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Distribution and trophic role of fairy shrimps (Anostraca) and microcrustaceans (Copepoda, Cladocera) in the astatic soda pans of the Carpathian Basin

— PhD Thesis —

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1. Introduction and aims

In the Carpathian Basin, sodic aquatic habitats are restricted to the lowland parts of Hungary (mainly the Great Hungarian Plain), the Austrian Seewinkel region (in Burgenland) and the province of Vojvodina in Northern Serbia (Boros 1999). Soda pans are shallow and astatic (i.e. temporary) waterbodies in these areas, generally drying out every summer and therefore naturally fishless. Soda pans in the Carpathian Basin can cover quite large areas (up to 100–200 ha), although their water depth is mostly below 1 m (Megyeri 1959). They generally have a vegetation-free lakebed and usually sparse or no vegetation along their shorelines. They have a high pH, due to large concentrations of dissolved sodium-hydrogen-carbonate, while salinity generally varies between hyposaline and mesosaline, depending on water level (Metz & Forró 1991; Boros 1999). The enormous productivity of the soda pans is largely due to nutrient enrichment derived from bird droppings by numerous large-bodied waterbirds (Boros et al. 2008b). Furthermore, high salinity, pH and permanent resuspension cause high remineralisation rates of phosphorus (Boros 2007), with total phosphorus concentration up to 34 mg l\(^{-1}\) (Boros 2007).

1.1. Distribution of Anostraca

Fairy shrimps (Anostraca) are important flagship species of temporary waters (Belk 1998). In the astatic soda pans of the Carpathian Basin, three Anostraca species were recorded: *Branchinecta ferox*, *Branchinecta orientalis* and *Chirocephalus carnuntanus* (Eder et al. 1997). They are mainly univoltine (Eder et al. 1997), generally occurring in early spring and disappearing as water temperature increases. Given the biogeographical importance of the Carpathian Basin in the distribution of the three species (meaning presumably the exceptional area of occurrence for *C. carnuntanus* and the main European patch for the *Branchinecta* species) and the former uncertainties regarding the taxonomy of the two *Branchinecta* species before Petkovski (1991), a comprehensive survey of this region was needed.

In our study, we aimed at updating knowledge on the distribution of the three anostracan species in the European astatic soda pans, assessing their current conservation status and determining the local, land use and spatial effects on their distribution.
1.2. Trophic role of crustaceans

With its diverse aquatic habitats, the Carpathian Basin has a major role in the migration routes of many waterbirds, providing important stopover sites for several species. Soda pans also attract many waterbird species in large numbers during their migration (e.g. Boros et al. 2006). In spring, anostracans (fairy shrimps) are the main or, in some pans, the sole aquatic macroinvertebrate food type for waterbirds (Winkler 1980; Boros et al. 2006). Apart from anostracans, only zooplankton are present in abundance, comprising mainly *Arctodiaptomus spinosus*, *Arctodiaptomus bacillifer* (Copepoda: Calanoida), *Daphnia magna* and *Daphnia atkinsoni* (Cladocera) (Megyeri 1959; Forró 1989; Boros et al. 2006, 2008a).

Regarding the role of crustaceans as a food source for waterbirds, Boros & Forró (1999) and Boros et al. (2006) have already noticed that zooplankton and anostracans can be important for ducks and waders e.g. avocets, but as these small-scale studies only dealt with the relationships between invertebrates and waterbirds, our knowledge on the relative importance of food compared to other habitat characteristics in the habitat choice of waterbirds is still very limited. Besides the importance of invertivorous birds as top predators in soda pans, large flocks of geese and other waterbirds that feed outside the pans also play a significant role in the nutrient cycling (by their high allochtonous input; Boros et al. 2008a, b; Vörös et al. 2008).

*Our objective here was to determine, over a large spatial scale, the relationships between abundance of invertebrate food and waterbirds in soda pans across the Carpathian Basin, including all natural representatives of this habitat type in our analysis. We aimed at comparing the occurrence of the different food types and other characteristics of the soda pans with bird densities during the period of spring migration, hypothesizing that the most abundant invertebrate prey types, anostracans and microcrustaceans, would have a strong effect on the composition of waterbird communities.*

1.3. Biodiversity–ecosystem functioning (BEF) relationships of zooplankton

Biodiversity–ecosystem functioning (BEF) relationships have recently developed to a central issue within both community ecology and conservation biology (Loreau et al. 2001; Balvanera et al. 2006). Initial studies focused on primary production as a function of species...
richness especially in terrestrial systems, while recently, emphasis has moved on to functional diversity, complex interactions and food webs. Many examples contributed to the increasing evidence that diversity generally promotes functioning while species loss causes malfunction (Loreau et al. 2002; Hooper et al. 2005; Balvanera et al. 2006; Cardinale et al. 2006).

Salinity represents a major structuring gradient in aquatic systems, affecting organisms directly (through osmotic regulation) and indirectly, as a determinant of other habitat characteristics, such as biotic interactions (e.g. fish predation) and the presence of biotic structuring elements (macrophytes). Although it has been known for a long time that salinity constrains diversity along natural salinity gradients, these patterns have received surprisingly little attention in terms of BEF research. In inland saline waters, zooplankton diversity generally declines with salinity, while only a few investigations have also looked at density, as estimation for zooplankton productivity. These few suggest that zooplankton productivity tends to decline with salinity, parallel with diversity. Such a negative relationship is in agreement with both an overall negative effect of increasing environmental stress, as well as with the negative effect of species loss.

In this study, we perform an analysis of zooplankton diversity and density along a natural salinity gradient represented by the astatic soda pans in the Carpathian Basin. By collecting a substantial large-scale dataset on biotic and abiotic variables in an extensive field sampling, our aim was to identify the main drivers of zooplankton species richness (S) and density, and we analysed patterns of density as a proxy for functioning and how it scaled with diversity along the stress gradient.

2. Methods

110 astatic soda pans in the Carpathian Basin were involved in our study, which constitute all representatives of this habitat type in the Basin and also in Europe. We considered a pan natural if it was of natural origin and was not strongly affected by human disturbance e.g. artificial inflow of freshwater and related fish stocking and semi-natural, if strong human disturbance was also absent but the pan was constructed/reconstructed in the former decades. 21 of the 110 habitats proved to be in a bad ecological state, having lost the characteristics of soda pans. These pans were only visited once and were not involved in
the analyses. 82 pans were categorised natural and 7 as semi-natural. Pans were visited both in early spring (between 4th March and 9th April 2010) and early summer (between 11th May and 20th June 2009 or between 12th May and 2nd June 2010). When water depth was too low for a representative sample in the summer of 2009 or the pan was already dry, sampling was repeated in the same period of 2010. In the Anostraca study, 91 astatic soda pans were studied. We included every pan, apart from reconstruction areas and those that had already lost all their sodic characteristics, mostly by severe anthropogenetic alteration. For the trophic role study, only the 82 natural soda pans were selected, while in the BEF study, we used data of 89 (all natural and semi-natural) pans.

We collected zooplankton samples (Copepoda, Cladocera) and push net samples for macroinvertebrates (Anostraca, Chironomidae, Heteroptera). For the trophic role study, only the densities of those large-bodied taxa were used that were present in considerable numbers in the early spring zooplankton samples. We also counted fairy shrimp nauplii and metanauplii in the zooplankton samples and used it for the Anostraca study. Densities in the push net samples were recorded for anostracans, chironomids and heteropterans, the only frequent macroinvertebrates. Data on all of these three groups were used in the trophic role study. Heteroptera densities were also included in the analyses of the BEF study and we used the densities of anostracans in all three topics (Anostraca, trophic role, BEF study).

Waterbirds were counted at each site before entering the pans for invertebrate sampling. For each waterbird species, density was calculated by dividing abundance by pan area. Waterbirds known to feed at least occasionally on anostracans and/or zooplankton were the focus of our analysis. Pied avocets (*Recurvirostra avosetta*) and northern shovelers (*Anas clypeata*) were chosen for a separate analysis, due to their different response to anostracans and calanoid zooplankton compared to the other birds in our study.

Water depth and Secchi disc transparency were measured at each sampling location, along with pH, conductivity and dissolved O$_2$ (DO) concentration. The concentration of total suspended solids (TSS), chlorophyll-$\alpha$ and total phosphorus (TP) were determined later in the lab. TP and chlorophyll-$\alpha$ were only measured in the summer samples, therefore, these latter data were only used in the BEF study.

Additional habitat characteristics were also determined. These included pan area, percentage of open water in the pan bed together with the percentage of reed coverage in
the pan bed (± 5%), two hydroperiod types (one for the permanence of open water in the lake bed and one for the frequency of drying out).

For land use effects, we used water supply (artificial inflow), drainage melioration channelling (artificial outflow), disturbance of the structure, grazing pressure, grazing time (from no grazing through a few spring months to year-round), and catchment characteristics as the ratios of natural habitats, agricultural fields and human settlements in the catchment area (determined with the help of information collected from managers of the pans).

For spatial effects, we calculated the nearest distance to the next pan, pan density (number of neighbouring pans within 10 or 20 km) and isolation (average distance of a pan from all the other sites).

3. Results and Discussion

1.a. We have found all three formerly reported Anostraca species, *Branchinecta orientalis*, *Branchinecta ferox* and *Chirocephalus carnuntanus* in the investigated astatic soda pans. The two *Branchinecta* spp. have disjunct distributions in Europe with very similar patterns. Their central European range in the Carpathian Basin is the greatest among the patches (Brtek & Thiéry 1995). This means that our data approximately covers the recent distribution of both species in their central European area, and particularly of *B. orientalis*, as this species here has only been documented in soda pans for a long time. The presumably exclusive area of *C. carnuntanus* is also the Carpathian Basin and this is the first large-scale study on its actual distribution in the region.

1.b. The distribution of the three anostracans was structured mainly by the salinity of the pans. The apparently halophilous *B. orientalis* tolerated higher salinities than the other species, which can be regarded as habitat-generalist halotolerants, showing a high preference for soda waters in Central Europe. Therefore, European soda pans with a wide range of different salinities constitute a suitable habitat for all the three species.

1.c. The density of Anostraca species was significantly affected only by local factors, while their occurrence was influenced also by pan isolation. Land use did not explain a significant amount of variation in either case. It seems to have no direct effect on the anostracans, though it can still have a role in changing local conditions, e.g. conductivity, which occurred in the case of the disturbed pans. Our results suggest that habitat
management, including grazing and agriculture which are quite regular in the case of most pans (especially in the Hungarian national parks), does not have a side effect on fairy shrimps as long as it does not alter any of the local environmental factors, e.g. salinity or turbidity. Protected areas with high number of pans – as Seewinkel (in Austria) or Kiskunság (in Hungary) – can play an essential role in the long-term conservation of these anostracans.

2.a. During the spring migration of waterbirds, only invertebrate food (Arctodiaptomus copepods and anostracans) and pan density proved to be significant determinants of the waterbird communities. Variance partitioning of the data revealed that aquatic invertebrate food supply (Arctodiaptomus and anostracans) had a greater effect than pan density on the investigated most frequent bird species, suggesting a dominant role of species sorting in structuring the communities.

2.b. Avocets and shovelers differed from the other species in their strong association with densities of anostracans (avocet) and Arctodiaptomus (shoveler). Our results suggest that shovelers and avocets, which were among the most frequent representatives of the studied waterbirds, are much more affected by crustacean food availability than the other waterbirds. The soda pans have very high concentrations of suspended solids, causing low transparency. High turbidity also means that filter feeding or tactile foraging strategies can obviously be more successful than feeding based on visual cues. Shovelers are indeed known to feed mainly by sieving water through the lamellae of their beaks (Guillemain et al. 2002), while avocets are able to use a range of strategies, including sweeping, which is the most common form of foraging in soda pans (Boros & Forró 1999).

2.c. Anostracans and microcrustaceans, especially the most frequent B. orientalis, A. spinosus and A. bacillifer can be regarded as keystone elements of this soda pan habitat because of their bottom-up role in structuring waterbird assemblages during spring migration. The high abundance and biomass of available crustacean prey in the soda pans means that they are available in such amounts that waterbirds cannot reduce their abundance significantly. Additionally, anostracan densities in soda pans are strongly determined by the local abiotic environment in spring (Horváth et al. 2013a). It seems likely that the regulating effect of predation is not as strong as the effect of other environmental
conditions. Our results therefore suggest that at least in spring, this trophic relationship between invertebrate-feeding waterbirds and aquatic invertebrates is regulated bottom-up.

2.d. Our study is the first large-scale evidence of other anostracans besides *Artemia* as bottom-up determinants of waterbird communities. Studies on the keystone feature of anostracans as prey have so far only included the brine shrimp, *Artemia* in spatially restricted studies. We have demonstrated the keystone nature of other anostracans, especially of *B. orientalis*, the most abundant anostracan species in the pans.

3.a. Diversity and density of microcrustacean zooplankton showed opposing trends along the salinity gradient. The most saline habitats had communities of one or two species only, with maximum densities well above 1000 ind l⁻¹. This seemed to be a unique feature of soda pans among inland saline lakes.

3.b. Trophy of soda pans also increased with salinity, but they both had significant positive partial effects on the density of communities. Therefore, increasing densities were not only the result of increasing trophy.

3.c. Top-down effects on zooplankton density can be excluded as main drivers of density patterns. Astatic soda pans are naturally fishless, and there are only a few invertebrate predators that would feed on zooplankton. The majority of them were very scarce in the pans during our study (e.g. *Chaoborus*, coleopterans, odonates). Only heteropterans were present in considerable numbers, but they showed a positive correlation with conductivity as did zooplankton density. We showed previously that the trophic relationship between zooplankton and invertivorous waterbirds is also bottom-up regulated (Horváth et al. 2013b). Hence, a top-down effect on zooplankton density can largely be excluded as a driver of the density pattern. This implies that zooplankton density largely reflects productivity in the pans.

3.d. Similarity of communities increased with salinity, with most of the highly saline communities being dominated by one highly tolerant calanoid copepod, *A. spinosus*, exhibiting extremely high densities. This calanoid copepod was at the same time the only endemic species found in these systems. Salinity obviously constrained species composition and resulted in communities of low complexity. The pattern suggests that environmental stress may result in highly constrained systems which exhibit high rates of functioning due to these key species, in spite of the very limited species pool.
4. References
HORVÁTH ZS. et al. (2013b) Freshwater Biology 58: 430–440.

5. Publications that served as a basis for the thesis

5.1. Papers in peer-reviewed journals

HORVÁTH ZS., VAD CS. F., VÖRÖS L., BOROS E. (2013) The keystone role of anostracans and copepods in European soda pans during the spring migration of waterbirds. FRESHWATER BIOLOGY 58: 430-440. [IF_{2011}: 3.29]

HORVÁTH ZS., VAD CS. F., TÓTH A., ZSUGA K., BOROS E., VÖRÖS L., PTACNIK R. (under revision) Opposing patterns of zooplankton diversity and functioning along a natural stress gradient: When the going gets tough, the tough get going. OIKOS [IF2011: 3.061]

5.2. Presentations at Hungarian and international conferences

HORVÁTH ZS. et al. (oral presentation) 32nd Congress of the International Society of Limnology (SIL). Budapest, Hungary, 2013 (accepted abstract).

HORVÁTH ZS. et al. (oral presentation) LIV Hydrobiological Days. Tihany, Hungary, 2012


HORVÁTH ZS. et al. (oral and poster presentation) LIII Hydrobiological Days, Tihany, Hungary, 2011

HORVÁTH ZS., BOROS E. (oral presentation) VIII Hungarian Aquatic Macroinvertebrate Research Conference (MaViGe). Jósvafő, Hungary, 2011

HORVÁTH ZS. et al. (oral presentation) Fresh Blood for Fresh Water: Young Aquatic Science. Lunz am See, Austria, 2010.
