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Epilithic diatom assemblages and environmental quality of the Su Gologone karst spring (central-eastern Sardinia, Italy)

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Abstract – Karst springs are considered among the most vulnerable groundwater-dependent ecosystems. Despite their ecological value and importance as strategic water sources, Mediterranean karst springs are still poorly investigated. The aim of this study was to analyse the epilithic diatom assemblages and to test their usefulness as indicators of environmental quality on the Su Gologone spring (central-eastern Sardinia, Italy), a biotope of great natural value and a precious source of drinking water. A total of 89 diatom taxa were found with 25 new records for Sardinian running waters. Species richness, Shannon-Wiener and Pielou indices showed good biotic integrity. The dominant taxa were alkaliphilous, halophobous-oligohalobous exigent, xeno-oligosaprobic and characteristic of oligotrophic waters. The eutrophication/pollution index − diatom based (EPI-D) and the *Navicula Nitzschia Surirella* indices indicated respectively an excellent/good biological water quality and a low physical disturbance. However, the biological and chemical oxygen demand, and the microbiological variables (*E. coli*, fecal and total coliforms) revealed an organic contamination of the water, although moderate. The judgment provided by the EPI-D should be verified after updating of the index. In fact, 10 taxa found in this study are not currently considered by the EPI-D method.

Keywords: Bacillariophyta, biological quality, diatom indices, groundwater-dependent ecosystems, karst springs, Mediterranean region, physical disturbance, Sardinia

Introduction

Springs are aquatic habitats with unique characteristics and a high ecological value (Odum 1971, Cantonati 2003, Cantonati et al. 2006). They belong to the group of groundwater-dependent ecosystems (GDEs) (Kløve et al. 2011) and provide contacts and connections between groundwater, surface water and terrestrial ecosystems (Webb et al. 1998, Scarsbrook et al. 2007, Cantonati et al. 2012a, b). Their nature of multiple ecotones creates a complex mosaic structure of different microhabitats (Weigand 1998) that makes them important hotspots of biodiversity (Cantonati et al. 2006, Scarsbrook et al. 2007, Ilmonen et al. 2012). Springs are considered insular biotopes (Mac Arthur and Wilson 1967, Whittaker et al. 2001) or water islands (Werum 2001) capable of hosting specific biocenoses because of their disjointed distribution within the landscape (Cantonati et al. 2012b). In addition, they show a greater stability of physico-chemical parameters than other surface aquatic ecosystems (Van der Kamp 1995, Glazier 1998). When pristine or still relatively sheltered from heavy human impacts, they are an important source of high quality water and can host endemic, rare, threatened and relict taxa (Botosaneanu 1995, Cantonati et al. 2006). Springs are among the most interesting aquatic environments for the study of algal microflora, especially diatoms, since they are often the dominant algae and are considered useful indicators of environmental quality, because they can reflect the ecological integrity of spring habitats (Cantonati and Lange-Bertalot 2010, Smol and Stoermer 2010).

The diatom flora of springs from south Europe was investigated in Spain (Aboal et al. 1998, Penalta-Rodríguez and López-Rodríguez 2007, Delgado et. al. 2013), Pyrenees (Sabater and Roca 1990, 1992), Slovenia (Menegalija and Kosi 2008), Bosnia and Herzegovina (Hafner 2008, Kapetanović

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and Hafner 2007), Republic of Macedonia (Stavreva-Veselinovska and Todorovska 2010), Greece (Economou-Amilli and Anagnostidis, 1981).

Studies on the diatom flora of Italian springs have been carried out, especially in recent years, and in particular in the Alpine region (Dell'Uomo 1975, Cantonati 1998a, b, 1999, 2001, 2003, Cantonati and Ortler 1998, Cantonati and Pipp 2000, Battegazzore et al. 2004, Falasco et al. 2012, Cantonati et al. 2006, 2012a, Cantonati and Spitale 2009, Angeli et al. 2010, Falasco and Bona 2011, Spitale and Cantonati 2011, Battegazzore 2012, Battegazzore and Morisi 2012, Spitale et al. 2012a, b) and in the Apennines (Dell'Uomo 1986, Dell'Uomo and Torrisi 2000, 2001, Torrisi and Dell'Uomo 2001, 2009). By contrast, very few studies have investigated springs of the Mediterranean region and, in particular, of the main islands, such as Sicily (Mannino 2007) and Sardinia (Lange-Bertalot et al. 2003). The diatom flora of karst springs, for example, has been explored only occasionally (Dell'Uomo 1990), despite these aquatic ecosystems are particularly important for the Mediterranean area (Civita 2008). Furthermore, this geographic area is one of the major hotspot of plant biodiversity (Myers et al. 2000, Zachos and Habel 2011). Karst springs are considered among the most vulnerable GDEs for both water quantity and quality (Leibundgut 1998). They respond quickly to heavy rainfall and drought periods (Meyer et al. 2003), which are typical of the Mediterranean climate, with wide variations in discharge (White 1988). Their load of suspended solids frequently varies with the discharge (Herman et al. 2007) and can increase significantly during the rainfall period, producing siltation events (Weigand 1998). Karst springs are also increasingly exposed to water abstractions and are particularly sensitive to pollution due to rapid infiltration, thin or absent soil cover, high flow velocity of the water and poor self-purification capacity of the karst aquifer (Sasowsky and Wicks 2000, Daly et al. 2002). These factors can significantly influence the composition and structure of their aquatic communities (Smith et al. 2003, Danehy and Bilby 2009).

Because of the natural scarcity of permanent surface freshwater (Fadda and Pala 1992), in Sardinia karst springs represent a precious and strategic source of drinking water. They are almost the exclusive source of drinking water for many urban centres (De Waele and Murgia 2001). Some studies have emphasized the importance of gaining a greater knowledge and understanding of the biocenoses and ecological dynamics of karst springs in Sardinia, also considering the significant potential vulnerability of these ecosystems (De Waele and Murgia 2001, De Waele 2003). Diatoms, as bioindicators, can provide important information on the environmental integrity of these ecosystems. Moreover, the geographic and ecological isolation of Sardinia, located in the middle of the Mediterranean Sea, is recognized as an important prerequisite for the potential presence of endemic diatom species (Lange-Bertalot et al. 2003).

This study focused on the Su Gologone spring, which is the most important spring in Sardinia, whose algal flora was investigated at the end of the 80s (Dell'Uomo 1990). The main objectives were: a) to describe the current taxonomic composition and structure of the epilithic diatom assemblages of the spring and compare them with data from the previous study; b) to document the presence of interesting taxa by light and scanning electron microscopy; c) to assess for the first time the environmental quality of the spring on the basis of physico-chemical and microbiological parameters and diatom indices.

Materials and methods

Study area

The Su Gologone spring is the most important spring system of Sardinia (Bianco 1993). It is the main resurgence of the Supramonte massif, a vast and complex karst system that extends in central-eastern Sardinia (Fig. 1) (De Waele 2008). The hydrogeological basin that feeds the spring system covers a total surface area of about 160 km^2 and is composed of Middle Jurassic-Upper Cretaceous dolostones and limestones covering a crystalline Palaeozoic basement made out of granites and metamorphic rocks. It is an aquifer of regional importance with a hydrodynamic behaviour that is still in need of proper understanding (De Waele 2008). The Su Gologone spring system is relatively isolated geographically due the surrounding impervious and largely inaccessible mountains. It is inside the Gennargentu and the Gulf of Orosei National Park (Presidential decree 30/03/1998), which, however, has never been operative due to the opposition of the local communities. The Su Gologone is also a Natural Monument (Regional decree 845/1998) and a Respect Zone (Legislative decree 152/1999). The potential sources of disturbance in this territory are mainly local animal farming and hiking activities.

The spring system (Fig. 1) is located in the Guthiddai Valley at the foot of the north-eastern slope of Mount Uddè (806 m) and is composed of two points of water emergence, Sa Vena Manna, a limno-rheocrene spring (104.5 m a.s.l.) and Sa Vena, a rheocrene spring (103.7 m a.s.l.). Sa Vena

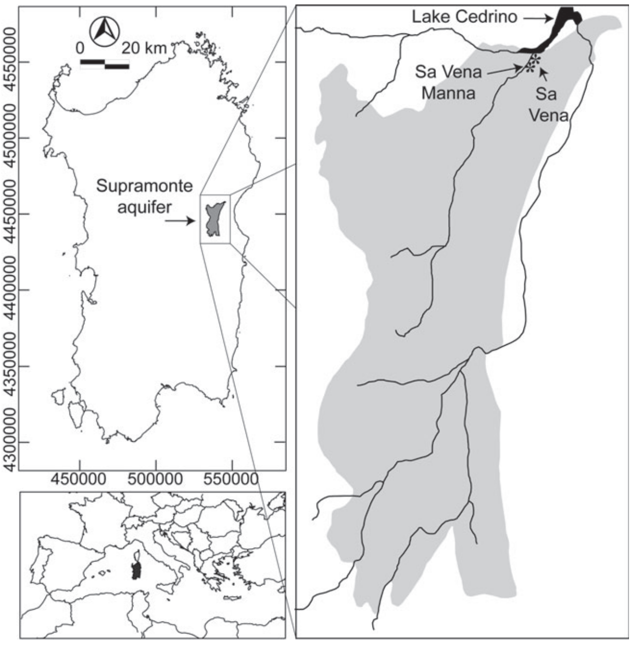

Fig. 1. Geographic location of the Su Gologone karst springs.

Manna, is the largest point of water emergence (Bianco 1993) and is a typical vauclusian spring (De Waele 2008). The water gushes permanently from a fracture in the limestone rock and forms at the opening a narrow and deep pool, trapped between two high and almost vertical rock walls. The water flows slowly in the initial portion and faster in the following stretch. The discharge is high but very irregular, rarely less than 200 L s^{-1} in the drought period (Bianco 1993) and up to 10 000 L s^{-1} during rainfalls (De Waele 2008). Sa Vena is the smallest of the two water emergences of the Su Gologone karst system and has a much more modest water discharge. The water emerges from a small rock from which it is abstracted by a small aqueduct (Bianco 1993) and immediately forms a small brook. Water abstraction from Sa Vena supplies drinking water to the municipalities of Oliena and Dorgali (about 16 000 inhabitants). Waters from both Sa Vena Manna and Sa Vena flow, after a short distance, into the Cedrino River and further downstream into Lake Cedrino, a eutrophic artificial lake, which supplies water to the downstream municipalities (about 20 000 inhabitants) (Bianco 1993, Padedda and Sechi 2008). When floods of the Cedrino River occur (on average twice a year), the spring system is submerged by the waters of the lake for a time ranging from a couple of hours to several days, making it impossible to supply drinking water (Bianco 1993, De Waele 2008).

Sampling

The sampling was carried out at Sa Vena between December 2010 and June 2011. Water samples for chemical and microbiological analyses were collected each month using 1 L polyethylene and glass bottles. The water samples were preserved in cold and dark conditions until the laboratory analyses were performed. Epilithic diatoms were collected in December 2010 and June 2011 by scraping the upper surface of hard natural substrates (five cobbles randomly selected in flowing water for a total surface of at least 100 cm^2) with a hard bristled toothbrush according to the methods of Kelly et al. (1998) and Ispra (2007). All diatom samples were preserved in 100 mL polyethylene bottles and fixed in situ with a formaldehyde solution (4% v/v).

Measurements and analyses

Temperature, pH, conductivity and dissolved oxygen were measured in situ with a multi-parameter probe (YSI MPS 556). Alkalinity, chlorides (Cl^-), hardness, biological oxygen demand (BOD), chemical oxygen demand (COD), soluble reactive phosphorus ($P\text{-}PO_4^{3-}$), total phosphorus (TP), ammonia nitrogen ($N\text{-}NH_4^+$), nitrites ($N\text{-}NO_2^-$), nitrates ($N\text{-}NO_3^-$), total nitrogen (TN), reactive silica (RSi), total suspended solids (TSS) and some ions (Ca^{2+}, Mg^{2+}, Fe^{2+} and Mn^{2+}) were determined in the laboratory according to standard methods reported by CNR-IRSA (1994) and APHA (1998). *Escherichia coli*, fecal and total coliforms were analysed using a membrane filtration method according to the CNR-IRSA (1994).

Diatom subsamples (50 mL) were treated in the laboratory, after natural decantation for 48 h. The organic matter was eliminated by boiling the samples in hydrogen peroxide (30%). Diluted hydrochloric acid (37%) was added to remove carbonates (ISPRA 2007). After being washed with distilled water, the cleaned frustules were mounted on permanent microscope slides using Styrax® resin (refractive index = 1.59).

Diatoms were examined using a Zeiss Axiovert 10 light microscope (LM) at a 1000× magnification. In the first stage, the identification of all species was done according to Krammer and Lange-Bertalot (1986, 1988, 1991a, b, 2000), Lange-Bertalot et al. (2003), Reichardt (2004), Werum and Lange-Bertalot (2004), Levkov (2009), Lange-Bertalot et al. (2011), Żelazna-Wieczorek (2011). Light microscope images were taken with an Axiocam Zeiss digital camera mounted on the microscope and connected to a computer. Afterwards, for each sample, at least 400 valves and/or frustules were counted. For the purpose of corroborating the LM study, several species were also examined using a Zeiss EVO LS10 environmental scanning electron microscope (SEM). Subsamples of the diatom suspension were air-dried on aluminium sheets and fixed on aluminium stubs that were sputter-coated with gold (Sputter Coater Edwards S-150A). The SEM identification of some diatom taxa was made after consultation of the current taxonomic literature available (e.g., Idei and Kobayasi 1986, Reichardt 2009, Van de Vijver et al. 2011, Jovanovska et al. 2013).

Data processing

All species observed in the samples were used to draw up a complete floristic list which was compared with that obtained in a previous study performed on the algal microflora of the Su Gologone spring at the end of the 80s (Dell'Uomo 1990). The ecological preferences of all diatom taxa were investigated primarily by referring to Dell'Uomo (2004), Torrisi and Dell'Uomo (2009) and Van Dam et al. (1994). In addition, the first indications on the vulnerability degree of the observed taxa were taken from the German Red List of threatened diatoms proposed by Lange-Bertalot and Steindorf (1996). This Red List, although compiled for the local diatom flora and not updated since 1996, is the only international reference currently available for the classification of diatoms based on their vulnerability.

The taxa present in the counts were used for the analysis of structure of the diatom assemblages and the evaluation of the environmental quality and physical disturbance of the spring. The abundance values of diatom taxa in each sample were transformed into relative abundance values which express the percentage contribution of each species compared to the total contribution of all species present in the count of each sample. The relative percentage abundance of each taxon was calculated by dividing the number of valves and/or frustules by the total number of valves and/or frustules of all the taxa counted in each sample and multiplying this quotient by 100.

The biotic integrity of the spring was estimated by calculating species richness, Shannon-Wiener diversity index, 2 based logarithm (H'), and evenness (J') (Shannon 1948, Shannon and Weaver 1949, Pielou 1975).

Synthetic ecological spectra of pH, salinity, organic matter and nutrients were obtained considering both the presence and the relative abundances of the taxa. The taxa with a wider ecological range were placed between the respective autecological levels, dividing equally the values of their relative percentage abundance.

Biological water quality was evaluated using the eutrophication/pollution index − diatom based (EPI-D) (Dell'Uomo 2004). The EPI-D index was chosen because it is the only index developed in Italy, after a long period of research conducted mainly on diatom communities of the central Apennines, but also of the Southern Alps and Apennines. Moreover, this index has already been applied with good results to different Italian springs, including karst springs such as the Clitunno springs (Torrisi and Dell'Uomo 2001). This index, based on the Zelinka and Marvan formula (Zelinka and Marvan 1961), considers the sensitivity (affinity/tolerance) of diatoms to nutrients, organic matter and degree of water mineralization, providing an estimation of the general quality of the water body. The values of the EPI-D were expressed in the original scale from 0 to 4, with values close to 0 indicating excellent quality and values close to 4 indicating very bad quality. The results around the threshold values were considered as transition classes (transition interval ± 0.05).

The degree of physical disturbance (siltation) was inferred by applying the *Navicula Nitzschia Surirella* indices (NNS and NNS') (Battegazzore et al. 2003, 2004, 2007). The NNS and NNS', based on the work by Hill et al. (2001) and Bahls (1993), provide an estimate of the physical disturbance in an aquatic ecosystem due to natural and anthropogenic factors assuming that siltation events determine an increase in the proportion of motile taxa within the community, both in terms of number of taxa and of number of individuals. The NNS (qualitative index) was calculated as a percentage ratio between the number of motile taxa belonging to the genera *Navicula*, *Nitzschia* and *Surirella* and the total number of taxa recorded in each sample. The NNS' (quantitative index) was calculated as a percentage ratio between the number of individuals belonging to three genera *Navicula*, *Nitzschia* and *Surirella* and the total number of individuals recorded in each sample. The values of both indices range from 0 to 100. Values close to 0 represent a low

Tab. 1. Monthly and average values of the physico-chemical and microbiological variables measured and analyzed in the karst spring Su Gologone (Sa Vena). D. L. – detection level; N/A – not available; BOD – biological oxygen demand; COD – chemical oxygen demand; TP – total phosphorus; TN – total nitrogen; TSS – total suspended solids; RSi – reactive silica; UFC – units forming colony.

Variables	D. L.	07/12/10	12/01/11	10/02/2011	07/03/11	18/04/11	18/05/11	13/06/11	Average
Temperature (°C)	-5 °C	12.4	12.0	12.0	12.0	12.0	13.0	13.0	12.3
pH (units)	0	7.6	7.9	8.0	7.8	8.3	8.1	7.5	7.9
Conductivity (µS cm^{-1})	0	356	254	353	336	338	322	344	329
Alkalinity (meq L^{-1})	N/A	2.7	2.7	2.9	2.7	2.6	2.8	2.6	2.7
Dissolved oxygen (mg L^{-1})	0	9.2	10.6	11.6	7.7	11.7	12.2	10.7	10.5
Oxygen saturation (%)	0	86	98	107	72	109	116	73	94
BOD (mg L^{-1})	N/A	3.6	1.6	3.3	0.9	2.9	4.6	2.9	2.8
COD (mg L^{-1})	5	–	< D. L.	< D. L.	18.1	< D. L.	9.2	23.7	17.0
Cl$^-$ (mg L^{-1})	5	21.3	14.2	14.2	12.4	16.0	13.5	17.7	15.6
Hardness (mg L^{-1})	5	120	105	143	133	145	143	155	135
P-PO$_4^{3-}$ (µg L^{-1})	4	4.0	4.0	4.0	< D.L.	4.0	4.0	< D.L.	4.0
TP (µg L^{-1})	4	14.0	38.0	11.0	12.0	13.0	13.0	12.0	16.0
N-NH$_4^+$ (µg L^{-1})	5	14.0	10.0	15.0	10.0	13.0	13.0	33.0	15.0
N-NO$_2^-$ (µg L^{-1})	1	< D. L.	< D. L.	< D. L.	1.0	< D. L.	< D. L.	1.0	1.0
N-NO$_3^-$ (µg L^{-1})	50	554.0	560.0	271.0	388.0	544.0	458.0	551.0	475.0
TN (µg L^{-1})	300	719.0	679.0	762.0	643.0	679.0	769.0	943.0	742.0
RSi (mg L^{-1})	0.05	1.8	2.1	1.8	1.5	2.0	2.0	2.0	1.9
Ca^{2+} (mg L^{-1})	N/A	40.1	40.1	88.0	52.0	44.0	46.0	49.0	51.0
Fe^{2+} (mg L^{-1})	0.001	0.008	0.005	0.011	0.013	0.008	0.007	0.013	0.009
Mg^{2+} (mg L^{-1})	N/A	4.9	1.2	19.0	1.0	8.5	6.6	7.9	6.9
Mn^{2+} (mg L^{-1})	0.01	< D. L.	< D. L.	0.011	0.019	< D. L.	0.010	0.023	0.016
TSS (mg L^{-1})	N/A	3.0	–	1.0	3.0	1.0	13.0	0.4	3.6
Escherichia coli (UFC 100 mL^{-1})	1	38	69	5	2	80	25	158	54
Fecal coliforms (UFC 100 mL^{-1})	1	51	115	20	1	103	170	342	115
Total coliforms (UFC 100 mL^{-1})	1	95	202	25	8	184	210	606	190

level of physical disturbance, whereas values close to 100 indicate a high level of physical disturbance.

All indices applied in this study, except the NNS and NNS', were calculated using the software OMNIDIA 7 V. 8.1 (Lecointe et al. 1993).

Statistical analyses

Principal component analysis (PCA) was performed using R 3.1.3 (Venables et al. 2015) on environmental variables to detect temporal differences among samplings and to better characterize the diatom samplings made in December and June. For the ordination analysis data were normalized using a (x-mean)/standard deviation. In the PCA analysis, we included temperature (Temp), conductivity (Cond), hardness (Hardn), biological oxygen demand (BOD), chlorides (Cl$^-$), nitrates (N-NO$_3^-$), ammonia nitrogen (N-NH$_4^+$), soluble reactive phosphorus (P-PO$_4^{3-}$).

Differences in the specific composition of the two seasonal diatom assemblages were analysed with a t-test performed on the abundances of the taxa present in each diatom sample, using R 3.1.0 (R CORE TEAM 2012).

Results

Environmental variables

The monthly and average values of the physico-chemical and microbiological variables are reported in Tab. 1. During our study, the water temperature varied from a minimum of 12 °C (January–April) to a maximum of 13 °C (May–June). Waters had a slightly basic pH (7.5–8.3), with an intermediate level of hardness (105–155 mg L^{-1} CaCO$_3$). Ca^{2+} was the most abundant cation and ranged between 40.1 mg L^{-1} (December–January) and 88.0 mg L^{-1} (February). Its highest concentration coincided with the maximum of Mg^{2+} (19.0 mg L^{-1}), which was always much lower in the other months (1.0–8.5 mg L^{-1}). Fe^{2+} and Mn^{2+}were present as trace elements in very small amounts. The conductivity ranged from 254 to 356 µS cm^{-1}. The percent water oxygenation was generally > 75%. Slight supersaturation was observed in February, April and May (107–116). P-PO$_4^{3-}$ showed values of 4.0 µg L^{-1} in almost all months, while TP values ranged from 11.0 to 38.0 µg L^{-1}. N-NO$_3^-$ was the most abundant inorganic nitrogen compound (271.0–560.0 µg L^{-1}). TN concentrations were in the range of 643.0–943.0 µg L^{-1}. BOD and COD values showed peaks in May (4.6 mg L^{-1}) and June (23.7 mg L^{-1}). The bacterial load varied widely with higher densities observed in the spring, from April to June. Suspended solids had very low concentrations with the exception of an isolated highest value in May (13 mg L^{-1}).

The PCA on environmental variables showed relationships among variables and between variables and samples (Fig. 2). The first axis explained 38.3% of the variance and the second 23.7%. N-NH$_4^+$ (0.48), water temperature (0.45) and hardness (0.44) were positively related to the first axis, characterizing the diatom sampling in June. The second axis was associated with N-NO$_3^-$ (0.54), Cl$^-$ (0.48) and P-PO$_4^{3-}$ (0.44) at opposite positions, characterizing the diatom sampling in December.

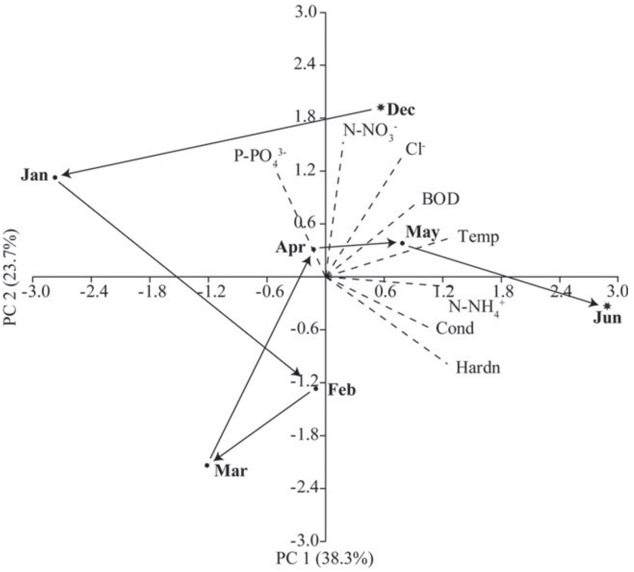

Fig. 2. Principal component analysis (PCA) biplot with the environmental variables. Temp – water temperature; Cond – conductivity; Hardn – hardness; BOD – biological oxygen demand; Cl$^-$ – chlorides; N-NO$_3^-$ – nitrates; N-NH$_4^+$ – ammonia nitrogen; P-PO$_4^{3-}$ – soluble reactive phosphorus. Dec – December; Feb – February; Mar – March; Apr – April; May – May; Jun – June.

Diatom assemblages

The complete list of taxa and their ecological preferences are reported (Tab. 2). The list is composed of a total of 89 diatom taxa, belonging to 36 genera. The genera with the highest number of species were *Navicula* (10), *Nitzschia* (9) and *Achnanthidium* (7), followed by *Amphora*, *Cocconeis*, *Diploneis* and *Gomphonema* (6). According to Van Dam et al. (1994), the majority of the species observed mainly occur in water bodies. Only 9 taxa (10% of the total) are not strictly linked to aquatic environments and belong to the categories "nearly exclusively occurring outside water bodies" and "mainly occurring on wet and moist or temporarily dry places". Of the total 89 taxa identified in our study, 52 (58% of the total) are included in different categories of the German diatom Red List (Lange-Bertalot and Steindorf 1996). However, only 5 taxa are classified as "in regression" and 2 taxa "extremely rare".

The diatom assemblages included 25 new records for the Su Gologone spring, and more for Sardinian running waters in general. LM and SEM images of some abundant, frequent, rare and occasional taxa, including some new records, were reported respectively in Figs. 5 A–P and Figs. 6 A–O.

Overall, there were 39 taxa present in the counts (44% of the total), belonging to 20 genera with 1 genus of Centrales (*Ellerbeckia*) and 19 genera of Pennales (38 taxa). They included 6 abundant taxa (relative abundance more than 5%), 10 frequent (relative abundance between 1.5 and 5%) and 23 rare (relative abundance less than 1.5%). *Achnanthidium subatomus*, *Amphora pediculus* and *Achnanthidium minutissimum* were the most abundant taxa in the

Tab. 2. List of the diatom taxa observed in the karst spring Su Gologone (Sa Vena) and their ecological preferences. All the acronyms used for pH, salinity, saprobity and trophic state are explained in the legend of the figure 3. MA – maximum relative abundance in at least one sample: r – rare: < 1.5%, f – frequent: 1.5 – 5%, a – abundant: > 5%; RL – Germain Red List of diatom taxa (Lange-Bertalot and Steindorf 1996): * – currently not considered endangered, ** – surely not endangered, 2 – highly endangered, 3 – endangered, D – insufficient data, G – considered at risk, V – in regression, R – extremely rare; Moisture: 1 – never, or only very rarely, occurring outside water bodies, 2 – mainly occurring in water bodies, sometimes in wet places, 3 – mainly occurring in water bodies, also rather regularly on wet and moist places, 4 – mainly occurring in wet and moist or temporarily dry places, 5 – nearly exclusively occurring outside water bodies. In bold: new diatom records for the Su Gologone karst spring. Taxa highlighted in gray color are not currently included in the eutrophication/pollution index − diatom based method.

Taxa	1985–1986	2010–2011	MA	RL	pH	Salinity	Saprobity	Trophic state	Moisture
Achnanthes coarctata (Brébisson) Grunow	X	X		* *	n	hb-oe	x-o	hypo-oligo	5
Achnanthidium acsiae Wojtal, E. Morales, Van de Vijver & Ector		X		–	–	–	–	–	–
Achnanthium affine (Grunow) Czarnecki	X			*					
Achnanthidium bioretii (H. Germain) O. Monnier, Lange-Bertalot & Ector		X		V	n	hb-oe	x-o	hypo-oligo	4
Achnanthidium exiguum (Grunow) Czarnecki		X		* *	ak	oe-ot	o-β	oligo-meso	3
Achnanthidium lineare W. Smith	X	X		–	n	–	–	–	–
Achnanthidium minutissimum sensu lato (Kützing) Czarnecki	X	X	a	–	n-ak	oe	o	oligo	3
***Achnanthidium* sp.**		X		–	–	–	–	–	–
Achnanthidium subatomus (Hustedt) Lange-Bertalot		X	a	*	–	–	–	–	–
Amphipleura pellucida (Kützing) Kützing		X	r	*	ak	oe	o-β	oligo-meso	2
Amphora indistincta Levkov		X		*	–	–	–	–	–
Amphora meridionalis Levkov		X		–	–	–	–	–	–
Amphora ovalis (Kützing) Kützing	X	X	r	* *	ak	ot	o-β	oligo-meso	1
Amphora pediculus (Kützing) Grunow ex A. Schmidt	X	X	a	* *	ak	ot	x-β	oligo-meso	3
Amphora vetula Levkov		X		–	–	–	–	–	–
Asterionella formosa Hassall		X		* *	ak	oe-ot	o-β	oligo-meso	1
Caloneis bacillum (Grunow) Cleve	X			* *					
Caloneis fontinalis (Grunow) Cleve-Euler		X	f	–	–	–	–	–	–
Caloneis lancettula (Schulz) Lange-Bertalot & Witkowski		X		–	–	–	–	–	–
Campylodiscus hibernicus Ehrenberg	X			*					
Cocconeis euglypta Ehrenberg	X	X	r	–	ak	ot-h	o-α	oligo-eu	2
Cocconeis neothumensis Krammer		X		V	akb	–	o	–	–
Cocconeis placentula sensu lato Ehrenberg	X	X	f	–	ak	ot	x-β	oligo-meso	2
Cocconeis placentula var. *lineata* (Ehrenberg) van Heurck	X	X		* *	ak	ot	x-β	oligo-meso	2
Cocconeis placentula var. *placentula sensu* Jahn et al. 2009		X		* *	–	–	–	–	–
Cocconeis pseudolineata (Geitler) Lange-Bertalot		X	f	D	ak	ot	x-β	oligo-meso	–
Cyclotella ocellata Pantocsek	X			*					
Cymbella affinis Kützing		X		*	ak	hb-os	o	oligo	2
Cymbella helvetica Kützing	X			V					
Cymbella lanceolata (Ehrenberg) Van Heurck	X			V					
Cymbopleura cuspidata (Kützing) Krammer	X			V					
Denticula tenuis var. *crassula* (Nägeli) Hustedt	X			V					
Diatoma hyemalis (Roth) Heiberg	X			*					
Diatoma mesodon (Ehrenberg) Kützing	X	X	r	*	n	hb-oe	o	oligo	2
Diploneis elliptica (Kützing) Cleve		X		*	a-k	oe	x-o	hypo-oligo	3
Diploneis minuta J.B. Petersen		X		R	–	–	–	–	5
Diploneis* cf. *oculata		X	f	–	–	–	–	–	–
Diploneis oculata (Brébisson) Cleve	X			*					
Diploneis separanda Lange-Bertalot		X	r	–	–	–	–	–	–
***Diploneis* sp. 1**		X	r	–	–	–	–	–	–
***Diploneis* sp. 2**		X		–	–	–	–	–	–

Tab. 2. – continued

Taxa	1985–1986	2010–2011	MA	RL	pH	Salinity	Saprobity	Trophic state	Moisture
Ellerbeckia arenaria (Moore ex Ralfs) R.M. Crawford	X	X	r	* *	a-k	hb	o	hypo	4
Encyonema elginense (Krammer) D.G. Mann	X			2					
Encyonema silesiacum (Bleisch) D.G. Mann		X		*	n	ot	β	meso	1
Encyonema ventricosum (C. Agardh) Grunow	X	X		*	n	oe	o	oligo	–
Encyonema vulgare Krammer		X		V	–	–	–	–	–
Eolimna minima (Grunow) Lange-Bertalot		X	r	* *	ak	h	α	eu	3
Epithemia argus (Ehrenberg) Kützing	X			*					
***Epithemia* sp.**		X		–	–	–	–	–	–
Eunotia mucophila (Lange-Bertalot, Nörpel & Alles) Lange-Bertalot	X			G					
Eunotia pectinalis (Kützing) Rabenhorst		X	r	V	ac	hb-oe	x-o	hypo-oligo	3
Eunotia sp.		X			–	–	–	–	–
Eunotia valida Hustedt	X			–					
Fallacia mitis (Hustedt) D.G. Mann		X	r	–	ak	oe	x	oligo	–
Fallacia subhamulata (Grunow) D.G. Mann		X	f	*	n	oe	o-β	meso	3
Fragilaria capucina Desmazières		X	r	–	n-ak	oe	o	oligo	–
Fragilaria mesolepta Rabenhorst	X			* *					
Fragilaria recapitellata Lange-Bertalot & Metzeltin		X		–	n	oe	o-β	meso	–
Frustulia vulgaris (Thwaites) De Toni		X		* *	ak	oe	o-β	meso	3
*Geissleria decussis (*Østrup) Lange-Bertalot & Metzeltin		X		* *	ak	oe	o	oligo	3
Gomphonema acuminatum Ehrenberg	X			* *					
Gomphonema angustius E. Reichardt		X		–	–	–	–	–	–
Gomphonema clavatum Ehrenberg		X		*	n-i	hb	x-o	oligo	3
Gomphonema elegantissimum E. Reichardt & Lange-Bertalot		X	r	–	–	–	–	–	–
Gomphonema micropus Kützing		X		*	ak	α-meso	β	eu	–
Gomphonema productum (Grunow) Lange-Bertalot & E. Reichardt	X			D					
Gomphonema pumilum (Grunow) E. Reichardt & Lange-Bertalot	X			*					
Gomphonema pumilum var. *rigidum* E. Reichardt & Lange-Bertalot		X		–	–	–	–	–	–
Gomphonema truncatum Ehrenberg	X	X		*	ak	ot	o-β	oligo-meso	–
Gomphosphenia grovei var. *lingulata* (Hustedt) Lange-Bertalot		X		–	–	–	–	–	–
Gyrosigma sciotense (Sullivant & Wormley) Cleve	X								
Halamphora montana (Krasske) Levkov		X		*	ak	oe	o-β	meso	4
Humidophila contenta R.L. Lowe et al.		X		* *	ak	oe-ot	o-β	oligo-meso	4
Karayevia clevei (Grunow) Bukhtiyarova		X	r	*	ak	oe	o-β	oligo-meso	1
Karayevia ploenensis var. *gessneri* (Hustedt) Bukhtiyarova		X	r	–	–	–	–	–	–
Luticola goeppertiana (Bleisch) D.G. Mann		X	r	* *	ak	h-β	α-p	eu-hyper	3
Melosira varians C. Agardh	X	X		* *	ak	ot	x-α	oligo-eu	2
Meridion circulare (Greville) C. Agardh	X	X	f	* *	ak	hb-oe	x-o	oligo	1
Navicula antonii Lange-Bertalot		X	r	* *	–	–	–	–	–
Navicula chiarae Lange-Bertalot & Genkal		X		–	–	–	–	–	–
Navicula cincta (Ehrenberg) Ralfs		X		* *	ak	h	α	eu	4
Navicula cryptocephala Kützing	X			* *					
Navicula cryptotenella Lange-Bertalot	X	X	f	–	ak	oe	o-β	oligo-meso	2
Navicula cryptotenelloides Lange-Bertalot		X	r	* *	ak	–	–	–	–
Navicula gregaria Donkin		X	r	* *	ak	h	α	meso	3
Navicula menisculus Schumann	X			V					
Navicula reichardtiana Lange-Bertalot		X		–	ak	oe	o-β	oligo-meso	–
Navicula tripunctata (O.F. Müller) Bory	X	X	a	* *	ak	hb-oe	o	oligo	3

Tab. 2. – continued

Taxa	1985–1986	2010–2011	MA	RL	pH	Salinity	Saprobity	Trophic state	Moisture
Navicula radiosa Kützing	X			* *					
Navicula veneta Kützing		X	r	* *	ak	h-β	α-p	eu-hyper	3
Navicula vilaplanii (Lange-Bertalot & Sabater) Lange-Bertalot & Sabater		X		R	–	–	–	–	–
Nitzschia commutata Grunow		X	r	*	–	–	–	–	–
Nitzschia dissipata (Kützing) Grunow	X	X	f	–	ak	ot	o-α	meso	3
Nitzschia fonticola (Grunow) Grunow	X	X	f	–	ak	oe	o-β	oligo-meso	1
Nitzschia frustulum (Kützing) Grunow		X		–	ak	ot-h	β-α	meso-eu	3
Nitzschia inconspicua Grunow		X	r	* *	ak	ot-h	β-α	meso-eu	3
Nitzschia linearis (C. Agardh) W. Smith	X	X	r	–	ak	ot-h	β-α	meso-eu	3
Nitzschia recta Hantzsch ex Rabenhorst		X		–	ak	ot	β	meso	1
Nitzschia sigmoidea (Nitzsch) W. Smith	X			* *					
Nitzschia sociabilis Hustedt		X	f	* *	n	ot-h	β-α	meso-eu	1
Nitzschia solgensis Cleve-Euler		X		V	ak	oe-ot	o-β	oligo-meso	4
Placoneis clementis (Grunow) E.J. Cox		X		*	ak	oe-ot	o-β	oligo-meso	3
Planothidium delicatulum (Kützing) Round & Bukhtiyarova	X			*					
Planothidium ellipticum (Cleve) M.B. Edlund	X			–					
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot		X	a	–	ak	hb-oe	α-poli	ind	–
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	X	X	a	–	ak	hb-oe	o	oligo	3
Platessa hustedtii (Krasske) Lange-Bertalot		X		*	ak	hb-oe	o	oligo	4
Pleurosigma elongatum W. Smith	X			*					
Reimeria sinuata (W. Gregory) Kociolek & Stoermer		X		* *	n	oe	o	oligo	3
Reimeria uniseriata S.E. Sala, J.M. Guerrero & Ferrario		X		–	–	–	–	–	–
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	X	X	r	* *	ak	oe	o-β	oligo-meso	2
Sellaphora sp.		X		–	–	–	–	–	–
Simonsenia delognei (Grunow) Lange-Bertalot		X		* *	–	ot	α	eu	3
Staurosira construens Ehrenberg		X			ak	oe	o	oligo	1
Ulnaria acus (Kützing) Aboal	X			*					
Ulnaria ulna (Nitzsch) Compère	X	X		*	ak	ot	β	meso	2

winter sample (respectively, 28%, 23% and 14%). Prevailing in the spring sample were *A. pediculus*, *A. subatomus* and *Planothidium frequentissimum* (26%, 15% and 9% respectively %).

The results of the biotic integrity indices are reported in Tab. 3. Species richness varied from 25 in winter to 31 in late spring and the diatom samples showed 20 taxa that were common to both seasons. The values of the Shannon-Wiener diversity index and the Pielou index (evenness) were respectively 3.27 and 0.70 in winter and 3.69 and 0.75 in late spring.

Tab. 3. Seasonal values of the biotic integrity indices applied to diatom assemblages of the karst spring Su Gologone (Sa Vena).

	Species richness	Diversity (H')	Evenness (J')
December 2010	25	3.27	0.70
June 2011	31	3.69	0.75

The t-test, performed on the abundances of the taxa present in each sample, indicated significant differences between the diatom assemblages in winter and late spring ($p < 0.01$).

Synthetic ecological spectra of pH, salinity, organic matter and nutrients are reported in Figs. 3A–D. The pH spectrum (Fig. 3A) showed a dominance of alkaliphilous species (69%), while circumneutral diatoms were present in insignificant amounts (4%). In the salinity spectrum (Fig. 3B), the halophobous and oligohalobous exigent (38%) prevailed over the oligohalobous tolerant diatoms (32%) that tolerate moderate concentrations of dissolved salts and, in particular, of chlorides. The halophilous forms that prefer water with a higher salt content were present in a negligible quantity (2%). The saprobic spectrum (Fig. 3C) revealed a greater presence of xenosaprobic and oligosaprobic taxa (43%) in respect to the β-mesosaprobic species (18%). The presence of the α-mesosaprobic (7%) and polysaprobic species (4%), which require a higher quantity of organic compounds, was negligible. With regard to preferences for the

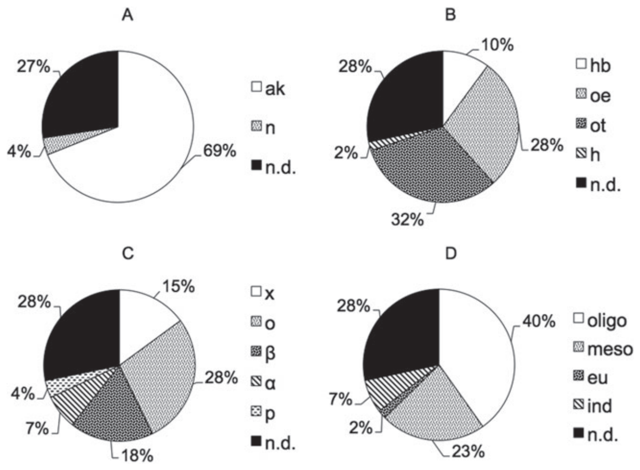

Fig. 3. Synthetic ecological spectra of the diatom taxa observed in the karst spring Su Gologone (Sa Vena): A) pH: ak – alkaliphilous, n – circumneutral; B) salinity: hb – halophobous, oe – oligohalobous exigent, ot – oligohalobous tolerant, h – halophilous; C) saprobity: x – xenosaprobic, o – oligosaprobic, β – β-mesosaprobic, α – α-mesosaprobic; p – polysaprobic; D) trophic state; taxa characteristic of environment: oligo – oligotrophic, meso – mesotrophic, eu – eutrophic; ind – indifferent taxa; for all spectra: n.d. – no data (ecological preferences poorly known).

trophic characteristics of the water bodies (Fig. 3D), the species characteristic of oligotrophic waters (40%) were most abundant in respect to the species characteristic of mesotrophic waters (23%). The eutraphentic taxa, characteristic of more nutrient-rich water bodies, were insignificant (2%) as were indifferent taxa with a wide ecological range (7%).

Diatom indices

The EPI-D values in winter (0.99) and in late spring (1.01) revealed an excellent/good water quality (class I–II) in both seasons (Fig. 4).

The NNS and NNS' indices revealed a low physical disturbance both in winter and in late spring. In particular, the NNS index showed a slightly greater number of mobile

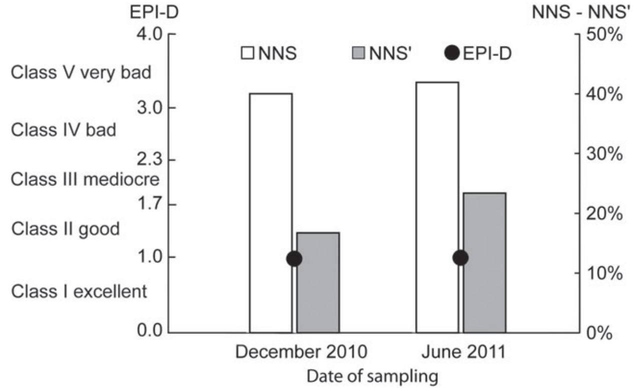

Fig. 4. Seasonal values of the indices EPI-D (the eutrophication/pollution index − diatom based, scale from 0 to 4), NNS and NNS' (the *Navicula Nitzschia Surirella* indices, scale from 0 to 100) used for the evaluation of the biological water quality and physical disturbance of the karst spring Su Gologone (Sa Vena).

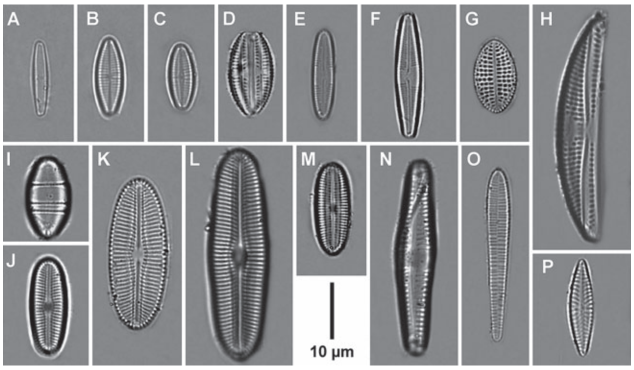

Fig. 5. Light microscopy. A) *Achnanthidium lineare* W. Smith; B-C) *Achnanthidium subatomus* (Hustedt) Lange-Bertalot; D) *Amphora indistincta* Levkov; E) *Caloneis fontinalis* (Grunow) Cleve-Euler; F) *Caloneis lancettula* (Schulz) Lange-Bertalot & Witkowski; G) *Cocconeis neothumensis* Krammer; H) *Amphora meridionalis* Levkov; I) *Diatoma mesodon* (Ehrenberg) Kützing; J) *Diploneis separanda* Lange-Bertalot; K) *Diploneis* sp. 1; L) *Diploneis* sp. 2; M) *Diploneis minuta* J.B. Petersen; N) *Gomphonema angustius* E. Reichardt, initial valve; O) *Meridion circulare* (Greville) C. Agardh; P) *Navicula vilaplanii* (Lange-Bertalot & Sabater) Lange-Bertalot & Sabater in Rumrich et al.. Scale bar = 10 µm.

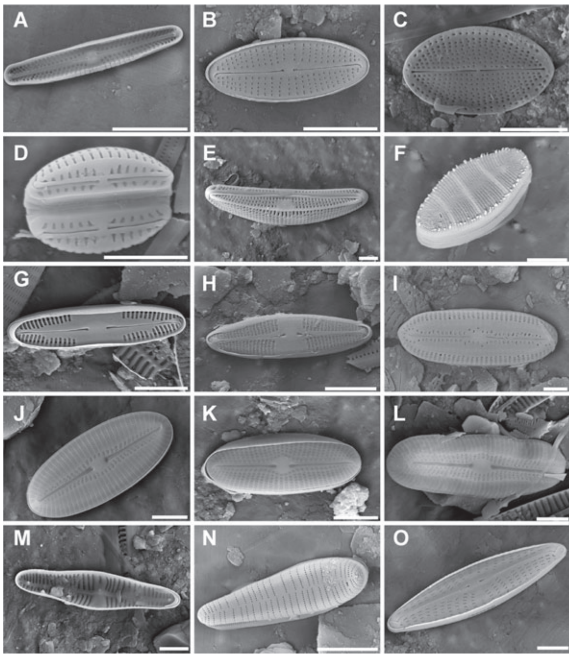

Fig. 6. Scanning electron microscopy. A) *Achnanthidium lineare* W. Smith, internal view of raphe valve; B) *Achnanthidium subatomus* (Hustedt) Lange-Bertalot, external view of raphe valve; C) *Cocconeis neothumensis* Krammer, external view of raphe valve; D) *Amphora indistincta* Levkov, external view; E) *Amphora vetula* Levkov, external view; F) *Diatoma mesodon* (Ehrenberg) Kützing, external view; G) *Caloneis fontinalis* (Grunow) Cleve-Euler, external view; H) *Caloneis lancettula* (Schulz) Lange-Bertalot & Witkowski, external view; I) *Diploneis minuta* J.B. Petersen, external view; J) *Diploneis* sp. 1, external view; K) *Diploneis separanda* Lange-Bertalot, external view; L) *Diploneis* sp. 2., external view; M) *Gomphonema angustius* E. Reichardt, internal view of initial valve; N) *Meridion circulare* (Greville) C. Agardh, external view; O) *Navicula vilaplanii* (Lange-Bertalot & Sabater) Lange-Bertalot & Sabater in Rumrich et al., external view. Scale bars = 5 µm.

taxa in spring (41.9%) than in winter (40.0%). Also the NNS' index showed a slightly higher number of individuals belonging to the mobile genera in late spring (23.4%) than in winter (16.7%).

Discussion

Despite its small dimensions, the Su Gologone spring at Sa Vena hosts a diatom flora with high total species richness, reflecting a high diversity of microhabitats and a moderate level of anthropic disturbance. The moderate current, due to the mild slope of the terrain, may have contributed to the high number of species found. In fact, according to many authors, the fast current generally present in the rheocrene springs may favour the rheophilous taxa, determining a loss in species number and diversity (Sabater and Roca 1992, Cantonati 1998a, 2001, Cantonati et al. 2012a). Moreover, rheocrene springs on carbonate substrate seem to be generally characterized by epilithic diatom communities with a lower diversity of species than rheocrene springs on siliceous substrate (e.g., Cantonati 1998a, Gesierich and Kofler 2010). The total species richness (89 taxa) was higher than that observed in the previous investigation carried out at the end of the 80s (49 taxa) (Dell'Uomo 1990), suggesting a possible role of the protective measures present since 1998 in the studied area. In fact, in other geographic areas, like for example the Alpine region, the species richness found in protected areas is higher than in non-protected areas (e.g., Falasco and Bona 2011).

The comparison between our list of species and that reported by Dell'Uomo (1990) indicated considerable differences in the Su Gologone spring after 25 years, with the presence of 21 taxa in common in both studies, 29 taxa detected only in the previous study and 68 taxa observed only in this study. The latter included 25 new records for the spring and more in general for running waters of Sardinia. The differences found seem mostly due to advances of taxonomic knowledge because several taxa like *Navicula antonii*, *Navicula vilaplanii*, *Diploneis separanda*, *Gomphonema angustius*, *Amphora indistincta*, *Amphora meridionalis*, *Amphora vetula*, were described some years after the study carried out in 1985–1986. Except for the temperature, previous environmental data of the spring are not available. However, differences found could be also related with environmental changes. In fact, species like *Campylodiscus hibernicus*, *Cymbella helvetica*, *Encyonema elginense*, *Cyclotella ocellata*, *Navicula radiosa*, (xenosaprobic and characteristic of hypotrophic or ultraoligotrophic environments) were not detected during our investigation, and nor were *Planothidium ellipticum* and *Diatoma hyemalis*, the only two taxa belonging to the nordic-alpine flora (Dell'Uomo 1990). In the central Apennines, *D. hyemalis* is reported as a species "at risk of extinction". It seems to be increasingly rare and exclusively located at high altitudes, in cold well-oxygenated waters, and with a fast current (Torrisi and Dell'Uomo 2009). A longer study could ascertain the real state of *P. ellipticum* and *D. hyemalis* in the Su Gologone spring, also taking into account a possible effect of global warming on these cold water stenotherm species. Instead, the new records found are probably explicable by the lack of specific studies on communities of benthic diatoms in the running waters of Sardinia.

In accordance with the results of the previous study, the diatom species of the Su Gologone spring have mostly a cosmopolitan and boreal-mountain distribution. The diatom assemblages showed some common features with some Alpine springs, like the presence of abundant and frequent taxa, such as *Achnanthidium minutissimum*, *Meridion circulare* and *Planothidium lanceolatum* (Cantonati 1998a, Battegazzore et al. 2004, Gesierich and Kofler 2010, Falasco and Bona 2011) and a high number of subdominant and rare taxa (e.g., Cantonati 1998a, Gesierich and Kofler 2010). However, a comparison with the floristic lists from other Italian springs showed a higher number of species in common with the diatom communities found in different springs and headwaters of rivers with the calcareous substrate found in the central Apennine region (e.g., Torrisi and Dell'Uomo 2009). Several taxa were also in common with diatom communities of springs in the Castellón province (Aboal et al. 1998), Pyrenean springs (Sabater and Roca 1992) and spring-fed streams on Majorca Island (Delgado et al. 2013).

Overall, the spring hosted a small group of taxa that are not closely linked to the aquatic environment according to Van Dam et al. (1994). For example, *Achnanthes coarctata* and *Diploneis minuta* were reported as species occurring nearly exclusively outside water bodies. Although this group of species accounted for 10% of the total species observed, they are part of the biodiversity of the spring and underline its nature of ecotone.

The vulnerable species according to the German Red List were *Achnanthidium bioretii*, *Cocconeis neothumensis*, *Encyonema vulgare* and *Nitzschia solgensis*, considered species "in regression", *D. minuta* and *N. vilaplanii* included as "extremely rare". All these species were occasional members of the diatom communities of the spring. This information has only a purely indicative and preliminary value for Sardinia. In fact, data on distribution and abundance of diatom taxa are recent and still scarce and prevent an extended comparison with the inland waters of the island. However, these first indications could integrate those from other regions and provide a contribution to a first possible diatom Red List for the Italian territory.

The indices of biotic integrity revealed high species richness and diversity and a balanced distribution of the species in the current diatom assemblages. The species richness and diversity were higher in late spring, probably due to a greater degree of light irradiation. In permanent springs with a constant temperature, the light regime is an important factor for the seasonal changes in the algal communities (e.g., Ward and Dufford 1979). Spring is also reported to be the season with the highest diatom diversity (e.g., Cantonati 1998a).

The t-test analysis indicated significant seasonal changes in the diatom assemblages, unlike what was reported for example for the Alpine springs on a carbonate substrate (Cantonati 1998a).

The observed diatom assemblages were dominated by *Achnanthidium subatomus* in winter and *Amphora pediculus* in late spring. As to *A. subatomus*, no information is available about its preference for organic matter, while with respect to its trophic preferences Krammer and Lange-Bertalot (1991b) indicated that it privileges oligo-mesotrophic waters. *Amphora pediculus* is a xeno-β-mesosaprobic species and prefers oligo-mesotrophic environments. Other dominant species were *A. minutissimum*, *Navicula tripunctata*, *Planothidium frequentissimum*, *Caloneis fontinalis*, *Nitzschia dissipata* and *P. lanceolatum*. Among these species, *N. dissipata* is an oligo-α-mesosaprobic species typical of mesotrophic environments, whereas *P. frequentissimum* is an α-meso-polisaprobic species and colonizes environments with different trophic states (Van Dam et al. 1994). All other species are oligosaprobic and typical of oligotrophic waters, except for *C. fontinalis*, about which no autecological information is available. In contrast, *Fragilaria mesolepta*, a species xeno-oligosaprobic and typical of hypo-oligotrophic waters, dominated the diatom assemblages in a previous study (Dell'Uomo 1990). The ecological preferences of the dominant species in the current assemblages suggest a possible slight deterioration of the water quality, especially of the organic type, over the years.

In general, the synthetic ecological spectra highlighted the dominance of species that prefer alkaline waters and low to moderate concentrations of dissolved salts, organic matter and nutrients. These results were very consistent with those of the physico-chemical analyses and EPI-D index. In fact, the water was characterized by a slightly basic pH and a medium hardness and degree of water mineralization. With respect to the nutrient concentrations, in particular of phosphorus, the Su Gologone spring may be considered an ecosystem with a low trophic level, which is comparable with that of the Alpine springs (Cantonati 1998a). However, their values and in particular nitrate values, do not reflect the condition of pristine environments. Moreover, BOD and especially COD, and microbiological variables indicated a not negligible degree of organic contamination in certain months. It is potentially attributable to pasture activities carried out in the surrounding area, the pollutant load of the Cedrino River and the periodic submersion of the spring by the Cedrino Lake. The highest densities of both *Escherichia coli* and fecal and total coliforms were observed in the spring season, probably due to the reduction of the discharge. This kind of contamination, although moderate, is important for the water quality, especially in view of its use as drinking water.

The EPI-D index revealed an excellent/good biological water quality (class I–II) in both seasons. However, a slight deterioration of the biological quality was observed in late spring, probably as a result of some peaks of BOD and COD in the months before the diatom sampling (June). The judgment provided by the EPI-D index seems fairly reliable and probably only slightly overestimates the quality of water. In our study *Eolimna minima* and *Navicula gregaria*, species strictly α-mesosaprobic and *Nitzschia inconspicua* and *Nitzschia sociabilis*, species β-α-mesosaprobic, showed very low relative abundances (0.1–1.6%). Among α-polysaprobic species *P. frequentissimum* was the taxon with higher relative abundance (5.1% in winter and 9.1% in late spring). The EPI-D index integrates the sensitivity of each species to salinity, organic matter and nutrients and it mediates the sensitivity of each species among different environmental variables. It provides a global judgment on the quality of the water body reflecting the interactions of several variables (Dell'Uomo 2004), rather than just organic matter. The PCA analysis performed on the environmental variables also indicated that the BOD was less important than other variables in December and June and may have affected to a lesser extent the structure of the diatom assemblages. However, 10 taxa found in this study (26% of the total), including *P. frequentissimum,* currently are not considered by the EPI-D method and their values of "i" and "r" are not present in the Omnidia software. For this reason, our result should be checked after the update of the EPI-D index in order to obtain a more accurate assessment. The NNS and NNS' indices indicated a low degree of physical disturbance, in accordance with the low concentrations of suspended solids observed throughout the period of the study. Both indices indicated a slight worsening in late spring, following an anomalous peak of the concentration of suspended solids in May. These results were also consistent with the low physical disturbance reported for the Su Gologone spring on the basis of the Disturbance Index for Karst environments (KDI) (De Waele 2009).

This study has contributed to a greater understanding of the diatom flora of the Su Gologone spring, which is the most important of Sardinia. It has led to an initial ecological characterization of the diatom assemblages, albeit limited to winter and late spring (wet period). Moreover it is the first work on the use of epilithic diatoms as indicators of biological quality and physical disturbance in the karst springs of Sardinia. Despite the presence of some potential impacts in the territory, the Su Gologone spring showed a high environmental quality, probably only slightly overestimated by the EPI-D index, and a good level of biotic integrity, reflecting the scarce human presence and the very high degree of naturalness of the hydrogeological basin. The high species richness and the presence of diatom taxa included in the German Red List also underline the importance of protecting and preserving this important biotope. However, the periodic submersion of the spring due to floods of the Cedrino River should be assessed more accurately in order to evaluate the role of floods as stressors for the ecosystem and their aquatic communities. In addition, the organic contamination detected in this study, albeit moderate, remains a problem that should no longer be neglected. The biocenoses and ecological dynamics of the Sardinian karst springs are still largely unknown. Yet these peculiar environments deserve greater consideration because of their high natural value and vulnerability and their strategic importance as sources of drinking water. Further investigations will contribute to the gathering of useful information for their long-term monitoring and management.

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References

Aboal, M., Angels Puig, M., Prefasi, M., 1998: Diatom assemblages in springs in Castellón province, Eastern Spain. Algological Studies 90, 79–95.

Angeli, N., Cantonati, M., Spitale D., Lange-Bertalot, H., 2010: A comparison between diatom assemblages in two groups of carbonate, low-altitude springs with different levels of anthropogenic disturbances. Fottea 10, 115–128.

APHA (American Public Health Association), 1998: Standard methods for the examination of water and wastewater. American Public Health Association, Washington, D.C.

Bahls, L. L., 1993: Periphyton bioassessment methods for Montana streams. Water Quality Bureau, Department of Health and Environmental Sciences, Helena, Montana.

Battegazzore, M., 2012: Environmental conditions of alpine springs of the upper Po River (NW Italy) on the basis of their epilithic diatom communities. Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano 153, 49–62.

Battegazzore, M., Bianco, L., Bona, F., Falasco, E., Fenoglio, S., Gastaldi, E., Morisi, A., Shestani, L., Badino, G., 2007: Diatomee e qualità dei corsi d'acqua in tre aree alpine e prealpine ad altimetria e substrato geologico differenti. Studi Trentini di Scienze Naturali, Acta Biologica 83, 111–116.

Battegazzore, M., Gallo, L., Lucadamo, L., Morisi A., 2003: Quality of the main watercourses in the Pollino National Park (Apennine Mts, S Italy) on the basis of the diatom benthic communities. Studi Trentini di Scienze Naturali, Acta Biologica 80, 89–93.

Battegazzore, M., Morisi, A., 2012: Environmental evaluation of springs in the intensely cultivated and industrialized inland plain of Cuneo (northwest Italy). Journal of Environmental Science and Engineering B1, 19–24.

Battegazzore, M., Morisi, A., Gallino, B., Fenoglio S., 2004: Environmental quality evaluation of alpine springs in NW Italy using benthic diatoms. Diatom Research 19, 149–165.

Bianco, L., 1993: S.O.S Su Gologone. Sardegna Speleologica 4-Dicembre 1993, 19–25.

Botosaneanu, L., 1995: Springs as refugia for geographic relicts. Crunoecia 4, 5–9.

Cantonati, M., 1998a: Diatom communities of springs in the Southern Alps. Diatom Research 13, 201–220.

Cantonati, M., 1998b: Le sorgenti del Parco Adamello-Brenta. Ricerche idrobiologiche su fonti non captate. Parco Documenti, 11. Parco Adamello-Brenta, Strembo.

Cantonati, M., 1999: Distribution and seasonality of the phytobenthos along two mountain spring streams in catchments of contrasting lithology. Atti del XIII Convegno del Gruppo per l'Ecologia di Base "G. Gadio", Venezia. Bollettino del Museo Civico di Storia Naturale di Venezia 49, 357–367.

Cantonati, M., 2001: The diatom communities of the liverwort *Chiloscyphus polyanthos* var. *rivularis* in a mountain spring-fed stream in the Adamello-Brenta Regional Park, Northern Italy. In: Jahn, R., Kociolek, J. P., Witkowsky, A., Compère P. (eds.), Lange-Bertalot-Festschrift-Studies on Diatoms. Dedicated to Prof Dr. Dr. h.c. Horst Lange-Bertalot on the occasion of his 65th birthday, 353–368. A.R.G. Gantner Verlag, Ruggell.

Cantonati, M., 2003: Le diatomee di tre sorgenti del Parco Naturale delle Prealpi Giulie (Italia nord-orientale). Gortania-Atti del Museo Friulano di Storia Naturale 25, 95–108.

Cantonati, M., Angeli, N., Bertuzzi, E., Spitale, D., Lange-Bertalot, H., 2012a: Diatoms in springs of the Alps: spring types, environmental determinants, and substratum. Freshwater Science 31, 499–524.

Cantonati, M., Füreder, L., Gerecke, R., Jüttner, I., Cox, E. J., 2012b: Crenic habitats, hotspots for freshwater biodiversity conservation: toward an understanding of their ecology. Freshwater Science 31, 463–480.

Cantonati, M., Gerecke, R., Bertuzzi, E., 2006: Springs of the Alps – sensitive ecosystems to environmental change: from biodiversity assessments to long-term studies. Hydrobiologia 562, 59–96.

Cantonati, M., Lange-Bertalot, H., 2010: Diatom biodiversity of springs in the Berchtesgaden National Park (north-eastern Alps, Germany), with the ecological and morphological characterization of two species new to science. Diatom Research 25, 251–280.

Cantonati, M., Ortler K., 1998: Using spring biota of pristine mountain areas for long term monitoring. Hydrology, water resources and ecology in headwaters. Proceedings of the HeadWater'98 Conference, Merano, 379–385.

Cantonati, M., Pipp, E., 2000: Longitudinal and seasonal differentiation of epilithic diatom communities in the uppermost sections of two mountain spring-fed streams. Internationale Vereinigung für Theoretische und Angewandte Limnologie: Verhandlungen 27, 1591–1595.

Cantonati, M., Spitale, D., 2009: The role of environmental variables in structuring epiphytic and epilithic diatom assemblages in springs and streams of the Dolomiti Bellunesi National Park (south-eastern Alps). Fundamental and Applied Limnology - Archiv für Hydrobiologie 174, 117–133.

Civita, M. V., 2008: An improved method for delineating source protection zones for karst springs based on analysis of recession curve data. Hydrogeology Journal 16, 855–869.

CNR-IRSA (Consiglio Nazionale delle Ricerche-Istituto di Ricerca sulle Acque), 1994: Metodi analitici per le acque. Istituto Poligrafico e Zecca dello Stato-Libreria dello Stato, Rome.

Daly, D., Dassargues, A., Drew, D., Dunne, S., Goldscheider, N., Neale, S., Popescu, I. C., Zwahlen, F., 2002: Main concepts of the "European approach" to karst-groundwater-vulnerability assessment and mapping. Hydrogeology Journal 10, 340–345.

Danehy, R. J., Bilby, R. E., 2009: Periphyton and macroinvertebrate assemblage responses to flow regime in spring-fed headwaters. Internationale Vereinigung für Theoretische und Angewandte Limnologie: Verhandlungen 30, 1210–1214.

Delgado, C., Ector, L., Novais, M. H., Blanco, S., Hoffmann, L., Pardo, I., 2013: Epilithic diatoms of springs and spring–fed streams in Majorca Island (Spain) with the description of a new diatom species *Cymbopleura margalefii* sp. nov. Fottea 13, 87–104.

Dell'Uomo, A., 1975: La flora e le associazioni algali della Sorgente di Salomone (Anterselva, Bolzano). Facies estiva. Giornale Botanico Italiano 109, 257–271.

Dell'Uomo, A., 1986: Diatoms and other algae from the thermal-sulphur springs of Triponzo (Central Italy). Algological Studies 73, 79–91.

Dell'Uomo, A., 1990: Chorological analysis of the algal flora of "Su Gologone" spring in Sardinia. Proceedings of the International Symposium on Biogeographical Aspects of Insularity, Rome, 417–427.

Dell'Uomo, A., 2004: L'indice diatomico di eutrofizzazione/polluzione (EPI-D) nel monitoraggio delle acque correnti. Linee Guida. Agenzia per la Protezione dell'Ambiente e per i servizi Tecnici, Rome.

Dell'Uomo, A., Torrisi M., 2000: Diatomées de sources et diatomées d'embouchures dans les cours d'eau de l'Apenin central (Italie). Cryptogamie Algologie 21, 247–248.

Dell'Uomo, A., Torrisi, M., 2001: Rheophile Apennine diatoms and their use as bioindicators of water quality. In: Seckbach, J., Kociolek, J. P. (eds.), The diatom world. Cellular origin, life in extreme habitats and astrobiology 19, 401–417. Springer, New York.

De Waele, J., 2003: I paesaggi carsici in Sardegna: tipologia, valenza e vulnerabilità. Atti del 2° Convegno Internazionale: L'ossidiana del Monte Arci nel Mediterraneo, Pau, 91–98.

De Waele, J., 2008: Interaction between a dam site and karst springs: The case of Supramonte (Central-East Sardinia, Italy). Engineering Geology 99, 128–137.

De Waele, J., 2009: Evaluating disturbance on mediterranean karst areas: the example of Sardinia (Italy). Environmental Geology 58, 239–255.

De Waele, J., Murgia, F., 2001: Le sorgenti e gli acquiferi carsici profondi della Sardegna. In: Bertocin, M., Sistu G. (ed.), Acqua, attori, territorio. IV Seminario Europeo di Geografia dell'Acqua "Conflitti per l'uso dell'acqua in ambito mediterraneo". [Water, stakeholders, territory. IV European Seminar on Geography of Water "Conflicts on water use in the Mediterranean area"], 131–136. Cooperativa Universitaria Editrice Cagliaritana, Cagliari.

Economou-Amilli, A., Anagnostidis, K., 1981: Periphyton algae and bacteria from springs of Levadia Boeotia, Greece. Hydrobiologia 80, 67–89.

Fadda, A. F., Pala, A., 1992: Le acque della Sardegna. Coedisar, Cagliari.

Falasco, E., Bona F., 2011: Diatom community biodiversity in an Alpine protected area: a study in the Maritime Alps Natural Park. Journal of Limnology 70, 157–167.

Falasco, E., Ector, L., Ciaccio, E., Hoffmann, L., Bona, F., 2012: Alpine freshwater ecosystems in a protected area: a source of diatom diversity. Hydrobiologia 695, 233–251.

Gesierich, D., Kofler, W., 2010: Epilithic diatoms from rheocrene springs in the eastern Alps (Vorarlberg, Austria). Diatom Research 25, 43–66.

Glazier, D. S., 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, 49–62. Backhuys Publishers, Leiden.

Hafner, D., 2008: Diatoms in the karstic springs of Herzegovina. In: Cantonati, M., Scalfi, A., Bertuzzi, E. (ed.), Abstract book of the 2nd Central European Diatom Meeting, p. 33. Trentino Nature & Science Museum, Trento, Italy.

Herman, E. K., Tancredi, J. H., Toran, L., White, W. B., 2007: Mineralogy of suspended sediment in three karst springs. Hydrogeology Journal 15, 255–266.

Hill, B. H., Stevenson, R. J., Pan, Y., Herlihy, A. T., Kaufmann, P. R., Johnson, C. B., 2001: Comparison of correlations between environmental characteristics and stream diatom assemblages characterized at genus and species levels. Journal of the North American Benthological Society 20, 299–310.

Idei, M., Kobayasi, H., 1986: Observations on the valve structure of fresh water *Diploneis* (Bacillariophyceae), *D. oculata* (Bréb.) Cleve and *D. minuta* Petersen. Japanese Journal of Phycology 34, 87–93.

Ilmonen, J., Mykrä, H., Virtanen R., Paasivirta, L., Muotka, T., 2012: Responses of spring macroinvertebrate and bryophyte communities to habitat modification: community composition, species richness, and red-listed species. Freshwater Science 31, 665–667.

ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale), 2007: Metodi biologici per le acque. Parte I. Agenzia per la Protezione dell'Ambiente e per i servizi Tecnici, Rome.

Jovanovska, E., Nakov, T., Levkov, Z., 2013: Observations of the genus *Diploneis* from Lake Ohrid, Macedonia. Diatom Research 28, 1–26.

Kapetanović, T., Hafner, D., 2007: Diatoms of wet habitats in the subalpine belt of Mt. Vranica (Bosnia and Herzegovina). In: Kusber, W. H., Jahn, R. (ed.), Proceedings of the 1st Central European Diatom Meeting, Berlin-Dahlem, 73–78.

Kelly, M. G., Cazaubon, A., Coring, E., Dell'Uomo, A., Ector, L., Goldsmith, B., Guasch, H., Hürlimann, J., Jarlman, A., Kavecka, B., Kwandrans, J., Laugaste, R., Lindstrøm, E.-A., Leitao, M., Marvan, P., Padisák, J., Pipp, E., Prygiel, J., Rott, E., Sabater, S., Van Dam, H., Vizinet, J., 1998: Recommendations for the routine sampling of diatoms for water quality assessments in Europe. Journal of Applied Phycology 10, 215–224.

Kløve, B., Ala-Aho, P., Bertrand, G., Boukalova, Z., Ertürk, A., Goldscheider, N., Ilmonen, J., Karakaya, N., Kupfersberger, H., Kvoerner, J., Lundberg, A., Mileusnić, M., Moszczynska, A., Muotka, T., Preda, E., Rossi, P., Siergieiev, D., Šimek, J., Wachniew, P., Angheluta, V., Widerlund, A., 2011: Groundwater dependent ecosystems. Part I: Hydroecological status and trends. Environmetal Science & Policy 14, 770–781.

Krammer, K., Lange-Bertalot, H., 1986: Bacillariophyceae 1. Naviculaceae. Süβwasserflora von Mitteleuropa, 2,1. Gustav Fischer Verlag, Jena.

Krammer, K., Lange-Bertalot, H., 1988: Bacillariophyceae 2. Bacillariaceae, Epithemiaceae, Surirellaceae. Süβwasserflora von Mitteleuropa, 2,2. Gustav Fischer Verlag, Stuttgart.

Krammer, K., Lange-Bertalot, H., 1991a: Bacillariophyceae 3. Centrales, Fragilariaceae, Eunotiaceae. Süβwasserflora von Mitteleuropa, 2,3. Gustav Fischer Verlag, Stuttgart.

Krammer, K., Lange-Bertalot, H., 1991b: Bacillariophyceae 4. Achnanthaceae. Kritische Ergänzungen zu *Navicula* (Lineolatae) und *Gomphonema*. Süβwasserflora von Mitteleuropa, 2,4. Gustav Fischer Verlag, Stuttgart.

Krammer, K., Lange-Bertalot, H., 2000: Bacillariophyceae 5. English and French translation of the keys. Süβwasserflora von Mitteleuropa, 2,5. Spektrum Akademischer Verlag GmbH Heidelberg, Berlin.

Lange-Bertalot, H., Bąk, M., Witkowski, A., Tagliaventi, N., 2011: *Eunotia* and some related genera. In: Lange-Bertalot, H. (ed.), Diatoms of Europe 6. A.R.G. Gantner Verlag K.G., Ruggell.

Lange-Bertalot, H., Cavacini, P., Tagliaventi, N., Alfinito, S., 2003: Diatoms of Sardinia. Rare and 76 new species in rock pools and other ephemeral waters. Iconographia Diatomologica, 12: 1–438. A.R.G. GantnerVerlag K.G., Ruggell.

Lange-Bertalot, H., Steindorf, A., 1996: Rote Liste der limnischen Kieselalgen (Bacillariophyceae) Deutschlands. Schriftenreihe für Vegetationskunde 28, 633–677.

Lecointe, C., Coste, M., Prygiel, J., 1993: "Omnidia": software for taxonomy, calculation of diatom indices and inventories management. Hydrobiologia 269/270, 509–513.

Leibundgut, C., 1998: Vulnerability of karst aquifers. Karst Hydrology. Proceedings of Workshop W2, Rabat, 45–60.

Levkov, Z., 2009: *Amphora* sensu lato. Diatoms of Europe, 5: 1–916. A.R.G. Gantner Verlag K.G., Ruggell.

Mac Arthur, R. H., Wilson, E. O., 1967: The theory of Island biogeography. Princeton University Press, Princeton.

Mannino, A. M., 2007: Diatoms from thermal-sulphur waters of "Fiume Caldo" (North-western Sicily). Cryptogamie Algologie 28, 385–396.

Menegalija, T., Kosi, G., 2008: Distribution of diatoms in springs in the Julian Alps (NW Slovenia). (In Slovenian). Natura Sloveniae 10, 21–37.

Meyer, A., Meyer, E. I., Meyer, C., 2003: Lotic communities of two small temporary karstic stream systems (East Westphalia, Germany) along a longitudinal gradient of hydrological intermittency. Limnologica 33, 271–279.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B., Kent, J., 2000: Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Odum, E. P., 1971: Fundamentals of ecology. W.B. Saunders co., Philadelphia.

Padedda, B. M., Sechi, N., 2008: Condizioni del Lago Cedrino e del suo bacino versante. Natura in Sardegna 13, 42–48.

Penalta-Rodríguez, M., López-Rodríguez M. C., 2007: Diatomeas y calidad del agua de los ríos del Macizo Central Gallego (Ourense, N.O. España) mediante la aplicación de índices diatomológicos. Limnetica 26, 351–358.

Pielou, E. C., 1975: Ecological diversity. Wiley, New York.

R Core Team, 2012: R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria, ISBN 3-900051-07-0. Retrieved September 30, 2011 from http://www.R-project.org/.

Reichardt, E., 2004: Eine bemerksenswerte Diatomeenassoziation in einem Quellhabitat im Grazer Bergland, Österreich. Ein Beitrag zur Kenntnis seltener und wenig bekannter Diatomeen. In: Lange-Bertalot, H. (ed.), Iconographia Diatomologica 13:419–479. A.R.G. Gantner Verlag K.G., Ruggell.

Reichardt, E., 2009: Silikatauswüchse an den inneren stigmenöffnungen bei *Gomphonema*-arten. Diatom Research 24, 159–173.

Sabater, S., Roca, J. R., 1990: Some factors affecting distribution of diatom assemblages in Pyrenean springs. Freshwater Biology 24, 493–507.

Sabater, S., Roca, J. R., 1992: Ecological and biogeographical aspects of diatom distribution in Pyrenean springs. British Phycological Journal 27, 203–213.

Sasowsky, I. D., Wicks, C. M., 2000: Groundwater flow and contaminant transport in carbonate aquifers. CRC Press/Balkema, Taylor & Francis Group, Rotterdam.

Scarsbrook, M. R., Barquín, J., Gray, D. P., 2007: New Zealand coldwater springs and their biodiversity. Science for conservation 278. Science & Technical Publishing Department of Conservation, Wellington.

Shannon, C.E., 1948: A mathematical theory of communication. Bell System Technical Journal 27, 379–423.

Shannon, C.E., Weaver, W., 1949: The mathematical theory of communication. University of Illinois Press, Urbana.

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

Smol, J. P., Stoermer, E. F., 2010: The Diatoms. Applications for the environmental and earth sciences. Cambridge University Press, Cambridge.

Spitale, D., Cantonati, M., 2011: Understanding the natural variability of diatom assemblages in springs of the Adamello-Brenta Nature Park (south-eastern Alps) on a temporal scale. Fundamental and Applied Limnology 179, 137–149.

Spitale, D., Leira, M., Angeli N., Cantonati, M., 2012a: Environmental classification of springs of the Italian Alps and its consistency across multiple taxonomic groups. Freshwater Science 31, 563–574.

Spitale, D., Lencioni, V., Cantonati, M., 2012b: Relative importance of space and time in determining the biotic structure in the upper part of spring-fed streams. Freshwater Science 31, 586–598.

Stavreva-Veselinovska, S., Todorovska A., 2010: Ecology of the diatomic flora in thermo-mineral springs of Katlanovska Banja in the Republic of Macedonia. Ecologia Balkanica 2, 1–6.

Torrisi, M., Dell'Uomo, A., 2001: Alcune diatomee (Bacillariophyta) rare o notevoli rinvenute nelle fonti del Clitunno (Umbria, Italia). Archivio Geobotanico 7, 11–15.

Torrisi, M., Dell'Uomo, A., 2009: Diatomee bentoniche del corso superiore di alcuni fiumi centro-appenninici. Studi Trentini di Scienze Naturali 84, 139–151.

Van Dam, H., Mertens, A., Sinkeldam, J., 1994: A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. Netherlands Journal of Aquatic Ecology 28, 117–133.

Van de Vijver, B., Ector, L., Beltrami, M. E., de Haan, E. M., Falasco, E., Hlúbiková, D., Jarlman, A., Kelly, M., Novais, M. H., Wojtal, A. Z., 2011: A critical analysis of the type material of *Achnanthidium lineare* W. Sm. (Bacillariophyceae). Algological Studies 136/137, 167–191.

Van der Kamp, G., 1995. The hydrogeology of springs in relation to the biodiversity of spring fauna: A review. Journal of the Kansas Entomological Society 68, 4–17.

Venables, W.N., Smith, D.M., R Core Team, 2015. An introduction to R. Notes on R: A programming environment for data analysis and graphics version 3.1.3. (2015-03-09). Retrieved March 8, 2015 from http://cran.r-project.org/manuals.html.

Ward, J. V., Dufford, R. G., 1979: Longitudinal and seasonal distribution of macroinvertebrates and epilithic algae in Colorado springbrook-pond system. Archiv für Hydrobiologie 86, 284–321.

Webb, D.W., Wetzel, M. J., Phillippe, L. R., Reed, P. C., Young, T. C., 1998: The macroinvertebrate biodiversity, water quality, and hydrogeology of ten karst springs in the Salem Plateau section of Illinois, USA. In: Botosaneanu, L. (ed.), Studies in crenobiology: The biology of springs and springbrooks, 39–47. Backhuys Publishers, Leiden.

Weigand, E., 1998: Limnologisch-faunistiche Characterisierung von Karstquellen, Quellbächen und unterirdischen Gewässern nach Choriotopen und biozönotischen Gewässerregionen. Nationalpark o.ö. Kalkalpen, Österreich.

Werum, M., 2001: Die Kieselalgen Gesellschaften in Quellen: Abhängigkeit von Geologie und anthropogener Beeinflussung in Hessen (Bundesrepublik Deutschland). Hessische Landesamt für Umwelt und Geologie, Wiesbaden.

Werum, M., Lange-Bertalot, H., 2004: Diatoms in Springs from Central Europe and elsewhere under the influence of hydrogeology and anthropogenic impacts. In: Lange-Bertalot, H. (ed.), Iconographia Diatomologica 13, 3–417. A.R.G. Gantner Verlag K.G., Ruggell.

White, W. B., 1988: Geomorphology and hydrology of karst terrains. Oxford University Press, New York.

Whittaker, R. J., Willis, K. J., Field, R., 2001: Scale and species richness: towards a general, hierarchy theory of species diversity. Journal of Biogeography 28, 453–470.

Zachos, F. E., Habel, J. C., 2011: Biodiversity hotspots. Distribution and protection of conservation priority areas. Springer-Verlag, Berlin-Heidelberg.

Żelazna-Wieczorek, J., 2011: Diatom flora in springs of Lódź Hills (Central Poland). Biodiversity, taxonomy, and temporal changes of episammic diatom assemblages in springs affected by human impact. Diatom Monographs 13, 1–419. A.R.G. Gantner Verlag K.G., Ruggell.

Zelinka, M., Marvan, P., 1961: Zur Präzisierung der biologischen Klassification der Reinheit fliessender Gewässer. Archiv für Hydrobiologie 57, 389–407.