

Diatom assemblages of non-living substrates in petrifying *Cratoneurion* springs from lower Belgium

LUC DENYS & PATRIK OOSTERLYNCK

Research Institute for Nature and Forest, Kliniekstraat 25, B-1070 Brussels
e-mail: luc.denys@inbo.be

Abstract: Diatom assemblages were examined from non-living substrates in thirteen springs supporting the Natura 2000 habitat type ‘Petrifying springs with tufa formation’ in the southern part of Flanders. More than 300 taxa were recorded, including some which appear to be particularly associated with this kind of environment. Even though a number of characteristic taxa and diatoms characterizing fast-flowing springs of more elevated terrain remained absent, local species richness was higher than reported from carbonate springs elsewhere in Europe. Relative abundance of ‘Red List’ taxa was, however, substantially lower. Taxon richness was not related to measured nutrients but increased with diatom-inferred trophic status. Assemblages consisted mainly of motile, small-celled species of *Achnanthes*, *Amphora*, *Caloneis*, *Planorbulina* and *Stauroneis*. Hygropetric rheocrenes presented a somewhat more distinct assemblage than helocrene and rheohelocrene settings, but overall, compositional metrics were similar among spring types. Assemblages were structured mainly along gradients of pH, major ions (in particular magnesium and potassium) and nitrate relating to calcite precipitation, aquifer and nitrogen supply. Besides substantiating broad similarities among *Cratoneurion* springs, the results provide arguments for a regionalized approach towards assessment of their diversity, community structure and conservation status.

Key words: habitats directive, tufa limestone, diversity, carbonate, nitrate, trophic status, regionalization, phytobenthos, crenobiota, Atlantic region

INTRODUCTION

Worldwide, spring diatoms have drawn considerable attention. This is mainly because the physical and hydrochemical characteristics of these comparatively isolated habitats render their assemblage composition distinct from more downstream parts of the drainage system and because diatoms are prominent primary producers in springs, contributing to conspicuous biogeochemical processes such as limestone formation. Also, because of their upstream location and often considerable hydrological age of the surfacing groundwater, springs may constitute rare havens for pollution-sensitive biota in regions where human impacts on surface water quality are pervasive, whilst environmental heterogeneity and ecocline structure warrant higher diversity (GERECKE et al. 2011; CANTONATI et al. 2012a).

Hydrogeological conditions for spring formation occur most frequently in (sub-)mountainous regions with surfacing hard bedrock and a series of recent diatom studies of carbonate springs in Europe have focussed on such areas (e.g., REICHARDT 1994, 1995,

2004; CANTONATI 1998; POULÍČKOVÁ et al. 2003; WERUM & LANGE-BERTALOT 2004; FRÁNKOVÁ et al. 2009; TORRISI & DELL’UOMO 2009; GESIERICH & KOFLER 2010; WOJTAŁ 2013). Springs also occur in less elevated sloping terrain, wherever groundwater is forced to the surface by less permeable strata, but unlike mid- and high-altitude regions, data on their diatom biodiversity are more restricted and often limited to more mineral-poor conditions. Although a number of previous studies considered diatoms from calcareous low-altitude springs, most concerned regions that are biogeographically distinct from Atlantic NW-Europe and sites at the foot of mountain ranges where the regional species pool is contiguous with headwaters at higher altitude (e.g. ABOAL et al. 1998; ANGELI et al. 2010; DELGADO et al. 2013). In addition, older surveys used a less detailed taxonomy than now customary, making comparisons less straightforward.

The most southern part of Flanders, the northern region of Belgium, where Quaternary loess or Tertiary sands superpose Tertiary aquitards at levels below 200 m above sea level, is not associated with orogenic terrain. Due to higher carbonate levels in the groundwater and its degassing at emergence, springs in this

area may present calcite precipitation as well as moss assemblages that allow them to be classified as the Natura 2000 habitat type 7220 'Petrifying springs with tufa formation (*Cratoneurion*)' (EUROPEAN COMMISSION 2007). Even though this habitat type remains less well-developed than its counterparts in more mountainous areas, such springs require particular protection, monitoring and reporting according to the European Habitats Directive. To resolve a general lack of information on the occurrence, abiotic environment and biotic characterisation of petrifying springs in Atlantic Flanders, OOSTERLYNCK & DE BIE (in press) recently presented a first region-wide survey, focusing primarily on hydrochemical aspects and bryophyte vegetation. This work also provided the opportunity for an initial, albeit more partial exploration of the diatom assemblages associated with the *Cratoneurion* in lower Belgium, aiming to elucidate its environmental and biological position better within the wider range of carbonate springs in western Europe and, finally, contribute to the assessment of the ecological quality of this habitat type. So far, only SYMOENS (1957, 1960) and ISERENTANT (1988) contributed more than cursory data on the diatom flora of Belgian carbonate springs, neither one considering the Flemish region.

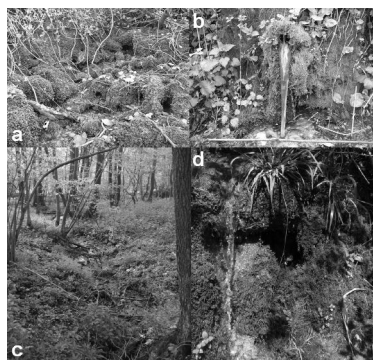


Fig. 1. Examples of *Cratoneurion* springs in Flanders: (a) Krindaal, rheohelocrene, (KRIND); (b) Boterhoek, tapped source of rheocrene (site BOTER); (c) Mabroek, rheocrene (MABRO); (d) Kwint, hydropetric rheocrene (KWINT).

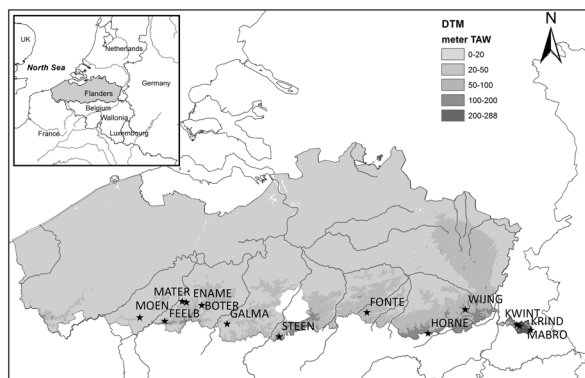


Fig. 2. Topographic map of Flanders with sample locations (TAW: mean sea-level at ebb for Oostende). Insert shows the situation in north-western Europe.

SPRING CHARACTERISTICS

Spring-associated limestone (SAL) systems (SANDERS et al. 2010) with *Cratoneurion* in Flanders vary from less well-defined areas with \pm diffuse seepage and limited carbonate deposition on plant debris and stones in discharging seeps (hillslope springs or rheohelocrenes), to distinct sources with encrustations or concretions of porous calcite (tufa limestone) in the bed of spring runs (rheocrenes) and modest (≤ 1.5 m high) mound-like limestone formations formed in small 'waterfall/creek' systems (hydropetric rheocrenes; Figure 1). Several types may occur at the same site, e.g. water from a hydropetric spring may join seepage of one or more rheohelocrenes within a single source area and transitional situations are common. Most spring waters are moderately alkaline (interquartile pH range 7.4–8), with pH typically increasing slightly downstream. EC_{25} values usually range between 714 and 950 $\mu S \cdot cm^{-1}$ and alkalinities from 4.9 to 6.6 $meq \cdot l^{-1}$ (OOSTERLYNCK & DE BIE in press). Bicarbonate and calcium predominate with levels (upper and lower interquartile ranges 300–403 and 133–169 $mg \cdot l^{-1}$, respectively; OOSTERLYNCK & DE BIE in press) that are well above the minimum for carbonate-depositing systems (PENTECOST 2005). Water chemistry varies somewhat with the nature of the Tertiary aquifer and therefore geographically, but overall such differences remain limited. Seasonal differences also occur, e.g. in calcium and ammonium. In some instances, elevated levels of ammonium, nitrate, sodium, potassium, chloride and sulphate suggest pollution. Nitrate leaching to the phreatic groundwater is generally quite considerable in southern Flanders (EPPINGER & THOMAS 2007), with atmospheric deposition (usually 21–35 $kg \cdot N \cdot ha^{-1} \cdot year^{-1}$; OVERLOOP et al. 2011) and agriculture as principal sources. *Palustriella commutata* (HEDW.) OCHYRA, and, quite occasionally, *Eucladium verticillatum* (BRIDEL) BRUCH et SCHIMPER are the more characteristic mosses representing the *Cratoneurion* in the stricter sense. These are usually accompanied by *Brachythecium rivulare* SCHIMPER, *Cratoneuron filicinum* (HEDW.) SPRUCE, *Oxyrrhynchium hians* (HEDW.) LOESKE, *Pellia endiviifolia* (DICKS) DUMORT. and *Plagiommium undulatum* (HEDW.) T.J. KOP.

MATERIAL AND METHODS

Samples were collected from thirteen springs (Figure 2) representing seven of the ten aquifers in Flanders that give rise to notable carbonate precipitation (Table 1). Only three aquifers were represented by more than one spring (Tielt 1, Vaals and Brusseliaan). Altitude varied from c. 30 to 200 m. In some cases, the source was confined by tubing or masonry. One of the sites, MOEN, was completely artificial, resulting from cutting a canal through the crest of a hill in the 1970's. Soils consisted of loam, sandy loam or occasionally, fen wood peat. All investigated springs, except for one, were under deciduous forest cover, usually dominated by *Fraxinus excelsior* L. and *Carpinus betulus* L. (*Carici remotae*-*Fraxinetum*, *Carpinion*).

Sampling sites were attributed to major spring types according to their local character (Table 1). In six springs, samples were positioned differently relative to the area of calcite formation. This was categorized as: (a) at the source, (b) just in front of the stretch with carbonate precipitation, or (c) within the main precipitation zone. Distance between the source and more downstream samples varied. Most sites

Table 1. Characteristics of sampled sites and variable transformation used for ordinations. a arcsinsqrt, l logarithm base 10.

sample	latitude (°)	longitude (°)	aquifer	spring type	soil	forest/fen type	altitude (m TAW)	bryophyte cover (%)	total cover (%)	tufa	shade	pH	EC ₂₅ (µS.cm ⁻¹)	ALK (eq.l ⁻¹)	HCO ₃ ⁻ (mg.l ⁻¹)	SO ₄ ²⁻ (mg.l ⁻¹)	Cl ⁻ (mg.l ⁻¹)	Ca ²⁺ (mg.l ⁻¹)	K ⁺ (mg.l ⁻¹)	Mg ²⁺ (mg.l ⁻¹)	Na ⁺ (mg.l ⁻¹)	Fe ²³⁺ (mg.l ⁻¹)	SRP (µg.l ⁻¹)	NO ₃ ⁻ -N (mg.l ⁻¹)	NH ₄ ⁺ -N (mg.l ⁻¹)	water samples	
BOTERa	50.8379	3.7759	Tielt 1	rheocene	sandy loam	oak-hornbeam	63	20	80	4	2	7.2	788	6.6	404	70.1	28.3	140.7	6.2	17.6	11.1	<0.05	32	0.03	0.086	3	
ENAMeA	50.8538	3.3759	Tielt 1	rheocene	sandy loam	ash-alder	54	50	60	3	5	7.7	995	6.3	385	96.7	53.1	184.4	2.1	18.0	13.6	<0.05	20	12.81	0.080	4	
ENAMeB	50.8535	3.6494	Tielt 1	rheohelo- cene	sandy loam	ash-alder	52	50	50	5	4	7.9	944	5.9	360	90.2	52.0	170.4	2.0	15.5	13.8	<0.05	8	13.26	0.039	4	
FEELBa	50.8379	3.6493	Tielt 1	rheocene	sandy loam	ash-alder	74	40	40	4	4	7.7	869	5.6	342	83.3	40.9	158.7	2.8	9.8	16.7	<0.05	17	11.38	0.081	4	
FEELBb	50.7720	3.5374	Tielt 1	hygropetric	sandy loam	oak-hornbeam	72	20	60	5	4	8.0	842	5.2	315	92.0	42.8	154.3	3.5	9.4	16.9	<0.05	12	10.54	0.039	3	
FEELBc	50.7721	3.5369	Tielt 1	hygropetric	sandy loam	oak-hornbeam	61	20	70	5	4	8.1	831	5.1	311	90.3	41.7	147.0	3.3	8.1	16.8	<0.05	15	9.77	0.039	2	
FONTEb	50.8100	4.8444	Brussel	rheocene	loam	oak-hornbeam	67	40	70	3	3	7.9	867	6.7	406	76.6	27.4	145.6	5.4	22.7	13.2	<0.05	39	7.71	0.097	3	
GALMAa	50.7623	3.9414	Tielt 3	rheocene	loam	ash-alder	46	50	60	2	5	7.8	953	6.9	423	120.2	33.8	182.2	4.3	16.6	13.2	<0.05	8	4.53	0.090	3	
GALMAb	50.7617	3.9419	Tielt 3	rheocene	loam	ash-alder	43	5	30	3	5	8.0	910	6.7	409	109.2	31.7	171.4	3.3	15.1	12.6	<0.05	8	4.85	0.039	4	
HORNEa	50.7217	5.2385	Heers	rheocene	sandy loam	oak-hornbeam	106	20	20	2	3	7.3	986	6.4	387	95.9	56.8	175.8	1.5	19.5	12.3	<0.05	8	9.70	0.081	4	
KRINDa	50.7457	5.8320	Vaals	rheohelo- cene	loam	ash-alder	154	20	60	3	5	7.8	685	5.5	333	46.7	23.3	143.4	2.7	2.7	7.2	<0.05	8	6.31	0.079	3	
KRINDb	50.7459	5.8311	Vaals	hygropetric	loam	ash-alder	153	50	50	4	4	7.8	710	5.2	315	40.9	20.8	135.8	1.7	2.6	6.7	<0.05	8	7.82	0.039	2	
KWINTa	50.7508	5.8124	Vaals	rheohelo- cene	peat	ash-alder	138	50	60	3	5	7.5	719	5.3	323	46.0	20.2	147.7	1.6	2.5	6.3	<0.05	15	12.51	0.076	3	
KWINTb	50.7513	5.8125	Vaals	hygropetric	peat	ash-alder	144	50	80	5	3	7.9	703	5.0	303	46.1	19.4	142.0	1.6	2.2	6.1	<0.05	8	12.47	0.051	3	
MABROa	50.7275	5.9013	Vaals	rheocene	loam	ash-alder	202	15	40	2	5	7.0	669	4.4	266	82.4	16.3	133.0	1.6	3.0	5.4	<0.05	30	8.31	0.073	3	
MABROb	50.7273	5.9015	Vaals	rheocene	loam	ash-alder	197	10	10	5	5	7.9	616	3.8	231	72.6	15.3	118.1	1.5	2.6	5.4	<0.05	27	9.51	0.051	4	
MATERb	50.8490	3.6767	Tielt 1	rheohelo- cene	sandy loam	ash-alder	49	80	90	3	2	7.3	1065	6.9	422	178.2	46.7	207.1	5.0	14.2	18.1	0.12	8	0.03	0.039	3	
MOENb	50.7118	4.2774	Moen	rheohelo- cene	loam	oak-hornbeam	29	5	40	3	1	8.0	956	4.9	300	200.1	45.1	133.4	24.5	25.8	27.3	<0.05	8	0.03	0.039	1	
STEENc	50.7854	3.3759	Brussel	rheocene	sandy loam	rich fen	73	35	90	3	5	8.1	713	5.5	334	78.6	23.8	133.5	1.2	10.3	11.9	<0.05	22	1.75	0.085	3	
WIJNGa	50.8167	5.4808	Borglo- on	rheocene	loam	ash-alder	106	70	90	1	5	7.2	865	6.8	416	64.7	32.4	156.0	1.2	17.0	16.9	<0.05	8	7.76	0.101	3	
median							73	38	60	3	4	7.8	854	5.5	338	82.8	32.1	147.3	2.4	12.2	12.9	<0.05	10	8.07	0.075		
average							94	35	58	-	-	-	834	5.7	349	89.0	33.6	154.0	3.9	11.8	12.6	<0.05	16	7.55	0.065		
st. dev.							54	20	22	-	-	0.3	131	0.9	55	41.2	13.4	22.5	5.2	7.6	5.6	-	8	4.52	0.022		
transfor- mation							a	a	a	-	-	-	1	1	1	-	1	1	-	1	1	-	-	-	1	1	

Table 2. Significant Pearson or Spearman correlations between environmental variables for all examined sites ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$).

	plant cover	pH	alkali- nity	SO ₄ ²⁻	Cl ⁻	K ⁺	Mg ²⁺	Ca ²⁺	Na ⁺	NO ₃ ⁻ -N	NH ₄ ⁺ -N
bryophyte cover	0.61	—	0.46	—	—	—	—	0.52	—	—	—
tufa		0.50	-0.47	—	—	—	—	—	—	—	-0.54
shade			—	—	—	-0.54	—	—	—	—	—
EC ₂₅			0.70	0.71	0.88	—	0.79	0.84	0.73	—	—
alkalinity				—	—	—	0.64	0.74	—	—	—
SO ₄ ²⁻					0.57	0.72	0.62	—	0.78	-0.54	—
Cl ⁻						—	0.64	0.69	0.70	—	—
K ⁺							0.55	—	0.69	-0.53	—
Mg ²⁺								—	0.73	—	—

were sampled two- or three-monthly for hydrochemistry from spring to autumn in a single year, but data were more limited for others (Table 1). Water samples were transported to the laboratory in firmly closed vials at 4 °C and analysed for pH, electrical conductivity (EC, WTW-multimeter), alkalinity (titration to pH 4.50 and 8.35), major ions and nutrients (calcium, magnesium, sodium, potassium, sulphate, chloride, soluble reactive phosphorus – SRP, nitrate and ammonium by ion chromatography with a Dionex ICS-3000) and total iron (inductively coupled plasma atomic emission spectrophotometry with Agilent Liberty II) according to standard protocols. EC and pH were also measured on site, but field readings were only retained for EC. The amount of tufa limestone was judged on a scale from 0 (none) to 5 (massive). The degree of shading in summer was also estimated ordinally from 0 (none) to 5 (full).

Only diatom assemblages from non-living substrates were analysed. Samples consisted of sediment (usually sand with some fine organic matter), carbonate concretions and pebbles from the bed of runs, or scrapings of the surface of more massive limestone formations. All diatom samples were taken on 16 and 17 October 2011. In the Alps, CANTONATI et al. (2012b) considered a single sample at the end of the vegetation season sufficient for assessing assemblage structure and typological differences.

Diatom samples were cleaned with concentrated hydrogen peroxide at 70 °C and carbonates were dissolved with hydrochloric acid. Strewn mounts (36 × 24 mm) were prepared using Naphrax® and examined with an Olympus BX 51 microscope equipped with differential interference optics. Up to 500 valves were counted along random transects at high magnification to calculate relative percentage abundances and additional taxa were identified by further screening the slides.

In addition to standard floras and monographs (e.g. REICHARDT 1999; KRAMMER 1997a, 1997b, 2000, 2002, 2003; KRAMMER & LANGE-BERTALOT 1999–2004; LANGE-BERTALOT 2001; LANGE-BERTALOT et al. 2003; LEVKOV 2009, LEVKOV et al. 2013), various thematic papers were used for identification.

Indicator Species Analysis (DUFRÈNE & LEGENDRE 1997) was used to identify characteristic taxa of predefined groups of samples. Similarity between assemblages was examined by

cluster analysis (Ward's method with Euclidian distances of square-root transformed abundances). Detrended Correspondence Analysis (DCA) was used to analyse species turnover and (constrained) Redundancy Analysis (RDA) with forward selection to elucidate the relation between assemblage composition and environmental variables. Only taxa with at least 1% relative abundance in one sample were included in ordinations with square-root transformation and down-weighting of rare taxa. Some environmental variables were transformed as log₁₀ and percentages as arcsine square root (Table 1). Samples were weighted proportionally by the ratio of their valve count to the standard total of 500 valves. Analyses were carried out using PC-ORD for Windows (McCUNE & MEFORD 1999) and CANOCO for Windows (TER BRAAK & SMILAUER 2003).

The 'Indice de Polluosensibilité' (IPS; CEMAGREF 1982), 'Trophie Index' (TI_{DIA}; ROTT et al. 1999) and relative abundance of taxa linked to anthropogenic alteration of springs in the marl-loess region of Hessen (% A; WERUM & LANGE-BERTALOT 2004, p. 103) were calculated, as well as the number (#) and percentage (%) of taxa considered endangered (E; categories 1, 2, 3 and G) or rare (R; categories R and unrecorded in Germany) on the Red List for Germany and Central Europe (LANGE-BERTALOT & STEINDORF 1996; CANTONATI & LANGE-BERTALOT 2010). Some more recently described taxa with very few records were added to the group of rare taxa (Table S1). The most xerotolerant taxa (X) were selected mainly from compilations by DENYS (1991) and VAN DAM et al. (1994). Differences between groups of samples were explored by means of Kruskal-Wallis Rank Sum tests and correlations between variables by the Pearson coefficient or, if one or both were ordinal, Spearman's rho. These tests were done with Tibco Spotfire S+ 8.2 for Windows.

RESULTS

Conductivity, pH, alkalinity and calcium showed relatively little variation (Table 1). Iron content was always low. Nitrate-nitrogen was often higher than what would be expected in pristine springs, remaining below measurable concentrations only for BOTER, MATER

Table 3. Characteristics and cluster attribution of diatom assemblages from springs with *Cratoneurion* in Flanders. FONTb and WIJNGa not considered in averages. % relative abundance, # number of taxa; A: indicators of anthropogenic alteration; R: Red List group rare; E: Red List group endangered; X: xerotolerant (see text).

sample	taxa	# R	% R	# E	% E	% X	IPS	TI _{DIA}	% A	group
BOTERa	72	9	4.4	9	2.6	21.0	16.6	2.7	3.4	I
ENAMEa	64	11	8.8	5	1.8	62.2	16.0	2.8	0.0	III
ENAMEb	61	6	2.0	7	2.8	18.8	17.0	2.3	1.4	II
FEELBa	50	5	1.4	3	1.0	20.0	16.2	2.5	1.8	II
FEELBb	57	6	3.4	5	0.8	21.8	15.6	2.7	1.6	II
FEELBc	64	4	3.2	5	0.8	17.4	16.0	2.7	0.8	II
FONTb	2	1	–	0	–	–	–	–	–	distinct
GALMAa	85	10	3.0	10	7.2	31.4	16.0	2.5	2.6	I
GALMAb	88	9	4.0	12	7.4	30.0	16.2	2.8	0.8	I
HORNEa	53	8	12.8	6	2.8	38.6	15.0	2.8	0.2	I
KRINDa	64	4	3.2	11	7.0	51.2	16.9	1.9	0.2	III
KRINDb	63	3	1.0	4	1.2	30.4	16.7	2.2	0.5	III
KWINTa	70	7	9.2	8	4.6	1	16.0	2.5	0.6	III
KWINTb	47	5	0.8	4	0.6	58.0	17.9	2.3	0.2	II
MABROa	76	11	5.4	9	6.6	58.0	15.6	2.7	1.0	I
MABROb	67	4	3.1	3	3.1	45.1	15.7	2.3	1.6	III
MATERb	89	10	3.0	8	4.0	24.6	13.9	2.9	23.1	I
MOENb	27	5	15.0	4	5.6	37.6	15.4	2.7	0.0	distinct
STEENc	49	6	1.8	5	0.6	36.8	15.2	2.5	1.6	II
WIJNGa	15	2	–	2	–	–	–	–	–	distinct
mean ± SD	63.6 ± 15.6	6.8 ± 2.6	4.8 ± 4.1	6.6 ± 2.8	3.4 ± 2.5	33.5 ± 16.5	16.0 ± 0.9	2.6 ± 0.3	2.3 ± 5.3	–

and MOEN and exceeding 10 mg.l⁻¹ in ENAME, FEELB and KWINT. Correlations between environmental variables for the sampled sites did not deviate from Flemish carbonate springs in general. Highly significant coefficients occurred mainly among EC, chloride, calcium, magnesium, sodium, sulphate and potassium (Table 2). Potassium also related inversely to shade, whilst ammonium tended to be lower where more calcite occurred. Nitrate correlated negatively to potassium and sulphate but varied independently from SRP.

In total, c. 310 diatom morphotaxa were recorded in the 20 samples (Table S1, with some illustrated in Figures 3–5). Almost half of them occurred in only one sample (Figure 6). Most taxa belonged to *Pinnularia* (40), followed by *Gomphonema* (27), *Navicula* s.s. (24), *Nitzschia* (19) and *Stauroneis* (16). The most constant species were *Halamphora normanii*, *Stauroneis thermicola* (90% of all samples), *Achnanthisdium jackii* (Figure 3 a), *Navicula cincta*, *Planothidium lanceolatum* (85%), *Achnanthisdium minutissimum*, *Humidophi-*

la brekkaensis (Figure 3 u), *Nitzschia linearis*, *Planothidium frequentissimum* (80 %), *Amphora pediculus* (Figure 3 c), *Eolimna tantula* (Figure 3 h), *Stauroneis parathermicola* (Figure 5 f–g) and *Surirella terricola* (75%). Some 36 taxa, mostly represented by fragments or very few valves, could not be identified confidently to species level. A few of them probably still require formal description.

Two samples contained extremely few and mostly corroded diatom valves. Extensive searching yielded only *Stauroneis muriella* (Figure 5 c–e) and *S. thermicola* with 2 valves each in FONTb. Merely 27 valves and 15 taxa could be counted from several slides of WIJNGa. Valve concentration was also very low for MABROb (total count 193 valves), but with 67 taxa, species richness was more in line with that of the remaining samples. Neglecting FONTb and WIJNGa, the average number of taxa per sample was 64, the highest number (89) occurring in MATERb (Table 3). On average 57 taxa were noted in hygropetric rheo-

crenes, increasing to 67 in rheocrenes, though differences in richness between geomorphological types were not significant (Table 4).

Several of the observed taxa were recorded rarely so far, and often mainly from other springs, e.g. *Cymboplectra diminuta*, *C. subaustriaca*, *Humidophila irata*, *Frustulia amosseana*, *Gomphonema extensum*, *Navicula arcotenelloides* (Figure 3 w), *Navicula* s.l. *harderi* (Figure 3 ae), *N.* s.l. *obsoleta* (Figure 3 ag), *N.* s.l. *parsura*, *Navicula* s.l. *subseminulum* (Figure 3 ah), *Neidium minutissimum*, *Nitzschia harderi* (Figure 4 c–d), *Pinnularia frauenbergiana* var. *caloneiopsis* (Figure 4 f) and *Pulchella minutissima* (Figure 4 v). Almost 14% of all taxa (43) was listed as rare and 11% (34) as endangered. On average there were 6.8 rare and 6.6 endangered taxa per sample (Table 3), both categories together representing 21.3 ± 5.8 % of all taxa in a sample. The relative abundance of RL taxa usually remained quite modest (8.4 ± 5.3 %). GALMAa, GALMAb, KWINTa and MABROa consistently scored above average for number as well as abundance of RL taxa. Most RL metrics also scored better than average for MATERb, in spite of the eutrophic character of its assemblage according to TI_{DIA} (Table 3). Interestingly the relative abundance of rare taxa was also more substantial in MOENb. The representation of RL taxa was lowest in hygropetric springs, but differences were again insignificant (Table 4).

The relative abundance of xerotolerant taxa varied strongly. KWINTa hardly contained any (1%), whereas they predominated in ENAMEa, KRINDa, KWINTb and MABROa (Table 3). Their average abundance was highest in rheocrenes (38.1 ± 14.8 %) and lowest in rheohelocrenes (26.6 ± 19 %), but also not significantly different between spring types (Table 4).

Considering only samples with adequate valve counts, IPS values showed little variation (average 16.0 ± 0.9 S.D.), ranging from 13.9 (MATERb) to 17.9 (KWINTb; Table 3). The average for Rott's trophic index was 2.5 ± 0.3 . It was close to 2 for KRINDa, KRINDb, ENAMEb, MABROb, which would be considered mesotrophic–eutrophic and at least 2.5 (eutrophic) for all other samples. MATERb obtained the highest score (2.9, euto–polytrophic) due to the abundance of *Planothidium frequentissimum* and *Gomphonema parvulum*. All spring types were very similar for both indices (Table 4). Only MATERb presented a substantial abundance of taxa considered to indicate anthropogenic alteration in Hessen (20.3%), resulting in a somewhat higher average for rheohelocrenes. Overall, the diatom results suggested the best water quality for KRIND, whereas considerable impairment was deemed likely for MATERb. The latter site was close to a road but chemically stood out only by a slightly higher iron content; SRP nor nitrogen levels were in any way exceptional.

Correlations between assemblage-derived metrics remained modest throughout (Table 5). In agree-

Table 4. Characteristics of springs with *Cratoneurion* in Flanders grouped by geomorphological type (average with standard deviation; none of the metrics differed significantly between spring types at $p \leq 0.05$). See Table 3 and text.

metric	hygropetric	rheocrene	rheohelocrene
taxa	57.3 ± 8.8	67.1 ± 14.8	63.0 ± 23.2
# R	4.5 ± 1.3	8.1 ± 2.6	6.4 ± 2.5
% R	2.1 ± 1.4	5.0 ± 3.7	6.5 ± 5.5
# E	4.5 ± 0.6	6.9 ± 3.2	7.6 ± 2.5
% E	0.9 ± 0.2	3.7 ± 2.7	4.8 ± 1.6
% X	31.9 ± 18.2	38.1 ± 14.8	26.6 ± 19.0
IPS	16.5 ± 1.0	15.8 ± 0.5	15.9 ± 1.3
TI_{DIA}	2.5 ± 0.3	2.6 ± 0.2	2.5 ± 0.4
% A	0.8 ± 0.6	1.4 ± 1.1	5.1 ± 10.1

Table 5. Significant Pearson or Spearman correlations between assemblage metrics and environmental variables ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$).

	# R	% R	# E	% E	IPS	% A
taxa	0.57	–	0.70	–	–	0.48
% R	–	1	–	–	–	–
# E	0.53	–	1	–	–	–
% E	–	–	0.78	1	–	–
TI_{DIA}	0.66	–	–	–	–0.64	–
% A	–	–	–	–	–0.59	1
bryophyte cover	–	–	–	–	–	0.56
tufa	–	–	–	–0.60	–	–
alkalinity	–	–	–	–	–	–0.52
Ca ²⁺	–	0.55	–	–	–	–0.48

ment with expectations, IPS correlated negatively to TI_{DIA} and the abundance of indicators for anthropogenic influence, but none of these indices related to obvious chemical clues for eutrophication. Notably, % A related positively to bryophyte cover, but decreased somewhat with higher alkalinity and calcium. Abundance of rare taxa, however, increased somewhat with calcium. There was also a weak positive relation between % A and taxa richness. The number of RL taxa increased with taxa richness, the number of endangered taxa, as well as the trophic score. The number of taxa in both RL groups correlated only marginally, but the number of endangered taxa followed their abundance rather closely. Yet, only % E declined significantly with more

tufa limestone. Although none of the well-illuminated sites showed more than 25% xerotolerant diatoms, their abundance was not correlated to vegetation cover or shade.

Again excluding FONTEb and WIJNGa, *Amphora pediculus* ($16.9 \pm 14.6\%$), *Planothidium lanceolatum* ($9.2 \pm 10.6\%$), *Achnanthyidium jackii* ($6.7 \pm 6.8\%$) and *P. frequentissimum* ($5.8 \pm 6.0\%$) were the only species with an average abundance of at least 5%. Besides these, *Caloneis lancettula* (Figure 3 e), *Cocconeis euglypta*, *Humidophila brekkaensis*, *H. perpusilla*, *Encyonopsis microcephala*, *Eolimna minima*, *Gomphonema parvulum*, *Halamphora normanii*, *Hippodonta neglecta* (Figure 3 t), *Navicula tripunctata*, *Nitzschia amphibia*, *N. sinuata*, *Pinnularia kuetzingii* (Figure 4 g–i), *Rhopalodia operculata* (Figure 4 z), *Stauroneis thermicola*, *Staurosira venter* and *Staurosirella pinnata* reached at least 10% in one or more samples. Overall, assemblages were dominated by smaller raphe-bearing taxa. Non-motile *Pseudostaurosira*, *Staurosirella* and *Staurosira* were only more prominent in KRINDb and, less so, KWINTa. Centrics were hardly present.

Most samples clustered in three groups (Table 4). Group I was characterized mainly by *Planothidium* spp., *Navicula veneta* and *Stauroneis separanda* (Figure 5j), group II by *Achnanthyidium jackii*, *Amphora pediculus*, *Gomphonema angustatum* and group III by *Humidophila irata*, *Luticola acidoclinata*, *Pinnularia obscura* morphotype2, *Pseudostaurosira brevistriata* and *Stauroneis borrichii* (Fig. 5a). Group III mainly comprised samples representing the Vaals aquifer, but clusters were not consistent with groundwater type, nor spring type or even site. Three samples were set apart, including both with skewed relative abundances due to low valve counts, as well as MOENb.

Indval analysis suggested a significant association of *Diploneis krammeri* (Indval 60%, $p = 0.04$), *D. separanda* (Indval 73%, $p = 0.03$), *Gomphonema micropus* (Indval 63%, $p = 0.04$; Figure 3n), *G. utae* (Indval 82%, $p = 0.02$), *Nitzschia alpina* (Indval 81%, $p = 0.01$; Figure 4b), *N. pusilla* (Indval 69%, $p = 0.04$), *Stauroneis pseudagrestis* (Indval 67%, $p = 0.05$; Figure 5i), *S. separanda* (Indval 69%, $p = 0.03$) and *S. smithii* (Indval 89%, $p = 0.03$) with the aquifer Tielt 3, but this was represented by only 2 samples from the same spring. The listing of *Gomphonema angustum* as the only indicative species (Indval 76%, $p = 0.02$) for the Vaals aquifer (6 samples) could be somewhat more robust. The aquifers Heers (HORNEa), Borgloon (WIJNGa) and Moen (MOENb) were represented by single samples. The first mainly had *Planothidium* spp., small *Stauroneis* and *Navicula veneta*, the second mostly *Frustulia amosseana*, *Stauroneis thermicola*, *Gyrosigma obtusatum* and *Humidophila brekkaensis*. MOENb differed distinctly by a prevalence of *Encyonopsis microcephala*, *Nitzschia amphibia*, *N. sinuata*, *Pinnularia kuetzingii* and *Rhopalodia operculata*.

Six taxa were indicative of hygroperitic rheo-

crenes: *Cocconeis euglypta* (Indval 68%, $p = 0.008$), *Navicula upsaliensis* (Indval 64%, $p = 0.02$; Figure 3 aa), *Caloneis lancettula* (Indval 75%, $p = 0.03$), *Hippodonta neglecta* (Indval 50%, $p = 0.03$), *Sellaphora pupula* (Indval 50%, $p = 0.03$) and *Amphora pediculus* (Indval 57%, $p = 0.04$), two of rheocrenes: *Stauroneis thermicola* (Indval 76%, $p = 0.03$) and *A. copulata* (Indval 55%, $p = 0.05$). Very few taxa indicated the longitudinal position of a sample within the drainage system: *Caloneis leptosoma* (mostly atypical linear forms with blunt apices; Figure 3f) and *Humidophila brekkaensis* occurred more where the water emerged (position A; Indval 66 and 69% respectively, $p = 0.04$), *Eolimna minima* was found mostly within the zone of carbonate precipitation (C sites; Indval 85%, $p = 0.02$). *Amphora pediculus* (Indval 57%, $p = 0.03$), *Cocconeis euglypta* (Indval 63%, $p = 0.02$) and *Sellaphora joubaudii* (Indval 55%, $p = 0.04$; Figure 4 ac) were more typical of sites with the highest amounts of calcite (categories 4–5), whereas *Diploneis separanda* (Indval 55%, $p = 0.03$), *Gyrosigma obtusatum* (Indval 50%, $p = 0.03$), *Hantzschia amphioxys* (Indval 71%, $p = 0.02$), *Muelleria terrestris* (Indval 50%, $p = 0.04$), *Nitzschia pusilla* (Indval 46%, $p = 0.04$), *Pinnularia isselana* (Indval 72%, $p = 0.03$) as well as *P. perirrorata* (Indval 51%, $p = 0.05$) occurred somewhat more where encrustation was very limited (categories 1 and 2).

DCA indicated limited species turnover (axis 1 2.9 S.D., axis 2 3.2 S.D.) and species–environment relations were explained best by RDA. Because its potassium concentration was at least four times higher (25.5 mg.l^{-1}) than in all other springs and levels of sulphate, magnesium and sodium were also the highest measured, the sample MOENb behaved as an extreme outlier in RDA. Consequently it was neglected in further analyses. Sample FONTEb was included passively because its very poor diatom content did not allow reliable abundance estimates, and samples MABROb and WIJNGa were down-weighted. Forward selection suggested that pH, nitrate and magnesium explained species composition best, accounting jointly for 32.9% of the variation (Table 6). The marginal contributions of nitrate and magnesium were similar and slightly smaller than for pH, with the response to nitrate depending more strongly on the condition of both additional variables (cf. λ_{1c}/λ_1 ; Table 7).

High pH was associated with more intense carbonate precipitation and higher abundance of *Amphora pediculus*, *Caloneis lancettula* and *Navicula upsaliensis*. With positive scores on the principal axis, magnesium aligned well with major anions, potassium, calcium and alkalinity. *Gomphonema parvulum* and *Planothidium* spp. were most abundant in more mineral-rich conditions. Also correlating positively with the first axis were phosphate and iron (the latter solely due to MATERb). Phosphorus will precipitate with calcium and iron as oxygenation increases and most of the samples away from the spring head indeed plotted on the left–

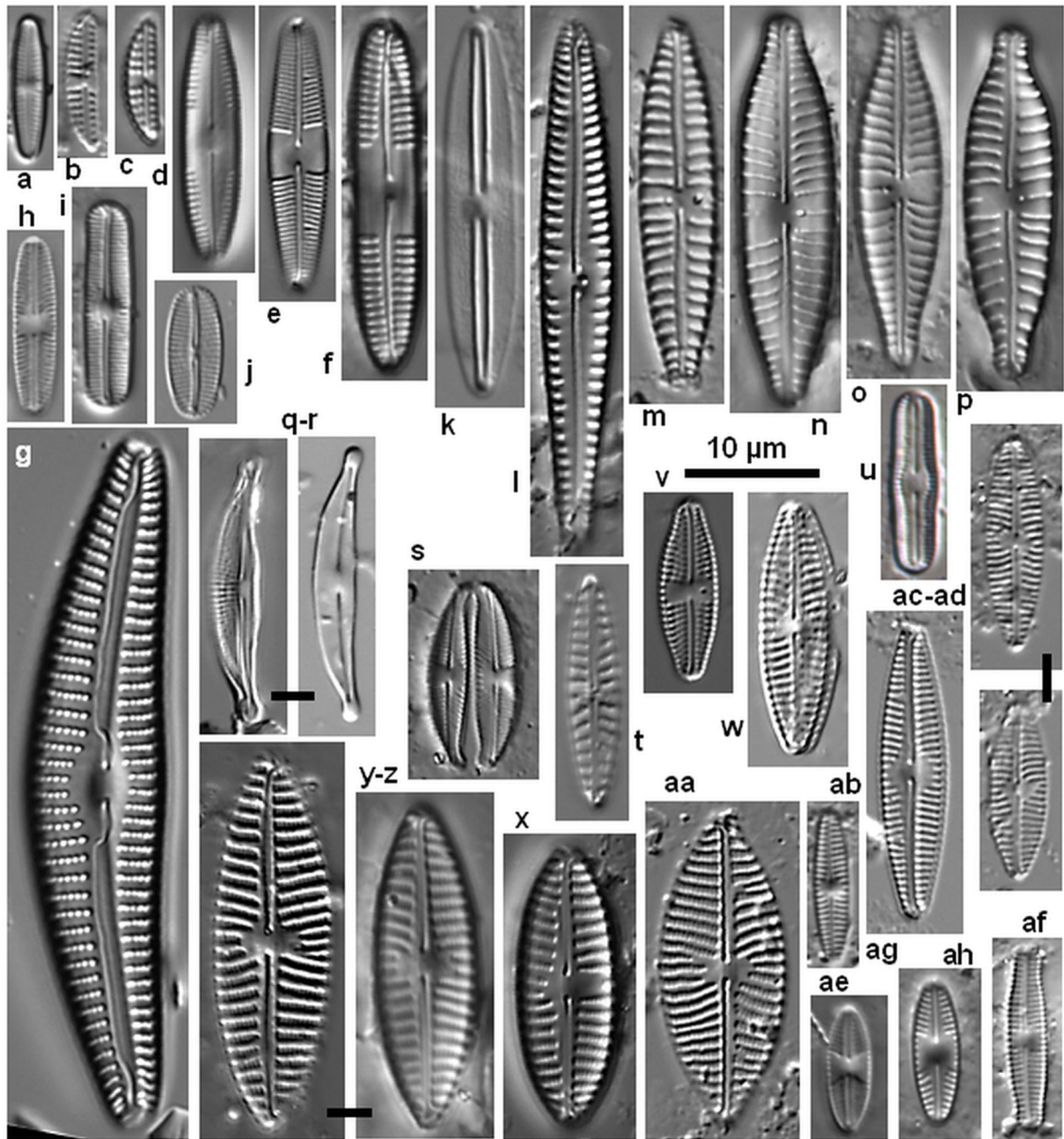


Fig. 3. (a) *Achnantheidium jackii*; (b) *Amphora indistincta*, (c) *A. pediculus*; (d) *Caloneis fontinalis*; (e) *C. lancettula*; (f) *C. leptosoma* (lack of triundulate margins and acute apices agree with *C. leptosoma* f. *grunowii* CLEVE-EULER, which may need taxonomic reconsideration); (g) *Cymbella alpestris*; (h) *Eolimna tantula*; (i) *Fallacia langebertaloti*; (j) *F. subluclidula*; (k) *Frustulia* sp. BOTERa; (l) *Gomphonema* cf. *designatum*; (m) *G. longilineare*; (n) *G. micropus*; (o) *G. micropus* morphotype “*aequalidictum*”; (p) *G. micropus* var. *aequale* (the distinctly curved raphe suggests it might be more appropriate to retain Gregory’s species rank); (q-r) *Halamphora oligotrappenta* (r. craticular valve); (s) *H.* sp. GALMAa; (t) *Hippodonta neglecta*; (u) *Humidophila brekkaensis*; (v) *Luticola paramutica*; (w) *Navicula arctotenelloides*; (x) *N.* aff. *catalanogermanica*; (y-z) *N. moenofranconica*; (aa) *N. upsaliensis*; (ab) *N.* aff. *veneta*; (ac-ad) *N.* sp. GALMAb; (ae) *Navicula* s.l. *harderi*; (af) *N.* s.l. ENAMEa; (ag) *N.* s.l. (*Chamaepinnularia*) *obsoleta*; (ah) *N.* s.l. sp. *subseminulum*.

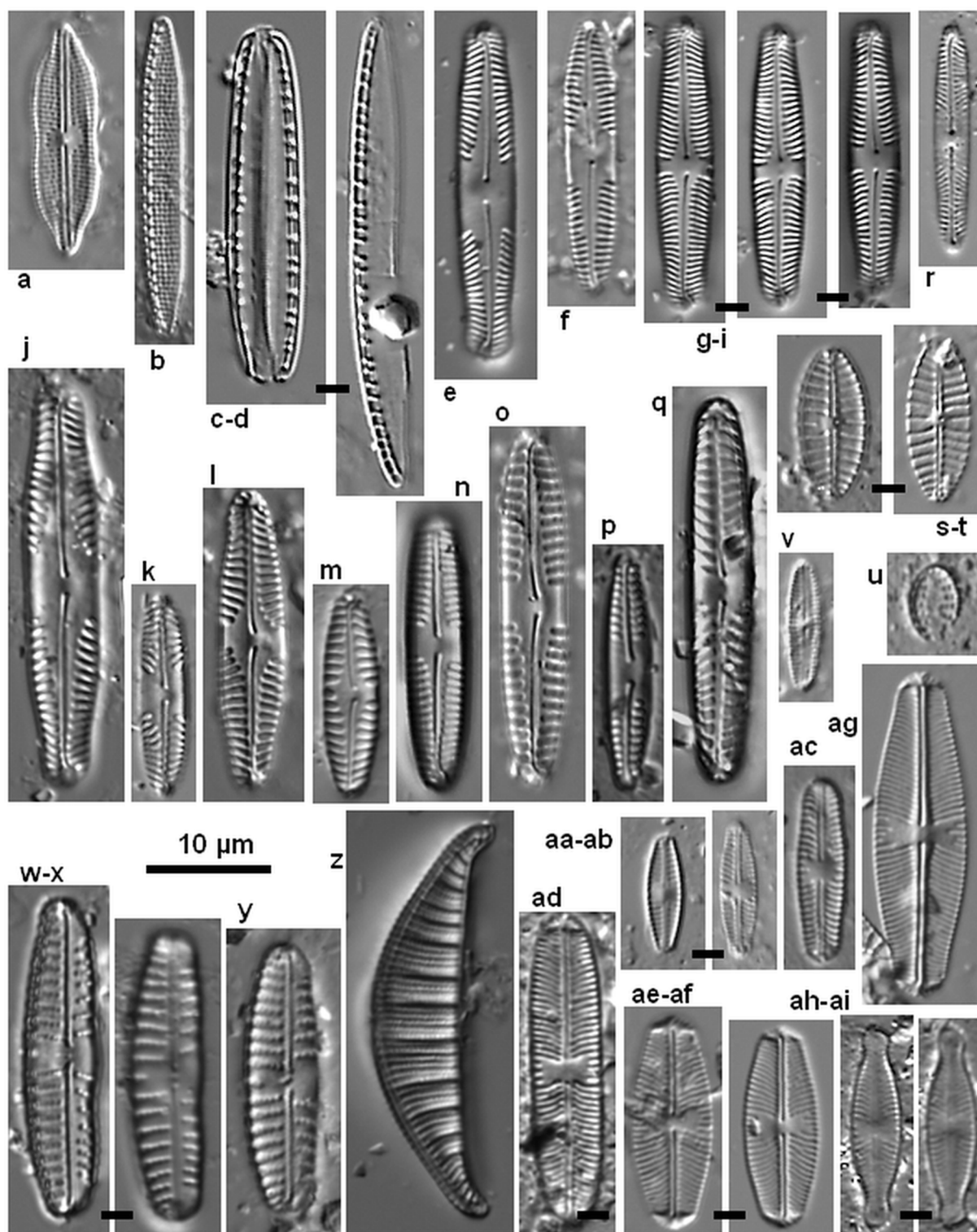


Fig. 4. (a) *Neidiomorpha binodiformis*; (b) *Nitzschia alpina*; (c–d) *N. harderi*; (e) *Pinnularia bertrandii* var. *orleansiana*; (f) *P. frauenbergiana* var. *caloneiopsis*; (g–i) *P. kuetzingii*; (j) *P. marchica*; (k) *P. osoresanensis*; (l) *P. pisciculus*; (m) *P. sp. BOTERa1* (distinct from *P. obscura*); (n) *P. sp. BOTERa2*; (o) *P. sp. FEELBa*; (p) *P. sp. FEELBc*; (q) *P. sp. GALMAa* (close to *P. palatina* LANGE–BERT. & KRÜGER); (r) *P. sp. HORNEa* (close to *P. acoricola* Hust. var. *linearis* WYDRZYCKA, LANGE–BERT. & METZELTIN); (s–t) *Placoneis* sp. KRINDb (note presence of a stigma); (u) *Pseudostaurosira* sp. HORNEa; (v) *Pulchella minutissima*; (w–x) *Reimeria uniseriata*; (y) *R. aff. uniseriata* (note double rows of areolae on dorsal side); (z) *Rhopalodia operculata*; (aa–ab) *Sellaphora* cf. *hafnerae*; (ac) *S. joubaudii*; (ad) *S. pseudopupula*; (ae–af) *S. pupula* phenodeme little; (ag) *S. pupula* phenodeme tidy; (ah–ai) *S. aff. sardiniensis*.

hand side. High nitrate samples tended to be from more shaded sites with lower mineral concentrations and had more *Humidophila contenta*, *Gomphonema angustum*, small fragilarioids (*Staurosira*, *Staurosirella*), as well as a good representation of *Amphora pediculus*. Away from this main gradient, a number of xerotolerant taxa (*Luticola* spp., *Pinnularia obscura*, *Stauroneis muriei*lla, *Tryblionella debilis*) clustered near the samples KWINTa and MABROa with high scores along the second ordination axis; opposite, *Achnanthisidium* species obtained negative scores. This second axis appeared to integrate mineral content (magnesium associated with potassium and sodium as chlorides and sulphates) and, to a lesser extent, plant cover (negatively) versus shade (positively).

In accordance with their inferred water quality, RDA positioned KRIND and MATERb oppositely, whilst TI_{DIA} scores for their closest satellites, MABROb and KWINTb and BOTERa, respectively, agreed well with a similar differentiation. Not all differences in TI_{DIA} concurred well with a similar ordination position, however, as evident from ENAMEb and KWINTb which shared the same value (2.3) and the samples from FEELB and GALMA with higher TI_{DIA} (2.5–2.8).

DISCUSSION

With 50 taxa/sample on average, excluding observations outside counts, and local (α) diversity exceeding 60 otherwise, local species richness was high compared to that of spring epilithon in the Italian Alps (average number 14, counts of 450 valves; SPITALE et al. 2012), Poland (18 with 300 valves counted; WOJTAŁ & SOBCZYK 2012), the German Spessart Muschelkalk

and Wetterau marl–loess regions (22 and about 30 in 400 valves, respectively; WERUM & LANGE–BERTALOT 2004), as well as central France (18; BERTRAND et al. 1999). In spite of intensive searching, CANTONATI & LANGE–BERTALOT (2010) observed only 12 to 37 taxa on various substrates in SE Germany. SABATER & ROCA (1992; see GIESERICH & KOFLER 2010 for studies with supporting observations), suggested that shear stress constrained species richness in springs and the lack of extreme flows probably contributed to the observed higher α diversity. Comparable flow conditions may also have resulted in similar taxon richness for all geomorphological types. On the other hand, ABOAL et al. (1998) reported that diversity increased with altitude. Species richness in springs is often considered to be lowered by impairment, although it is not always clear whether this is due to reduced habitat heterogeneity from morphological alteration (TAXBÖCK & PREISIG 2007), altered water quality (BERTRAND et al. 1999), or both. No relation to IPS, TI_{DIA} or physical–chemical indications of eutrophication was observed in the present study. Rather, α diversity tended to increase along with the abundance of taxa associated with impact in comparable German springs. This shows that higher species richness is not an unequivocal sign of more natural or oligotrophic conditions and should be considered strictly within its proper geographical context.

Although physical–chemical conditions were largely similar among sites, regional taxonomic richness (γ diversity) of calcareous spring habitats in Flanders also appeared to be high compared to other regions. SABATER & ROCA (1992), for example, noted slightly less than 200 taxa in 28 presumably pristine Pyrenean springs on limestone, granite and slates. Seventeen Swiss springs, also presenting a wider conduc-

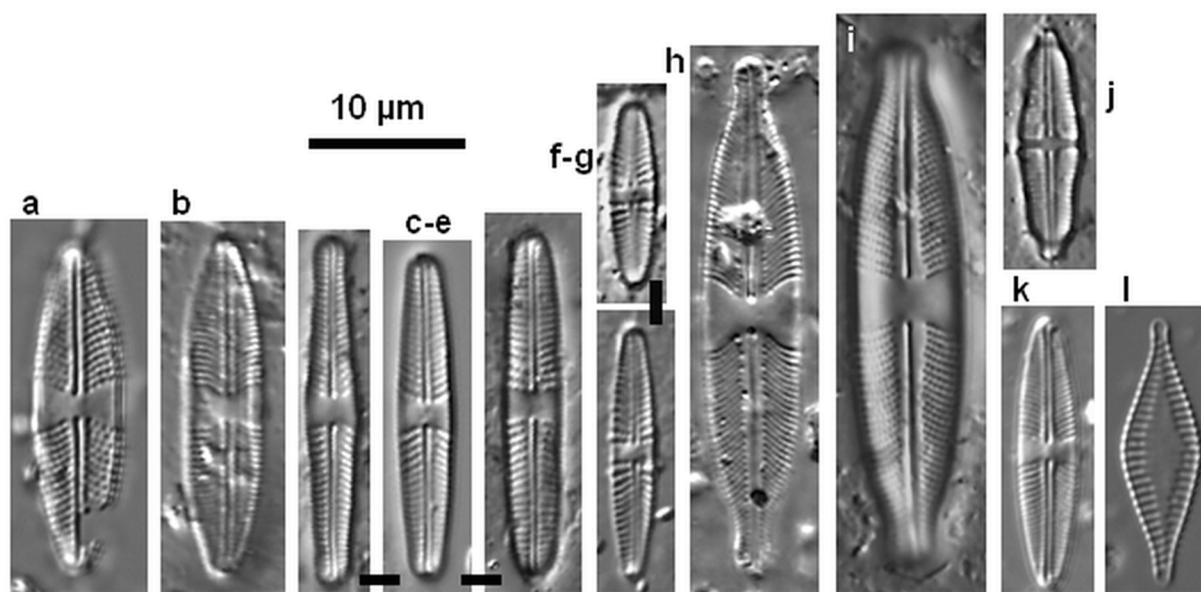


Fig. 5. (a) *Staurosira borrichii*; (b) *S. lundii*; (c–e) *S. muriella*; (f–g) *S. parathermicola*; (h) *S. pseudagrestis*; (i) *S. schimanskii*; (j) *S. separanda*; (k) *S. sp. FEELBa*; (l) *Synedrella parasitica*.

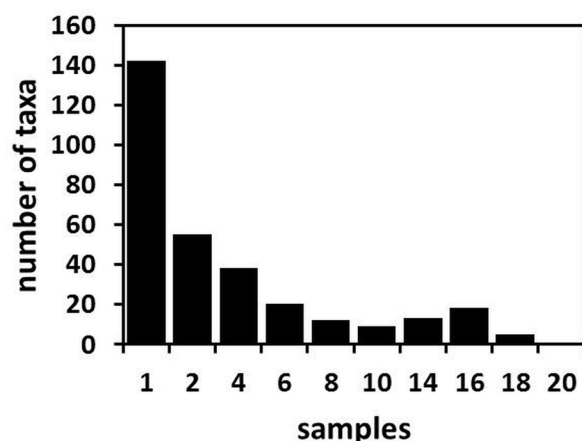


Fig. 6. Frequency distribution of taxa.

tivity range and no obvious signs of pollution, yielded only 136 taxa (TAXBÖCK & PREISIG 2007) and 197 taxa were noted in 52 samples from 27 Austrian springs and spring streams (GESIERICH & KOFLER 2010). Thirteen springs in the Western Carpathians yielded 188 taxa, with 114 occurring in calcareous fens (FRÁNKOVÁ et al. 2009). WERUM & LANGE-BERTALOT (2004) examined 97 springs from regions with varying geology in SW-Germany (Hessen) but recorded only one hundred taxa more than the 300+ tallied here. Although comparisons of species numbers are always hampered by differences in methodology and adherence to counts of a few hundred valves leads to severe truncation of diatom inventories to more abundant taxa (see also GERECKE et al. 2011), the differences in richness seem genuine, taxonomic resolution also offering only a partial explanation. Strong heterogeneity of spring assemblages is well established (BERTRAND et al. 1999; CANTONATI et al. 2012b) and the very low frequency of a large majority of all taxa leaves little doubt that γ diversity of carbonate-precipitating springs in Flanders is far from accounted completely. The present observations were restricted to a single substrate type and occasion and therefore neglected much of the heterogeneity in local habitat conditions. Epipellic and bryophytic assemblages are likely to differ in composition (e.g. FRÁNKOVÁ et al. 2009). In northern Italy, CANTONATI et al. (2012b) found the highest numbers of diatom taxa on bryophytes, a substrate offering diverse microhabitats as well as pockets with accumulative conditions and little scouring, and significant substrate preference for a considerable number of taxa. Other studies, however, found that species richness on mosses was quite similar to that of epilithon (e.g. WOJTAŁ & SOBCZYK 2012), or even lower (HUSTEDT 1945). We observed no relation between bryophyte abundance and species richness, but expect that their examination will yield additional taxa. Seasonal variation is also not without possible consequence for assemblage composition (e.g. CANTONATI 2001, BATTEGAZZORE et al. 2004) but was not yet addressed, either. In addition, *Cratoneurion* springs

representing the aquifers Lede, Tielt 4 and Hannuit await investigation. Some of these show a more distinct chemical composition, e.g. water emanating from the formation of Lede presents higher chloride levels than observed in any of the springs examined here.

Two samples probably offered a very incomplete picture of local diversity. The very low valve concentrations in these samples may have various reasons and it is not clear which contributed exactly. CANTONATI & SPITALE (2009) and CANTONATI et al. (2012b) made similar observations in a few iron-rich springs, but this was not an apparent feature here. FRÁNKOVÁ et al. (2009) observed poor valve silicification in petrifying springs, concurring with somewhat lower silica concentrations than in less mineral-rich fens. The solubility of biogenic silica increases markedly at pH > 8.5 and such values may develop in carbonate-precipitating biofilms (BISSET 2008). Also, primary productivity of springs can be relatively low in comparison to other lotic habitats due to low light and nutrient availability or transient water supply. In such conditions, the phytobenthos may become depleted more easily by grazing or scouring. Moreover, extensive covering by leaf litter until shortly before sampling would limit diatom growth and, perhaps, even enhance microbial decomposition facilitating valve dissolution (EHRlich et al. 2010).

In agreement with many alkaline springs elsewhere in Europe (e.g. ROUND 1957; REICHARDT 1994; FRÁNKOVÁ et al. 2009; GESIERICH & KOFLER 2010; TAXBÖCK & PREISIG 2007; WERUM & LANGE-BERTALOT 2004; WOJTAŁ & SOBCZYK 2012), the core assemblage consisted of a series of more or less ubiquitous taxa (*Achnanthes jackii*, *A. minutissimum*, *Amphora pediculus*, *Cocconeis placentula* group, *Planothidium frequentissimum*, *P. lanceolatum*). Relative to other European spring areas, *Stauroneis*, but also *Caloneis* and *Gomphonema* appeared to be particularly abundant. In spite of the more alkaline conditions, *Pinnularia* species were not uncommon, either. In particular, small-celled taxa of these genera occurring widely in subaerial environments were well represented, e.g. *Caloneis fontinalis*, *Gomphonema micropus*, *Stauroneis thermicola*, *S. muriella*, *Pinnularia isselana* and *P. obscura*. Several of the species considered typical for limestone formation by REICHARDT (1994) were frequent companions (*Caloneis fontinalis*, *Fallacia langebertalotii*, *Gomphonema angustum*), but *Caloneis alpestris*, *Diploneis minuta* and *Cymboplectra subaequalis* remained rare. Of the taxa occurring more often in the marl-loess than in the Musschelkalk parts of Hessen according to WERUM & LANGE-BERTALOT (2004), *Caloneis bacillum sensu auct. nonnull.*, *Diploneis* spp., *Navicula veneta*, *Nitzschia dissipata* and *Stauroneis smithii* were encountered regularly, but so were *Achnanthes jackii*, *Amphora copulata*, *Fallacia langebertalotii* and *Navicula s.l. harderi* for which the reverse was observed. Overall *Cymbella*, *Denticula*, *Encyonema*,

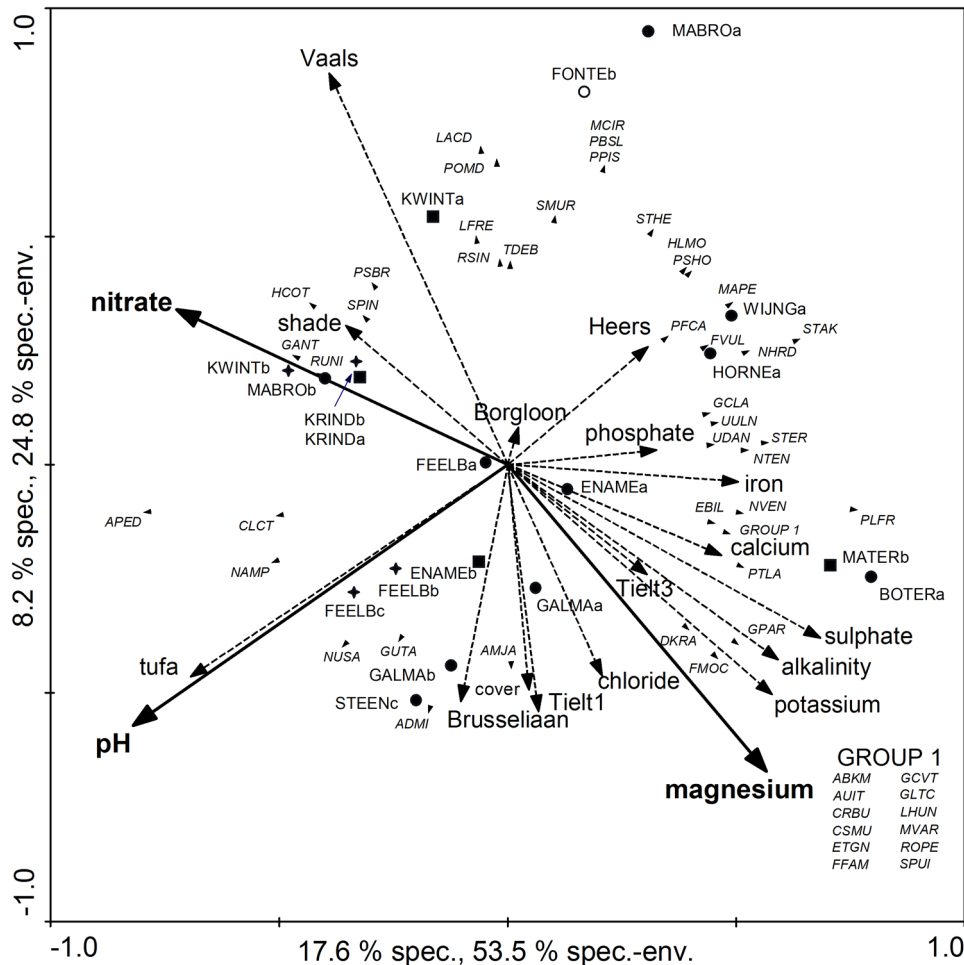


Fig. 7. Ordination plot of samples (♦ hydropetric rheocrene, ● rheocrene, ■ rheohelocrene), taxa (arrow heads), active variables (full-line arrows) and selected passive variables (dashed line arrows) for the two principal RDA axes. Only taxa with fit $\geq 20\%$ are shown. (AMJA) *Achnanthes jackii*; (ADMI) *A. minutissimum*; (ABKM) *Adlafia brockmanni*; (APED) *Amphora pediculus*; (AUIT) *Aulacoseira italica*; (CRBU) *Craticula buderi*; (CLCT) *Caloneis lancetula*; (CSMU) *Chamaepinnularia submuscicola*; (DKRA) *Diploneis krammeri*; (ETGN) *Epithemia turgida* var. *granulata*; (EBIL) *Eunotia bilunaris*; (FMOC) *Fallacia monoculata*; (FFAM) *Fragilaria famelica*; (FVUL) *Frustulia vulgaris*; (GANT) *Gomphonema angustum*; (GCLA) *G. clavatum*; (GCVT) *G. clavatum*; (GLTC) *G. laticollum*; (GPAR) *G. parvulum*; (GUTA) *G. utae*; (HCOT) *Humidophila contenta*; (HLMO) *Halimophora montana*; (LHUN) *Lemnicola hungarica*; (LACD) *Luticola acidoclinata*; (LFRE) *L. frequentissima*; (MAPE) *Mayamaea atomus* var. *permitis*; (MVAR) *Melosira varians*; (MCIR) *Meridion circulare*; (NHRD) *Navicula* s.l. *harderi*; (NTEN) *Navicula tenelloides*; (NUSA) *N. upsaliensis*; (NVEN) *N. veneta*; (NAMP) *Nitzschia amphibia*; (PBSL) *Pinnularia borealis* var. *sublinearis*; (PFCA) *P. frauenbergiana* var. *caloneiopsis*; (POMD) *P. obscura* morphotype 2; (PPIS) *P. pisciculus*; (PSHO) *P. schoenfelderi*; (PSBR) *Pseudostaurosira brevistriata*; (PLFR) *Planothidium frequentissimum*; (PTLA) *P. lanceolatum*; (RSIN) *Reimeria sinuata*; (RUNI) *R. uniseriata*; (ROPE) *Rhopalodia operculata*; (SPUI) *Sellaphora pupula* phenodeme little; (SMUR) *Stauroneis muriei*; (STAK) *S. tackei*; (STHE) *S. thermicola*; (SPIN) *Staurosirella pinnata*; (STER) *Surirella terricola*; (TDEB) *Tryblionella debilis*; (UDAN) *Ulnaria danica*; (UULN) *U. ulna*.

Encyonopsis and *Psammothidium* were poorly represented. *Brachysira*, *Diatomella*, *Rossethidium* and *Tetracyclus* lacked entirely, as well as *Achnanthes pyrenaicum* (HUST.) KOBAYASHI, *Diatoma hiemale* (ROTH) HEIB. and *D. mesodon* (EHR.) KÜTZ. These taxa often dominate in more elevated regions and high-flow conditions (e.g. HUSTEDT 1945; SABATER & ROCA 1992; GESIERICH & KOFLER 2010). *Psammothidium grischunum* (WUTHRICH) BUKTH. & ROUND and *Planothidium dubium* (GRUN.) ROUND & BUKTH., both frequent on limestone in Germany (WERUM & LANGE-BERTALOT 2004), also remained absent, similar to many taxa common in *Cratoneurion* habitat elsewhere (e.g. *Caloneis tenuis* (GREG.) KRAMMER, *Delicata delicatula* (KÜTZ.)

KRAMMER, *Encyonopsis falaisensis* (GRUN.) KRAMMER, *Epithemia argus* (EHR.) KÜTZ., *Eucoconeis flexella* (KÜTZ.) CL., *E. laevis* (KÜTZ.) MEISTER, *Eunotia arcubus* NÖRPEL-SCHEMP & LANGE-BERT., *Hannea arcus* (EHR.) PATRICK; ECTOR & ISERENTANT 1988; ISERENTANT 1988; FRÁNKOVÁ et al. 2009; CANTONATI & LANGE-BERTALOT 2010). Together with the better representation of fragilarioid taxa, this resulted in a somewhat stronger resemblance to assemblages found in limnocrenes (WOJTAL et al. 2009). Many of the above-mentioned species occur elsewhere in Flanders, e.g. in calcareous fens, rendering their apparent lack in these springs unexpected. Differences in thermal regime or trophic conditions could be involved, but it should be noted

Table 6. Characteristics of RDA for diatom assemblages from calcareous springs in lower Belgium with pH, nitrate and magnesium as active variables (MOENb excluded, FONTEb passive, MABROEb and WIJNGa down-weighted).

axis	1	2	3
eigenvalue (λ)	0.18	0.08	0.07
species–environment correlation	0.91	0.81	0.89
% species relations explained	17.6	25.8	32.9
% species–environment explained	53.5	78.3	100

Table 7. Characteristics of constrained RDAs of diatom assemblages from calcareous springs in lower Belgium with pH, nitrate or magnesium as active variables (MOENb excluded, FONTEb passive, MABROEb and WIJNGa down-weighted).

	marginal effects				conditional effects				
	λ_1 (%)	F	P	λ_1/λ_2	λ_{1c} (%)	F	p	$\lambda_{1c}/\lambda_{2c}$	λ_{1c}/λ_1
pH	14.5	2.7	0.003	1.03	11.5	2.4	0.002	0.87	0.79
NO ₃ [−] –N	12.8	2.3	0.004	0.68	7.9	1.7	0.038	0.60	0.62
Mg ²⁺	11.0	2.0	0.025	0.53	9.0	1.9	0.009	0.68	0.82

that TI_{DIA} values were similar to those on marl in Germany and largely within the range observed in northern Italy (ANGELI et al. 2010). Overall, and in analogy of their moss vegetation (OOSTERLYNCK & DE BIE in press), it appears that, the diatom flora of these springs presents a pauperized facies of the *Cratoneurion* elsewhere as far as ‘boreo–alpine’ taxa are concerned, somewhat dampening expectations regarding their possible role as least-impaired habitat relicts (CANTONATI et al. 2012a).

In line with the previous, the representation of taxa featuring on the Central European Red List was lower than reported for springs in many other regions (e.g. CANTONATI 1998; TAXBÖCK & PREISIG 2007; ANGELI et al. 2010; WERUM 2001; CANTONATI & LANGE–BERTALOT 2010; GESIERICH & KOFLER 2010). Still, this was more a matter of relative abundance than taxa numbers, as more than 70 (c. 25%) of all taxa might be considered rare or endangered, making up about 1/5 of all taxa in a sample, but only 1/12 of all individuals. Absence of base–line data on springs in the region so far also prevents to conclude that their relative scarcity reflects a more impacted condition. Notably, the historical ‘background’ representation of such taxa in standing waters within the same region is quite low (DENYS 2000). The Red List predominantly focusses on oligotraphentic taxa occurring either in mineral–poor waters, or where phosphorus availability is much reduced by formation of non–soluble precipitates, such as carbonates. The percentage abundance of rare taxa indeed increased somewhat with calcium content. The conservation value of hygropetric helocrenes was not exceptional with regard to their diatom composition.

Neither was this the case for sites where more limestone occurred, which even showed a lower abundance of endangered taxa. This suggests that, although an essential criterion to delimit habitat type 7220, the amount of already deposited carbonate may not be very informative to assess its local conservation status in this region. On the other hand, the calcium content of the emerging water regulates the nutrient status, buffering against eutrophication.

Achnanthidium minutissimum, *Amphora pediculus*, *Caloneis lancettula*, *Cocconeis euglypta*, *Navicula upsaliensis*, *Nitzschia amphibia* and *Sellaphora joubaudii* characterized more intensive calcite formation, whereas taxa with an upright stature (*Eunotia bilunaris*, *Gomphonema clavatum*, *Ulnaria* spp.), growing in mucilaginous colonies (*Frustulia vulgaris*, *Gyrosigma obtusatum*, *Mayamaea atomus* var. *permitis*), or adapted to semi–terrestrial conditions (*Halamphora montana*, *Hantzschia amphioxys*, *Stauroneis thermicola*) favoured the opposite. So far, it remains unclear to which extent subtle differences in water chemistry, flow or substrate characteristics contributed to this distinction. Hygropetric springs presented a somewhat higher number of characteristic taxa, which appears to align with their regional paucity (SPITALE et al. 2012).

In general, xerotolerant diatoms (e.g. *Humidophila* spp., *Halamphora* spp., *Hantzschia* spp., *Luticola* spp.) were common. Their abundance in springs may relate to periodic desiccation, e.g. due to water abstraction (WERUM & LANGE BERTALOT 2004; CANTONATI et al. 2012b), but is also expected to be higher in rheohelocrenes where slope erosion and small–scale variation in wetness should be most pronounced, as

well as in hygroscopic rheocrenes where the water film is thin and often intermittent. Although, the representation of xerotolerant diatoms was actually the lowest in rheohelocrenes and highest in rheocrenes, within-type variation overruled possible differences. If not merely due to unrepresentative sampling, conditions may well have been too similar to result in a clear pattern. Likewise, a relation to vegetation cover could only be hinted at. However, more precise estimators of soil inputs may be required, as cover only presents a crude proxy.

Exploration of environmental relations was hampered in the present study by limited data and conclusions should be drawn cautiously. Clearly, the artificial site MOEN was the most prominent example of environmental species sorting. In spite of its recent nature, the assemblage comprised a range of 'typical spring diatoms', including several rare ones, illustrating that even sparsely occurring taxa will rapidly colonize suitable habitat. Although EC was not substantially different from several other springs, the main constituents of its assemblage were different from all other investigated springs and included several species that are considered to indicate higher conductivities (e.g. *Nitzschia amphibia*, *N. sinuata*, *Pinnularia kuetzingii*, *Rhopalodia operculata*). However, concentrations and ratios of specific ions were unique, presumably due to the mineralogical composition of the local aquifer of Tertiary marine deposits.

For the remaining sites, only pH, nitrate and magnesium related to some extent with assemblage composition. These variables, or close correlates, were repeatedly shown to structure diatoms of springs and headwaters, substantiating that the detected pattern was not spurious. We did not include direct measures of current but physical effects were probably less influential than in torrential springs (SABATER & ROCA 1990, 1992). Where pH was highest and limestone formation most prominent, assemblage composition was closer to that of Alpine rheocrenes on limestone with nitrate enrichment or shading, or similar rheocrenes with periodic dessication where abundance of *Humidophila* spp. was higher (CANTONATI et al. 2012b). Whereas *Achanthidium jackii* and *A. minutissimum* obtained similar ordination scores in the present analysis, WERUM & LANGE-BERTALOT (2004) found that these taxa differentiated springs of the Muschelkalk region presenting water with a higher mineral content ($EC_{25} > 700 \mu S \cdot cm^{-1}$) from those on loam-marl in Hessen. Such differences in the response of individual taxa once again illustrate the importance of geographic regionalization.

Although shade was found to be important elsewhere (CANTONATI 1998; CANTONATI & PIPP 2000; CANTONATI & SPITALE 2009), it was not singled out as such in this case. Its alignment with nitrate prevented to establish a clear response, but it was also fairly intense at most places. Nitrate sensitivity was demonstrated for the diatom phytobenthos in many spring systems (e.g. CANTONATI 1998; FRÁNKOVÁ et al. 2009; ANGELI et

al. 2010; HÁJKOVÁ et al. 2011). Nitrate in spring water is considered to indicate anthropogenic impact but is modulated by the degree of retention and transformation in the soil and subsoil, as well as biological uptake before and after emergence (NIEDERMAYR & SCHAGERL 2010). Of the common indicators for nitrate enrichment in other regions (CANTONATI et al. 2007, 2012b), only *Amphora pediculus* reacted more or less consequently. Together with other taxa tolerating organic pollution (*Craticula buderi*, *Fallacia monoculata*, *Gomphonema parvulum*, *Lemnicola hungarica*, *Navicula veneta*, *Planothidium frequentissimum*), *Planothidium lanceolatum* showed a negative relationship to inorganic nitrogen but aligned with higher mineral content (viz. chloride, potassium and sulphate). This suggests that, although it might be possible to identify the predominant form of nitrogen and cause of pollution from its diatom signature, generalisations about the indicative meaning of individual taxa may be less straightforward.

Several issues remaining unresolved, the results of this preliminary survey and considerably more extensive studies carried out abroad point out that the microphytobenthos, with diatoms in particular (GERECKE et al. 2011), represents a major source of information on the functional and structural integrity, future prospects and regional peculiarities of petrifying springs. This may be especially welcome where their morphological development is more marginal and characteristic bryophyte communities less diverse, hampering a comprehensive elaboration of the favourable conservation status and environmental reference values of this endangered habitat type from more readily accessible macroscopic features.

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Supplementary material

the following supplementary material is available for this article:

Table S1. List of all observed taxa with their relative abundance and frequency, total valve count and attribution of samples to cluster groups. FONTEb and WIJNGa not considered in average and standard deviation. + outside count, * Red List group rare (R), ** Red List group endangered (E).

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