Diatom communities and vegetation of springs in the south-western Alps

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Abstract – Springs are unique but understudied habitats. Diatom communities have received some attention but have remained largely unknown in the south-western Alps. We therefore studied the springs of the south-western extreme of the Alpine mountain range. We analysed epilithic and epiphytic assemblages in 48 springs of different ecomorphological types, located on contrasting lithological substrata (carbonate/siliceous). Moreover, phytosociological relevés were carried out for carbonate springs. The diatom flora consisted of 223 taxa. Most (198) of the taxa were included in the Red List, and 12.5% belonged to threatened categories. Characteristic spring taxa (crenophiles) were present. The ecological preferences of crenophilous diatom species described in the eastern Alps were confirmed. Diatom species characteristic of the lake-littoral zone were found in pool springs. We observed no significant differences in species richness and diversity between epilithic and epiphytic assemblages, but some species showed a preference for bryophytes, and live occurred in the epibryon only. As regards moisture conditions, 15% of the taxa occurred on wet or temporarily dry sites, and 4% lived mostly outside water bodies. The main environmental factors influencing diatom assemblages were pH, conductivity, altitude, and shading. The carbonate-substratum crenic vegetation was composed of a mixture of vascular plants and bryophytes, which find their ecological optimum in springs. Bryophyte cover was dominant, with the most abundant taxa belonging to the genus *Palustriella*. The vegetation corresponded to the *Cratoneuretum commutati* association.

Keywords: bryophyte, diatoms, epibryon, epilithon, Ligurian Alps, phytosociology, springs, vascular plant

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Introduction

Springs provide a habitat for specialised organisms that are e.g. adapted to the relatively constant environments found in permanent springs (Thienemann 1924, Odum 1971, Ellenberg 1996). There are many different spring types, and the early diatom studies mostly concentrated on specific habitats: thermal (Bíly 1934, Brábež 1941, Dell’Uomo 1986, Lederer et al. 1998, Kášťovský and Komárek 2001, Hindák and Hindáková 2006, 2007), saline (Kadlubowska 1985), tufa (Pentecost 1991, 1998) and acid (Cambra and Hindák 1998). More recently springs and especially Alpine springs have attracted the attention of biologists and conservationists due to the high share of endangered species and specialists found in their communities (e.g., Cantonati 1998, Peintinger et al. 2003, Cantonati et al. 2006, Warner and Asada 2006, Hájek et al. 2006, 2007, Payne and Mitchell 2007).

High-integrity springs provide an opportunity to describe naturally preserved freshwater habitats (We rum 2001). However such intact springs are becoming increasingly rare. These ecosystems are threatened by nutrient enrichment, exploitation for drinking water and hydropower generation, climate change, pollution, etc.

Springs should be carefully monitored as many of them are exposed e.g. to extreme environmental conditions (Cantonati and Spitale 2009), and human exploitation (e.g., Fráňková et al. 2009). In terms of biomass production, these ecosystems are dominated by vascular plants and bryophytes (e.g., Hájková and Hájek 2003). Although microscopic diatoms are not major contributors to biomass in springs, they are abundant (Poulicková et al. 2004), and play a key role in the functioning of these systems (Cantonati et al. 2006).

Benthic diatoms are included in the Water Framework Directive (EU-WFD 2000) and are regularly used as biological indicators for the environmental assessment of river water quality. The analysis of diatom communities can as well be a useful tool for the correct development of guidelines for an ecological and sustainable use, and preservation, of water resources. The diatom microflora of oligotrophic environments has received renewed attention only in the last fifteen years. Lange-Bertalot and Metzeltin (1996) found as many as 800 taxa in a small number of samples from three oligotrophic lakes at a time when the total diatom flora of Central Europe was estimated to comprise 1600 taxa.

A Red List has been proposed for Central Europe by Lange-Bertalot in 1996. In high mountains, springs often show rich biodiversity due to low nutrient content, moderate water flow, and constant temperature (permanent springs). These habitats provide shelter to endangered and rare taxa (Cantonati 1998, Cantonati and Spitale 2009).

The main factors found to influence diatom communities are: pH, geochemistry of the substratum, conductivity, current velocity, temperature and shading (Cantonati et al. 2006). On the other hand, the relative importance of substratum type (lithic or plants) has been debated (Cantonati et al. 2012 b). Fránková et al. (2009), for instance, hypothesized a specific relationship between Sphagnum and diatom species.

The aim of this study is to enhance our knowledge and our understanding of spring diatom communities in the south-western Alps, providing important information concerning the ecological preferences in relation to the most important environmental variables, and on the occurrence of endangered and rare taxa.

**Materials and methods**

The study area is located in the Ligurian Alps, at the south-western extreme of the Alps (Fig. 1). The springs sampled were located in the Pesio and Tanaro Valleys Nature Park (Fig. 1), a protected area of 67.7 km², and in the Ellero and Corsaglia Valleys that are not included in this Nature Park. Karst phenomena are widespread and prominent in the Park. The most important mountains are: Marguareis (2651 m a.s.l.), Mongioie (2630 m a.s.l.), and Mount of Saline (2612 m a.s.l.). The lithology is very diverse: porphyroids, schists, different types of limestones, rhyolites, quartzites.

The chemical composition of groundwater reflects the bedrock chemistry, varying from calcareous waters rich in calcium and magnesium, to acidic waters rich in silica.

**Morphological, physical, and chemical factors**

In the summers of 2009–2010 we sampled 48 springs, representing different morphological types: 34 rheocrenes, 9 helocrenes, and 5 limnocrenes, located between 1500 and 2000 m a.s.l. (Tab. 1). The location of each spring was recorded using a GPS. The per cent cover of each substratum (sand, gravel, cobbles, boulders, and detritus) was estimated visually in each spring.

Temperature, pH, and conductivity were measured in the field with a multiparametric probe. Chemical analyses including all major ions were carried out by two laboratories: IRIDE of Tortona (Italy), and ARPA (Regional Environmental Protection Agency) of Cuneo (Italy). Chemical analyses are not available for the following three springs: RF 1477, LG 1510, LG 1500.

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Concentrations of SiO₂, Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻ are in mg L⁻¹, and of NO₃⁻ in μg L⁻¹.
Diatom sampling, preparation, identification, and statistical analyses

When possible, we collected diatom communities from different substrata. We sampled epilithon (44 samples), and epibryon (21 samples). Epipelon was sampled with a pipette in only one site (IC 1300), in which no cobbles and bryophytes were found. The epilithon was sampled by randomly collecting at least five cobbles or small boulders (Kelly et al. 1998) in each spring. To study epiphytic communities we sampled along a transect across the dominant bryophytes cutting entire portions of plants that were identified to the species level. The collected material, including the bryophytes, was treated with hydrogen peroxide (100 vol.), and hydrochloric acid (EN 13946, 2003). Cleaned valves were mounted in Naphrax, two permanent mounts were prepared for each sample, and copies of all slides were deposited in the diatom collection of the Science Museum – MUSE in Trento (Italy). About 400 valves were counted in each sample. The identification and nomenclature mainly followed Krammer and Lange-Bertalot (1986–1991), Lange-Bertalot and Krammer (1989), Lange-Bertalot (2001), Krammer (1997a, b, 2000–2003), Krammer and Lange-Bertalot (2004), and Werum and Lange-Bertalot (2004). For single taxa the following were further used: Jüttner et al. (2011), Levkov (2009), Levkov et al. (2013), Lowe et al. (2014). Light microscope observations and micrographs were made using a Zeiss Axioskop 2 (Zeiss, Jena, Germany) with an Axiocam ICC 1 digital camera. Scanning electron microscope (SEM) observations were made primarily at the Department of Life and Environmental Science (DISAV) of the A. Avogadro University of Eastern Piedmont, Alessandria (Italy), using an ESEM (environmental scanning electron microscope) Quanta 200 (FEI, Oland). Further SEM observations were done at the Science Museum – MUSE in Trento, using a LEO XVP (Carl Zeiss SMT Ltd., Cambridge, UK).

To investigate diatom ecology, preferences of the species with respect to pH, moisture, and trophism were determined consulting van Dam et al. (1994), and the diversity of assemblages was quantified using the Shannon-Wiener diversity index (Shannon 1948). The conservation status of diatom species was evaluated with the German Red List (Lange-Bertalot 1996). To understand the relationships among environmental parameters and diatom communities, canonical correspondence analyses (CCA) were carried out with the Canoco 4.5 software (Ter Braak 1998). The biological matrix for the CCA included epiphytic and epilithic samples, excluding taxa with maximum abundance lower than 0.5%. CCA significance was verified using Monte Carlo permutations test.

Vegetation sampling, identification, and statistical analyses

The study of vegetation was carried out following the classical phytosociological method (Braun-Blanquet 1964, Westhoff and van der Maarel 1973). The methodological details are found in Petraglia and Tomaselli (2007). In the summers of 2010–2011, we performed 28 vegetation relevés in the three morphological types of springs (but on carbonate substratum only) located between 1500 and 2000 m a.s.l. The identification of vascular plants followed Pignatti (1983), Tutin et al. (1993, 1964–1980), Cortini-Pedrotti (2001; 2006), Smith (2004) and Aleffi et al. (2008) for bryophytes. Syn-tax 2000 (Podani 2000) was used to work out sample groups with similar species composition, and to relate these sample groups to the environmental conditions at the sampling sites.
Results

Morphology and hydrochemistry

Most of the springs are rheocrenes, and are seasonally influenced by pastures. Four are intercepted with pipes for drinking water. The contrasting lithology (carbonate vs. siliceous) results in sharp differences in geochemical variables (Tab. 1). Most of the springs (33) emerge on carbonate substratum, with conductivity values from 105 to 640 μS cm⁻¹ (median: 203 μS cm⁻¹), pH values from 7.2 to 8.7, and low silica values (around 2.5 mg L⁻¹). Fifteen springs emerge on siliceous substratum, and show low conductivity values (from 20 to 180 μS cm⁻¹, median: 66 μS cm⁻¹), pH values from 6.6 to 8.4, and silica values around 5.5 mg L⁻¹. As expected, the calcium and magnesium concentrations were very low in siliceous springs (medians: 10.4 mg L⁻¹ Ca²⁺, 1.3 mg L⁻¹ Mg²⁺), and higher in the carbonate springs (medians: 39.0 mg L⁻¹ Ca²⁺, 1.6 mg L⁻¹ Mg²⁺). Nitrate values were remarkably low (median: 230 μg L⁻¹ N-NO₃⁻), the only exception being represented by ME1906, which showed a nitrate value of 1805 μg L⁻¹ N-NO₃⁻.

Diatoms: Assemblage composition, ecological preferences, and species richness

Overall, 223 diatom taxa belonging to 61 genera were found (On-line Supplement Tab. 1). The genera with the highest number of taxa were: Gomphonema (24), Navicula (14), Eunotia and Nitzschia (13), Cymbella (12), Encyonema (9), Achnanthidium and Psammothidium (7), Pinnularia and Surirella (6), Planothidium, Diploneis, and Fragilaria (5). The most frequent and abundant species were (Fig. 2): Achnanthidium minutissimum (Fig. 2c), A. pyrenaicum (+ A. rostropyrenaicum), Diatoma mesodon (Fig. 2j) (max abundances > 70%); Cocconeis lineata (Fig. 2g) (max abundance > 60%); Achnanthidium lineare (Fig. 2b), Amphora micra (Fig. 2d), Cymbopleura subaequalis (Fig. 2i), Encyonema silesiacum (Fig. 2k), Gomphonema angustatum (Fig. 2o), Meridion circulare (Fig. 2p), Planothidium lanceolatum (Fig. 2m), Tetracyclus rupestris (Fig. 2z) (max abundances 30–50%); Achnanthidium inconspicuum, A. affine, Amphora pediculus, Caloneis fontinalis (Fig. 2e), Cymbella excisiformis (Fig. 2h), Denticula tenuis (Fig. 2f), Cymbella subhelvetica, Humidophila contenta (+ H. paracontenta), Humidophila perpusilla, Encyonema lance-bertaloti, Encyonopsis microcephala, Encnotia minor (Fig. 2n), Gomphonema olivaceoides, Gomphonema parvulum, Navicula cryptotenella (Fig. 2I), Navicula menisculus, Psammothidium bioretii (Figs. 2t–u), Psammothidium chlidanos, Psammothidium oblongellum (Figs. 2r–s), Reimeria uniseriata (Fig. 2v) (max abundances 10–30%). All other species had max abundances < 8%. The Achnanthidium minutissimum species group was the most widespread taxonomic unit (64 samples, maximum abundance 75.8%), accompanied by the rheophilic Achnanthidium pyrenaicum (+ A. rostropyrenaicum) (55 samples, maximum abundance 71.1%) and by the crenophilous Diatoma mesodon (46 samples, maximum abundance 80%).

Diatom species characteristic of the lake-littoral zone (Figs. 3a–b) were found in limnocrenic springs (e.g., Encyonema caespitosum, Diatoma ehenbergii (Fig. 3a), Navicula subalpina, Planothidium distinctum). Typical mire taxa (Figs. 3c–e), e.g. Encyonema lunatum (Fig. 3c), Frustulia crassinervia (Fig. 3d), Fragilariforma virescens (Fig. 3e), Navicula angusta) were found in helocrenic springs, and typical stream taxa (Figs. 3f–i), e.g., Achnanthidium pyrenaicum (Fig. 3i), A. rostropyrenaicum (Figs. 3g–h), Cymbella lange-bertalotii, Hannaeas arcus (Fig. 3f) in rheocrenic springs with medium and high discharge.
Moisture-condition preference indicator values could be found for 131 species in the list of Van Dam et al. (1994): 50% (67 taxa) mainly occurred in water bodies, but also rather regularly on wet and moist surfaces, while 15% occurred on wet and moist or temporarily dry substrata (Figs. 3j–o, 18 taxa, e.g., Adlaflia minuscula, Caloneis tenuis, Humidophila contenta (Fig. 3i) (+ H. paracontenta, Fig. 3m), Eunotia minor, Platessa montana, Psammothidium bioretii), 4% of the species were found to be not strictly bound to the aquatic environment and to live nearly exclusively outside water bodies; these pseudaelia species include e.g.: Adlaflia bryophila (Fig. 3k), Humidophila laevissima, H. perpusilla (Fig. 3n), Encyonema alpinum (Fig. 3j), Orthoseira roeaeana (Fig. 3o).

Out of the taxa found, 198 could be classified according to the German Red List for diatoms (Lange-Bertalot 1996) (Fig. 4): 9% were found to be in the category endangered (e.g., Cavinula pseudoscutiformis (Fig. 4b), Cymbella lancettula, Eucocconeis flexella (Fig. 4f), Eunotia curtagunowii, E. intermedia (Fig. 4h), E. nymanniana, Navicula angusta, Neidium alpinum, Platessa montana (Fig. 4a), Rossithidium petersenii (Fig. 4m)), 13% were on the decrease and 4% were extremely rare (Adlaflia suchlandtii, Cymbella tridentina, Epi-theemia goeppertiana, Gomphonema parallelstitratum) (On-line Supplement Tab. 1). The species that could not be classified with the German Red List include several recently discovered or described species (e.g., Geissleria gereckei, Eunotia glacialispinosa).

Diatom species recently described from springs in the eastern Alps were found (e.g., Cymbella cantonatii (Fig. 4g), C. tridentina (Fig. 4j), Encyonema sublangebertalotii (Fig. 4k), Eunotia glacialispinosa (Fig. 4n), Geissleria gereckei (Fig. 4i), Sellaphora perhibita).

The classification of trophic preferences according to the list of Van Dam et al. (1994) could be made for 134 of 223 taxa, and yielded the following results: 23% oligotrophentic, 12% oligo-mesotrophentic, 13% mesotrophentic, 15% meso-eutrophentic, 21% eutrophentic, 15% oligo- to eutrophentic, and 1% hypereutrophentic.

According to Hustedt in Van Dam et al. (1994), the pH preferences of 159 of the taxa identified were as follows: 2% acidobiontic, 11% acidophilous, 35% circumneutral, 47% alkaliphilous, 5% alkalibiontic.

Diatom species diversity of the springs

The Shannon-Wiener diversity index showed no significant differences among the three spring types: the highest diversity index value was found for the helocrenes (median: 3.4), followed by limnocrenic springs (3.2), and rheocrenic sources (3.0). The t-test analyses revealed a statistically significant difference between helocrenic and rheocrenic springs (t = 2.14, P = 0.05).

There were no significant differences in diversity index between carbonate (median = 3.1) and siliceous springs (median = 3.0). The maximum taxa number (44 taxa) was found in epilithic samples from helocrenic carbonate springs (SE 2140, and SE 2105), while the minimum (15 taxa) was found in epilithic samples from carbonate rheocrenic springs (SE1510). On siliceous substrata the highest and lowest taxa numbers were 34 and 17 respectively. There were no significant differences in the number of taxa or the diversity index values between epilithic and epiphytic assemblages but the difference between bryophyte samples taken from siliceous and carbonate substratum (3.3 on carbonate, and 2.7 on siliceous substrata) was highly significant.
Diatom communities and environmental variables

The significance of the CCA shown in Fig. 5 was tested with Monte Carlo permutation tests (999 permutations under full model), and found to be highly significant (test of significance of first canonical axis: eigenvalue = 0.289, F-ratio = 3.628, P-value = 0.0140; test of significance of all canonical axes: trace = 0.809, F-ratio = 1.651, P-value = 0.0010). The proportion of variance explained by Axis 1 was 35.7%, whilst the proportion of variance explained by Axis 2 was 23.5%.

Conductivity (–0.53) and pH (–0.93) were significantly correlated to CCA Axis 1, while altitude (–0.79), shading (0.66), and temperature (0.60) were significantly correlated to CCA Axis 2 (Fig. 2). The left part of the biplot contains taxa typical of circumneutral (e.g., *Achnanthidium minutissimum*, *Cymbopleura subaequalis*, *Diatoma mesodon*) and alkaline (*Achnanthidium pyrenaicum*, *Amphora pediculus*, *Navicula cryptotenella*) waters. The right part collects acidophilous taxa (e.g., *Eunotia implicata*, *E. intermedia*, *E. minor*).

Altitude was negatively correlated with shading (–0.71). Temperature and altitude were not always negatively correlated, because karstic phenomena are very important in this area and drain the water to lower altitudes. Nitrate concentrations were higher at lower altitudes due to human impacts. Nevertheless, high values were sometimes recorded also at high altitudes, probably because of the impact of pastures.

Fig. 5. Canonical correspondence analysis (CCA): springs’ sampling sites – environmental variables biplot. See table 1. for details about investigated sampling sites.
Vegetation: Analysis and classification

In the 28 relevés, the bryophyte component was dominant, as usual in the Cratoneurion commutati alliance (On-line Supplement Tab. 2). Besides Palustriella commutata, the presence of additional elements belonging to this alliance was also observed (i.e. Saxifraga aizoides, Equisetum variegatum). Some species belonging to the Caricetalia davallianae order were found as well.

Cluster analysis (not shown) revealed two main groups. The first represented, in general, communities dominated by bryophytes growing on constantly moist or intermittently submerged substrata. Within this first group, two sub-groups could be identified: a. the typical aspect of this community, and b. its Palustriella falcata variant. The second group was related to coenoses which colonize the fringe of springs reached only by water spray and only occasionally directly influenced by water. Multivariate analyses (not shown) suggested that the distribution of relevés was related to the presence of water: in particular a group, referred to the first group mentioned above, included species colonizing the submerged area (vascular plants mainly found in water bodies, e.g. Veronica anagallis-aquatica and Calla palustris).

Discussion

Our study extended and improved knowledge gained on spring diatom communities in the south-eastern Alps to the south-western Alps, and added several further useful observations. In particular spring diatom communities of the south-western Alps were found to include: 1) crenophilous species (part of which were relatively recently described in the south-eastern Alps); 2) many threatened Red List species; 3) diatom species characteristic of other freshwater habitats (lakes, mires, streams) according to spring typology (limnocrenes, helocrenes, rheocrenes). Patterns and features outlined by means of the analysis of diatom communities were in good agreement with those suggested by explorative analyses on the vascular plant and bryophyte vegetation of carbonate springs.

The water quality of the springs sampled is relatively high in most cases. Mineral concentrations are low: average values of conductivity were 66 $\mu$S cm$^{-1}$ on siliceous and 203 $\mu$S cm$^{-1}$ on carbonate substrata. As confirmed by the comparison of nitrate values with land-use information, the main impact on water quality derives from pastures.

The relatively high species richness (223 taxa in 48 springs) generally confirms the findings of other studies carried out in the Alps (250 taxa in 30 sampling sites, CANTONATI 1998; 131 taxa in 21 sampling sites, CANTONATI and SPITALE 2009; 197 taxa in 54 sites, GESIERICH and KOFLER 2010; 174 taxa in 19 sampling sites, FALASCO and BONA 2011). In agreement with studies carried out in similar habitats located on comparable lithological substrata (CANTONATI 1998, CANTONATI et al. 2007, GESIERICH and KOFLER 2010, FALASCO and BONA 2011, CANTONATI et al. 2012) the most frequent and abundant taxa were: Achnanthidium minutissimum species complex, and Diatoma mesodon.

We found many species that showed a strong affinity to springs and could therefore be classified as crenophiles (e.g.: Caloneis alpestris, Campylodiscus hibernicus, Cymbella laevis, Delicatea minutata, Diatoma hyemalis, Diatoma mesodon, Encyonopsis cesatii, Eu- cocconeis flexella, Eunotia exigua, Eunotia minor, Eunotia tenella, Meridion circulare, Suriella spiralis). Species recently described in the eastern Alps as typical for springs from carbonate bedrock were found, and their ecological preferences could be confirmed: Cym-
bella tridentina (CANTONATI et al. 2010), Encyonema sublangebertalotii and Eunotia glacialispinosa (CANTONATI and LANGE-BERTALOT 2010), Geissleria gereckei (CANTONATI and LANGE-BERTALOT 2009).

Most of the springs studied are rheocrenes, the most common spring type in the Alps according to DI SABATINO et al. (1997). The biodiversity and richness analysis of the three spring types yielded high values, e.g. average Shannon-Wiener index value: helocrenes (3.4), limnocrenes (3.1), rheocrenes (3.0). Helocrenes were thus confirmed to be the most species rich spring type (CANTONATI 1998, GERECKE et al. 2011).

In good agreement with previous studies (CANTONATI and SPITALE 2009), we found typical mire taxa (e.g., Encyonema lunatum, Frustulia crassinervia, Fragilariforma virescens, Navicula angusta; CANTONATI et al. 2011 b) in helocrenic springs, and typical stream taxa (e.g., Achnanthidium pyrenaicum, Cymbella lange-bertalotii, Hannaea arcus) in rheocrenic springs with medium and high discharge. Moreover, taxa typical of lake shores (e.g.: Cymbella subhelvetica, Diatoma ehrenbergii, Encyonema caespitosum, Navicula subalpina; CANTONATI and LOWE 2014) could also be observed in this study in limnocrenic springs.

The presence of aquatic plants, especially of bryophytes, increases the heterogeneity of microhabitats, and allows for colonization by high numbers of taxa, confirming the role of springs as hot spots for biodiversity (CANTONATI 2004, FRÁNKOVÁ et al. 2009).

As already observed by BERTRAND et al. (2004), no significant differences in diversity and species richness between epilithic and epiphytic assemblages could be found. However, we observed that, whilst several species occurred on all substrata, some showed a clear preference for bryophytes, e.g.: Caloneis fontinalis, Diatoma hyemalis, Diploneis krammeri, Encyonopsis cesatii, Eunotia glacialispinosa, Tetracyclus rupestris. Five species even occurred in the epibryon only: Encyonopsis falaisensis, Eunotia meisteri, Adlafla bryophila, Tabellaria flocculosa, Surirella spiralis.

Among the diatom taxa found, 223 were included in in the German Red List (LANGE-BERTALOT 1996), 84 were classified as »endangered« or »decreasing«. This figure would have been even higher if a more updated version of the Red List had been available. The still high percentage of taxa with unknown conservation status shows the potential of regional studies in the Alpine area to contribute to the improvement of biodiversity-conservation oriented knowledge.

Geochemical factors were found to be the most important variables for the ordination of the springs studied. The CCA results indicated that diatom community composition was mainly determined by pH, conductivity, temperature, altitude, and shading. Similar results were obtained by other authors, e.g.: CANTONATI (1998) and CANTONATI et al. (2012 b) for springs in the south-eastern Alps (pH, altitude, and shading), POULÍCKOVÁ et al. (2004) for helocrenic springs in the western Carpathians, KILROY et al. (2006) for freshwater habitats in the subalpine zone in New Zealand. NOVÁKOVÁ (2002), studying mires in the Czech Republic, identified pH and shading as the major environmental determinants; in particular the number of species increased with increasing pH, while shading decreased the number of species. GESIERICH and KOFLER (2010) studied the springs in the central Alps in Austria, and found that pH, altitude, conductivity, and nitrates, were the most relevant differentiating variables of diatom assemblage composition.

As concerns the part of the study devoted to vegetation analysis, it must be underlined it was limited to springs on carbonate substratum. We were able to attribute the vegetation...
studied to the *Montio Cardaminetea* class, typical of springs on carbonate rocks. This class includes the order *Montio Cardaminetalia* and the assemblage *Cratoneurion commutati*. The identified plant association is *Cratoneurum commutati*, with the constant presence of the characteristic species *Palustriella commutata*, which is an Asiatic bryophyte colonizing carbonate substrata (Dierssen 2001), often with other bryophytes such as *Palustriella falcatata*, *Brachyteceum rivulare* and *Ptychostomum pseudotriquetrum*, forming dense carpets. The *Cratoneuretum commutati* is an association occurring on permanently moist ground, irrigated by base-rich, calcareous, from oligotrophic to mesotrophic waters. Some species belonging to the *Caricetalia davallianae* order, which groups plant communities typical of neutral-alkaline bogs (Miserere et al. 2001), were found as well.

Spring habitats show very peculiar ecological features such as low and stable water temperature, high air humidity and high oxygen concentration. These ecological characteristics have a very strong influence on spring bryophyte and vascular plant life (Zechmeister and Mucina 1994). The constancy of these habitat conditions led to the establishment of stenothermic plants, many of them representing relicts of past climatic periods (Wilmanns 1989, Ellenberg 1996). The presence of bryophytes was an important component of the habitats we studied, in accordance with Vitt and Wieder (2009). The main finding of the vegetation study was the identification of the *Cratoneuretum commutati* association, as reported also by Tomaseelli et al. (2011) for the south-eastern Alps and Miserere et al. (2001) for the Aosta Valley Region. *Cratoneuretum commutati* is a widely-distributed coenosis, even on ample elevation gradients. Its *Palustriella falcata* variant colonizes the higher elevations, preferring oligotrophic waters (Tomaseelli et al. 2011). *Palustriella falcata* is a circumboreal bryophyte, which can colonise wet calcareous rocks, springs, and fens (Dierssen 2001).

Even though vascular plant species were present in higher numbers, bryophytes showed a higher cover value. Only occasionally, particularly on muddy substrata, did species like *Chaerophyllum hirsutum*, *Petasites albus*, and *Cirsium montanum*, show higher cover values (Tomaseelli et al. 2011). A species richness increase, characteristic of spring margins, where tall vascular plants inhibit bryophyte growth (Tomaseelli et al. 2011), could be noted in our data.

In conclusion, the high diatom and plant biodiversity found in spring habitats of the western Alps underlines the importance of their preservation, the main impacts being pastures causing nutrient inputs and trampling damage by cattle during the summer.

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References


MOGNA M., CANTONATI M., ANDREUCCI F., ANGELI N., BERTA G., MISERE L.


CANTONATI, M., LANGE-BERTALOT, H., 2010: Diatom biodiversity of springs in the Berchtesgaden National Park (northern Alps, Germany), with the ecological and morphological characterization of two species new to science. Diatom Research 25, 251–280.

CANTONATI, M., LANGE-BERTALOT, H., DECET, F., GABRIELI, J., 2011 b: Diatoms in very-shallow pools of the site of community importance Danta di Cadore Mires (south-eastern Alps), and the potential contribution of these habitats to diatom biodiversity conservation. Nova Hedwigia 93, 475–507.


CANTONATI, M., TAXBOCK, L., MOGNA, M., SPITALE, D., 2011 a: A first comparison among the diatom communities in spring habitats of carbonate mountain ranges of the Alps. 5th Central European Diatom Meeting (CEDIATOM5), Szczecin, Poland, 70–71.


DIATOMS AND VEGETATION OF SW ALPS SPRINGS


