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ORIGINAL ARTICLE

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Comparative ecology of Ostracoda (Crustacea) in two rheocrene springs (Bolu, Turkey)

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Abstract A total of ten taxa belonging to the class Ostracoda of the Crustacea (*Darwinula stevensoni*, *Candona neglecta*, *Cypria ophthalmica*, *Ilyocypris bradyi*, *Prionocypris zenkeri*, *Herpetocypris chevreuxi*, *Psychrodromus olivaceus*, *Heterocypris incongruens*, *Scottia pseudobrowniana*, *Eucypris* sp.) were collected from two rheocrene Darwinula stevensoni springs (Çetin Bey and Çaygökpınar springs) on 15 separate occasions between November 2002 and November 2004. Almost all of the species identified exhibit cosmopolitan distributions – at least in the Holarctic region. The presence of *Scottia pseudobrowniana* represents the second recording of this species in the ostracod fauna of Turkey. The dominant taxa in both springs was *Cy. ophthalmica*, *I. bradyi*, *Pr. zenkeri* and *Ca. neglecta*. Correlation analyses suggested a significant positive relationship in relative abundance between *I. bradyi*, *Pr. zenkeri* and *Cy. ophthalmica*. Species composition differed significantly between the upper and lower study sites for each spring, but differences could not be detected between sites at the same elevation across sites. Environmental tolerance index (ETI) values suggest that species with high optima and tolerance ranges show cosmopolitan characteristics.

Keywords Cosmopolitan · Environmental tolerance index (ETI) · Ostracoda · Pseudorichness · Springs

Introduction

Springs are ecologically important because they act as ecotones between hypogean and epigean habitats (Smith et al. 2003) and are also strongly influenced by anthropogenic activities (Särkkä et al. 1997). Studies on the water quality and faunal diversity of springs provide invaluable knowledge on the past, present and future conditions of both surface and underground waters (Gooch and Glazier 1991). The water conditions of springs are nearly stable and, therefore, they can provide the opportunity to explain speciation in both an evolutionary and ecologically context (Glazier 1998; Külköylüoğlu and Vinyard 1998; Külköylüoğlu 2003a). For example, old springs often have more endemic species than newly formed one (Hynes 1970; Roca and Baltanás 1993), most likely because such stable conditions facilitate changes in the phenology, habitat preferences, life histories and ecological tolerances of spring-dwelling species. However, in order to determine these changes, we need to carry out long-term comparative analyses of springs to obtain data that will enable the effects of environmental factors on species occurrence to be evaluated (Glazier 1991).

Ostracods are bivalved crustaceans that can be found in all types of water bodies. They can be used as indicator species in aquatic habitats (Delorme 1983; Külköylüoğlu 1999; Mezquita et al. 1999, 2001) because each species prefers specific ecological conditions, which can be assessed based on the level of the response of the species to environmental changes (Geslin et al. 2002; Külköylüoğlu 2004; Ruiz et al. 2004). The concept of indicator species depends on a knowledge of the ecological requirements of individual species (Külköylüoğlu 2003a); however, little information is available for most ostracod species, with the exception of some cosmopolitan species with broad tolerance levels to environmental fluctuations (Külköylüoğlu 2005a). Given that spring habitats are natural laboratories with stable conditions, any change in spring water quality may be reflected first as changes in the relative abundance and species composition – in the favor of cosmopolitan species. In such a case, an increase in the relative abundance of cosmopolitan species may imply effects of disturbance and pollution, resulting in a reduction in the relative abundance of native species (Külköylüoğlu 2005b). Thus, the ratio between cosmopolitan and native species can be used as a signal of changes in spring habitat quality; this

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change is called ‘‘pseudorichness’’ (Külköylüoğlu 2004). However, testing the merits of pseudorichness requires long-term detailed studies of different habitats.

The aim of this study was to provide answers to the following questions: (1) How do the ostracod communities in two ecologically similar springs differ? (2) Is there any correlation between environmental variables and the species composition of ostracods? (3) How does seasonality affect ostracod occurrence in springs? (4) Do cosmopolitan species have higher tolerance and optimum values than native species? (5) Can pseudorichness describe the relationship between species composition and habitat quality?

Materials and methods

The species composition of the ostracod The composition and physical characteristics of two permanent rheocrene springs (Fig. 1), located in the Bolu region of Turkey, were compared in terms of their physical characteristics and the species composition of the ostracod populations in the springs. Çetin Bey spring (hereafter denoted simply as Çetin Bey; 40°42′570″N 31°31′856″E) is located in the western part of Bolu city, near the Gölköy reservoir, at 730 m a.s.l. Çaygökpınar spring (= Çaygökpınar; 40°42′342″N 31°1′819″E) is located in the southern part of Bolu city at about 760 m a.s.l. The distance between the springs is about 18 km. Çetin Bey flows from west to east, while Çaygökpınar flows from south to north. Both springs flow about 60 m down from the spring head (eucrenal site). At that point, Çetin Bey reaches the Lake Gölköy reservoir, whereas Çaygökpınar joins a tributary of Büyüksu creek, which flows north of Bolu. Çetin Bey was a limnocrene spring with a pool full of water until the end of 2001, when the spring was covered by the city’s environmental protection foundation with a concrete structure to provide water for public use (Külköylüoğlu 2003a). As a consequence of this intervention, the spring changed from a limnocrene to a rheocrene spring with a slow water flow, and it has remained so to the present. In contrast, the lower parts of Çaygökpınar, 60 m away from the eucrenal site, have been heavily disturbed by the lime dust, but there have been no other obvious sources of disturbance or contamination.

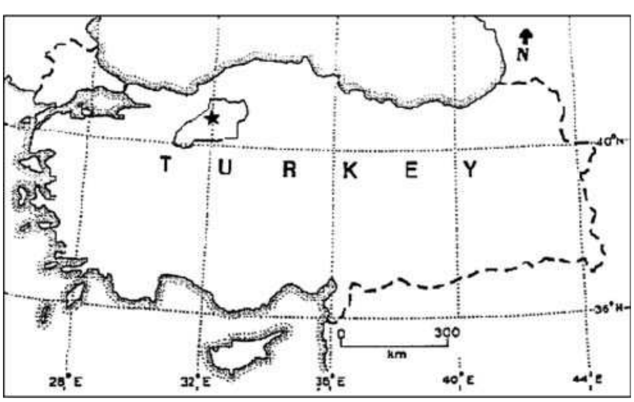

Fig. 1 Map of study region (Bolu region of Turkey). *Star* study site

Two stations were selected at both study sites – one at the eucrenal site and the second 60 m downstream. The springs were visited before 1200 hours on 15 separate occasions. During each visit, pH, redox potential (Eh, in mV), temperature (T, in °C), electrical conductivity (EC, in µs/cm), dissolved oxygen (DO, in mg/l), salinity (ppt) and percentage oxygen saturation (%Sat) were measured in situ. Redox potential (Eh) was calculated for the value of the standard hydrogen electrode (SHE). A Hanna model HI-98150 pH/ORP meter was used to measure the pH and redox potential, and aYSI-85 model oxygen-temperature meter was used to measure the remaining five variables. Additional water samples were also collected and returned to the laboratory to determine biological oxygen demand (BOD) for each station, following the standard methods of American Public Health Association (APHA 1989).

Geographical data (elevation, latitude, longitude) were recorded with a global positioning system (Garmin 45 XL model). Both sediment and water samples were collected from the spring source (eucronal) and downstream sites using a plankton net (mesh size 0.25 mm). These samples were put into 250-ml glass jars containing 70% of ethanol. In the laboratory, samples were first washed with pressurized tap water, then filtered through four standard-sized sieves (mesh size: 0.25, 0.50, 1.0 and 2.0 mm) and finally stored in 70% ethanol. The identification of ostracod species was based on the soft body parts and valves in accordance with the systematic keys of Bronshtein (1947) and Meisch (2000) and on Kempf’s index and bibliography of non-marine Ostracoda (1980, 1997). Ostracod species were identified under an Olympus BX-51 model microscope after they were permanently mounted in lactophenol solution on slides.

Statistical analyses

Two similarity indices (Jaccard and Sorenson similarity indices) based on species occurrence were used to compare the community structure of the springs (Magurran 1988). Values of tolerance (tk) and optima (uk) were calculated for each of nine ostracod species for six environmental variables (Ter Braak and Barendregt 1986; Külköylüoğlu and Dügel 2004). The environmental tolerance index (ETI) was used to estimate the relative tolerance range of each species for different environmental variables (Curry 1999). Unweighted pair group mean averages (UPGMA) was used to determine the clustering relationships among species based on their occurrence, which was later correlated with their ecological preferences. Chi-squared tests were performed on $\log_{(e)}$-transformed variables in UPGMA within the MVSP ver. 3.1 multi-variate statistical package (Kovach 1998). A non-parametric Spearman’s correlation analysis was performed to outline associations among species and environmental variables. During the analyses, singletons (i.e. species occurring at only one site, such as *Eucypris* sp.) were not entered into multivariate ordinations. A *t* test for equal variances was used to compare possible variations among environmental variables using $\alpha = 0.05$ as the significance level. A graphical representation of the Ostracoda watch model (OWM) (Külköylüoğlu 1998) was used to show the seasonal distributions and monthly occurrences of each species.

Results

A total of ten ostracod species [*Darwinula stevensoni* (Brady and Robertson, 1870), *Candona neglecta* Sars, 1887, *Cypria ophthalmica* (Jurine, 1820), *Ilyocypris bradyi* Sars, 1890, *Prionocypris zenkeri* (Chyzer and Toth, 1858), *Herpetocypris chevreuxi* (Sars, 1896), *Psychodromus olivaceus* (Brady and Norman, 1889), *Heterocypris incongruens* (Ramdohr, 1808), *Scottia pseudobrowniana* Kempf, 1971 and *Eucypris* sp.] were collected from both springs during a 15-month sampling period (Tables 1, 2, 3, 4). Nine species showed almost cosmopolitan distribution in the Holarctic region. Seven and nine species were found from Çetin Bey and Çaygökpınar, respectively. The number of species in both springs was lower at the source than at the downstream sites. At the first station (headwater) of Çetin Bey, we found 20 individuals belonging to three species; in contrast; at the second station there were about 310 individuals belonging to seven species. Similarly, at the first station of Çaygökpınar we found 67 species belonging to seven species, while at the second station there were 346 individuals belonging to seven species (except *Eucypris* sp.). Of the species identified, *D. stevensoni* and *S. pseudobrowniana* were only collected from Çaygökpınar spring; for *S. pseudobrowniana* (five individuals), this is only its second recording in Turkey. Although only rarely present in the first station of both springs, *Pr. zenkeri* and *I. bradyi* were generally abundant in the respective downstream stations (but see Discussion). In contrast, *Hc. chevreuxi* was only found in the downstream station of Çetin Bey (42 individuals). *Cypria ophthalmica* was collected from Çaygökpınar spring's first and second stations, while *Ca. neglecta* and *Ps. olivaceus* were found in almost all stations of both springs. A well-known cosmopolitan species, *Het. incongruens*, was the most widely distributed species in that it was found at all sites except for the headwater station of Çetin Bey. In both springs, the mean values (Table 5) were generally lower at the source than at the downstream sites.

The UPGMA analysis revealed a clustering of four main groups of species (Fig. 3). The first group includes only *Pr. zenkeri*, which was the most abundant species encountered from all stations – with the exception of the headwater of Çetin Bey, the second clustering group consists of five species (*Het. incongruens, Eucypris* sp., *S. pseudobrowniana, D. stevensoni* and *Her. chevreuxi*) and both the third and fourth groups have two species each, *Ps. olivaceus* and *Ca. neglecta*, and *I. bradyi* and *Cy. ophthalmica*, respectively.

The results of the UPGMA mean averages analysis were supported by the results of Spearman's correlation analyses, with both sets of results revealing positive

Table 1 The monthly measured variables[a] and the species recorded at Station 1 (Çetin Bey spring, first station) (*n.a.* not available)

Date	Month	pH	Eh (mV)	DO (mg/l)	EC (μs/cm)	%Sat.	Temperature (°C)	Salinity (ppt)	Species[b]
23.11.02	N02	7.62	176.07	7.89	630	75.5	13.2	0.3	
04.05.03	M03	7.45	190.03	6.26	679	72.9	15.1	0.3	
18.10.03	Oc03	7.57	182.89	7.48	600	73.6	14.7	0.3	
15.11.03	N03	n.a.	214.86	6.13	652	59.2	12.9	0.3	Cn (1), Ib (1)
13.12.03	De03	7.7	177.9	6.6	n.a.	61.2	11	0.3	
17.01.04	Ja04	7.47	189.22	4.36	594	44.1	12.2	0.3	Cn (1)
13.03.04	Ma04	7.3	196.83	6.98	536	66.5	12.8	0.2	Po (6)
17.04.04	Ap04	7.12	205.82	8.13	638	80	14.2	0.3	Cn (1), Ib (1)
15.05.04	M04	7.03	211.68	6.32	293	60.8	14.1	0.1	
12.06.04	J04	6.7	230.93	4.97	628	50	15.1	0.3	
09.07.04	Jl04	6.8	208.42	4.32	591	46.4	16.5	0.3	Ib (1)
27.08.04	Ag04	7.03	205.91	3.81	679	41.9	19.9	0.3	
18.09.04	S04	6.76	219.52	2.33	647	24.9	18.2	0.3	Cn (6)
17.10.04	Oc04	6.78	220.67	1.83	674	19.6	17.5	0.3	Cn (1)
13.11.04	N04	6.76	232.09	1.74	381.6	17.1	14.4	0.2	Po (1)
	Mean	7.14	204.19	5.27	587.3	52.9	14.7	0.2	
	Minimum	6.7	176.07	1.74	381.6	17.1	11	0.1	
	Maximum	7.62	232.09	8.13	679	80	19.9	0.3	

[a] Variables measured: pH; Eh, redox potential; DO, dissolved oxygen; EC, electrical conductivity; %Sat., saturation; temperature, temperature of the water
[b] All species encountered during this study. Numbers of individuals are given in parenthesis. Cn, *Candona neglecta*, Ib *Ilyocypris bradyi*, Po *Psychrodromus olivaceus*, Pz *Prionocypris zenkeri*, Hc, *Herpetocypris chevreuxi*; Hi, *Heterocypris incongruens*; Co, *Cypria ophthalmica*; Sp, *Scottia pseudobrowniana*; Ds, *Darwinula stevensoni*; El, *Eucypris* sp.

Table 2 The monthly measured variables and the species recorded at Station 2 (Çetin Bey, second station) (*n.a.*, not available)

Month	pH	Eh (mV)	DO (mg/l)	EC (µs/cm)	%Sat.	Temperature (°C)	Salinity (ppt)	Species
N02	8.07	147.0	13.86	614	132.6	12.9	0.3	Po (1)
M03	7.64	179.9	7	680	60.8	17.2	0.3	Pz (45), Po (2), Cn (4), Ib (16), Hc (4)
Oc03	8.18	145.8	4.78	381.7	51.2	16.2	0.2	Pz (22), Cn (4), Ib (12), Hc (2), Co (1), Hi (6)
N03	n.a.	214.8	9.22	462.9	87.3	12.9	0.2	Pz (43), Po (3), Cn (5), Ib (30), Hc (2), Hi (1)
De03	7.99	163	6.49	622	56.6	9	0.3	Pz (5), Ib (4), Hc (1)
Ja04	8.22	148.8	9.86	558	89.1	10.7	0.3	Pz (39), Cn (8), Ib (16), Hc (5)
Ma04	7.62	181.6	9.23	600	85.7	12	0.3	
Ap04	7.46	188.1	10.68	640	104.7	14.6	0.3	
M04	7.29	198.6	8.71	631	83.9	13.9	0.3	
J04	7.15	203.5	6.37	589	65.9	17.1	0.3	
Jl04	7.06	204.4	5.57	628	61.2	19.2	0.3	
Ag04	7.22	196.9	4.05	702	45.3	20.6	0.3	
S04	7.02	209.0	2.33	680	26.7	21.7	0.3	
Oc04	7.43	186.7	2.18	671	23.8	19.5	0.3	Ib (3), Hc (23)
N04	7.37	201.9	2.11	598	21.4	16	0.3	Pz (3), Ib (1), Hc (5)
Mean	7.55	184.7	6.83	603.8	66.4	15.5	0.2	
Minimum	7.02	145.8	2.11	381.7	21.4	9	0.2	
Maximum	8.22	214.8	13.86	702	132.6	21.7	0.3	

For definitions of variables and species names, see the footnote to Table 1

Table 3 The monthly measured variables and the species recorder at Station 3 (Çaygökpınar, first station) (*n.a.*, not available)

Month	pH	Eh (mV)	DO (mg/l)	EC (µs/cm)	%Sat.	Temperature (°C)	Salinity (ppt)	Species
N02	7.26	191.0	9.76	671	101.3	16.9	0.3	Pz (30), Po (4)
M03	7.14	205.8	5.38	717	56.5	17.5	0.4	
Oc03	7.14	204.6	6.03	556	63.4	17.6	0.3	Co (3)
N03	n.a.	212.7	6.38	343.8	64.7	16.2	0.2	Co (3)
De03	7.23	200.4	5.81	690	59.6	15.7	0.3	Co (5)
Ja04	7.03	208.8	6.39	625	64.2	15.9	0.3	
Ma04	7.25	202.6	6.66	633	67.1	15.8	0.3	
Ap04	7.04	209.8	6.01	685	61.8	17.2	0.4	Co (2), Sp (4)
M04	6.83	226.8	6.01	692	62.8	17.6	0.4	Sp (1), Hi (3)
J04	6.91	216.2	4.38	600	46.1	18	0.3	Hi (5)
Jl04	6.81	222.3	4.06	624	4.6	15.3	0.3	Co (1)
Ag04	6.9	217.5	4.38	627	44.8	16	0.3	Co (3), Cn (2), Ds (1)
S04	7	213.9	2.89	508	19.8	14.7	0.3	
Oc04	6.79	220.6	2.29	584	20.2	14.6	0.3	
N04	6.72	231.0	1.95	682	20.2	17.3	0.3	
Mean	7.00	212.3	5.22	611.9	50.4	16.42	0.3	
Minimum	6.72	191.0	1.95	343.8	4.6	14.6	0.2	
Maximum	7.26	231.0	9.76	717	101.3	18	0.4	

For definitions of variables and species names, see the footnote to Table 1

relationships among some of the species but not showing significant correlations between species and environmental variables (Table 6). Accordingly, *I. bradyi* had a positive relationship with *Pr. zenkeri* and *Cy. ophthalmica* ($P < 0.01$) and *Her. chevreuxi* ($P < 0.05$). The associations among the three species (*Her. chevreuxi*, *Pr. zenkeri* and *I. bradyi*) may be related to their similar ecological preferences.

The ostracod watch model identified seven species with differing seasonal distributions. *Prionocypris zenkeri* and *I. bradyi* showed similar life cycles and were detectable throughout most of the year (Fig. 3). In contrast, *Ca. neglecta* and *Cy. ophthalmica* were detectable only from July to May (except December and March, respectively; sampling was also not possible in February), *D. stevensoni* only from August to January, *Her. chevreuxi* only from October to January (and May) and *S. pseudobrowniana* only from April to May.

Optima (uk), tolerance (tk) and ETI values for each species are listed in Table 7. The cosmopolitan species generally showed relatively high tolerance levels and had different optima estimates than the noncosmopolitan

Table 4 The monthly measured variables and the species recorded at Station 4 (Çaygökpınar, second station) (*n.a.*, not available)

Month	pH	Eh (mV)	DO (mg/l)	EC (μs/cm)	%Sat.	Temperature (°C)	Salinity (ppt)	Species
N02	7.86	159.3	13.46	658	137.7	17	0.3	Co (7), Pz (16), Ib (2), Po (17)
M03	7.79	182.9	5.53	688	67.5	24.4	0.3	Co (17), Pz (19), Ib (1), Po (6)
Oc03	7.88	161.9	7.8	364.3	83.1	18.3	0.2	Co (2), Pz (21), Cn (2)
N03	n.a.	216.2	9.5	656	87.4	10.7	0.3	Co (3), Pz (10), Cn (2), Po (2), Ds (6)
De03	7.67	179.2	7.6	614	69.2	12	0.3	Co (11), Pz (30), Ib (17), Ds (1), El (1)
Ja04	7.86	168.0	7.94	272.9	74.2	12.3	0.1	Co (13), Pz (3), Cn (5), Ib (5), Ds (6)
Ma04	8.27	146.6	7.2	510	65.5	11	0.2	Cn (5), Po (1)
Ap04	7.48	184.4	12.17	695	129.8	18.7	0.4	Pz (3)
M04	7.36	193.1	6.14	697	63.2	17	0.4	Pz (11), Ib (1), Po (1)
J04	7.08	206.3	5.47	708	60.2	19.5	0.3	Pz (30), Ib (4)
Jl04	7.15	203.	5.75	638	59.2	16.2	0.3	Co (2), Pz (5), Cn (3), Hi (1)
Ag04	7.38	190.8	4.31	581	45.4	17.5	0.3	Co (2), Ib (2), Po (1), Ds (2)
S04	7	211.2	3.64	598	38.7	18.2	0.3	Co (7), Pz (9), Ib (1), Po (1), Ds (5), El (1)
Oc04	7.37	190.8	2.46	565	26.7	18.6	0.3	Co (9), Pz (2), Ib (2), Ds (2)
N04	7.31	194.4	2.55	666	26.8	18.1	0.3	Ds (1)
Mean	7.53	185.0	6.77	594	68.97	16.63	0.28	
Minimum	7	146.6	2.46	272.9	26.7	10.7	0.1	
Maximum	8.27	216.2	13.46	708	137.7	24.4	0.4	

For definitions of variables and species names, see the footnote to Table 1

Table 5 Monthly mean values of eight variables at four stations in both springs (*n.a.*, not available)

Month	pH	Eh (mV)	DO (mg/l)	EC (μs/cm)	%Sat.	Temperature (°C)	Salinity (ppt)	BOD	*T*(°C)(a)
N02	7.70	168.37	11.24	643.2	111.7	15	0.3	n.a.	12
M03	7.50	189.71	6.042	691	64.42	18.55	0.32	n.a.	30.3
Oc03	7.69	173.82	6.522	475.5	67.82	16.7	0.25	4.61	18
N03	n.a.	214.68	7.807	528.6	74.65	13.17	0.25	4.45	11.8
De03	7.64	180.14	6.625	481.5	61.65	11.92	0.3	3.57	6.2
Ja04	7.64	178.74	7.137	512.4	67.9	12.77	0.25	3.1	7.1
Ma04	7.61	181.94	7.517	569.7	71.2	12.9	0.25	3.9	5
Ap04	7.27	197.08	9.247	664.5	94.07	16.17	0.35	5.52	17.4
M04	7.12	207.55	6.795	578.2	67.67	15.65	0.3	1.85	10.7
J04	6.96	214.27	5.297	631.2	55.55	17.42	0.3	1.96	27.2
Jl04	6.95	209.70	4.925	620.2	42.85	16.8	0.3	1.42	29.2
Ag04	7.13	202.82	4.137	647.2	44.35	18.5	0.3	1.8	34.1
S04	6.94	213.44	2.797	608.2	27.52	18.2	0.3	0.63	24.3
Oc04	7.09	204.74	2.19	623.5	22.57	17.55	0.3	0.88	25.2
N04	7.04	214.87	2.087	581.9	21.37	16.45	0.27	0.16	22.3
Mean	7.3	196.79	6.02	590.4	59.69	15.85	0.29	2.60	18.7
Minimum	6.94	168.37	2.087	475.5	21.37	11.92	0.25	0.16	5
Maximum	7.70	214.87	11.24	664.5	111.7	18.55	0.3	5.52	34.1

BOD, Biological oxygen demand
For definitions of variables and species names, see the footnote to Table 1

species. Because the ETI values lie between zero and one, species with ETI values nearer to one appear to be better adapted to the ecological conditions in these springs.

A *t* test for two samples with similar variances was applied to the environmental variables among stations. The first two stations of Çetin Bey and Çaygökpınar are significantly different ($P < 0.05$) for pH and redox potential, but there were no differences between the remaining variables ($P > 0.05$).

Both Jaccard's and Sorenson indices based on recorded species occurrence and environmental variables, resprectively (Table 8) indicated high similarities among Stations 2, 3 and 4. In contrast, the similarity was very slight between Station 1 and the other three stations.

Discussion and conclusion

A higher ETI value corresponds with a higher tolerance level for that species (Table 7). The UPGMA analysis revealed a clustering of four main groups of species, which was supported by the results of the Spearman's correlation analyses, indicating a positive relationship for some of the species shown in Table 6. The UPGMA analysis clustered *I. bradyi* and *Cy. ophthalmica* in the

Table 6 Spearman's correlation shows a relationship between seven environmental variables and the nine Ostracoda species

	pH	Eh (mV)	DO (mg/l)	EC (µs/cm)	% Sat.	Temperature (°C)	Salinity (ppt)	Co	Pz	Cn	Ib	Po	Hc	Ds	Sp	Hi
pH	1.000	−0.952[b]	0.71[b]	−0.253	0.754[b]	−0.552[a]	−0.242	0.385	0.467	0.027	0.439	0.155	0.178	−0.180	−0.043	−0.149
Eh (mV)		1.000	−0.514[a]	0.132	−0.554[a]	0.329	0.039	−0.469	−0.256	−0.084	−0.208	−0.017	−0.017	0.254	0.036	0.232
DO (mg/l)			1.000	−0.121	0.979[b]	−0.671[a]	−0.094	0.022	0.399	0.115	0.115	0.251	0.279	0.239	0.374	0.043
EC (µs/cm)				1.000	−0.082	0.643[b]	0.791[b]	0.029	−0.177	−0.262	−0.179	0.251	−0.285	−0.289	0.205	−0.315
% Sat.					1.000	−0.568[a]	−0.131	0.031	0.422	0.193	0.153	0.289	−0.239	−0.248	0.332	0.066
Temperature (°C)						1.000	0.485	0.042	−0.168	0.005	−0.047	0.036	−0.035	−0.083	−0.133	0.034
Salinity (ppt)							1.000	0.143	−0.069	−0.443	−0.118	−0.036	−0.319	−0.356	0.440	−0.244
Co								1.000	0.512	0.308	0.703[b]	−0.001	0.483	0.382	−0.381	−0.397
Pz									1.000	0.152	0.715[b]	0.206	0.251	−0.028	−0.169	0.301
Cn										1.000	0.203	0.103	0.196	0.447	−0.289	−0.062
Ib											1.000	−0.082	0.597[a]	0.367	−0.370	0.109
Po												1.000	−0.155	−0.060	−0.138	−0.275
Hc													1.000	0.430	−0.344	−0.177
Ds														1.000	−0.344	−0.330
Sp															1.000	0.108
Hi																1.000

For definitions of variables and species names, see the footnote to Table 1

[a] Significance (alpha two-tailed) = 0.05

[b] Significance (alpha) = 0.01

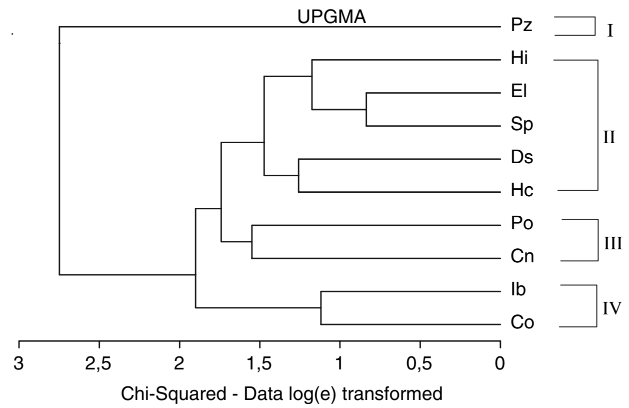

Fig. 2 Unweighted pair group mean averages shows the clustering relationships of ten ostracods collected from four stations of two springs. *Pz Pr. zenkeri*, *Hi Het. incongruens*, *El Eucypris* sp., *Sp S. pseudobrowniana*, *Ds D. stevensoni*, *Hc Her. chevreuxi*, *Po Ps. olivaceus*, *Cn Ca. neglecta*, *Ib I. bradyi*, *Co Cy. ophthalmica*

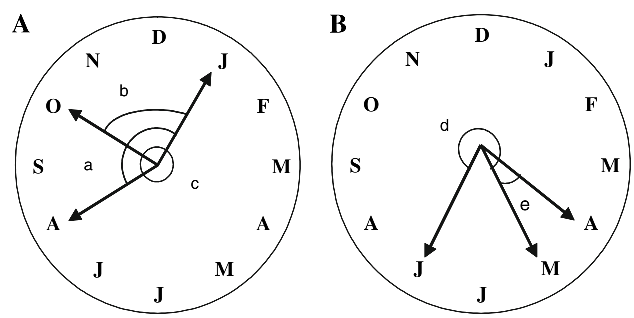

Fig. 3 Graphical representation of ostracoda watch model (OWM) showed seasonal distribution of species during study period. *Angles* indicate the occurrence of species in certain months (stenochronal distribution), *full circle* shows eurychronal distribution. **a** *a D. stevensoni b Her. chevreuxi**, *c Pr. zenkeri*, *I. bradyi*. **b** *d Ca. neglecta*** and *Cy. ophthalmicae****, *e S. pseudobrowniana*. For details see Tables 1, 2, 3, 4. Abbreviations for each month consist of the first letter of the (e.g. *J* January; *D* December, etc.). *Single asterisk* Additional occurrence in May, *two asterisks* except December and February, *three asterisks* except February and March

fourth group of the UPGMA dendrogram (Fig. 2). Such a relationship seems to be related to the ecological preferences of these two species, with both exhibiting a similar optimum and tolerance level for at least some of the variables (e.g., dissolved oxygen). The euchronal occurrence of *I. bradyi* may be related to its wide tolerance levels to fluctuations in some environmental variables. In general, wide tolerance levels can increase the chance of a species surviving over the long term in habitats where it occurs all around the year. Indeed, adults of *I. bradyi* survive year-round in a variety of European (Mezquita et al. 1999; Meisch 2000) and North American (Great Basin area) waters (Külköylüoğlu and Vinyard 2000). We found *I. bradyi* at salinities ranging from 0.1 to 0.4 ppt. In contrast to earlier reports, *I. bradyi* also seems to tolerate a wide range of DO values (2.11–9.86 mg/l) (Tables 1, 2, 4, 5).

Table 7 Descriptive statistics, including the optima (uk), tolerances (tk) and ETI values for mean pH, redox potential, dissolved oxygen, electrical conductivity, saturation and temperature of the water, for the nine Ostracoda species

	pH	Eh (mV)	DO (mg/l)	EC (μs/cm)	%Sat.	Temperature (°C)
C. ophthalmica						
Average	7.35	193.93	6.24	590.5	61.78	15.94
SD	0.31	15.94	2.68	76.73	26.76	2.38
Maximum	7.70	214.68	11.24	691	111.77	18.55
Minimum	6.94	168.37	2.19	475.5	22.57	11.92
Range	0.75	46.311	9.05	215.5	89.2	6.62
ETI[a]	1	0.99	0.98	1	0.98	1
Optima	7.44	190.27	6.14	581.7	60.83	15.57
Tolerance	0.26	14.67	2.29	81.02	22.47	2.65
P. zenkeri						
Average	7.29	197.47	6.05	587.71	59.98	15.58
SD	0.31	17.21	2.67	69.19	26.64	2.1
Maximum	7.70	214.87	11.24	691	111.77	18.55
Minimum	6.94	168.37	2.08	475.5	21.37	11.92
Range	0.75	46.50	9.15	215.5	90.4	6.62
ETI[a]	1	1	1	1	1	1
Optima	7.49	190.40	7.08	576.63	70.55	15.35
Tolerance	0.26	17.34	1.96	80.24	19.06	2.35
C. neglecta						
Average	7.31	196.67	5.83	594.11	57.73	16.13
SD	0.3	14.86	2.28	70.33	22.57	2.33
Maximum	7.69	214.6	9.24	691	94.07	18.55
Minimum	6.94	173.82	2.19	475.5	22.57	12.77
Range	0.74	40.86	7.05	215.5	71.5	5.77
ETI[a]	0.98	0.87	0.77	1	0.79	0.87
Optima	7.40	195.13	6.20	567.31	60.46	15.20
Tolerance	0.30	16.10	1.82	64.10	29.50	2.43
D. stevensoni						
Average	7.25	201.35	4.68	569.07	45.71	14.97
SD	0.31	15.71	2.46	62.28	22.52	2.79
Maximum	7.64	214.87	7.80	647.25	74.65	17.55
Minimum	6.94	178.74	2.08	481.5	21.37	11.92
Range	0.70	36.13	5.72	165.75	53.27	5.62
ETI[a]	0.92	0.77	0.62	0.76	0.58	0.84
Optima	7.27	201.70	5.38	564.17	52.25	15.08
Tolerance	0.30	15.17	2.26	53.30	20.94	2.62
H. incongruens						
Average	7.18	204.00	6.26	566.78	61.71	15.95
SD	0.34	17.14	1.16	65.05	12.59	1.67
Maximum	7.69	214.68	7.80	631.25	74.65	17.42
Minimum	6.95	173.82	4.92	475.5	42.85	13.17
Range	0.73	40.86	2.88	155.75	31.8	4.25
ETI[a]	0.97	0.87	0.31	0.72	0.35	0.64
Optima	7.28	197.58	6.17	555.80	62.82	16.51
Tolerance	0.33	18.571	0.78	67.80	8	1.05
I. bradyi						
Average	7.28	197.85	5.91	591.96	58.87	15.79
SD	0.30	16.6	2.61	68.35	25.94	2.13
Maximum	7.70	214.87	11.24	691	111.77	18.55
Minimum	6.94	168.37	2.08	475.5	21.37	11.92
Range	0.75	46.50	9.15	215.5	90.4	6.62
ETI[a]	1	1	1	1	1	1
Optima	7.52	192.69	6.62	559.12	65.32	14.85
Tolerance	0.21	15.85	1.42	80.06	13.19	2.58
P. olivaceus						
Average	7.29	199.17	6.05	606.04	60.37	15.58
SD	0.30	17.35	2.99	52.04	29.00	2.28
Maximum	7.70	214.87	11.24	691	111.77	18.55
Minimum	6.94	168.37	2.08	528.67	21.37	12.7
Range	0.75	46.50	9.15	162.32	90.4	5.85
ETI[a]	1	1	1	0.75	1	0.88
Optima	7.58	182.77	8.76	624.49	87.10	15.20
Tolerance	0.18	16.83	2.55	49.99	25.59	1.94

Table 7 (Contd.)

	pH	Eh (mV)	DO (mg/l)	EC (µs/cm)	%Sat.	Temperature (°C)
H. chevreuxi						
Average	6.26	193.81	5.48	556.36	54.34	14.76
SD	0.29	17.45	2.35	79.71	22.46	2.62
Maximum	7.69	214.87	7.80	691	74.65	18.55
Minimum	7.04	173.82	2.08	475.5	21.37	11.92
Range	0.65	41.05	5.72	215.5	53.27	6.62
ETI[a]	0.86	0.88	0.62	1	0.58	1
Optima	7.24	199.83	3.71	596.81	37.37	16.11
Tolerance	0.24	12.44	2.20	57.84	21.33	2.29
S. pseudobrowniana						
Average	7.20	202.31	8.02	621.37	80.87	15.91
SD	0.10	7.4	1.73	60.98	12.59	1.67
Maximum	7.61	207.55	9.24	664.5	94.07	16.17
Minimum	7.27	197.08	6.79	578.25	67.67	15.65
Range	0.33	10.46	2.45	86.25	26.4	0.52
ETI[a]	0.44	0.22	0.26	0.40	0.29	0.07
Optima	7.24	199.17	8.75	647.25	88.79	16.07
Tolerance	0.13	4.18	0.98	34.45	10.56	0.21

[a] ETI, Environmental tolerance index

Külköylüoğlu (2005c) showed a strong negative correlation between *I. bradyi* and pH in a man-made lake in Turkey. We did not find such a correlation during our study, although *I. bradyi* was common in waters with a pH of 6.8–8.22.

With its wide ecological tolerance (Meisch 2000), *Cy. ophthalmica* is known to occur in almost all aquatic habitats with almost cosmopolitan distribution. We found this species to be most common from July to May (Fig. 3b), but it can be encountered all year round (Meisch 2000) and in waters that exhibit a large variability in conductivity (272.9–690.0 µs/cm), DO (2.46–13.46 mg/L) (Table 4), oxygenated water conditions (26.7–137.7%) and water temperature (10.7–24.4°C). We found a paucity of species at Station 4, where aquatic plants (primarily watercress) were covered with lime dust.

The third group in the UPGMA dendrogram (Fig. 2) was a cluster of two species (*Ps. olivaceus*, *Ca. neglecta*). These species have already been shown to be exhibit similar patterns of occurrence (Külköylüoğlu and Yılmaz 2006). As indicated by its high ETI values, *Ps. olivaceus* seems to be able to tolerate a wide range of conditions, as expressed in terms of physical and chemical variables (Table 7). Roca and Baltanás (1993) found that this species was able to survive at varying temperatures (6.5–16.4°C), DO levels (8.0–9.9 mg/L), pH (6.9–7.4) and conductivity (108–636 µs/cm) in a spring. Similarly, Külköylüoğlu and Yılmaz (2006) reported that for this species living in the springs the optimum pH was 7.75, the optimum conductivity 111 µs/cm, the optimum level of dissolved oxygen 8.84 mg/l and the optimum temperature of water 10.9°C. Unlike these earlier studies, we found that *Ps. olivaceus* has a relatively low tolerance (with high optimum) for pH and electrical conductivity. Hence, the species is classified as an oligothermophilic, indicating its preference for cold water (Meisch 2000). The second species of this group, *Ca. neglecta*, was found almost all year around (but see Tables 1, 2, 3, 4 for exceptions), showing higher tolerances to pH and DO than *Ps. olivaceus*. We found *Ca. neglecta* in waters with a pH value of 6.9–7.69; in contrast, Roca and Baltanás (1993) found this species in water with a pH of 6.8–9.4, and Külköylüoğlu (2005c) in water with a pH of 6.3. Overall, these studies suggest that *Ca. neglecta* has wide pH tolerances as well as tolerance to a broad range of DO concentrations (Meisch, 2000; Külköylüoğlu 2003b). We collected *Ca. neglecta* from all stations, at minimum (1.83 mg/l) and maximum (9.86 mg/l) DO levels (Tables 1, 2). Consequently, our results strongly support the contention that this species can survive even under conditions of hypoxia and that it can be found year round, as reported by Külköylüoğlu and Dügel (2004) in a man-made lake. According to Meisch (2000), *Ca. neglecta* is an oligothermophilic species, it can also persist in a wide range of temperature values, from 3.8°C in a helocrene spring (Külköylüoğlu and Yılmaz 2006) to over 28°C in heavily polluted lake (Külköylüoğlu et al. 2007).

Five taxa clustered in the second group of UPGMA dendrogram, including *S. pseudobrowniana, Her. chevreuxi, D. stevensoni, Het. incongruens* and *Eucypris* sp. (Fig. 2). Whereas the two well-known cosmopolitan species *Het. incongruens* and *D. stevensoni* are distributed widely over the world, *Her. chevreuxi* and *S. pseudobrowniana* occur throughout the Holarctic region. All of the species of this group are usually found at the bottom of lentic habitats (Külköylüoğlu 2005b). In addition, *S. pseudobrowniana* is known to be a semi-terrestrial species tolerant to desiccation (Külköylüoğlu 1999; Smith et al. 2002).

Heterocypris incongruens and *D. stevensoni* had high ETI values for all of the environmental variables measured in this study except DO and saturation levels. *Darwinula stevensoni* was collected only once from Çaygökpınar and has been known to exhibit wide tolerance levels (Külköylüoğlu 2004), but the calculated ETI values for DO and oxygen saturation were relatively low (Table 7). The OWM (Fig. 3) also indicated the occurrence of this species from August to January, in accordance with the results of Yılmaz and Külköylüoğlu (2006). In contrast, Külköylüoğlu (1999) collected the species from the springs of Nevada from February to November. In another study, Külköylüoğlu (2005c) suggested that *D. stevensoni* can exist throughout the year, depending on water conditions. Although *Het. incongruens* has wide ranges of tolerances as a cosmopolitan species, it was not found frequently during our study. Another species of the second group of the UPGMA dendrogram, *Her. chevreuxi*, was only collected from the Çetin Bey spring (Fig. 3a) during this study. This species showed high ETI values for all environmental variables except DO and saturation values (Table 7); the optimum and tolerance values of this species are also low for DO and saturation.

The first group clustered in the UPGMA dendogram is represented by one species, *Pr. zenkeri*, which has wide tolerance levels for all ecological conditions calculated and was also the most abundant species, except in the source water of Çetin Bey. Adults can occur throughout the year (Fig. 3A). Spearman's correlation indicated that *Pr. zenkeri* had a strongly positive relationship to *I. bradyi* (Table 6), possibly due to the bottom-dependent, non-swimming characteristics of these species.

The data obtained from the *t* test for equal variances between the two stations of each spring are significantly different for both redox potential and pH. However, there was no difference between the headwater stations of both springs, nor between the downstream stations. There was a significant difference in both pH and redox potential values between the two stations of each spring (Table 6). Sorenson and Jaccard's similarity indices revealed that similarities among the four stations ranged from 40 to 80% and from 25 to 66%, respectively (Table 8). This wide range of similarities may be related to the effects of environmental factors; for example, the headwater sites of each spring have not been greatly disturbed, while the downstream sites have experienced severe negative effects due to human activities (Külköylüoğlu 2003a).

In summary, our results imply that ostracod species (especially cosmopolitans) have high tolerance levels to a variety of environmental variables for which they can be used as indicator species of aquatic habitats. Külköylüoğlu (2004) suggested that increasing numbers of cosmopolitan species, which may correspond with a decrease in the quality of waters, can cause biotic homogenization (Olden 2006; Olden and Rooney 2006). Cosmopolitan species with a wide tolerance range may not always represent a specific habitat, but their presence and/or dominancy may, however, reflect changing conditions in that habitat. In addition, noncosmopolitan species may not be tolerant of any reduction in the quality of the waters and eventually disappear. In such a case, if decreasing water quality shows a parallel trend with a reduction of noncosmopolitan species but an elevation in the numbers (or frequency) of cosmopolitan species, cosmopolitans can be used as indicator species for low water quality. To underline the situation, this state is called 'pseudorichness', where the low ratio between the numbers of noncosmopolitan and cosmopolitan species suggests a decrease in the quality of water. If we consider that almost all of the species reported here have a cosmopolitan distribution in the Holarctic region at least, one may presume that these two springs have a low water quality. This is in fact true: pseudorichness is higher at the downstream sites where the effects of disturbances are higher than at the headwaters. Although the ratio of pseudorichness explains some of the ecological characteristics of these springs, long-term biomonitoring studies are required to support such approach.

Table 8 Similarity values (%) calculated with Jaccard's (in parentheses) and Sorenson indices among the four stations

	Station 2	Station 3	Station 4
Station 1	42 (60)	25 (40)	33 (54.5)
Station 2		55 (71)	66 (80)
Station 3			66 (80)

Stations 1 and 2 are in Çetin Bey spring; Stations 3 and 4 are in Çaygökpınar spring

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References

APHA (1989) Standard methods for the examination of water and wastewater, American public health association, Washington D.C.

Bronshtein ZS (1947) Fauna SSSR. Crustacea. vol 2, no: 1. Ostracodes des Eaux Douches. Inst Zool'Acad Sci URSS Nouvelle Ser 31:1–339

Curry BB (1999) An environmental tolerance index for ostracodes as indicators of physical and chemical factors in aquatic habitats. Palaeogeogr Palaeoclimatol Palaeoecol 148:51–63

Delorme LD (1983) Lake Erie oxygen: the prehistoric record. Can J Fish Aquat Sci 39:1021–1029

Geslin E, Debenay JP, Duleba W, Bonetti C (2002) Morphological abnormalities of foraminiferal tests in Brazilian environments: comparison between polluted and non-polluted areas. Mar Micropaleontol 45:151–168

Glazier DS (1991) The Fauna of North American temperate cold springs: patterns and hypotheses. Freshw Biol 26:527–542

Glazier DS (1998) Springs as model systems for ecology and evolutionary biology: a case study of *Gammarus minus* Say (Amphipoda) in mid-Appalachian springs differing in pH and ionic content. In: Botosaneanu L (ed) Studies in crenobiology: the biology of springs and springbrooks. Backhuys, Leiden, pp 41–54

Gooch JL, Glazier DS (1991) Temporal and spatial patterns in mid-Appalachian springs. Mem Ent Soc Can 155:29–49

Hynes HBN (1970) The ecology of running waters. Liverpool University, Liverpool, p 555

Kempf EK (1980) Index and bibliography of marine Ostracoda, part 1, vol 35. Index A. Sonderveröffentlichungen des Geologischen Instituts der Universität zu Köln, Cologne

Kempf EK (1997) Index and Bibliography of Nonmarine Ostracoda, part 6, vol 109. Index A, Supplement. Sonderveröffentlichungen des Geologischen Instituts der Universität zu Köln, Cologne

Kovach W (1998) Multi-variate statistical package, ver. 3.0. Kovach computer services, Pentraeth, UK

Külköylüoğlu O (1998) Freshwater Ostracoda (Crustacea) and their quarterly occurrence in Şamlar Lake (Istanbul, Turkey). Limnologica 28:229–235

Külköylüoğlu O (1999) Seasonal distribution of freshwater Ostracoda (Crustacea) in springs of Nevada. Geosound 35:85–91

Külköylüoğlu O (2003a) A new report and the loss of *Scottia pseudobrowniana* KEMPF, 1971 (Ostracoda) from a limnocrene spring in Bolu, Turkey. Crustaceana 76:257–268

Külköylüoğlu O (2003b) Ecology of freshwater Ostracoda (Crustacea) from lakes and reservoirs in Bolu-Turkey. J Freshw Ecol 18:343–347

Külköylüoğlu O (2004) On the usage of ostracoda (Crustacea) as bioindicator species in the different aquatic habitats in the Bolu region, Turkey. Ecol Indic 4:139–147

Külköylüoğlu O (2005a) Factors effecting Ostracoda (Crustacea) occurrence in Yumrukaya Reedbeds (Bolu, Turkey). Wetlands 25:224–227

Külköylüoğlu O (2005b) Ecological requirements of freshwater Ostracoda (Crustacea) in two limnocrene springs (Bolu, Turkey). Ann Limnol Int J Lim 41(4):237–246

Külköylüoğlu O (2005c) Ecology and phenology of freshwater ostracods in Lake Gölköy (Bolu, Turkey). Aquatic Ecol 39:295–304

Külköylüoğlu O, Dügel M (2004) Ecology ad spatiotemporal patterns of Ostracoda (Crustacea) from Lake Gölcük (Bolu, Turkey). Arch Hydrobiol 160:67–83

Külköylüoğlu O, Vinyard GL (1998) New bisexual form of *Cavernocypris subterranea* (Wolf, 1920) (Crustacea, Ostracoda) from Idaho. Great Basin Nat 58:380–385

Külköylüoğlu O, Vinyard GL (2000) Distribution and ecology of freshwater Ostracoda (Crustacea) collected from springs of Nevada, Utah, and Oregon: a preliminary study. West North Am Nat 60:291–303

Külköylüoğlu O, Yılmaz F (2006) Ecological requirements of Ostracoda (Crustacea) in three types of springs in Turkey. Limnologica 36:172–180

Külköylüoğlu O, Dügel M, Kılıç M (2007) Ecological requirements of Ostracoda (Crustacea) in a heavily polluted shallow lake, Lake Yeniçağa (Bolu, Turkey). Hydrobiologia 585:119–133

Magurran AE (1988) Ecological diversity and its measurement. Princeton University, Princeton

Meisch C (2000) Freshwater Ostracoda of Western and Central Europe. Süsswasserfauna von Mitteleuropa 8/3. Spektrum Akademischer Verlag, Heidelberg

Mezquita F, Hernández R, Rueda J (1999) Ecology and distribution of ostracods in a polluted Mediterranean river. Palaeogeogr Palaeoclimatol Palaeoecol 148:87–103

Mezquita F, Griffiths HI, Domínguez MI, Lozano-Quilis MA (2001) Ostracoda (Crustacea) as ecological indicators: a case study from the Iberian Mediterranean brooks. Arch Hydrobiol 150:545–560

Olden JD (2006) Biotic homogenization: a new research agenda for conservation biogeography. J Biogeogr 33:2027–2039

Olden JD, Rooney TP (2006) On defining and quantifying biotic homogenization. Glob Ecol Biogeogr 15:113–120

Roca JR, Baltanás A (1993) Ecology and distribution of Ostracoda in phyrenean springs. J Crust Biol 13:165–174

Ruiz F, González-Regalado ML, Borrego J, Abad M, Pendón JG (2004) Ostracoda and foraminifera as short-term tracers of environmental changes in very polluted areas: the Odiel Estuary (SW Spain). Environ Pollut 129:49–61

Särkkä J, Levonen L, Mäkelä J (1997) Meiofauna of springs in Finland in relation to environmental factors. Hydrobiologia 347:139–150

Smith H, Wood PJ, Gunn J (2003) The influence of habitat structure and flow permanence on invertebrate communities in karst spring systems. Hydrobiologia 510:53–66

Smith RJ, Matzke-Karasz R, Kamiya T, Ikeda Y (2002) *Scottia birigida* sp. nov. (Cypridoidea, Ostracoda) from western Honshu, Japan and a key to the subfamily Scottiinae Bronshtein, 1947. Zootaxa 126:1–20

Ter Braak CJF, Barendregt LG (1986) Weighted averaging of species indicator values: its efficiency in environmental calibration. Math Biosci 78:57–72

Yılmaz F, Külköylüoğlu O (2006) Tolerance, optimum ranges, and ecological requirements of freshwater Ostracoda (Crustacea) in Lake Aladağ (Bolu, Turkey). Ecol Res 21:165–173