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Research Paper

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Environmental factors shaping assemblages of ostracods (Crustacea: Ostracoda) in springs situated in the River Krąpiel valley (NW Poland)

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Abstract – We investigated the ostracod assemblage structure in springs of a small lowland River Krąpiel valley. Environmental factors and the potential of ostracods as indicators of spring type were analysed. The analysis involved the type of spring, substrate, physicochemical parameters, and the river valley's hydrological status as environmental factors. A total of thirty ostracod species were recorded. The average count of ostracod individuals in a sample amounted to 100. The assemblages were dominated by juvenile *Candona* sp., juvenile *Psychrodromus* sp., *Cypria ophtalmica*, and *Cypridopsis vidua*. The spring species accounted for less than 1% of the individual counts, except for juvenile *Psychrodromus* sp. with 21% and *Eucypris pigra* with 4%. The average number of taxa per spring was 5. The ostracod assemblages were significantly influenced by limnocrene springs, spring flooding by the river overflow, the presence of coarse leaf litter, the presence of fine organic matter, a high content of NH_4 as well as by BOD_5, conductivity, pH, Fe, the river habitat modification index (RHM), the habitat modification score (HMS), and the river habitat quality (RHQ). The habitat conditions for the spring species appeared to be enhanced by the domination of mineral substrate over fine particulate organic matter, an elevated pH, the presence of leaf-derived organic matter, and the absence of flooding by the river. The spring species showed no association with the Krąpiel valley hydrological factors.

Keywords: Benthos / substrate / spring type / physicochemical parameters / species distribution

Résumé – Facteurs environnementaux façonnant les communautés d'ostracodes (Crustacea: Ostracoda) dans les sources situées dans la vallée de la rivière Krąpiel (nord-ouest de la Pologne). Nous avons étudié la structure d'assemblage des ostracodes dans les sources de la Krąpiel une rivière de plaine. Les facteurs environnementaux et le potentiel des ostracodes en tant qu'indicateurs du type de source ont été analysés. L'analyse a porté sur le type de source, le substrat, les paramètres physico-chimiques et l'état hydrologique de la vallée fluviale en tant que facteurs environnementaux. Un total de trente espèces d'ostracodes a été enregistré. Le nombre moyen d'individus d'ostracodes dans un échantillon s'élevait à 100. Les assemblages étaient dominés par les jeunes *Candona* sp., les jeunes *Psychrodromus* sp., *Cypria ophtalmica* et *Cypridopsis vidua*. Les espèces de printemps représentaient moins de 1% des comptages individuels, à l'exception des Psychrodromus sp. juvéniles avec 21% et des Eucypris pigra avec 4%. Les assemblages d'ostracodes ont été fortement influencés par les sources limnocrènes, les inondations printanières dues au débordement de la rivière, la présence de litière de feuilles, la présence de matière organique fine, une forte teneur en NH_4 ainsi que par la BOD_5, la conductivité, le pH, le Fe, l'indice de modification de l'habitat fluvial (RHM), le score de modification de l'habitat (HMS) et la qualité de l'habitat fluvial (RHQ). Les conditions de l'habitat des espèces de source semblaient être améliorées par la domination du substrat minéral sur la matière organique en fines particules, un pH élevé, la présence de matière organique provenant des feuilles et l'absence d'inondation par la rivière. Les espèces de source n'ont montré aucune association avec les facteurs hydrologiques de la vallée de la Krąpiel.

Mots clés : Benthos / substrat / type de source / paramètres physico-chimiques / distribution des espèces

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1 Introduction

Environmental factors are crucial in shaping assemblages of animals, including aquatic invertebrates. The invertebrate community structure is a result of a continuous sorting process through environmental filters ranging from regional or catchment-wide processes, involving speciation, geological history and climate, to small-scale characteristics of individual patches, such as the local predation risk, substratum porosity and current velocity (Malmqvist, 2002). Lowland springs are often characterized by stable habitats, with modest changes in hydrological, thermal, chemical, and biological characteristics (De Luca *et al*., 2014; Rossetti *et al*., 2020). However, they may be affected by destabilizing factors, such as drought, flooding, and human impact (Rossetti *et al*., 2005, 2020; Pieri *et al*., 2007; Pakulnicka *et al*., 2016; Zawal *et al*., 2018). Individual springs can support a mosaic of microhabitats which results in a high species richness, for which reason springs are sometimes referred to as biodiversity hotspots (Cantonati *et al*., 2012; Rosati *et al*., 2014). Springs can be considered distributional islands due to their spatial isolation, with dispersal between springs expected to be limited (Stutz *et al*., 2010; Rosati *et al*., 2014). Despite the wide range of environmental factors which can be taken into account when studying springs in the context of ostracod assemblage structure controls, few researchers have undertakes a holistic ecological study. Examples include Külköylüoğlu and Yılmaz (2006) as well as Sarı and Külköylüoğlu (2008) who considered only physical and chemical water properties as environmental factors. In contrast, the holistic approach is represented by Rosati *et al*. (2014), Rossetti *et al*. (2005), Zhai *et al*. (2015), Mezquita *et al*. (1999), as well as Roca and Baltanás (1993). The research potential of springs situated in the valley of the River Krąpiel has been noted by Pakulnicka *et al*. (2016) who studied aquatic beetles, by Zawal *et al*. (2018) who investigated ecological determinants of the water mite occurrence, and by Bankowska *et al*. (2015) who discovered new and rare water mite species.

The traditional classification of springs is based on flow velocity at the source: in limnocrenic springs the water forms a nearly still pool, with the water flowing out at the bottom. Helocrenic springs form a swampy zone, with the water flowing out – relatively sluggishly – over a relatively large area, where as in rheocrenic springs the water flows out rapidly, at a single point (Steinmann, 1915; Thienemann, 1922). However, the distinction between different types of springs is not always so clear, intermediate types being frequent. The nature of a spring is strongly influenced by its surroundings. The presence of trees in the close vicinity of the spring greatly enriches it with organic material from fallen leaves and can cause strong shading, which may reduce or even eliminate spring vegetation. Highly important is the presence of other water bodies from which fauna can migrate and thereby substantially alter the nature of the spring fauna (Pakulnicka *et al*., 2016; Stryjecki *et al*., 2016; Zawal *et al*., 2017). On the other hand, as small, isolated habitats, springs are highly susceptible to degradation associated with pollution, drainage, and land use in the catchment (Rossetti *et al*., 2005, 2020; Pieri *et al*., 2007). Springs in a river valley may be flooded by the river when it overflows, resulting in exchange of fauna between these habitats as well as in alteration of the spring water chemistry. The fauna of springs is also influenced by their location with respect to the river; a close proximity enhances migration of river fauna (Buczyńska *et al*., 2016; Zawal *et al*., 2018). Under the current climate change, the groundwater level is often lowered, causing some springs to become ephemeral and thus facilitating survival (*e.g.* via resting eggs or dispersal) of species adapted to such conditions.

The aim of this study was to determine the structure of ostracod assemblages in springs situated in the valley of a small lowland river, to identify environmental factors (and the extent of their influence) shaping these assemblages, and to investigate the potential of ostracods as indicators of spring habitats.

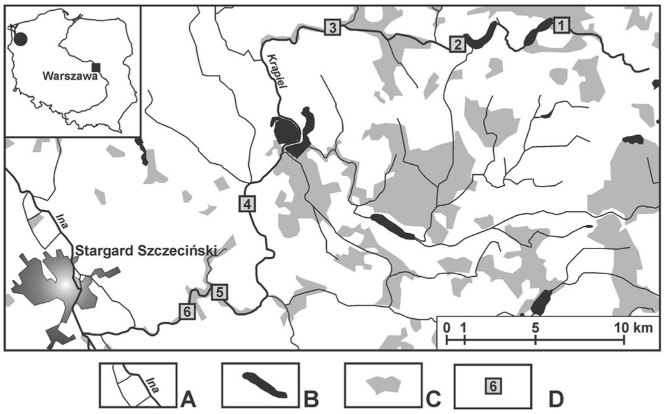

Fig. 1. Location of the sampling sites. (A) Rivers. (B) Lakes and fish ponds. (C) Forests. (D) Locations of springs (Z1–Z6).

2 Materials and methods

2.1 Study sites

The River Krąpiel is a small, lowland river, about 60 km in length, located in north-western Poland. The study area and specifically the Krąpiel, as well as the water bodies in the river valley, were described by Stryjecki *et al*. (2016) and Zawal *et al*. (2017). The samples for the present study were taken from springs situated in the Krąpiel valley. Six sampling sites along the entire length of the valley, 4.5–15 km away from one another, at locations with the highest numbers of springs, were visited. The sites were designated Z1 (53_28010.6300N 15_21041.7900E), Z2 (53_27036.9700N 15_16033.200E), Z3 (53_27041.4700N 15_12022.9400E), Z4 (53_2106.400N 15_1105.2300E), Z5 (53_ 20029.5600N 15_9015.0400E), and Z6 (53_19058.1400N 15_7057.5400E) (Fig. 1). The springs at a given site were located less than 50 m apart and fed a common stream. The number of springs sampled depended on the habitat diversity at each particular locality; two springs were sample dat Z4, three at Z1, and four each at Z2, Z3, Z5 and Z6. For each spring, its type, distance from the river, depth, flow type (permanent vs temporary), predominant sediment type, surrounding and submerged vegetation, flooding status (present or absent), and dryness (drying up vs. not drying up) were recorded.

2.2 Fauna sampling

In 2011, when the study was conducted, the Krąpiel water level was a long-term average, so in each season the valley was flooded or dry to an extent typical of that resulting from long-term observations (A. Zawal and A. Szlauer- Łukaszewska, pers. obs., 2008–2012). The samples were taken during and after flooding, except that no samples were collected if the spring was completely dried up. The samples were taken in May, July, and November 2011, but the springs were monitored throughout the seven-month period from May to November to find out whether they were flooded or dried up. To avoid destruction of the springs, which were very small, only a single sample was taken from an area of about 0.25 m^2 in each spring, using a hand net, at least at 3 spots in the spring to capture its microhabitat diversity. The spring surface area ranged from 1 to 10 m^2, with the predominance of smaller springs. A total of 42 samples were collected (one sample from each of the 14 springs $\times$ 3 sampling events). Due to thevery small surface area of the springs, the numbers of ostracods collected were unusually low. Therefore, the material should be regarded as representing a 'general community' rather than a statistical sample from this community, which justifies the data processing approach adopted. Therefore, extrapolating the findings to other locations should be done, if at all, with a great caution.

2.3 Environmental parameters

The following environmental variables characterizing the springs were measured: water temperature (temp., °C), insolation (insolati, %), water pH (pH), conductivity (cond., µS/cm), total hardness (hardness, mg $CaCO_3$/L), oxygen content (O_2, mg/L), ferric ions (Fe_3C, mg/L), phosphates (PO_3, mg/L), ammonia nitrogen (NH_4, mg/L), nitrate nitrogen (NO_3, mg/L), concentration of solids (mg/L), BOD_5 (O_2, mg/L), mineral sediment contribution (mineral, %), organic sediment contribution (organic, %), mean sediment grain size (M, mm), sediment sorting (W, mm), and density of aquatic vegetation (plants, on a scale from 0 to 5, where 0 indicates the complete absence of plants and 5 means total overgrowth). The water parameters, *i.e.* temperature, pH, electrolytic conductivity and dissolved oxygen content, were measured using an Elmetron CX-401 multiparametric sampling probe; the flow rate was determined with a SonTek acoustic FlowTracker flowmeter (m/s); BOD_5 was determined using Winkler's method; insolation was measured with a CEM DT-1309 light meter, and the remaining parameters were determined with a Slandi LF205 photometer. On each sampling occasion, three measurements were taken and the median was used for further analysis. The hydrological status of the Krąpiel valley was assessed by performing the standard River Habitat Survey (RHS) at each site (Z1-Z6), which ensures that the results can be compared with those of other studies (Szoszkiewicz and Gebler, 2012). For the purpose of this study, the RHS methodology was modified in that the assessments were performed along 100-m sections of the river rather than along the standard 500-m long ones. The following indices were calculated from the data (Szoszkiewicz and Gebler, 2012): river habitat modification (RHM), habitat modification score (HMS), river habitat quality (RHQ), and habitat quality assessment (HQA).

2.4 Data analysis

The data were analyzed with the STATISTICA ver. 10 INC StatSoft, PAST ver. 2.17c (Hammer *et al.*, 2001), and CANOCO v. 4.5 (TerBraak and Šmilauer, 2002) software packages.

Diversity metrics, *i.e.* the number of taxa (S), dominance (D), density, Simpson index (1–D), Shannon index (H), and Buzas and Gibson's evenness index (e^H/s), were calculated for different types of springs. Significance of differences in the diversity metrics between the spring types was tested with the Kruskal-Wallis test (Sokal and Rohlf, 1995).

The non-metric multidimensional scaling (NMDS), with Simpson's similarity coefficient, was used to look for patterns in spring grouping and correlation in the species' occurrence. The data for the multivariate analyses were not transformed, and the taxa which appeared in the samples once or twice were excluded.

The CANOCO v. 4.5 software was used to analyse relationships between ostracod species composition and habitat variables. The Canonical Correspondence Analysis (CCA) (TerBraak, 1986) was run to analyse patterns of species distribution in relation to environmental variables; it was preceded bythe Detrended Correspondence Analysis (DCA) which defined the structure of the data (Jongman *et al.*, 1987). The stepwise variable selection was used to determine the significance ($p \leq 0.05$) of the effect of each environmental variable on the species diversity. The Monte-Carlo permutations test with 499 permutations was applied to determine which variables were significant above a given threshold. The proportion of variation in the ostracod composition explained by the type of spring, substrate type, and hydromorphological parameters was expressed as the ratio of the sum of all canonical eigenvalues to the value of the total variance (total inertia), converted to a percentage. The proportion of variation in the ostracod composition explained by individual variables was calculated from the ratio of Lambda A to the total variance (total inertia), converted to a percentage.

Spearman's rank correlation coefficient was used to express the extent of correlation between the abundance of individual ostracod taxa and the habitat type, chemical water variables, spring type, and substrate variables.

3 Results

The materials collected from the Krąpiel valley springs yielded a total of 34 ostracod taxa, including 30 species. The numbers of individuals in the springs were very low, averaging 100 per sample. The ostracods were dominated by juvenile *Candona* sp., juvenile *Psychrodromus* sp., and the eurytopic species *Cypria ophtalmica* and *Cypridopsis vidua*. The high proportion of *Cyprois marginata* was due to its extremely high numbers in just two springs in samples collected in May (Tab. 1).

The mean number of taxa present in the springs was very low (5), which was reflected in the low values of other metrics based on the taxon richness (Tab. 2).

The two-dimensional NMDS plot (Fig. 2) shows three clusters, with area 1 grouping limnocrenes (denoted by L) and

Table 1. The number of ostracods collected, SD, dominance, frequency, and the number of Krąpiel valley springs supporting individual taxa.

Taxon	Abb.	Abundance	SD	Dominance %	Frequency %	Number of springs
Bradleystrandesia reticulata	**Bra ret**	1.00	4.7	0.99	5.7	2
Candona candida	Can can	5.74	14.9	5.70	40.0	13
Candona juv.	Can juv	17.02	40.5	16.90	57.1	17
Candonopsis kingslei	Can kin	0.05	0.3	0.05	2.9	1
Candonopsis scourfieldi	Can sco	0.36	1.4	0.35	11.4	3
Candona weltneri	Can wel	0.05	0.2	0.05	5.7	2
Candoninae juv.	Canae	2.55	16.3	2.53	2.9	1
Cryptocandona vavrai	**Cry vav**	0.50	2.0	0.50	11.4	3
Cyclocypris laevis	Cyc lae	2.98	7.5	2.95	37.1	11
Cyclocypris ovum	Cyc ovu	3.45	9.3	3.43	48.6	13
Cyprois marginata	Cyp mar	6.76	42.7	6.71	5.7	2
Cypria ophtalmica	Cyp oph	12.67	36.6	12.57	57.1	14
Cypridopsis vidua	Cyp vid	8.71	22.2	8.65	42.9	12
Eucypris pigra	**Euc pig**	3.79	13.4	3.76	22.9	8
Eucypris virens	Euc vir	3.64	23.3	3.62	2.9	1
Fabaeformiscandona acuminata	Fab acu	0.38	2.3	0.38	5.7	2
Fabaeformiscandona brevicornis	**Fab bre**	0.52	2.0	0.52	11.4	2
Fabaeformiscandona fabaeformis	Fab fab	0.10	0.5	0.09	5.7	2
Fabaeformiscandona fragilis	Fab fra	0.76	4.1	0.76	11.4	4
Fabaeformiscandona hyalina	Fab hya	0.02	0.2	0.02	2.9	1
Fabaeformiscandona protzi	Fab pro	0.45	2.2	0.45	5.7	2
Limnocythere inopinata	Lim ino	0.07	0.5	0.07	2.9	1
Neglecandona neglecta	Neg neg	2.57	8.3	2.55	22.9	7
Potamocypris juv.	Pot juv	0.10	0.6	0.09	2.9	1
Potamocypris zschokkei	**Pot zsc**	0.81	2.8	0.80	22.9	7
Pseudocandona albicans	Pse alb	0.26	0.7	0.26	20.0	6
Pseudocandona compressa	Pse com	0.05	0.3	0.05	2.9	1
Pseudocandona juv.	Pse juv	2.19	4.4	2.17	54.3	15
Pseudocandona sarsi	**Pse sar**	0.10	0.6	0.09	2.9	1
Psychrodromus fontinalis	**Psy fon**	0.07	0.5	0.07	2.9	1
Psychrodromus juv.	**Psy juv**	21.43	134.7	21.27	8.6	3
Psychrodromus olivaceus	**Psy oli**	0.60	2.8	0.59	8.6	3
Schellencandona belgica	**Sch bel**	0.02	0.2	0.02	2.9	1
Scottia pseudobrowniana	**Sco pse**	0.88	3.1	0.87	25.7	8
Total		100.74	202.8			22

Taxa in bold are known to be associated with springs (Meisch, 2000).

Table 2. Ostracod diversity metrics in the Krąpiel valley springs.

	Mean	SD
Taxa S	5.379	2.311
Individuals	120	160
Dominance D	0.418	0.109
Simpson 1-D	0.582	0.109
Shannon H	1.110	0.289
Evenness e^H/S	0.667	0.213

area 2 grouping mainly helocrenes (denoted by H). Area 3 groups springs flooded by the river overflow (F).

In the species-based NMDS plot (Fig. 3), the taxa known to be associated with springs are grouped in areas 1 and 2, whilethe eurytopic species and those associated with small-water bodies, usually with mud deposits, are grouped in between areas 1 and 2.

Positive Spearman's rank correlation coefficients were found between spring species pairs: *Candonopsis scourfieldi* and *Psychrodromus fontinalis* (k = 0.49), *Eucypris pigra* and *Potamocypris zschokkei* (k = 0.44), *Eucypris pigra* and *Fabaeformiscandona brevicornis* (k = 0.52), and *P. zschokkei* and *Psychrodromus olivaceus* (k = 0.32). *Scottia pseudobrowniana* and *Cryptocandona vavrai* were not significantly correlated with any other spring species.

The DCA results revealed the gradient length represented by the first ordination axis to be higher than 3 SD, for which reason the direct CCA ordination was performed. The cumulative percent variance of the species data on the first and second axis explains 27% of the total variance in the species composition (Tab. 3).

3.1 Types of springs and substrates

The CCA performed on the ostracod assemblages and spring types showed the variables used in the ordination to

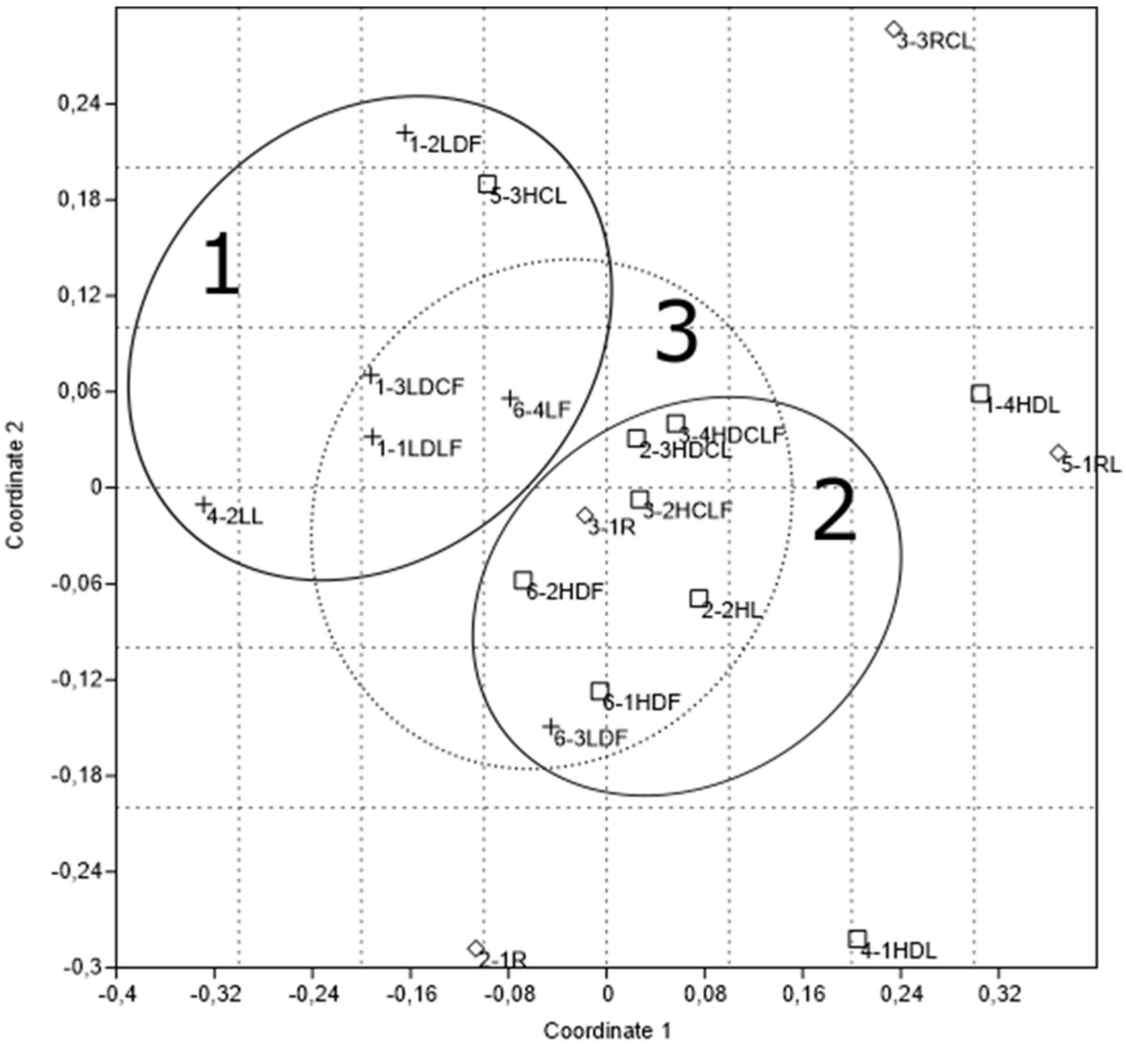

Fig. 2. Two-dimensional NMDS plot for each spring. Coding: Two digits – spring number; L, H or R – limnocrene, helocrene or rheocrene, respectively; D – drying up, C – presence of *Cardamine amara*, L – presence of leaf litter, F – flooded.

explain 28% of the total variance. The stepwise selection of environmental variables showed only three out of the six environmental parameters to statistically significantly ($p < 0.05$) explain the moderate range of total explained variance in the species occurrence. The presence of limnocrenes, flooding, and leaf litter were responsible for 9.4, 6.9, and 3.5% of the variance in the ostracod assemblages, respectively. As shown by the CCA ordination plot, the presence of leaf litter is related to the area 1 species association in which *Fabaeformiscandona brevicornis*, *Psychrodromus olivaceus*, *Scottia pseudobrowniana*, *Cryptocandona vavrai*, *Eucypris pigra*, *Potamocypris zschokkei*, and *Candonopsis scourfieldi* are known for being associated with springs. Area 2 brings together species associated with limnocrenes (Fig. 4, Tab. 1).

A summary of the ostracod occurrence in individual spring types is given in Table 4. The diversity metrics showed no significant differences between limnocrenes, helocrenes, and rheocrenes. Significant were the differences in the Evennesse^H/S and the number of individuals between springs with and without leaf organic matter as well as between drying-up and not drying-up springs. Significant correlations were found between the total number of ostracods and springs without leaf litter (k = −0.41, $p < 0.05$), flooded springs (k = 0.34), and springs drying up in the warm part of the year (k = 0.4, $p < 0.05$).

The water flow rate in the springs was low, ranging from 0 to 0.181 m/s. The insolation depended primarily on the season and was linked to shading by trees. The plant cover consisted mainly of *Cardamine amara* and *Carex acutiformis*. The bottom sediment was mainly mineral. The plant cover correlated positively with the sediment organic matter content and sorting coefficients M and W (Tab. 5).

The CCA run on the ostracod assemblages and the substratevariables showed the variables used in the ordination to explain 17.7% of the total ostracod variance. The stepwise selection of environmental variables showed only two of the seven environmental parameters proved significant ($p < 0.05$) in explaining the moderate range of the total variance in the species occurrence. Substrates dominated by the mineral and

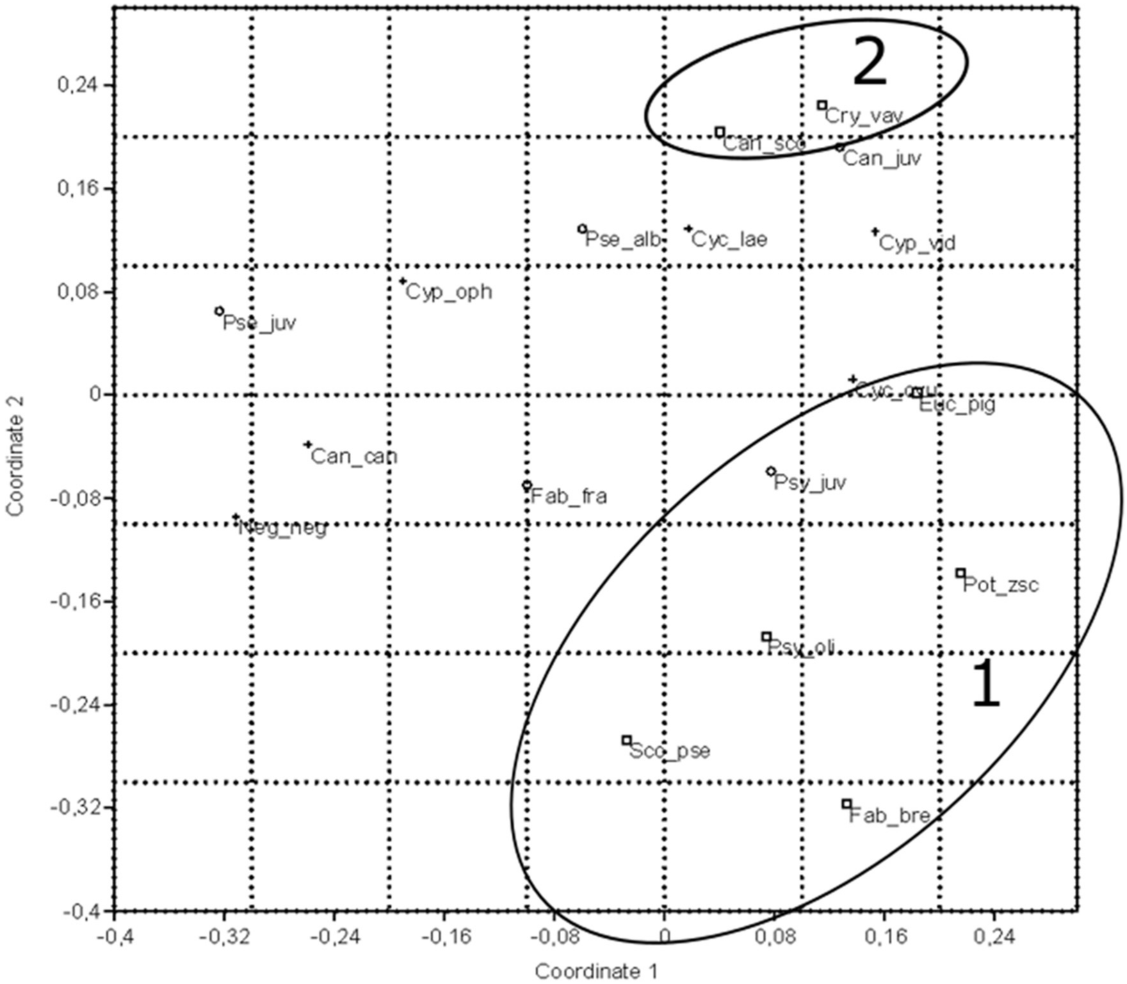

Fig. 3. Two-dimensional NMDS plot for species. For abbreviations see Table 1.

Table 3. Summary of DCA for samples from the Krąpiel valley springs.

Axis	1	2	3	4	Total inertia
Eigenvalue	0.816	0.563	0.404	0.211	5.089
Length of gradient	4.036	4.454	3.504	2.576	
Cumulative percentage variance of species data	16.0	27.0	35.0	39.2	
Sum of all eigenvalues					5.089

organic content accounted for 7.2 and 7.4% of the variance in the ostracod assemblages, respectively. As shown by the CCA plot, the presence of mineral sediment is related to the occurrence of species typical of springs (Fig. 5, Tab. 1). Organic matter-dominated substrates were related only to juvenile *Psychrodromus* sp. individuals and, to a lesser extent, to *Candona fragilis* and *C. candida*.

3.2 Physical and chemical water properties

The oxygen content in some of the springs was low due to the low water flow rate and a high leaf litter supply. The leaf litter content was negatively correlated with the mineral content, the correlations with BOD_5 and flow rate being positive. The water pH fluctuated between neutral and acidic. The mineral and organic matter contents varied extensively between the springs (Tab. 6).

The abundance of *Candona candida* correlated positively with PO_4 (k = 0.43, $p < 0.05$), Fe (k = 0.66), and turbidity (k = 0.43); *Cypridopsis vidua* correlated positively with PO_4 (k = 0.39) and NH_4 (k = 0.62); *Eucypris pigra* correlated positively with BOD_5 (k = 0.34); *F. brevicornis* correlated positively with conductivity (k = 0.33) and NO_3 (k = 0.52); juvenile *Psychrodromus* sp. correlated positively with PO_4 (k = 0.6), NH_4 (k = 0.83), and turbidity (k = 0.38). On the other hand, reverse correlations were found between the abundances of *Psychrodromus olivaceus, Scottia pseudobrowniana,* and *F. fragilis* and pH (k = −0.37, k = −0.41, k = −0.35, respectively).

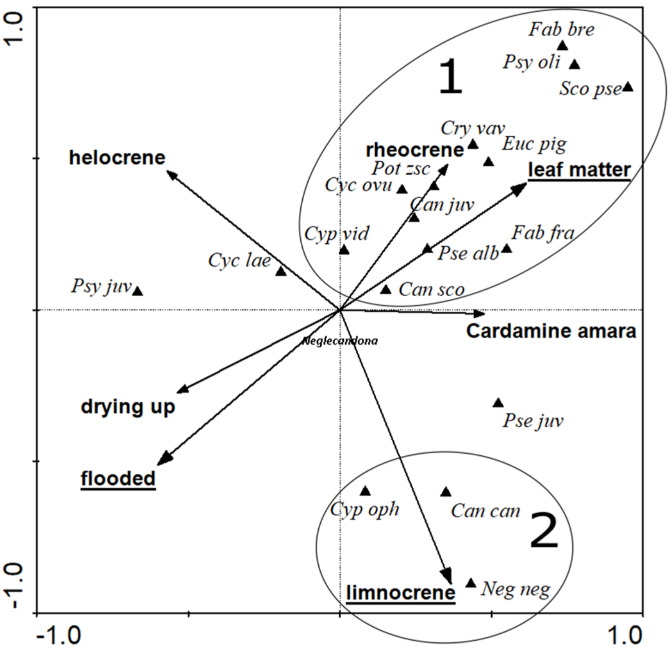

Fig. 4. Ordination plot for species and environmental variables (spring type) on the first two CCA axes for samples from the Krąpiel valley springs. For abbreviations see Table 1.

Table 4. The mean number of ostracods, mean number of species, Shannon H, Evenness e^H/S, and dominants in individual Krąpiel valley spring types.

Spring type	Abundance	Number of species	Shannon H	Evenness e^H/S	Dominant species
Limnocrenes	92	5	1.044	0.713	Canae
Helocrenes and rheocrenes	80	5	1.032	0.702	Can juv, Psyjuv, Cyp vid
Leafliter-rich	44	4	1.899	0.774	Can juv, Cyp vid
Leafliter-poor	80	6	1.157	0.063	Psyjuv, Can juv, Cypoph, Cyp mar
Flooded	135	5	1.072	0.664	Psyjuv, Can juv, Cypoph, Cyp mar
Not flooded	44	4	0.980	0.746	Can juv, Euc pig, Cyp vid
Drying up	130	5	0.571	1.112	Can can, Can juv, Cyp vid, Cypoph
Not dryingup	48	4	0.454	0.892	Cancan, Canjuv, Cypvid

For abbreviations see Table 1.

Table 5. Average values of substrate-associated environmental parameters in the Krąpiel valley springs in different seasons.

	May			July			November		
	Average	Max	Min	Average	Max	Min	Average	Max	Min
Flow rate	0.017	0.178	0.000	0.037	0.181	0.000	0.013	0.087	0.000
Insolation	24.27	42.46	1.41	2.87	6.25	0.95	20.00	73.68	0.58
Plant cover	1.19	4.00	0.00	0.80	3.00	0.00	1.29	5.00	0.00
Organic matter	15.42	46.69	0.75	5.42	15.89	1.43	24.19	79.05	0.26
Mineral matter	80.29	99.25	12.74	94.58	98.57	84.11	75.81	99.74	20.95
M	2.14	3.07	1.11	2.19	3.07	1.55	2.04	3.07	0.58
W	1.55	2.00	1.25	1.42	1.57	1.33	1.38	1.88	0.93

Table 6. Physical and chemical water properties in the Krąpiel valley springs in different seasons.

	May			July			November		
	Average	Max	Min	Average	Max	Min	Average	Max	Min
O_2	4.89	9.80	0.30	5.30	7.90	0.80	5.91	11.20	2.60
pH	7.17	7.89	6.48	6.54	6.92	5.80	6.74	7.90	5.30
Temperature	13.25	20.50	10.30	17.86	26.70	10.90	11.47	13.50	8.90
Conductivity	194	342	141	274	374	187	200	343	176
NH_4	0.536	3.100	0.100	0.465	0.673	0.272	0.215	0.522	0.079
NO_3	14.03	60.00	0.10	10.96	24.51	1.21	4.59	39.42	0.10
PO_4	0.299	2.148	0.010	0.120	0.266	0.022	0.311	1.751	0.010
Fe	0.204	1.200	0.020	0.132	0.520	0.020	1.022	6.500	0.020
Turbidity	31	115	0	21	35	2	29	122	0
Hardness	194	433	47	259	402	159	179	280	62
BOD_5	2.09	5.10	0.30	3.38	5.00	0.60	2.64	6.50	1.30

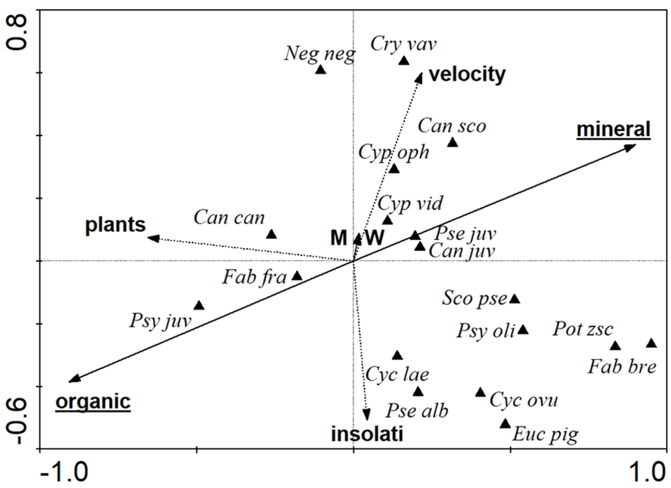

Fig. 5. Ordination plot for species and environmental variables (substrate) on the first two CCA axes for samples from the Krąpiel valley springs. For abbreviations see Table 1.

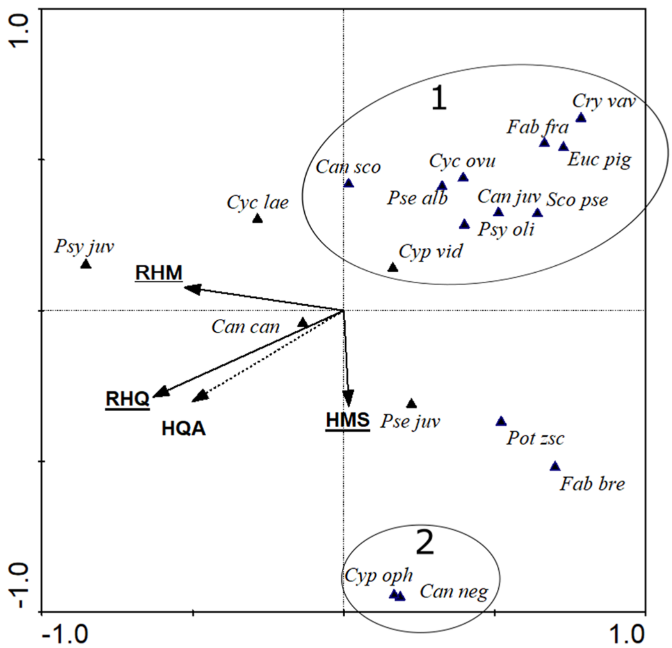

Fig. 6. Ordination plot for species and environmental variables (hydromorphological factors) on the first two CCA axes for samples from the Krąpiel valley springs. For abbreviations see Table 1.

The total number of ostracods showed significant correlations with the contents of iron (k = 0.41) and organic matter (k = 0.49).

The results of CCA for ostracod assemblages and hydromorphological parameters indicated that the variables used in the ordination explained 22% of the total variance in Ostracoda. Stepwise selection of environmental variables showed that three of the four environmental parameters statistically significantly ($p < 0.05$), explained the moderate range of total variance in the occurrence of species. The river habitat modification index (RHM) was responsible for 6% of the variance in ostracod assemblages, the habitat modification score (HMS) for 6%, and the river habitat quality index (RHQ) for 5.5%. In the CCA ordination diagram the RHM is related to juvenile *Psychrodromus* sp., and HMS is related to the species in area 2. The species concentrated in area 1 are no trelated to any of the hydromorphological parameters, which may indicate that the transformations of the river habitat are not severe enough to affect the assemblages of these ostracods in the springs (Fig. 6).

3.3 Taxonomic remarks

According to Meisch (2000), *Cypria ophtalmica* has two forms: *C. ophtalmica* f. *ophtalmica* and *C. ophtalmica f. lacustris*. Morphological features, coloration and the habitat of occurrence showed the form *lacustris* to be represented in our materials. *Cypria ophtalmica f. lacustris* occurs almost exclusively in springs (Meisch *et al.*, 2019).

4 Discussion

The 22 springs sampled in this study yielded 30 ostracod species, including 8 known from the literature to prefer springs or to be found in water bodies associated with springs (Tab. 1). In addition, there were also two stygobiontic species: *Schellencandona belgica* and *Cryptocandona vavrai* (Meisch, 2000). The remaining species were mainly either eurytopic or associated with small water bodies or wetlands. For comparison, Rossetti *et al*. (2005) found 12 species (including 3 spring ones) in 31 lowland springs of Northern Italy, while Rossetti *et al*. (2020) recorded 19 (5) taxa from 50 springs in the same region. The spring species in out study accounted for 30% of the total species richness, about 25% being reported by the studies cited. In our study, the number of taxa per spring ranged from 2 to 9 and averaged 5. This is consistent with results of Rossetti *et al*. (2020) who reported the mean number of taxa per site to be 2.5–2.2.

In this study, the dominants and/or the most frequent taxa were *Cypria ophtalmica, Cypridopsis vidua, Candona candida, Cyclocypris laevis*, juvenile *Candona* sp., juvenile *Psychrodromus* sp., and juvenile *Pseudocandona* sp. Literature data on the distribution of ostracods in springs concern mainly from mountain areas. Despite the differences in the altitude, the mountain spring ostracod assemblages were dominated by the same species as those listed above (Roca and Baltanás, 1993; Scharf *et al*., 2004; Külköylüoğlu and Yılmaz, 2006; Rosati *et al*., 2014; Zhai *et al*., 2015). In limnocrenes of lowland Italy, the most common species were *C. ophtalmnica, Cyclocypris leavis* (Rossetti *et al*., 2005), and *C. ophthalmica, Cypridopsis vidua, Prionocypris zenkeri* (Rossetti *et al*., 2020). Crenophilic and spring-related species showed usually a low prevalence and low frequency. One of such species, *Scottia pseudobrowniana*, may also be semi-terrestrial (Meisch, 2000; Rossetti *et al*., 2020). Differences in the ecological preferences of *S. pseudobrowniana* and the stygobiontic *C. vavrai* were confirmed by the correlation analysis which showed those species not to be significantly correlated with other spring species. A very similar pattern among crenobionts was reported by Rossetti *et al*. (2020) who found single such species at individual sites (1–7).

4.1 Effects of spring type and substrate

With respect to the spring type, the present study showed *C. ophtalmica* to be the most common taxonin both helocrenes and limnocrenes; while *C. laevis*, *C. ovum,* and *C. vidua* were most common in helocrenes, *C. candida* and *N. neglecta* being most common in limnocrenes. *C. ophtalmica, C. laevis,* and *C. ovum* are remarkably tolerant of a wide range of environmental condition (Meisch, 2000). *C. vidua* is often associated with a plant-derived substrate (Meisch, 2000), present in 54% of the helocrenes. The dominance of adult specimens of *C. candida* and *N. neglecta* in limnocrenes may have been related to the fact that these springs did not dry up, for which reason the species could complete their life cycles there; on the other hand, helocrenes, highly prone to drying-up, showed numerous *Candona* spp. larvae. No comparative literature data could be found on lowland helocrenes. In the case of limnocrenes, Rossetti *et al*. (2005) indicated *C. ophtalmica* and *C. laevis*as frequently occurring and *C. vidua* as very numerous there. Their recent research (Rossetti *et al*., 2020) showed *C. ophthalmica, C. vidua,* and *Prionocypris zenkeri* as the most frequent species.

The average species richness in our study was 5 per spring. Rosati *et al*. (2014) reported 4.7 and 2.1 species for helocrenes of the Palearctic rheo-limnocrenic springs. Rossetti *et al*. (2020) found 3.6, 2.5, 2.0, and 2.2 species in 2004, 2015 (in Lombardy), 2001, and 2015–2016 (in Emilia-Romagna), respectively. Thus, the species richness data collected so far show the Krąpiel valley springs to support quite high numbers of aquatic invertebrate species, which may be taken as evidence of the springs' high ecological status.

In no other ostracod study have the spring habitat properties such as the presence of leaf litter, drying-up in the warm part of the year, and flooding been taken into account. In our study, the number of ostracod species in springs without leaf litter was twice as high as that in springs with leaf-derived organic material. The species richness in flooded springs was higher than in not flooded ones by a factor of 3. In addition, the number of species in springs that were intermittently drying up was higher than in those not affected by drying by a factor of 3 as well. Consequently, the high number of ostracod species was associated with the absence of leaf litter, with flooding, and with intermittent drying up. A large amount of leaf litter generates oxygen deficiency in water with low flow rate, which clearly did not act in favor of the ostracod fauna. Spring dry-up, in turn, enhances sediment-bound organic matter mineralization and sediment oxygenation. In addition, desiccation is often necessary for ostracod resting eggs to develop. Flood water can remove the leaf litter burden from a spring and facilitate faunal exchange between the spring and the river bed.

4.2 Effects of physical and chemical water properties

It is interesting to find out whether the differences in species richness could be associated with differences in physical and chemical water properties. The species richness in our study was twice that reported by Rossetti *et al*. (2020). The oxygen status of the springs studied by Rossetti *et al*. (2020) was much lower and hypoxia was often observed. The temperature ranges in both studies were similar, particularly with respect to the Alpine springs. While our springs showed a slightly lower mineralization, their phosphate and nitrate levels were 10 and 3 times higher, respectively, that the springs studied by Rossetti *et al*. (2020). The latter were alkaline, while ours were neutral or slightly acidic. This comparison allows to infer that the oxygen content could have affected the number of ostracod species, while the mineral salt content was irrelevant. However, Rossetti *et al*. (2020) concluded that it was the water temperature and mineralization level that were the variables most important for structuring the ostracod communities. Mezquita *et al*. (1999) found ostracod assemblages to be mainly affected by water chemistry, with organic content and oxygen concentration playing a secondary role. Ostracod assemblages studied by Zhai *et al*. (2015) were significantly influenced by the mineral content and TOC. In this study, the total number of ostracods correlated significantly with the contents of iron and organic matter. The number of ostracods was probably not affected by the iron content in itself, and the correlation between the iron content and that of organic matter was more important. However, the association between the organic matter and ostracod abundance may be due to the ostracod feeding on i.a. dead plant fragments, including leaf litter, where by the litter decomposition is facilitated and contributes to the dissolved organic matter pool.

There is no literature evidence on the relationship between ostracod assemblages and the hydrological assessment of a river valley (River Habitat Survey, RHS). Such analyses have been performed for aquatic beetles (Pakulnicka *et al.*, 2016) and water mites (Zawal *et al.*, 2018) in the Krąpiel valley. In the case of beetles, hydrological parameters were responsible for 13.3% of the total variance in species composition, while anthropogenic transformation of the river valley (RHM and HMS) had a positive influence on the crenobiontic water mite fauna. For ostracods of this study, environmental variables explained 22% of the total variance in the species composition, and parameters defining anthropogenic transformations also significantly influenced the species assemblages (12% of the variance). It appears, however, that this group of environmental factors was more important for eurytopic than for most of the typical spring species.

5 Conclusions

Ostracod assemblages were most affected by the spring type (particularly limnocrenes), spring flooding by the river, the presence of leaf litter and fine particulate organic matter, a high content of NH_4, BOD_5, conductivity, pH and Fe. The total number of ostracods correlated significantly with the contents of iron and organic matter. The ostracod abundance in springs without leaf litter was twice that in springs supporting leaf-derived coarse organic matter. In flooded springs, the abundance was three times that found in not flooded springs. The springs subject to periodic dry-out supported ostracod abundances three times higher than those in springs that did not dry up. Similarly, a higher number of species was associated with the absence of leaf litter, with flooding, and with drying up. Springs with a substrate dominated by mineral material rather than fine organic matter, with a higher pH, the presence of leaf litter, and the absence of flooding provided apparently more suitable habitat conditions for spring species. The hydrological factors within the river valley (River Habitat Survey, RHS) showed no effect on the spring species.

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