm$ 1.4	8.4 $\pm$ 0.9	2.0 $\pm$ 0.4
	S_{excl}	0	*	4	2	6	* (4)	* (2)	1.3 $\pm$ 0.4 (1)	1.6 $\pm$ 0.5 (5)

Implications for conservation

This study, focusing on the ecology of large branchiopods and cladocerans performing a key role in ecosystem functioning, allows us to formulate some considerations that should be taken into account for a better protection of temporary pools, the biological diversity they house and their ecological integrity. These considerations should apply to various temporary pool ecosystems worldwide.

- Pools with an increasing duration of inundation were characterized by a richer local species pool of Cladocera. Inadequate filling of the pools owing to dry weather conditions resulted in an impoverished local species pool. A reduction of the length of the aquatic phase of pools is expected to adversely affect the diversity of cladoceran communities through interference with natural community development and mechanisms that promote species coexistence throughout the growing season – temporal niche segregation and possibly temporal habitat selection. Additionally, reductions in pool size will interfere with spatial niche segregation. Through trophic interactions, such changes are expected to also affect diversity at other trophic levels and ultimately interfere with the functioning of these ecosystems (Downing & Leibold 2002). Variation in hydromorphological conditions of pools due to natural climatic variation is inevitable. However, to protect the regional cladoceran species pool, any human impact on this natural variation, especially drainage for agricultural purposes, should be strongly restricted. In relatively long-lived pools, large branchiopods were only observed during the earliest succession stages. Due to their fast growth and reproduction, they are adapted to more short-lived habitats. Large branchiopod diversity, therefore, is not particularly protected by the conservation of long hydroperiod pools.
- Large branchiopods and cladocerans will not equally benefit from the protection of long hydroperiod pools. From this we conclude that different groups of temporary pool inhabitants might not be equally protected by certain conservation measures. In a large scale study on the biodiversity of shallow lakes at different trophic levels (from bacteria to fish and macrophytes), Declerck et al. (2005) likewise concluded that taxon richness at the ecosystem level is a multidimensional feature. In their study, richness of the different taxonomic groups responded differently to environmental gradients of productivity and macrophyte cover, implying that conservation measures designed to increase species richness in one group may be inefficient or counteractive for the management of other groups.
- In order to maximize regional species diversity of branchiopods in general, we strongly promote the maintenance of a diversity of habitat types in the landscape. Variation in surface

area, depth and duration of pools promoted regional species diversity both in the large and small branchiopods' assemblages. Variation in transparency and vegetation cover, furthermore, contributed to variation in large branchiopod communities. Analogously to promoting species richness at the local scale (i.e. within habitats), habitat heterogeneity may favor regional species coexistence at the landscape scale, as also demonstrated for various other taxa (Weibull et al. 2000, Romero-Alcaraz & Ávila 2000, Benton et al. 2003; aquatic macroinvertebrates: Stoks & McPeck 2003, Verberk et al. 2006; zooplankton: De Bie et al. 2008, Timms & Sanders 2002). Additionally, small habitats with a short duration may be locally species poor, but are of considerable importance in maintaining metacommunity dynamics and stability (Semlitsch 2000, Holyak et al. 2005, Tarr et al. 2005). In addition to cladocerans and large branchiopods, various other groups of aquatic organisms are likely to benefit from a regional diversity of pool types. Likewise, Declerck et al. (2005) suggested maintaining a regional variety of lake types as taxon richness of different organism groups could not be ensured by protecting a single lake type or environmental condition.

- Small, muddy pools may be little attractive to the general public, but they are likely to house species of particular conservation concern. Wheel tracks, for example, probably make part of historical dispersal trajectories and have been colonized by large branchiopod species that are highly specialized to the unpredictable nature and disturbance degree of their habitat. The nature and conservation value of a habitat therefore should not be measured merely by its esthetic value.
- The species composition of active cladoceran communities was highly variable throughout the growing season. Also the presence of large branchiopods varied through time. For example, in meadow pools their appearance was restricted to the early succession stages. In order to reliably estimate local species diversity in temporary aquatic habitats, simple community snap shots are not recommended as a lot of temporal variation will be missed. Active samplings should preferably cover an early, middle and late stage after pool inundation. Additionally, for branchiopod species richness assessment the analysis of dormant egg banks and especially the identification of hatchlings will not be reliable in all systems and for all taxa. In such cases, the method should rather be used to complement active community samplings.

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SUMMARY

The protection of relatively small freshwater wetlands such as ponds and temporary pools could play a major role in ensuring the integrity and biodiversity of the changing European landscape. Not only do these habitats support a high regional biodiversity, but through the functions they perform in the landscape (e.g. flood control and water quality improvement), they are of great value to human society. Ecological studies are needed to contribute to a better understanding of their functioning and the factors structuring their inhabiting communities. Based on such studies, effective management and conservation strategies can be formulated for these valuable habitats. Our study especially focused on ecological processes that structure keystone invertebrate communities – large branchiopods and cladocerans - through space and time in a set of pristine temporary pools in Kiskunság (Hungary). We conducted an intensive monitoring of large branchiopods in a total of 82 pools belonging to five distinct habitat types (meadow pools, clay pits, pools in arable land, orchard pools and wheel tracks) during the spring of 2005 and 2006. We studied local and spatial factors explaining the distribution of the most common species. To study processes structuring Cladocera communities, we focused on a subset of 18 meadow pools and clay pits, located in three pool clusters and with a variable duration of inundation. They were subsequently monitored during the spring of 2005. Due to low winter and spring precipitation and relatively high spring temperatures in 2007, only four pools contained water and could be sampled. Additionally, the dormant communities were collected and their species richness and hatching dynamics were characterized in the laboratory.

The high regional species richness and occurrence frequency of large branchiopods, a taxon that is internationally recognized as in need of protection, underline the considerable nature value of the temporary freshwater pools in Kiskunság. Our study pools were furthermore amongst the most species rich temporary freshwater habitats with respect to Cladocera when compared to a total of 36 studies carried out worldwide. We defined Kiskunság as a “hot spot” of both small and large branchiopod diversity and as such stressed the need for their protection.

High habitat diversity contributed to the high regional diversity both of small and large branchiopods. Variation in the species composition of large branchiopod assemblages was related to differences in local habitat characteristics such as size, transparency and vegetation cover. The significant effect of local habitat characteristics was furthermore indicative of species sorting mechanisms structuring the large branchiopod metacommunities in our study pools. The extent to which the spatial structure in species distribution was linked to dispersal limitation or hidden environmental gradients remains unknown. A comparative

literature study indicated that the high level of regional cladoceran species richness was related to the high degree of variation in habitat size and duration.

The temporary study pools were highly variable through time. Throughout the growing season, conductivity increased and we observed vegetation and algae growth in the pools, which gradually became more turbid. As habitat quality changed with time, cladoceran communities became dominated by small and/or plant-associated species. We argued that the presence of large-bodied zooplankton species such as *Daphnia* and anostracans exclusively early in succession, as well as their overall small contribution to the branchiopod communities in meadow pools and clay pits, was due to size-selective invertebrate predation. The high regional cladoceran species richness of Kiskunság when compared to various studies worldwide was partly also attributed to the relatively long hydroperiod of our study pools. Even in the most short-lived meadow pools and clay pits, constraints on development time were relaxed and colonization by cladocerans was not limited. However, in pools that dried earlier, seasonal succession was truncated and the cumulative local species pool was significantly reduced when compared to pools that were inundated longer.

In addition to intraseasonal variation, we also assessed interseasonal variation in cladoceran community composition. In May 2007, which was a dry year, cladoceran communities were impoverished and the pools supported only small populations when compared to the previous wet year (end of April 2005). If the currently ongoing trends of decreasing total annual and winter precipitation and increasing frequency of droughts in Hungary are going to continue in the future, hydrological conditions of the Kiskunság pools are expected to be altered, eventually resulting in smaller pools with on average shorter and more variable hydroperiods. Although temporary pool species naturally have a relatively high tolerance to environmental variation, they might not be able to adapt fast enough to such changes. Not only the impoverishment of communities in the small pool volumes during the dry year, but also the truncation of seasonal community development in early drying pools suggests that species richness of cladoceran communities could be adversely affected by such predicted hydrological changes. Given the importance of cladocerans in the food web of temporary waters, alterations in their communities may ultimately interfere with the diversity at different trophic levels and the ecological quality of these ecosystems.

Through controlled laboratory experiments, we detected a species-specific hatching response of cladocerans to combined temperature and photoperiod cues with maximal hatching fractions corresponding to the seasonal timing of maximal abundance in the field. Such response is suggestive of temporal habitat selection and could allow species to initiate populations mainly when their relative fitness is expected to be high. Moreover, the observed hatching behavior may result in a more important contribution of hatching to active

community patterns than merely the initiation of active populations at the onset of the growing season, as is generally assumed.

Finally, we assessed the efficiency of the identification of hatchlings retrieved after incubation of the dormant egg banks for cladoceran species richness assessment in our study pools. We also made use of results obtained in the first chapter where this method was used to complete the large branchiopod species list from active community samplings. In previous studies in permanent lakes, this method proved efficient. Our results, however, in combination with some previous studies carried out in temporary wetlands, suggested that the method might not be equally effective in all types of temporary aquatic systems. Especially in shallow temporary pools, the identification of hatchlings retrieved from the sediment might not allow a reliable estimation of the local species pool, in contrast to the deeper temporary water bodies where it is more likely to be effective.

Based on our results, we formulated some implications for conservation that should apply to various temporary pool ecosystems worldwide. In order to reliably estimate local species diversity, biodiversity monitoring schemes in shallow temporary systems should make use of active community samplings rather than dormant egg analysis. Additionally, active samples should preferably be collected at moments spread in time, such as during an early, middle and late inundation stage of pools. To maintain high regional diversity of Cladocera and large branchiopods, we stressed the need of maintaining high habitat diversity especially with regards to size, transparency and vegetation cover. Various other groups of aquatic organisms are likely to benefit from this measure. Less appealing and therefore often neglected habitats such as mud pools and wheel tracks should be protected as they are likely to house species of particular conservation concern.

SAMENVATTING

De bescherming van relatief kleine watersystemen zoals meertjes, vijvers en tijdelijke poelen is belangrijk om de integriteit en biodiversiteit van het veranderende Europese landschap te verzekeren. Deze habitatten onderhouden niet alleen de regionale biodiversiteit, maar doordat zij instaan voor het bufferen van overstromingen en het verbeteren van de waterkwaliteit, kunnen ze ook van nut zijn voor de maatschappij. Enkel op basis van ecologische studies die peilen naar de werking van deze ecosystemen en de factoren die hun levensgemeenschappen structureren, kunnen efficiënte beheersmaatregelen voor deze waardevolle habitatten worden geformuleerd. Onze studie was voornamelijk gericht op ecologische processen die het voorkomen van ongewervelde sleuteltaxa verklaren in ruimte en tijd in een set van pristiene tijdelijke poelen in Kiskunság (Hongarije). We voerden een intensieve veldsurvey uit van de grote branchiopoden tijdens de lente van 2005 en 2006, en dit in een totaal van 82 poelen behorende tot vijf verschillende habitattypes (weidepoelen, kleiputten, karrensporen en poelen in akkers en boomgaarden). We bestudeerden lokale en ruimtelijke factoren die het voorkomen van de meest algemene soorten verklaarden. Om processen te bestuderen die de watervlooiengemeenschappen structureren, concentreerden we ons op een subset van 18 weidepoelen en kleiputten. Deze poelen, gesitueerd in drie poelclusters en met een variabele duur van de vullingfase (hydroperiode), werden opeenvolgend bemonsterd in de lente van 2005. Omwille van lage neerslaghoeveelheden tijdens de lente en voorgaande winter en relatief hoge lentetemperaturen in 2007, waren slechts vier poelen gevuld en konden bijgevolg worden bemonsterd. Bijkomend werd de dormante branchiopodengemeenschap (rusteierenbank) verzameld en in het laboratorium tot ontluiking gebracht om de soortenrijkdom verder in te schatten en ontluikingsvereisten van soorten te bestuderen.

De hoge regionale soortenrijkdom en frequentie van voorkomen van grote branchiopoden, een taxon waarvan de bescherming internationaal als een hoge prioriteit wordt erkend, onderschrijven de natuurwaarde van de tijdelijke zoetwaterpoelen in Kiskunság. Onze studiepoelen behoorden verder tot de meest soortenrijke tijdelijke zoetwaterhabitatten met betrekking tot Cladocera in vergelijking met een totaal van 36 studies die wereldwijd werden uitgevoerd. We definieerden Kiskunság daarom als een “hot spot” zowel van kleine als grote branchiopoden en benadrukten het belang van hun bescherming.

De hoge regionale branchiopodendiversiteit kwam tot stand door de hoge habitatdiversiteit. Variatie in de soortensamenstelling van de grote-branchiopodengemeenschappen was gerelateerd aan verschillen in lokale habitatkarakteristieken zoals grootte, helderheid en de graad van bedekking door vegetatie.

Het significante effect van lokale habitatkarakteristieken vormde bovendien een aanwijzing voor 'species sorting' als een belangrijk structurerend mechanisme van de metagemeenschappen van grote branchiopoden in onze studiepoelen. De mate waarin de ruimtelijke verspreiding van soorten het resultaat is van dispersielimitatie of ongekende omgevingsgradiënten blijft voorlopig onduidelijk. Een vergelijkende literatuurstudie gaf aan dat de hoge regionale soortenrijkdom aan watervlooien gerelateerd was aan de hoge graad van variatie in habitatgrootte en inundatieduur.

De tijdelijke studiepoelen en hun levensgemeenschappen vertoonden variatie in de tijd. Tijdens het groeiseizoen nam de conductiviteit van het water toe en observeerden we groei van waterplanten en algen, die resulteerden in een hogere troebelheid. Naarmate de habitatkwaliteit veranderde, werden de watervlooiengemeenschappen gedomineerd door kleine soorten en/of soorten die doorgaans geassocieerd zijn met waterplanten. We vermoeden dat de aanwezigheid van relatief grote zooplanktontaxa zoals *Daphnia* en anostraken enkel vroeg in de successie, evenals hun kleine proportie in de branchiopodengemeenschappen van weidepoelen en kleiputten, in het algemeen te wijten was aan grootte-selectieve predatie. De hoge regionale soortenrijkdom aan watervlooien in Kiskunság in vergelijking met diverse studies wereldwijd werd gedeeltelijk ook toegewezen aan de relatief lange hydroperiode van de poelen. Zelfs in poelen die voor een vrij korte periode gevuld waren, was voor verscheidene soorten watervlooien immers voldoende tijd beschikbaar om hun levenscyclus te vervolledigen en was hun kolonisatiesucces daardoor niet beperkt. In poelen die vroeger opdroogden, kwamen tijdens een volledige vullingfase echter gemiddeld minder soorten voor omdat de seizoenale opeenvolging en vervanging van soorten eerder werd onderbroken.

Naast intraseizoenale variatie, bestudeerden we ook de interseizoenale variatie in de samenstelling van de watervlooiengemeenschap. Tijdens een droog jaar (2007) waren minder soorten watervlooien aanwezig in de poelen en slechts kleine populaties in vergelijking met het voorgaande natte jaar (2005). Indien de huidige trends van verminderde neerslag en toenemende droogte zich voortzetten in de toekomst, valt te verwachten dat poelen in Kiskunság op termijn gemiddeld kleiner zullen worden en voor kortere periodes gevuld zullen blijven. Hoewel tijdelijke-poelbewoners variatie in hun omgeving relatief goed kunnen tolereren, zijn ze mogelijk niet in staat om zich op lange termijn snel genoeg aan zulke veranderingen aan te passen. Niet alleen de verarming van de gemeenschappen in de kleinere poelvolumes tijdens het droge jaar, maar ook de onderbreking van seizoenale successie in poelen die eerder opdrogen, geeft aan dat de soortenrijkdom aan watervlooien negatief zou worden beïnvloed door zulke hydrologische veranderingen. Gezien watervlooien een belangrijke schakel zijn in de voedselketen van deze ecosystemen, kunnen

wijzigingen in hun gemeenschappen nefaste gevolgen hebben voor de diversiteit op verschillende trofische niveaus en de ecologische waarde van deze ecosystemen.

Aan de hand van gecontroleerde laboratoriumexperimenten observeerden we een soortspecifieke ontluikingsrespons van watervlooien op gecombineerde temperatuur- en fotoperiodebehandelingen waarbij maximale ontluikingsfracties voorkwamen onder condities die overeenkwamen met het moment in het seizoen waarop soorten in het veld ook een maximale abundantie vertoonden. Zulke respons wijst mogelijk op een mechanisme van temporele habitatselectie dat soorten in staat zou stellen om populaties op te starten voornamelijk wanneer ze verwacht worden een hoge relatieve fitness te hebben. Doorgaans wordt aangenomen dat ontluiking enkel bijdraagt tot het opstarten van actieve zoöplanktonpopulaties in het begin van het groeiseizoen. De respons die wij observeerden zou echter kunnen resulteren in een belangrijkere bijdrage van ontluiking tot seizoenale patronen in de actieve gemeenschappen.

Tenslotte evalueerden we de efficiëntie van de identificatie van organismen ontloken uit rusteieren voor het inschatten van de lokale diversiteit aan watervlooien in onze studietoelen. We maakten bovendien gebruik van resultaten uit het eerste hoofdstuk waarbij deze methode werd gebruikt om de soortenlijst van grote branchiopoden aan te vullen. In eerdere studies in permanente meren bleek deze methode efficiënt. Onze resultaten, in combinatie met eerdere studies in tijdelijke wetlands, gaven echter aan dat de methode niet even efficiënt is in alle types tijdelijke aquatische systemen. Vooral in ondiepe tijdelijk poelen vormt rusteierenbankanalyse een minder betrouwbare methode voor het inschatten van de lokale soortenrijkdom, terwijl in diepere tijdelijke systemen de methode betere resultaten gaf.

Op basis van onze bevindingen formuleerden we enkele nuttige richtlijnen voor een duurzaam beheer van tijdelijke zoetwaterhabitaten. Om in de eerste plaats de lokale soortenrijkdom aan branchiopoden op een betrouwbare manier in te schatten zijn actieve stalen te verkiezen boven de identificatie van organismen ontloken uit ruststadia. Actieve stalen dienen verder bij voorkeur genomen te worden tijdens momenten gespreid in de tijd, zoals een vroege, midden en late vullingfase. Teneinde een hoge regionale diversiteit aan watervlooien en grote branchiopoden te bewaren, is een hoge habitatdiversiteit aangewezen, vooral met betrekking tot habitatgrootte, helderheid en bedekking met vegetatie. Verscheidene andere groepen aquatische organismen zijn waarschijnlijk ook gebaat bij zulke maatregel. Modderpoelen en karrensporen waren een voornaam habitat voor grote branchiopoden. Dergelijke habitaten die weinig aantrekkelijk lijken voor de mens en daardoor vaak verwaarloosd worden, dienen daarom bewaard te blijven in het landschap.

APPENDIX 1

Overview of pool coordinates of meadow pools and clay pits located in the three pool clusters (Cluster I, II and III).

Pool code	Cluster	Coordinates	
SOL 1	I	N 46° 48' 02.4"	E 19° 15' 53.7"
SOL 2	I	N 46° 47' 58.8"	E 19° 16' 05.9"
SOL 3	I	N 46° 48' 03.3"	E 19° 16' 05.5"
SOL 4	I	N 46° 48' 16.8"	E 19° 16' 23.8"
SOL 5	I	N 46° 48' 15.2"	E 19° 16' 26.4"
SOL 6	I	N 46° 48' 14.3"	E 19° 16' 17.9"
SOL 7	I	N 46° 48' 08.2"	E 19° 16' 09.9"
SOL 8	I	N 46° 48' 05.1"	E 19° 16' 19.5"
SOL 10	I	N 46° 48' 03.7"	E 19° 16' 20.8"
SOL 11	I	N 46° 48' 06.0"	E 19° 16' 09.8"
SOL 12	I	N 46° 48' 11.5"	E 19° 15' 17.6"
SOL 13	I	N 46° 47' 36.8"	E 19° 15' 35.3"
FUL 1	II	N 46° 49' 53.8"	E 19° 12' 47.7"
FUL 2	II	N 46° 49' 54.7"	E 19° 12' 57.7"
FUL 3	II	N 46° 50' 03.3"	E 19° 13' 06.8"
FUL 4	II	N 46° 50' 12.3"	E 19° 13' 01.5"
FUL 5	II	N 46° 49' 42.1"	E 19° 13' 35.3"
FUL 6	II	N 46° 49' 07.6"	E 19° 12' 32.5"
FUL 7	II	N 46° 49' 24.4"	E 19° 13' 40.2"
FUL 8	II	N 46° 49' 25.4"	E 19° 13' 37.7"
FUL 9	II	N 46° 49' 27.9"	E 19° 13' 27.2"
FUL 10	II	N 46° 49' 29.5"	E 19° 13' 21.2"
FUL 11	II	N 46° 49' 31.7"	E 19° 13' 17.2"
FUL 12	II	N 46° 49' 33.1"	E 19° 13' 17.9"
KISK 1	III	N 46° 41' 15.9"	E 19° 16' 34.9"
KISK 2	III	N 46° 41' 14.6"	E 19° 16' 25.9"
KISK 3	III	N 46° 41' 11.7"	E 19° 16' 16.5"
KISK 4	III	N 46° 40' 55.3"	E 19° 16' 16.5"
KISK 5	III	N 46° 41' 06.7"	E 19° 16' 17.3"
KISK 6	III	N 46° 40' 55.3"	E 19° 16' 14.5"
KISK 7	III	N 46° 40' 51.9"	E 19° 16' 12.7"
KISK 8	III	N 46° 40' 29.2"	E 19° 16' 58.7"
KISK 9	III	N 46° 40' 06.1"	E 19° 16' 48.9"
KISK 10	III	N 46° 40' 28.7"	E 19° 17' 02.6"
KISK 11	III	N 46° 41' 58.1"	E 19° 16' 47.3"
KISK 12	III	N 46° 41' 32.8"	E 19° 16' 43.3"

APPENDIX 2

Overview of macroinvertebrate taxa found in the study pools in Kiskunság in the spring of 2005 and 2007.

Turbellaria

Oligochaeta

Hirudinea

Haemopsis sanguisuga

Erpobdella

Acari

Gastropoda

Crustacea

Crangonyx pseudogracillis

Crangonyx subterraneus

Asellus aquaticus

Collembola

Ephemeroptera

Baetis

Caenis

Odonata

Lestes barbarus, *L. dryas*, *L. sponsa*, *L. viridis*

Sympecma fusca

Coenagrion puellum

Ischnura elegans, *I. pumilio*

Anax imperator

Aeschna affinis

Sympetrum sanguineum, *S. striola/vulgatum*

Hemiptera

Hebridae *Hebrus*

Gerridae *Gerris*

Naucoridae *Ilyocoris*

Nepidae *Nepa cinerea*

Ranatra linearis

Pleidae *Plea*

Notonectidae *Notonecta glauca*, *N. maculata*

Corixidae *Corixa affinis*, *C. panzeri*, *C. punctata*

Hesperocorixa linnei

Sigara distincta, *S. falleni*, *S. striata*

Coleoptera

Halplidae *Halplus furcatus*

Noteridae *Noterus clavicornis*, *N. crassicornis*

Dytiscidae *Bidessus unistriatus*

Coelambus impressopunctatus, *C. parallelogrammus*

Hydroporus memnonius, *H. striola*

Graptodytes

Agabus

Hydaticus

Graphoderus cinereus

	<i>Acilius</i>
	<i>Dytiscus</i>
	<i>Cybister lateralimarginalis</i>
Hydraenidae	<i>Ochthebius</i>
	<i>Hydraena</i>
Hydrochidae	<i>Hydrochus megaphallus</i>
Hydrophilidae	<i>Hydrobius fuscipes</i>
	<i>Enochrus coarctatus</i> , <i>E. quadripunctatus</i>
	<i>Hydrophilus piceus</i>
	<i>Berosus spinosus</i>
Dryopidae	<i>Dryops</i>
Trichoptera	
Polycentropodidae	
Limnephilidae	
Leptoceridae	
Lepidoptera	
Diptera	
Psychodidae	
Culicidae	
Chaoboridae	
Dixidae	
Chironomidae	
Ceratopogonidae	
Limoniidae	
Tipulidae	
Tabanidae	
Stratiomyidae	
Sciomyzidae	
Ephydriidae	
Muscidae	

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