

Water quality and diversity of the Recent ostracod fauna in lowland springs from Lombardy (northern Italy)

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Abstract The Po river plain (northern Italy) is delimited to two mountain ridges, the Alps and the Apennines. It hosts peculiar lowland man-modified springs, locally known as “fontanili”, which originate from natural resurgences occurring along the alluvial fans of the main water-courses, namely in the transition zone from the higher to lower plain which is characterized by

changes in slope profile and sediment granulometry. These habitats usually show low variation in hydrological, hydrochemical and thermal conditions throughout the year. Twenty-eight springs, located in the provinces of Lodi and Cremona (Lombardy) in the alpine sub-catchment of the Po river were sampled in summer and autumn of 2004. Twenty-three of them were typical alluvial-fan springs, while the remaining five were terrace springs. The two groups of springs showed marked differences in their hydrochemical and hydrological characteristics. Sixteen ostracod species in three families (Candonidae, Ilyocyprididae, and Cyprididae) were identified. The most frequent species were *Cypria ophthalmica* (19 sites), *Herpetocypris reptans* (16), and *Prionocypris zenkeri* (13). Five species were found only once: *Chlamydotheca incisa*, *Scottia pseudobrowniana*, *Pseudocandona compressa* and *Candona neglecta*. Up to 6 taxa were recorded from a single site and the average number of taxa for each site was c. 3. The associations among ostracod taxa and their occurrence in relation to environmental factors were examined. Finally, the results of this survey were compared with a similar study previously conducted in 31 alluvial fan springs of the apennine sub-catchment of the Po river.

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Introduction

The “fontanili” are man-modified springs characteristic of the lowland plains of northern Italy (Minelli, 2001). These springs typically occur along lines corresponding to the alluvial or paleoalluvial fans of rivers flowing from the Alps and the northern Apennines, at the boundary between higher and lower plains. The change in the valley slope profile favours the deposition of clay and sand layers; the presence of these less permeable sediments forces the groundwater coming from deep aquifers upwards. To facilitate the water outflow, small basins are artificially excavated and perforated metal tubes are usually inserted into the ground; in addition, periodic removal of bottom sediment and plant biomasses is required to guarantee the hydraulic effectiveness of springs. Another type of springs is also common in this area. They occur in eroded river terraces and are in direct contact with the underlying water table.

Previously, these springs were used as sources of drinkable water and for crop irrigation. They were properly managed and gave origin to an important network formed by a vast system of lentic waters, streams and channels which sustained a rich freshwater biodiversity (Bracco et al., 2001). Changes in the agricultural practices and in the captation methods, associated with the expansion of transportation infrastructures and economic activities, have progressively led to the disappearance of many springs and a reduction in

the ecological connectivity between lowland aquatic habitats, with negative consequences on their biological communities. In addition, pollution of agricultural origin is a growing concern for spring water quality. Nevertheless, only in few cases, protection areas have been established to safeguard springs of particular conservation interest.

Here, we report on the results of a monitoring programme on water quality and ostracod fauna of 23 alluvial-fan and five terrace springs in the Po river plain of Lombardy (northern Italy). The major objectives were to analyse the associations among ostracod taxa and to assess possible relationships between their occurrence and environmental parameters. The data are compared with those obtained from a previous investigation carried out in 31 springs located south of the present study area (Rossetti et al., 2005).

Materials and methods

Study area

All the springs analysed in the present study are located in the provinces of Lodi and Cremona (Fig. 1). The sites were selected on the basis of a cartographic search using both historical cadastral records (1904) and the 1:10000 scale Technical Regional Maps produced by the Lombardy Region in 1999. The actual presence of active springs was then verified during field surveys. A

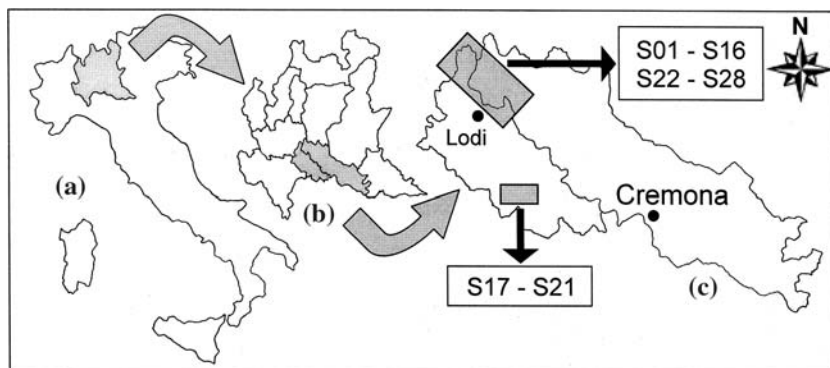


Fig. 1 Maps of Italy (a), Lombardy (b) and the provinces of Lodi and Cremona (c). Location of sampling sites is also shown (see Table 1 for codes)

total of 28 springs, considered to be representative for the area, were eventually included in this study (Table 1); 23 of them are typical alluvial-fan springs (S01–S16, S22–S28), and the remaining five are terrace springs (S17–S21). Nine of the studied springs (S12, S17–S20, S22–S25) are subjected to protection measures under different conservation programmes.

In the study area, alluvial-fan springs are clustered in the northern part, while the terrace springs occur in the southern part along the Adda river, one of the major tributaries of the Po river.

Alluvial-fan springs are fed by deep confined aquifers of alluvial valleys which guarantee a relatively constant flow, transparent waters and little seasonal fluctuation in water temperature. In the discharging part, they can either be small ponds or heads of channels with several abstraction points; the water depth rarely exceeds 1 m. Different springs may join together to form streams with large water flows. Terrace springs,

on the other hand, are supplied by groundwater intersecting the land surface in eroded river banks; aquifers are mainly recharged by the areal precipitation and are particularly susceptible to contamination by percolation of polluted surface waters. These springs appear as small channels or ditches, and their outflows are usually modest and variable throughout the year.

The resurgence points have no direct connection to other surface waters, except for S05 which receives waters from an adjacent river (Addetta) through a small drain. S23 has concrete banks, while all the other springs have vegetated banks.

Sampling and analytical methods

Each spring was sampled twice, in summer (late June-early September) and autumn (November) 2004. Water temperature was measured in situ using a digital thermometer (ILC). Water samples were collected close to the discharge points and

Table 1 Geographic location of the studied springs

Spring	Code	Lat N	Long E	Municipality	Province
Quattro Ponti	S01	45°27'26"	09°24'56"	Lavagna	Lodi
Sorgente a T	S02	45°27'34"	09°25'45"	Lavagna	Lodi
Cavo Marocco	S03	45°27'01"	09°25'48"	Lavagna	Lodi
Casolate	S04	45°23'51"	09°26'41"	Zelo Buon Persico	Lodi
Lavagna	S05	45°27'53"	09°27'17"	Lavagna	Lodi
El Rio	S06	45°21'39"	09°30'33"	Boffalora d'Adda	Lodi
Fontana 2	S07	45°21'17"	09°30'16"	Boffalora d'Adda	Lodi
Fontana 1	S08	45°21'20"	09°30'14"	Boffalora d'Adda	Lodi
Fontana 0	S09	45°21'23"	09°30'13"	Boffalora d'Adda	Lodi
Galluppina	S10	45°21'29"	09°29'57"	Boffalora d'Adda	Lodi
Comazzo	S11	45°26'53"	09°28'26"	Comazzo	Lodi
Merlo Giovane	S12	45°26'22"	09°30'43"	Rivolta d'Adda	Cremona
Falconetta nord	S13	45°25'47"	09°30'06"	Rivolta d'Adda	Cremona
Nord Cascina Maleo	S14	45°25'55"	09°30'06"	Rivolta d'Adda	Cremona
Falconetta 2	S15	45°25'53"	09°30'02"	Rivolta d'Adda	Cremona
Falconetta sud	S16	45°25'33"	09°30'17"	Rivolta d'Adda	Cremona
Fontanile 1	S17	45°08'46"	09°38'56"	Somaglia	Lodi
Fontanile 2	S18	45°08'49"	09°39'23"	Somaglia	Lodi
Fontanile 3	S19	45°08'49"	09°39'33"	Somaglia	Lodi
Fontanile 4	S20	45°08'49"	09°39'33"	Somaglia	Lodi
Madonna del Fontanone	S21	45°09'32"	09°35'29"	Ospedaletto Lodigiano	Lodi
Francavalla	S22	45°21'12"	09°31'55"	Dovera	Cremona
Aliprandia	S23	45°21'52"	09°31'16"	Dovera	Cremona
Cimitero	S24	45°22'20"	09°32'23"	Dovera	Cremona
El Rì	S25	45°22'58"	09°31'41"	Dovera	Cremona
Mozzanica	S26	45°22'51"	09°28'40"	Spino d'Adda	Cremona
Fontanina	S27	45°23'06"	09°29'25"	Spino d'Adda	Cremona
Meraviglia	S28	45°20'33"	09°31'57"	Lodi	Lodi

were kept refrigerated until analysed in the laboratory. Hydrochemical and physical variables and parameters were determined as follows: pH by potentiometry (TIM 90, Radiometer); total alkalinity by potentiometric end-point titration at pH 4.5 and 4.2 (TIM 90, Radiometer) and linearization according to Rodier (1978); electric conductivity at 20°C by conductometry (CDM 83, Radiometer); chemical oxygen demand by titration with ammonium iron(II) sulphate (A.P.H.A., 1998); nitrate (Rodier, 1978), ammonia (A.P.H.A., 1998), nitrite (A.P.H.A., 1998), soluble reactive phosphorus (Valderrama, 1981), dissolved reactive silica (Golterman et al., 1978), and chlorophyll-*a* (Golterman et al., 1978) by spectrophotometry (Beckman DU 65). The full data set of hydrochemical data is available as online appendix to the article.

Ostracod samples were collected using a 250 µm net equipped with a long handle pulled close to the sediment and swept through the vegetation. Living samples were transferred to the laboratory, where specimens were sorted under a binocular microscope and then fixed in ethanol. The specific allocation of the collected material was based on adult specimens; in few cases (*Herpetocypris* sp. and *Pseudocandona* sp.) the specific identification remained uncertain due to the presence of immature stages only. Both soft parts (dissected in glycerine and stored in sealed slides) and valves (stored dry in micropal slides) were checked for species identification, using Fox (1965) for *Chlamydotheca incisa* and Meisch (2000) for the remaining species.

Data analysis

The ordination of abiotic and biotic data was carried out through Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA) using the statistical package CANOCO version 4.5 (ter Braak & Šmilauer, 2002), and Cluster Analysis (CA) using PAST ver. 1.06 (Hammer et al., 2001). PCA was performed on the correlation matrix of measured physical and chemical water variables after log ($x + 1$) transformation (except for pH) to normalize the distributions. The concentration of dissolved inorganic nitrogen (DIN) was the sum

of nitrate, nitrite, and ammonia. The same matrix and the ostracod absence/presence data were used to assess possible relationships between ostracod distribution and environmental data by CCA. Monte Carlo permutation tests (9999 permutations) were used to assess the significance of the canonical axes and of the environmental variables that were selected in the forward selection procedure. Similarity between species assemblages was assessed by Unweighted pair-group average CA using the Raup-Crick index (Raup & Crick, 1979) for absence/presence data with a Monte Carlo randomization procedure (200 replicates).

Results

Water temperature ranged between 14.4 and 20.0°C in summer and between 12.3°C and 19.0°C in autumn; the difference between summer and autumn values in each spring was usually less than 2°C, except for S04, S13, S14, and S27 where the differences were slightly larger (from 4.2°C to 4.6°C). Conductivity generally varied between 300 µS cm⁻¹ and 500 µS cm⁻¹, although in the southern springs values were rather higher especially in autumn, with a maximum of 908 µS cm⁻¹ recorded in S17. The lowest values were measured in S05 (206 and 238 µS cm⁻¹ in summer and in autumn, respectively), indicating a dilution effect due to the inflow of waters from the river Addetta. In the study area pH values showed a relatively low variability in both space and time, ranging from 6.85 (S13) to 7.47 (S02) in summer and from 6.80 (S27) to 7.25 (S05) in autumn. Also total alkalinity values were typical of well-buffered waters, with a minimum of 2.06 meq l⁻¹ in S05 (summer) and a maximum of 4.80 meq l⁻¹ in S20 (autumn); marked seasonal differences were chiefly observed in the terrace springs, with higher values in autumn. Soluble reactive phosphorus content was highly variable among sites; higher concentrations were generally measured in summer (up to a maximum of 38 µg l⁻¹ in S06); in autumn, values were under the detection limit of 5 µg l⁻¹ in 19 out of 28 springs, and a maximum of 30 µg l⁻¹ was found in S06. Even though there was a considerable variability between sites, nitrate

was the most abundant form of dissolved inorganic nitrogen in all springs; concentrations were $\leq 11 \text{ mg l}^{-1}$, except in S21 with 21.1 mg l^{-1} in summer and 22.2 mg l^{-1} in autumn. Nitrite concentrations ranged between below the detection limit ($5 \text{ } \mu\text{g l}^{-1}$) and $16 \text{ } \mu\text{g l}^{-1}$, with two isolated autumn peaks of 55 and $27 \text{ } \mu\text{g l}^{-1}$ in S17 and S20. Ammonia, with few exceptions, showed higher concentrations in summer samples (up to a maximum of $171 \text{ } \mu\text{g l}^{-1}$ in S23), while in autumn values remained below $12 \text{ } \mu\text{g l}^{-1}$ in all springs except for S04 ($31 \text{ } \mu\text{g l}^{-1}$) and S17 ($43 \text{ } \mu\text{g l}^{-1}$). Dissolved reactive silica had concentrations comprised between 0.6 and 9.2 mg l^{-1} ; the highest values were observed in the terrace springs. In most of the sites, chemical oxygen demand concentrations were $\leq 20 \text{ mg l}^{-1}$; values $\geq 40 \text{ mg l}^{-1}$ in at least one sample were measured in S01, S03, and S13. Chlorophyll-*a* concentrations, apart from S10 ($6.2 \text{ } \mu\text{g l}^{-1}$ in autumn), were always $\leq 2.2 \text{ } \mu\text{g l}^{-1}$.

PCA was used to summarize variation patterns in physical and chemical characteristics of waters among the studied springs (Fig. 2). The first two axes account for 72.7% of total cumulative variance. Total alkalinity and conductivity have the highest correlation with the first principal component ($r = -0.87$ and $r = -0.81$), soluble reactive phosphorus and chemical oxygen demand with the second principal component ($r = 0.67$ and $r = -0.59$, respectively). Most of the alluvial-fan spring samples are grouped around the origin of the axes and in general no clear seasonal pattern is evident. Also summer samples from terrace springs tend to partially overlap with this main cluster, while samples collected in autumn, characterized by more mineralised waters and higher concentrations of dissolved inorganic nitrogen, are restricted to the upper left quadrant. A third group of samples is located in an opposite position, consisting of the samples collected in S05 and S15 (summer and autumn) and S13 (only autumn) which have a low-ionic content. Finally, the summer sample from S16 occupies a rather isolated position in the upper right quadrant due to its low conductivity and total alkalinity values associated with high soluble reactive phosphorus concentration (Fig. 2).

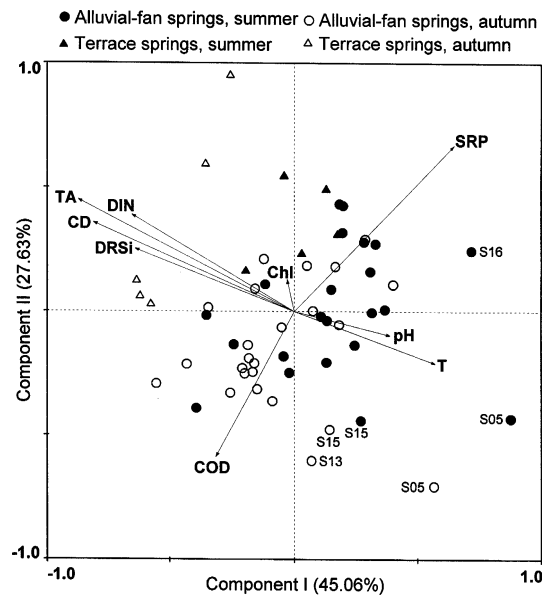


Fig. 2 PCA biplot of springs and environmental variables in the plane defined by the first two axes. To make the graph more readable, only six samples are labelled. T: temperature; CD: conductivity; TA: total alkalinity; SRP: soluble reactive phosphorus; DIN: dissolved inorganic nitrogen; DRSi: dissolved reactive silica; COD: chemical oxygen demand; Chl: chlorophyll *a*

Sixteen ostracod taxa in three families (Candonidae, Ilyocyprididae, and Cyprididae) were identified (Table 2). The most frequent species were *Cypria ophthalmica* (19 sites), followed by *Herpetocypris reptans* (16), and *Prionocypris zenkeri* (13). Four taxa (*Scottia pseudobrowniana*, *Pseudocandona compressa*, *Candona neglecta* and *Herpetocypris* sp.) were recorded in one sampling site only. Nine taxa were present in both seasons, while *Ilyocypris bradyi*, *Chlamydotheca incisa*, *Heterocypris reptans*, *Pseudocandona compressa*, and *Candona neglecta* were collected only in summer, *Herpetocypris* sp. and *Pseudocandona* sp. only in autumn. A maximum of six taxa were recorded from a single site (S12), while in three springs (S01, S18, and S20) only one species was found; the average number of species per site was 3.32 (Fig. 3). The highest ostracod diversity was recorded in the Comazzo area, where eleven species were identified (Tables 1 and 2).

In the CCA, the Monte Carlo permutation test showed that all the canonical axes are not

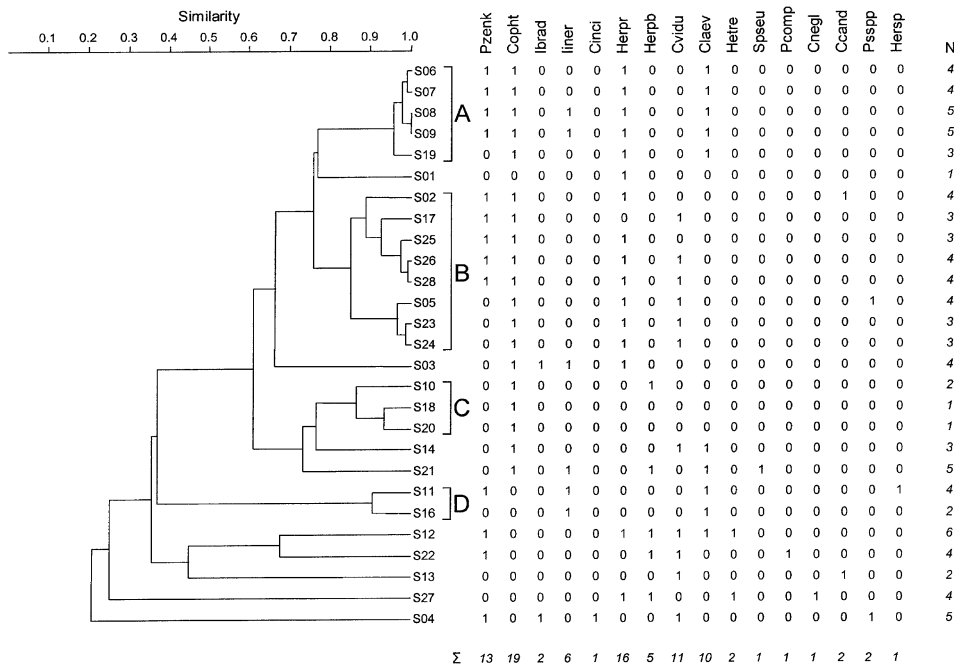


Fig. 3 Dendrogram obtained from the CA showing the similarity level between ostracod communities. Presence and absence (indicated as 1 and 0, respectively) of ostracod taxa in each spring is also indicated. Pzenk: *Prionocypris zenkeri*; Copht: *Cypria ophthalmica*; Ibrad: *Ilyocypris bradyi*; Iiner: *Ilyocypris inermis*; Cinci: *Chlamydotheca incisa*; Herpr: *Herpetocypris reptans*; Herpb: *Herpetocypris*

brevicaudata; Cvidu: *Cypridopsis vidua*; Claev: *Cyclocypris laevis*; Hetre: *Heterocypris reptans*; Spseu: *Scotia pseudobrowniana*; Pcomp: *Pseudocandona compressa*; Cnegl: *Candona neglecta*; Ccand: *Candona candida*; Pspsp: *Pseudocandona* sp.; Herhp: *Herpetocypris* sp.; Σ: number of total record for each taxon; N: number of taxa in each spring

significant ($P > 0.05$), indicating that the available data do not support a causal relationship between species occurrence and hydrochemical features.

CA was performed using the data matrix of presence/absence of taxa to examine patterns in ostracod assemblage composition. At a similarity value of approximately 0.8, four major groups were evidenced (Fig. 3). The first cluster (A) consists of five springs characterized by a relatively high number of taxa (3–5) and by the presence of *Cypria ophthalmica*, *Herpetocypris reptans* and *Cyclocypris laevis*. Four of these springs (S06, S07, S08 and S09) are located in the area of Boffalora d'Adda (Table 1). The second cluster (B) is formed by eight sites in which three or four taxa were found. Here, *Cypria ophthalmica* is alternatively associated with *Herpetocypris reptans*, *Cypridopsis vidua* and *Prionocypris zenkeri*, i.e., the species which are more common in the study area. Springs forming this cluster are spread across different geographic areas

(Table 1); one of them (S17) is a terrace spring. The third (C) and fourth (D) cluster are each composed of a limited number of springs: cluster C includes two terrace springs (S18 and S20) and an alluvial fan spring (S10) which show a low-ostracod diversity; cluster D contains only two springs (S11 and S16) characterized by the simultaneous presence of *Ilyocypris inermis* and *Cyclocypris laevis*.

Discussion and conclusions

Compared to the total number of lowland springs included in historical cadastral registers, only few of them are still active in the study area. This accelerated loss is mainly due to inappropriate management of water bodies or even to their active destruction to meet the needs of large-scale agricultural practices. Another issue of concern is the water quality of the remaining springs.

Table 2 Comparison between ostracod taxa found in lowland springs of the southern (Rossetti et al., 2005) and the northern (this study) sub-catchment of the Po river

	Rossetti et al., 2005	This study
Family Candonidae Kaufmann, 1900		
<i>Candona candida</i> (O.F. Müller, 1776)		X
<i>Candona neglecta</i> Sars, 1887		X
<i>Pseudocandona</i> sp.		X
<i>Pseudocandona compressa</i> (Koch, 1838)	X ^a	X
<i>Cypria ophthalmica</i> (Jurine, 1820)	X	X
<i>Cyclocypris ovum</i> (Jurine, 1820)	X	
<i>Cyclocypris laevis</i> (O.F. Müller, 1776)	X	X
Family Ilyocyprididae Kaufmann, 1900		
<i>Ilyocypris decipiens</i> Masi, 1905	X	
<i>Ilyocypris inermis</i> Kaufmann, 1900	X	X
<i>Ilyocypris bradyi</i> Sars, 1890	X	X
Family Notodromadidae Kaufmann, 1900		
<i>Notodromas persica</i> Gurney, 1921	X	
Family Cyprididae Baird, 1845		
<i>Prionocypris zenkeri</i> (Chyzer & Toth, 1858)	X	X
<i>Herpetocypris</i> sp.		X
<i>Herpetocypris brevicaudata</i> Kaufmann, 1900	X	X
<i>Herpetocypris reptans</i> (Baird, 1835)		X
<i>Heterocypris reptans</i> (Kaufmann, 1900)		X
<i>Scottia pseudobrowniana</i> Kempf, 1971		X
<i>Cypridopsis vidua</i> (O.F. Müller, 1776)	X	X
<i>Potamocypris fulva</i> (Brady, 1868)	X	
<i>Chlamydotheca incisa</i> (Clauss, 1812)		X
Number of taxa	12	16

^a *Pseudocandona* gr. *compressa*

Indeed, the results of this study reveal that waters are severely impacted by chemicals leaching into groundwater from the recharge zone or locally washed off from crop fields. In particular, high nitrate concentrations measured in several springs (with peaks over 20 mg l⁻¹) confirm the progressive contamination of aquifers in northern Italy mainly caused by the excessive use of fertilizers (Russo & Zavatti, 2001). Farming (including livestock) in lowlands is probably responsible for the high levels of chemical oxygen demand frequently observed in the studied springs. Obviously, springs situated in protected areas (usually restricted to a very limited surface) are also affected by these kinds of impact. Other variables reflecting the trophic state of spring waters (e.g., soluble reactive phosphorus and chlorophyll *a*) show, with few exceptions, low concentrations, most likely because of the development of dense stands of aquatic macrophytes and/or algal mats. In general, there was no clear pattern in seasonal variation of hydrochemical characteristics of alluvial-fan springs. In terrace areas, on the contrary, autumn samples showed notably higher conductivity and total alkalinity values than those

collected in summer, as the superficial groundwater feeding the springs is directly influenced by the rainfall regime and by the agricultural cycles.

The studied sites usually host relatively simple ostracod communities, as it is expected in spring habitats (see the literature revised by Rossetti et al., 2005). Most of the species identified in this study are known for their high tolerance to different hydrochemical and ecological factors and have a wide geographic distribution or are cosmopolitan. Nevertheless, the records of *Scottia pseudobrowniana* in S21 and *Chlamydotheca incisa* in S04 are of particular interest. The subfamily Scottiinae consists of (semi-) terrestrial species; *Scottia* is the only genus within the tribe Scottiini and has a Holarctic distribution (Martens et al., 2004); *S. pseudobrowniana* is the most common representative of the genus and, although not rare, it is known for a limited number of localities (Meisch, 2000). To our knowledge, in Italy this species was previously found only in a spring of the central-eastern Alps (Meisch, 2006), while it has never been reported for the Mediterranean area (Meisch, 2000). The specimens collected in S21 were very scarce and

they were exclusively retrieved from the sediment of the spring channel within few meters from the resurgence point. All the attempts to recover animals from leaf litter and mosses (i.e., semi-terrestrial habitats) along the channel banks failed, as well as from other sampling points located downstream.

Chlamydotheca incisa, a South American species, has repeatedly been found in Italy in ricefields (Rossi et al., 2003) and in small water bodies of the Po river plain (Rossetti, unpubl.), although it has not been reported yet from other European countries (<http://www.faunaeur.org>). Its occurrence in contrasting habitats from several locations is a paradigmatic example of a species of “ospiti esteri” (*sensu* McKenzie & Moroni, 1986) which has successfully colonized the Italian waters.

The overall prevalence of euryecious ostracods in the studied springs probably accounts for the lack of strong relationships between species occurrence and physical and chemical variables. For example, apart from *Scottia pseudobrowniana*, all other taxa found in terrace springs were also present in alluvial-fan springs. No significant differences in species richness between protected and unprotected springs were observed. As evidenced by CA, similarities in community composition do not necessarily reflect the geographic proximity between springs, with the exception of the area of Boffalora d’Adda (Table 1, Fig. 2).

A previous study performed in 2001 in 31 alluvial-fan springs of the Apennine sub-catchment of the Po river (Rossetti et al., 2005) revealed the presence of 12 taxa in 9 genera; eight species and 7 genera were found in both areas (Table 2). In Lombardy, 5 species with ≥ 10 records were found, while in the other group of springs *Cypria ophthalmica* was overwhelmingly the most common species. The average number of taxa per site was 3.32 in the first case, and only 1.97 in the second case. Although such divergent figures can be partly due to a different sampling effort (the springs situated on the Apennine side were sampled only once), the comparison of the results obtained from the two studies seems to indicate that the springs of the province of Lodi and Cremona possibly support a more diversified ostracod fauna.

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