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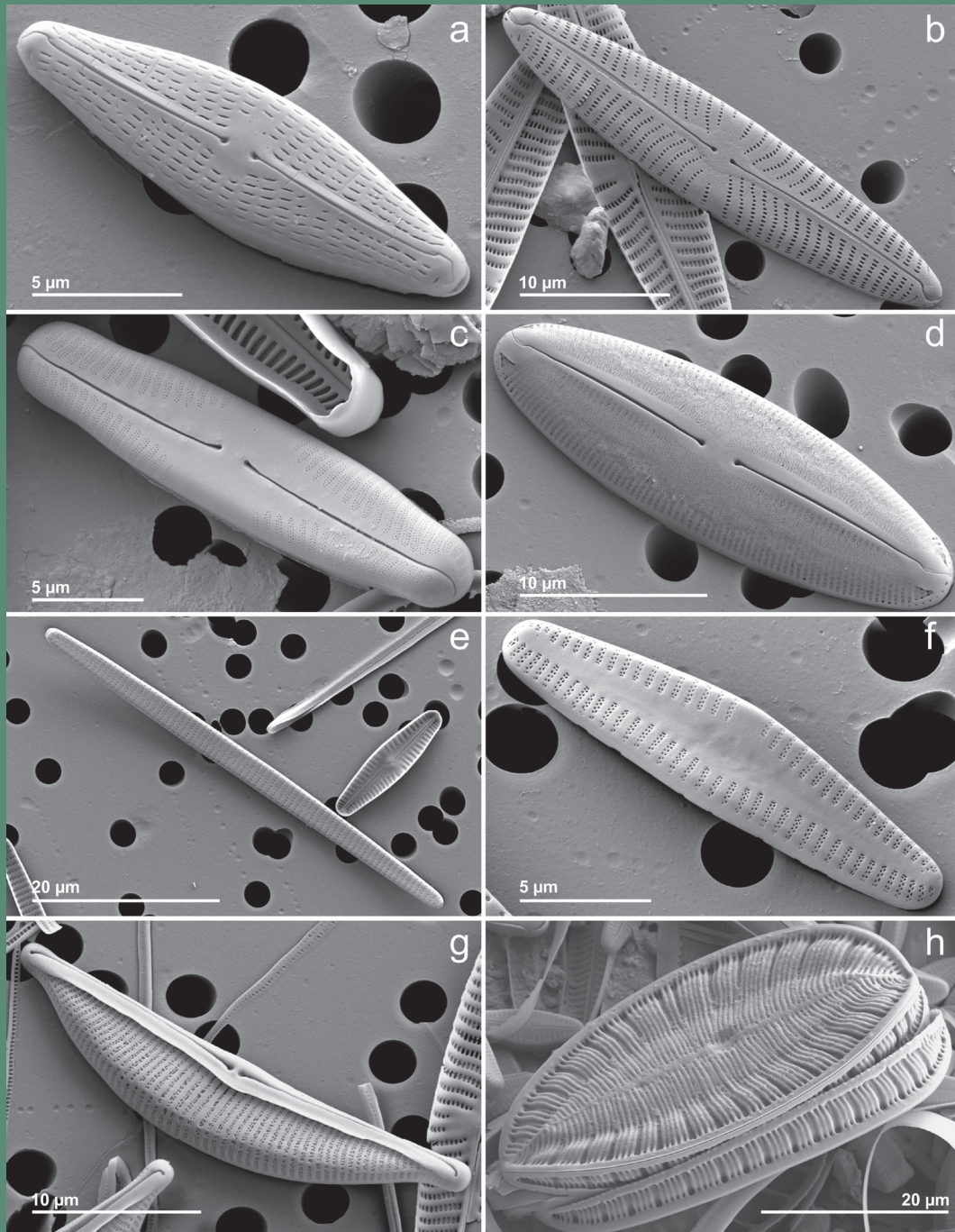
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# BIOM

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Sainte Marguerite (Saint-Maurice-ès-Allier, Massif central, France)

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# Biodiversity and ecology of diatoms in mineral springs of the area of Sainte Marguerite (Saint-Maurice-ès-Allier, Massif central, France)

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## Abstract

This study investigated the diatom flora from mineral springs in Auvergne (France). Samples were collected from rock/cobbles and fine sediments in 17 springs between November 2014 and April 2015 in which a total of 58 taxa were found. Among the different taxa present in some of the studied springs, two have been recently described: *Navicula sanctamargaritae* and *Sellaphora labernardierei*. *Crenotia thermalis* was observed in all sites except in the Nid de l'Epervier spring. Multivariate analyses revealed differences in species abundance among the springs due to the physical and chemical characteristics and mainly to the presence or absence of nutrients. Petit Jean spring is separated from the other springs and is characterized by a lower calcium concentration. In this site, *Pinnularia kuetzingii*, *Navicula veneta* and *Fallacia pygmaea* were the dominant species. This study brings a better knowledge of the diatom biodiversity of mineral springs and the ecology of each species present.

## Résumé

Cette étude a porté sur la flore de diatomées provenant de sources minérales d'Auvergne (France) et plus précisément sur le secteur de Saint-Maurice-ès-Allier. Des échantillons de diatomées ont été pris sur des roches / galets et des sédiments fins dans 17 sources entre novembre 2014 et avril 2015, dans lesquelles 58 taxons au total ont été trouvés. Parmi les différents taxons, deux présents dans certaines des sources étudiées ont été récemment décrits: *Navicula sanctamargaritae* et *Sellaphora labernardierei*. *Crenotia thermalis* était présent sur tous les sites sauf dans la source Nid de l'Epervier. Des analyses multivariées ont révélé des différences dans l'abondance des espèces entre les différentes sources en raison de leurs caractéristiques physiques et chimiques et principalement en lien avec la présence ou non de nutriments en forte concentration. La source Petit Jean était séparée des autres sites et caractérisée par une concentration en calcium inférieure à celle des autres sources. *Pinnularia kuetzingii*, *Navicula veneta* et *Fallacia pygmaea* étaient les espèces dominantes sur ce site. Cette étude a permis de mieux connaître la biodiversité des diatomées des sources minérales et l'écologie de chaque espèce présente.

### Keywords

Mineral springs  
Diatoms  
Biodiversity  
Ecology

### Mots-clés

Sources minérales  
Diatomées  
Biodiversité  
Écologie

## Introduction

Mineral springs are isolated habitats (Werum 2001) in the landscape (Cantonati et al. 2012). They have been widely studied hydrogeologically but the communities that inhabit them are still poorly known. These environments, influenced by deep groundwater upwelling and surface water (i.e. Boineau & Maisonneuve 1972 ; Cantonati et al. 2012) also known as groundwater dependent ecosystems (GDE) (Kløve et al. 2011), host important biodiversity and specific biocenoses (species adapted to the constraints imposed by these ecosystems) (Wojtal 2013 ; Beauger et al. 2015, 2016 ; Lai et al. 2019). These specificities may be related to the high stability of the physicochemical parameters in these habitats (Van der Kamp 1995).

Springs remain fragile when exposed to human impacts. When they are very or largely isolated, they can harbour endemic, rare, endangered and even relict taxa (Botosaneanu 1995 ; Cantonati et al. 2006). Whatever the spring, living organisms are often unique, with very specific traits and distribution patterns (Wojtal 2013 ; Beauger et al. 2015, 2016, 2017 ; Segadelli et al. 2015). Despite these very specific features, these habitats are still poorly considered in habitat monitoring systems since the EU Water Framework Directive (EU WFD 2000) does not explicitly consider them. However, pressures caused by various human activities degrade aquatic biodiversity (Strayer & Dudgeon 2010) while it is the keystone of the functioning of the ecosystems from which human societies derive goods and services essential for their development. It is therefore important to study particularly these peculiar ecosystems, to specify the biocenoses and their ecological and heritage characteristics.

Among the communities thriving in these habitats are the diatoms that are microscopic and photosynthesising algae characterized by a siliceous skeleton (frustule). Also found in almost every aquatic environment including fresh and marine waters, soils, in fact almost anywhere moistly, they are very useful indicators of the environmental quality, reacting with a high sensitivity especially to elements of geogenic origin, but also to the nutritive load that can result from contamination (Segadelli et al. 2015). In mineral springs, these organisms are studied by diatomists all over the world (e.g. Ector & Iserentant 1988 ; Werum & Lange-Bertalot 2004 ; Potapova & Ponader 2008 ; Želazna-Wieczorek 2011 ; Solak & Wojtal 2012). These ecosystems are characterized by a wide range of physical and chemical conditions that differ from spring to spring. For example, in Auvergne, the conductivity ranges from 100 to 123,200  $\mu\text{S}\cdot\text{cm}^{-1}$  (at la Poix spring near Clermont-Ferrand). This results in a wide variability of habitats for diatoms (Sabater & Roca 1992 ; Angeli et al. 2010 ; Wojtal 2013). In the springs, biodiversity is high and new species are regularly identified (Cantonati & Lange-Bertalot 2006, 2011 ; Reichardt 2006 ; Wojtal 2009, 2013 ; Želazna-Wieczorek 2011).

In the Massif central, few studies have been conducted on diatoms in mineral springs (Héribaud 1893, 1920 ; Chaouite 1987 ; Chaouite & Romagoux 1989 ; Tudesque 1996) and they have often been limited to specific emergences of interest for human usages (bottling, thermal activities). Thus, these studies did not address the inventory of the biodiversity of these algae. As a consequence, new taxa have been recently discovered in

Auvergne springs, such as *Navicula sanctamargaritae* Beauger in Beauger et al. (2015), *Sellaphora labernardierei* Beauger, C.E.Wetzel & Ector in Beauger et al. (2016), *Craticula lecohui* Beauger, C.E.Wetzel & Ector in Beauger et al. (2017) and *Pseudostaurosira bardii* Beauger, C.E.Wetzel & Ector in Beauger et al. (2019). The presence of new species highlights the importance of protecting these unique reservoirs of threatened or declining species. The same observation was done in Poland, where Želazna-Wieczorek (2011) noted that 2% of the 456 taxa identified in the springs studied were classified as endangered (Siemińska et al. 2006). Moreover, new taxa found in a particular spring or group of springs in a small geographical area could be considered endemic and habitat protection should be organized as springs are largely ignored by the surveillance network. As a result, it seems necessary to study the biodiversity of these environments, the ecology of the species present in mineral springs, and understand the anthropogenic pressure they are exposed to.

This paper describes the diatom biodiversity of mineral springs situated in the southern-east part of the Massif central along the Allier River and the ecology of the observed species.

## Materials and methods

### Study sites

The volcanic history of the Massif central lead to the emergence of many mineral springs. Indeed, the internal activity of this region associated with carbon dioxide emissions allows the groundwater to rise to the surface via faults in the rocks. On their way, the waters increase their concentrations in chemical elements.

The longitudinal reach of the Allier River situated between Longues (Vic-le-Comte town) and Sainte Marguerite (St Maurice-ès-Allier town) shelters well known springs on each bank (Fig. 1). The area belongs to the French Massif central and more precisely to the southern part of the Limagne d'Allier basin. Mineral waters emerging into the Limagne d'Allier basin are strongly influenced by their deep circulation in the crystalline basement and the presence of deep  $\text{CO}_2$  sources (Fouillac 1983 ; Gal et al. 2012). Before reaching the surface, water can be mixed in varying proportions with the water surface layers when the fractures have tree-like aspect (Labernardière, personal communication).

In the area of interest, we have identified 16 emergences on the two river banks. At Sainte Marguerite, on the right bank of the river, different springs are present such as Tennis, Vallois and Ile springs. A geyser is also present that gushes out every 15 to 20 minutes. The water that gushes out forms a basin with stagnant water. Both the water that gushes out and the basin were sampled. The sparkling mineral water of Sainte Marguerite has been used since the Antiquity and is presently commercialized. Sainte Marguerite area is also characterized by the presence of travertine deposits formed by water degassing at spring emergence (Fouillac 1983 ; Casanova et al. 1999 ; Rihs et al. 2000). Near the former bottling factory, a drilling is present and the water runs in an open pipe. On the left bank, different springs are also present such as the Tambour, Nid de l'Epervier and Petit and Grand Saladis springs. Grand Saladis appears as a large pond (21 m maximum length and 11 m maximum width) with travertine banks. A seepage is

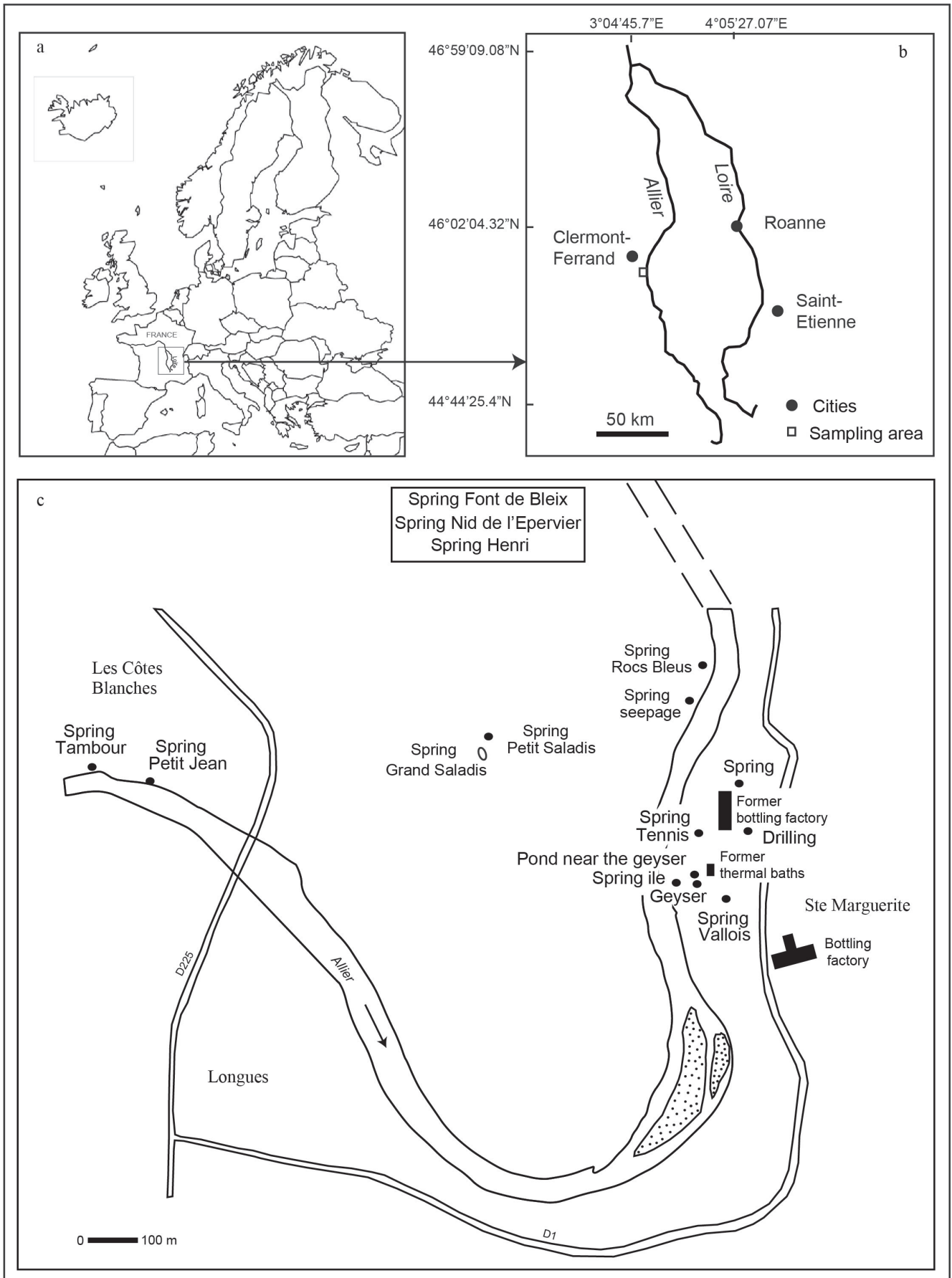


Figure 1 - Map of the studied mineral springs. **a**: map of Europe. **b** and **c**: location of the different mineral springs on each bank of the Allier River (the spring "Basin of the geyser" is not presented on the map as it is at the same place as Geysers).

also present near Rocs Bleus spring and emerges in the alluvial terrace.

### Physical and-chemical analyses and diatom sampling

The sampling survey was carried out between 15<sup>th</sup> November 2014 and 16<sup>th</sup> April 2015 (Fig. 2). The geographical position of the sampling site was measured using a DGPS Trimble Geo7x, in Lambert 93. Conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ), pH (pH units), and water temperature ( $^{\circ}\text{C}$ ) were measured at each sampling site, using a multi-parameter WTW FC probe 340i. The oxygen was measured in most sites using the oximeter Ysi ProODO. At each site, a water sample was collected for further chemical analysis in the laboratory and was analysed using the high pressure ion chromatography technique. First, these samples were filtered using Whatmann GF/C filters. For the cation analysis, a Thermo Scientific Dionex ICS1100 system was used, whereas for the anions, a Thermo Scientific Dionex DX120 system was used. The concentrations ( $\text{mg L}^{-1}$ ) in lithium ( $\text{Li}^+$ ), sodium ( $\text{Na}^+$ ), ammonium ( $\text{NH}_4^+$ ), potassium ( $\text{K}^+$ ), magnesium ( $\text{Mg}^{2+}$ ), calcium ( $\text{Ca}^{2+}$ ), fluorine ( $\text{F}^-$ ), chloride ( $\text{Cl}^-$ ), nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{3-}$ ) and sulphate ( $\text{SO}_4^{2-}$ ) were measured in the laboratory. At last, carbonate concentration ( $\text{HCO}_3^-$ ) was measured using a Hach carbonate kit.

In 2019, an analysis of the radon content of some of these springs was conducted in order to better characterize, both physically and chemically, the water at the sampling points. Ambient gamma-ray dosimetry measurements (expressed in nanoSieverts per hour ( $\text{nSv}\cdot\text{h}^{-1}$ )) were also conducted *in situ* using a Colibri radiometer (Mirion Technology). In the French Massif central, spring water radioactivity is mainly coming from gaseous dissolved radon ( $^{222}\text{Rn}$ ) and its short-lived daughters, most notably  $^{214}\text{Pb}$  and  $^{214}\text{Bi}$  (Boineau & Maisonneuve 1972). Radon activity was determined by gamma-spectrometry at LPC using a germanium detector (Courtine et al. 2008).

For each spring, diatom sampling was adapted according to the sediments present at the different sites, and then vials were named and dated (Tab. 1): 1) in case of fine sediments, the substrate was recovered by scraping the first millimeters of mud

directly with the vial, 2) in case of stones, travertine and metal pipe, the diatoms were sampled by brushing the substrates using a toothbrush that was then rinsed in the vials. One sample was collected per spring.

Table 1 - Substrates collected for each spring and their location along the Allier River.

Springs	Substrates	Bank
Tennis	stone landscaping	right
Ile	stone landscaping	right
Spring	fine sediments	right
Petit Jean	fine sediments	left
Tambour	stone landscaping	left
Petit Saladis	stone landscaping	left
Les Rocs bleus	fine sediments	left
Drilling	stone landscaping	right
Font de Bleix	stone landscaping	left
Seepage	travertine	left
Geyser	metal pipe	right
Basin of the geyser	fine sediments	right
Vallois	stones	right
Pond near the geyser	fine sediments	right
Henri	fine sediments	left
Nid de l'Epervier	fine sediments	left
Grand Saladis	travertine	left

### Slide preparation and microscopy

For each spring, a small fraction of the sample (2 ml) was prepared for light microscopy (LM) observation following the method described in Prygiel & Coste (2000). Samples were cleaned using hydrogen peroxide ( $\text{H}_2\text{O}_2$ , 35%) and hydrochloric acid (HCl), and rinsed several times with distilled water. Cleaned material was diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Then, a drop of dried, clean material was mounted in Naphrax®. LM observations and morphometric measurements were done using a Leica® DM2700M microscope with a 100x oil immersion objective using a differential interference contrast. For each slide, 400 valves were counted. Once the counting was complete, the relative abundances of diatom species were calculated.

Diatom species were identified according to Krammer & Lange-Bertalot (1997a, 1997b, 2000a, 2000b, 2004), Krammer (2000, 2002, 2003), Lange-Bertalot (2001) and Werum & Lange-Bertalot (2004).

We also, analyzed the percentage of teratological individuals (i.e. identification of the deformations) to identify the impact of these extreme habitats. For this purpose, the following protocol was applied on each slide: 300 different individuals valves were observed and classified either as teratological or normal. Only individuals in valve view were considered in this protocol.



Figure 2 - Sampling process. a: sampling of the diatoms at Tambour spring. b: physical measures in situ at Petit Jean spring and c: biofilm observations at Les Rocs bleus spring.

For the scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered with additional deionized water through a 3  $\mu\text{m}$  Isopore polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminum stubs and coated with platinum using a Modular High Vacuum Coating System BAL-TEC MED 020 (BAL-TEC AG, Balzers, Liechtenstein). An ultrahigh-resolution analytical field emission (FE) scanning electron microscope, Hitachi SU-70 (Hitachi High-Technologies Corporation, Japan), was operated at 5 kV and 10 mm distance for image analysis. SEM images were taken using the lower (SE-L) and upper (SE-U) detector signal and sometimes tilted up to an angle of 28°.

## Data analysis

The environmental data structure and their relationships were explored by Principal Component Analysis (PCA, Goodall 1954) based on 14 physical and chemical variables.

The species response to the environmental gradients, regardless of the measured parameters, was tested using Detrended Correspondence Analysis (DCA, Hill & Gauch 1980). The length of the maximum gradient of the first two DCA axes was nearly 4 SD (4.063), which indicates that unimodal methods should be further applied for multivariate analysis of diatom assemblages. Then, a Correspondence Analysis (CA, Greenacre 1984) was performed to reveal changes in diatom species composition in all examined mineral springs, using taxa representing 1% or more of the total diatom population in at least one sample. At last, a Canonical Correspondence Analysis (CCA) was carried out to assess diatom species-environmental relationships (Borcard et al. 1992 ; Birks 2012). Densities were log-transformed, prior to analyses, to normalize and homogenize the variance. PC-Ord 6 statistical tools were used (McCune & Mefford 2006).

## Results

### Physical and chemical characteristics of the mineral springs

In this area, the conductivity ranged from 1344  $\mu\text{S}\cdot\text{cm}^{-1}$  at Font de Bleix spring to 8580  $\mu\text{S}\cdot\text{cm}^{-1}$  at Vallois spring and pH from 6.42 at Font de Bleix to 7.48 pH units at the seepage while the water temperature ranged between 4.3 at Grand Saladis and 29.1°C at the drilling. Considering the dissolved oxygen, it ranged between 0.71% at Tennis spring and 73.4% at the geyser basin.

Figure 3 displays the result of a Principal Component Analysis using the spring physical and chemical variables. PCA axes 1 and 2 explained a total of 68 and 12% respectively of the variance in the environmental data. No difference appears between the left and right bank. In the right part of the first factorial plan, a first group appeared with the springs highly mineralized (conductivity >7000  $\mu\text{S}\cdot\text{cm}^{-1}$ ) with low nitrate and phosphate concentrations. These springs are also carbonated ( $\text{HCO}_3^- > 2000 \text{ mg}\cdot\text{L}^{-1}$ ) and known to be salty with concentrations of the order of 1600  $\text{mg}\cdot\text{L}^{-1}$  for sodium and 1700  $\text{mg}\cdot\text{L}^{-1}$  for chloride. In this group, Grand Saladis spring and the seepage have higher pH (>7.40) while it varies between 6.42 and 7.18 for the other springs.

On the left part of the first factorial plan, a second group gathers Henri, Nid de l'Epervier and Font de Bleix springs displaying lower conductivity (< 2000  $\mu\text{S}\cdot\text{cm}^{-1}$ ). Nid de l'Epervier and Font de Bleix share high nitrate concentration (>30 $\text{mg}\cdot\text{L}^{-1}$ ). At last, the geyser basin is characterized by a high conductivity (>8000  $\mu\text{S}\cdot\text{cm}^{-1}$ ) but 2  $\text{mg}\cdot\text{L}^{-1}$  of nitrates. For this last sampling site, the other ionic concentrations were lower than the other springs of the first group.

Table 2 displays the radioactivity measurements done on the

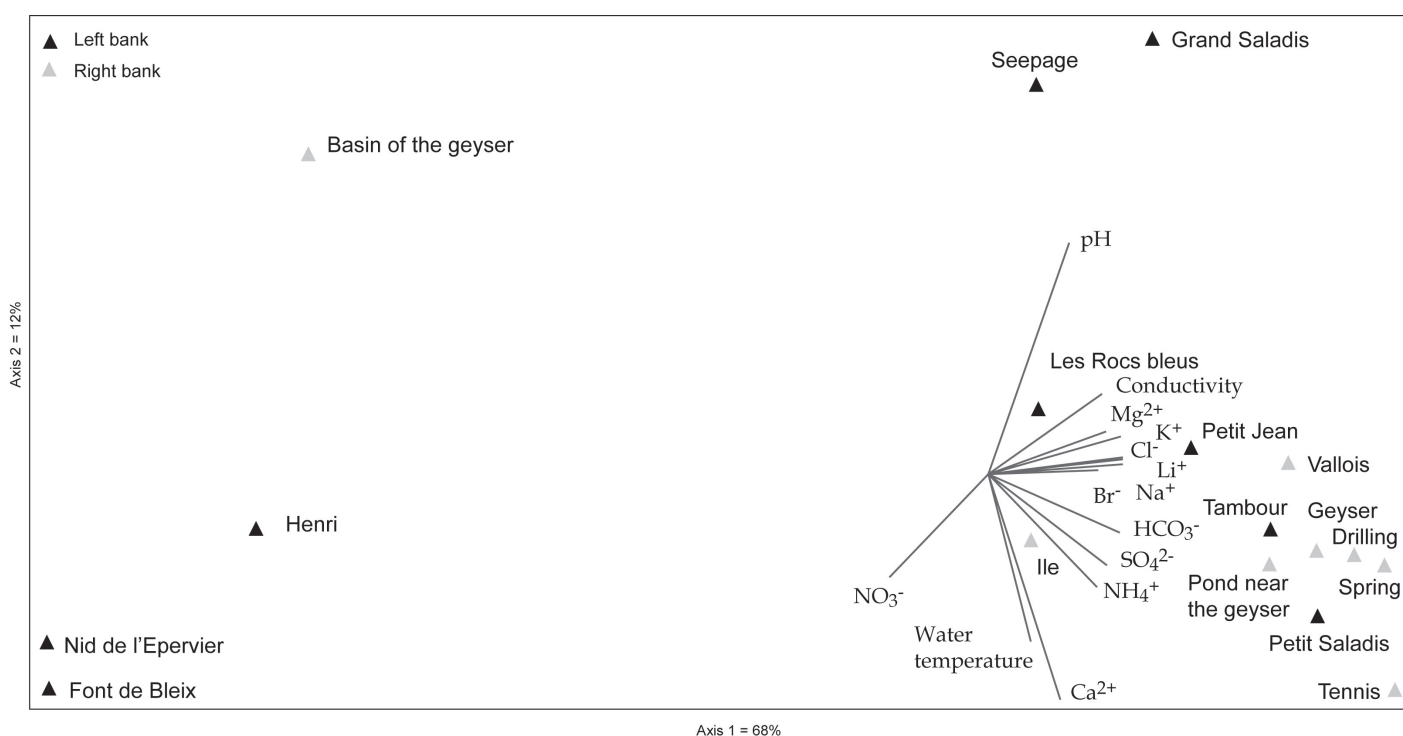


Figure 3 - Ordination plot (F1 X F2) of a Principal Correspondence Analysis of the samples based on the physical and chemical variables.

Table 2 - Measure of radioactivity and percentage of teratological forms: the second column correspond to the range of ambient equivalent doses measured at contact between the COLIBRI radiometer and the spring sediments, travertines or water. The third and fourth column provide the radon activity in spring water together with the rate of teratological shapes.

Springs	Gamma (nSv.h <sup>-1</sup> )	Radon (Bq.L <sup>-1</sup> )	Rate of teratological shape (%)
Tennis	390-840	10-177	1
Ile	/	/	2
Spring	/	/	2
Petit Jean	195-450		7
Tambour	250-370		3
Petit Saladis	/	/	0.5
Les Rocs bleus	150-210	11.2	3
Drilling	/	/	4
Font de Bleix	200	32.2	9
Seepage	/	/	4
Geyser	/	/	3
Basin of the geyser	/	/	4
Vallois	/	/	2
Pond near the geyser	/	/	0
Henri	/	/	5
Nid de l'Epervier	/	/	6
Grand Saladis	330-510	10.2	3

different springs together with the rate of diatom deformation. It is interesting to note that ambient equivalent doses can reach up to 850 nSv.h<sup>-1</sup>, significantly above the 150-200 nSv.h<sup>-1</sup> background in Auvergne. This is due to the presence of radioelements from <sup>238</sup>U decay chain in spring sediments and travertines. Correlation between radon activity and diatom deformation has been recently observed in very radioactive springs (Millan et al. 2019). According to Table 2, the highest concentrations of radon measured in the sampled springs are below 200 Bq.L<sup>-1</sup>, and no

correlation is observed with the diatom deformation rate.

### Diatom assemblages

A total of 58 species were identified in the 17 springs (Figs. 4-6). The highest richness was observed at Les Rocs bleus, Petit Jean and Font de Bleix (Fig. 4). Some species were uniquely observed at Les Rocs bleus: *Amphora copulata* (Kützing) Schoeman & R.E.M.Archibald, *Luticola mutica* (Kützing) D.G.Mann, *Mastogloia*

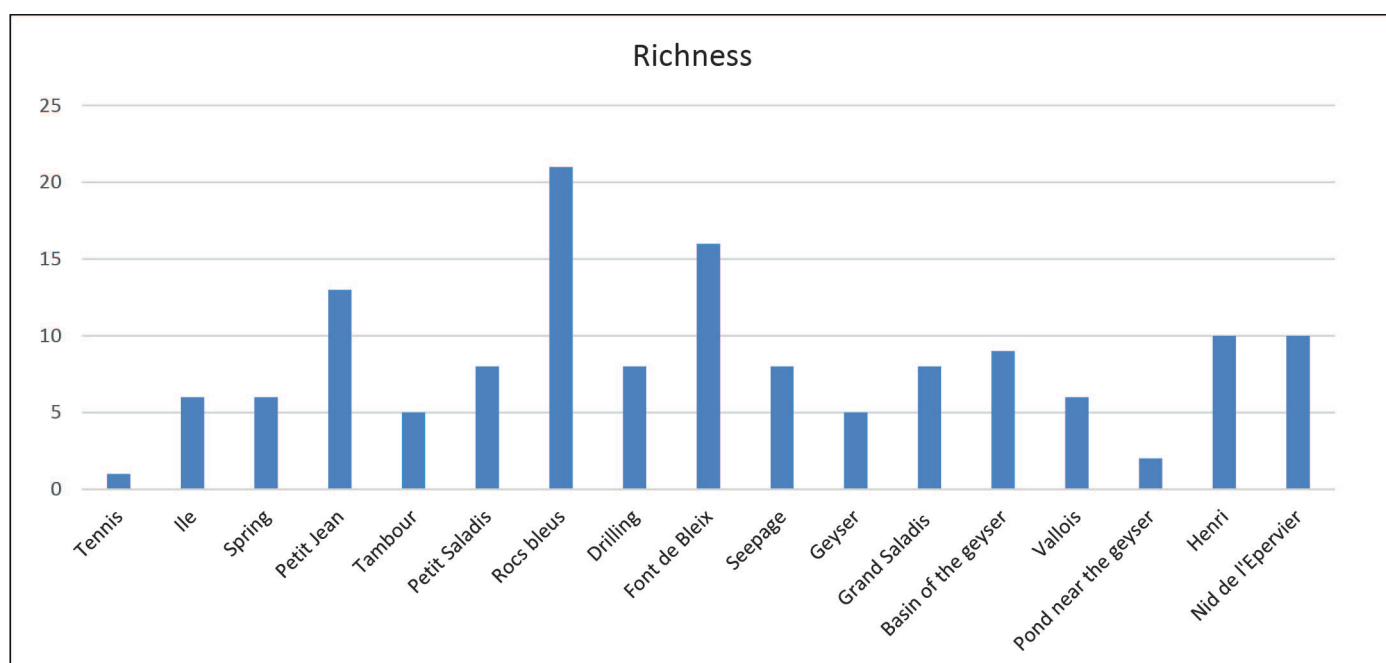


Figure 4 - Species richness observed per spring.



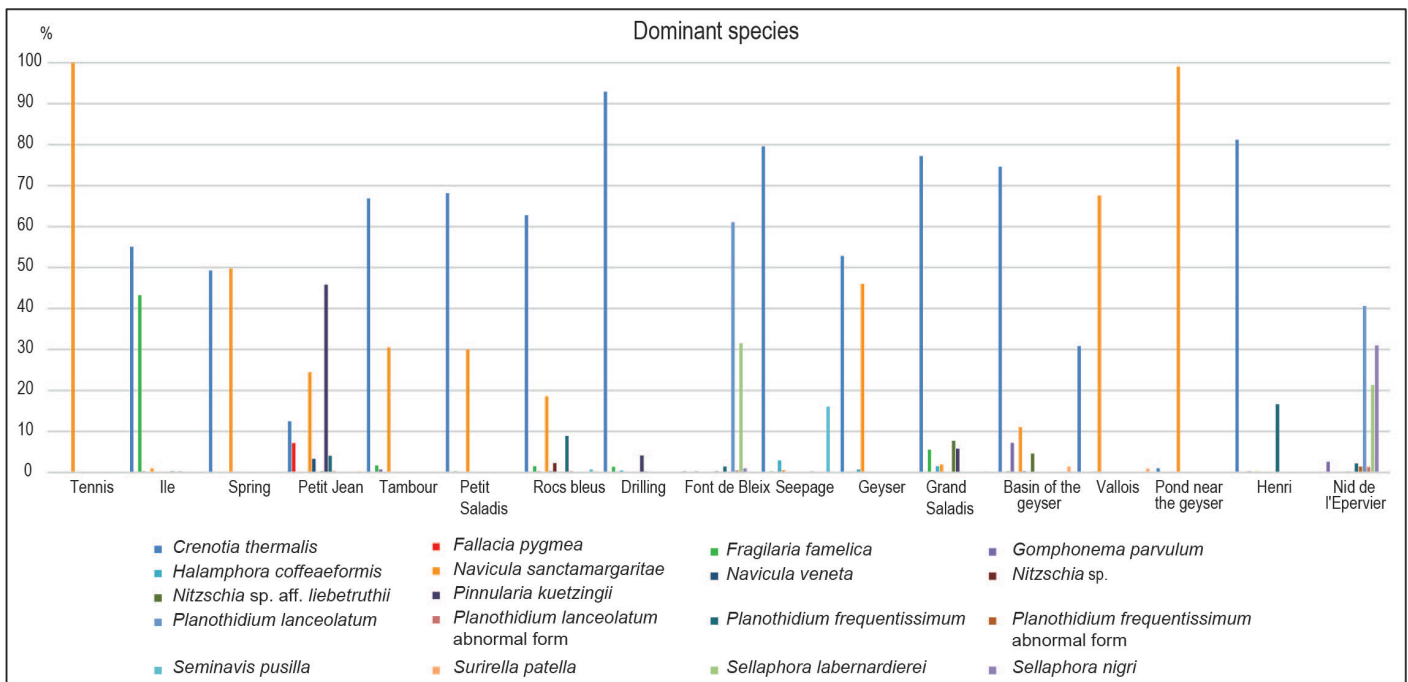


Figure 5 - Dominant species in the 17 springs. Only species with relative abundance > 1% are considered.

*smithii* Thwaites ex W.Smith, *Rhopalodia brebissonii* Krammer and different *Nitzschia* such as *N. supralitorea* Lange-Bertalot.

Only 16 diatom species reached the minimum relative abundance of 1% in at least one sample: *Crenotia thermalis* (Rabenhorst) Wojtal, *Fallacia pygmaea* (Kützing) Stickle & D.G.Mann, *Fragilaria famelica* (Kützing) Lange-Bertalot, *Gomphonema parvulum* Kützing, *Halamphora coffeaeformis* (C.Agardh) Levkov, *Navicula sanctamargaritae*, *Navicula veneta* Kützing, *Nitzschia* sp., *Nitzschia* sp. aff. *liebetruithii*, *Pinnularia kuetzingii* Krammer, *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot normal and abnormal forms, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot normal and abnormal form, *Sellaphora labernardierei*, *Sellaphora nigri* (De Notaris) C.E.Wetzel & Ector in Wetzel et al., *Seminavis pusilla* (Grunow) E.J.Cox & G.Reid, *Suriella patella* Kützing. Some of these species were characterized using SEM observations (Fig. 6). In general, *Crenotia thermalis* was the most abundant and the most frequently observed species in the springs (Fig. 5): its relative abundance averages at 47%. This species was present in all sites except in Nid de l'Epervier spring. In the drilling, it dominated the community with 92% of relative abundance, while at Tennis, it was almost absent with *Navicula sanctamargaritae* reaching up to 99% of the relative abundance (Supplementary Table).

Figure 7 displays the Principal Correspondence Analysis of the diatom species: CA axes 1 and 2 explained 36 and 15% of the variance, respectively. In Font de Bleix and Nid de l'Epervier springs, different species were present as *Planothidium lanceolatum* normal and abnormal forms, *S. labernardierei* and *S. nigri*. These two springs were on the right part of the first factorial plan. On the left part, the other springs were grouped with taxa such as *H. coffeaeformis* and *N. sanctamargaritae*.

At last, a CCA was performed and the first two axes explained a total of 48% of the variance with respectively 33% and 15%

for axes 1 and 2 (Fig. 8). The eigenvalues of the first two CCA axes were 0.73 and 0.32 respectively. The species-environment correlation was 0.999 for axis 1 and 0.993 for axis 2 respectively ( $p=0.03$ ). The first axis represents the mineral-content gradient on its positive part, as it is strongly correlated to conductivity, lithium, sodium, chloride ( $r=0.872$ ,  $r=0.804$ ,  $r=0.801$ ,  $r=0.801$ ). On its negative part, it is related to the trophic gradient. Indeed, nitrates are also associated with the first axis ( $r=-0.962$ ). The second axis is only associated with calcium ( $r=0.394$ ).

Figure 8 shows the CCA biplot of environmental variables and diatom scores for the first two axes. No correlation is observed with the spring location either on the right or left bank. Most of the taxa are located on the right part of the CCA, i.e. they are related to intermediate to high levels of mineral content. The species associated with higher values of the mineral-content gradient (i.e. with a more extreme position in the right part of the first CCA axis) were *Fragilaria famelica*, *Halamphora coffeaeformis*, *Nitzschia* sp. and *Crenotia thermalis*. On the upper right quadrant, *Suriella patella*, *Navicula sanctamargaritae* and *Seminavis pusilla* were associated with high mineral content but also calcium-rich water. In the lower-right quadrant *Pinnularia kuetzingii*, *Navicula veneta* and *Fallacia pygmaea* were associated with Petit Jean spring, characterized with less calcium content.

Other species were associated with the left part of the CCA and they are related to high trophic status (high nitrate concentration) and less mineralized water. Indeed, Nid de l'Epervier and Font de Bleix were apart (as for the PCA) and associated with *Planothidium lanceolatum*, *Sellaphora nigri* and *S. labernardierei*.

*Planothidium frequentissimum* was near the center of the first factorial plan as this species was present in different springs (Ile, Petit Jean, Les Rocs bleus, Drilling, Font de Bleix, Henri and Nid de l'Epervier).

Considering the diatom deformations observed at the different springs, there were differences among the sites (Tab. 2). Indeed,

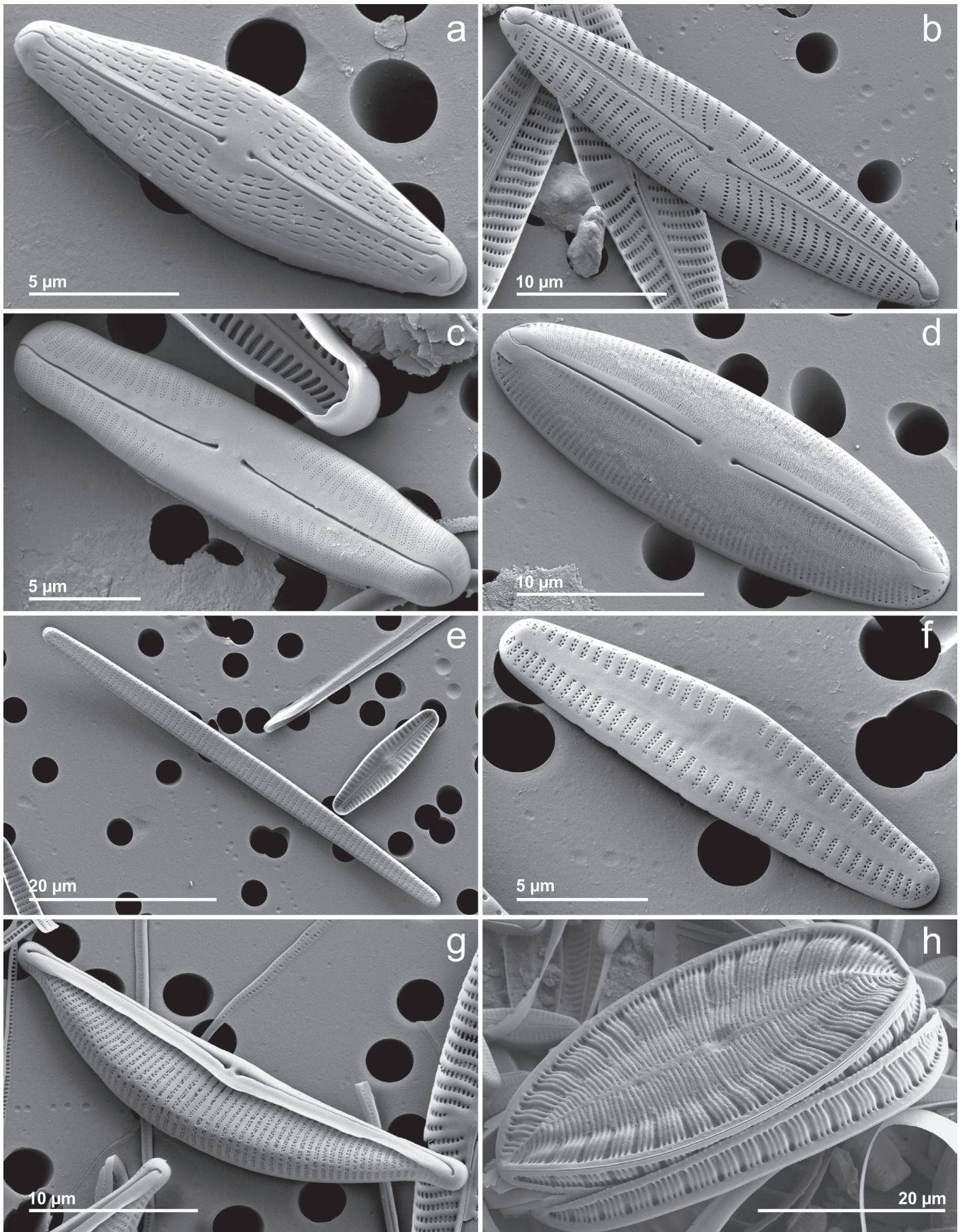


Figure 6 - SEM observations of (a) *Navicula veneta*; (b) *Navicula sanctamargaritae*; (c) *Pinnularia kuetzingii*; (d) *Fallacia pygmaea*; (e) *Fragilaria famelica*; (f) *Crenotia thermalis*; (g) *Halamphora coffeaeformis* and (h) *Surirella patella*.



Figure 7 - Ordination plot of the first factorial plane (F1 X F2) of a Correspondence Analysis based on the taxa. Only species with a relative abundance  $\geq 1\%$  in at least one sample are considered.

the highest percentage of teratological individuals was observed at the Font de Bleix spring with 9%, at Petit Jean (7%) and Nid de l'Epervier (6%) springs. The abnormal forms observed had deformed valve outline (loss of symmetry relative to both axes; abnormal outline as bent) and changes in striation patterns (Falasco et al. 2009a, 2009b).

## Discussion

Although the Auvergne volcanoes have been extinct for thousands of years, geothermal activity stimulates the penetration of water in the rocks (Boineau & Maisonneuve 1972). These superficial infiltrations as well as the characteristic volcanic footprint is at the origin of a halophilic flora rarely encountered in continental environment. Thus, organisms associated with springs and communities are often unique, with very specific traits and distribution patterns (Beauger et al. 2015, 2016, 2017, 2019 ; Segadelli et al. 2015). Indeed, diatoms were observed in all studied springs, including those characterized by high water temperature and high mineralization, factors generally considered to limit diatom growth (e.g. Żelazna-Wieczorek et al. 2015). Moreover, due to the geology of the area, diatoms lived in acidophilous to slowly neutrophilous water (pH from 6.42 at Font de Bleix to 7.48 at the seepage). At the seepage, the pH increases when  $\text{CO}_2$  is

degassing as mineral water is running on the travertine present at this spring.

Springs are an interesting biotope given their location at the interface of groundwater and surface water. In the area of Sainte Marguerite, two groups of springs appeared: emergences with a low mineralization and those highly mineralized. In this last group, Petit Jean spring was apart.

Font de Bleix and Nid de l'Epervier springs both display low conductivity ( $< 2000 \mu\text{S}\cdot\text{cm}^{-1}$ ) and high diatom species richness. At Font de Bleix spring, superficial water inputs are confirmed by the low lithium (Michard 1990) and high nitrate concentration, also observed in Nid de l'Epervier spring. High nutrient contents are mainly related to agricultural activities in the catchment and could account for the deformations observed on diatoms (Falasco et al. 2009a). In these peculiar physical and chemical conditions, the main species are *Planothidium lanceolatum*, *Sellaphora nigri* and *S. labernardierei* recently described (Beauger et al. 2016). According to Wojtal (2013), *P. lanceolatum* was reported as a sensitive indicator of calcium ions and nutrient enrichment in springs studied in Poland. *Sellaphora nigri* is also a diatom species with a wide ecological range, but is generally more abundant in degraded habitats (Lange-Bertalot et al. 2017) reinforcing our

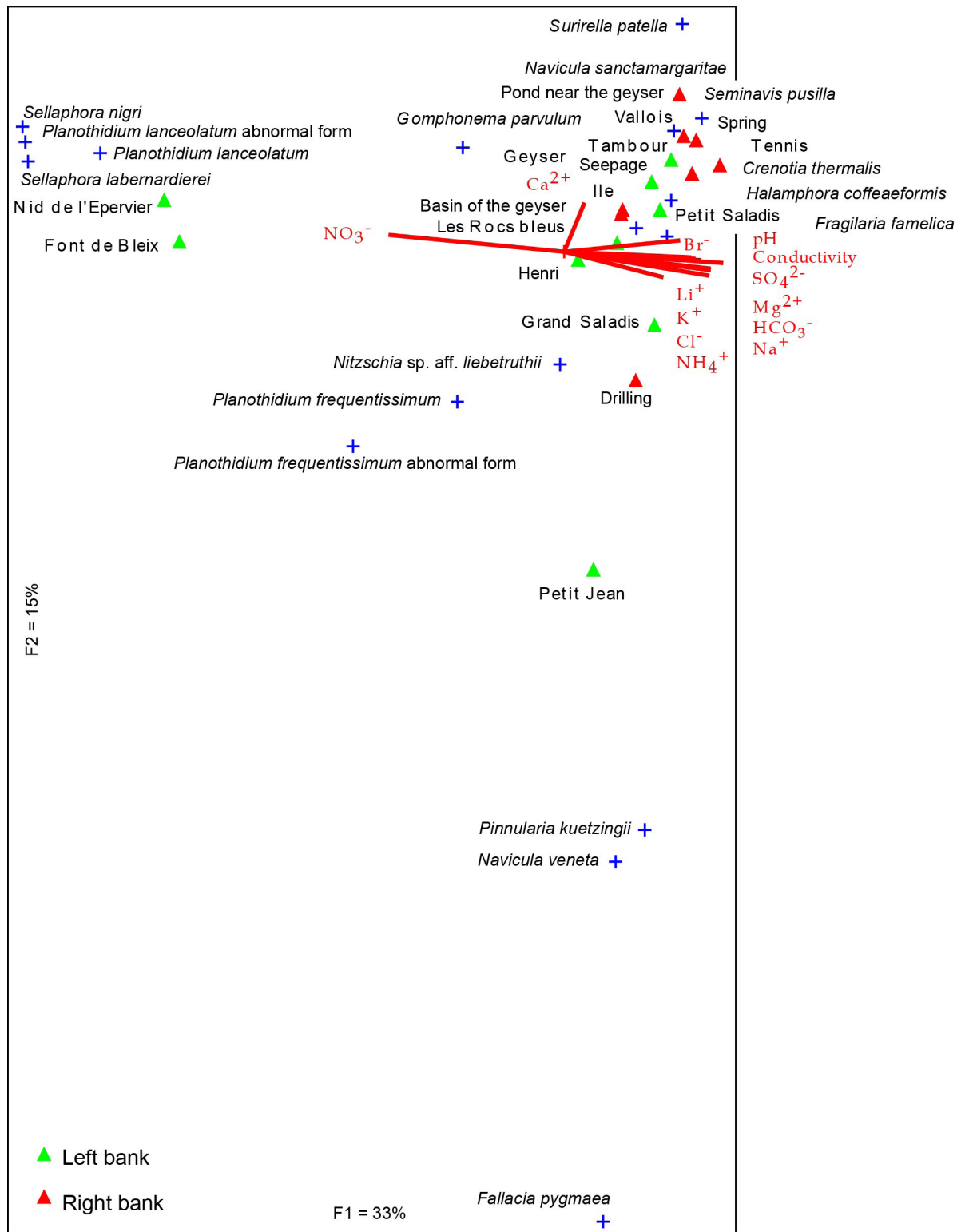


Figure 8 - Ordination plots of the first factorial plane (F1 X F2) of a Canonical Correspondence Analysis based on the physical and chemical data and the taxa with a relative abundance  $\geq 1\%$  in at least one sample.

observations as this species was found in the presence of a higher concentration of nitrates. *Planothidium frequentissimum* was found in these springs but also in five other springs (Ile, Petit Jean, Rocs bleus, seepage and Henri) presenting different physical and chemical characteristics (conductivity ranging between 1344 and 8340  $\mu\text{S}\cdot\text{cm}^{-1}$ ) confirming that this species has a wide ecological range (Van Dam et al. 1994). In few mineral springs of Sardinia (Italy), displaying comparable physico-chemical conditions to Font de Bleix and Nid de l'Epervier springs, some common

species were observed such as *Diploneis elliptica* (Kützing) Cleve and *Nitzschia linearis* (C.Agardh) W.Smith (Lai et al. 2019).

The other springs studied were highly mineralized with a conductivity ranging between 5170  $\mu\text{S}\cdot\text{cm}^{-1}$  at Ile spring and 8580  $\mu\text{S}\cdot\text{cm}^{-1}$  at Vallois spring while the water temperature varied between 4.3°C at Grand Saladis and 29.1°C at the drilling. In this group of springs, the richness varied between 2 at Tennis spring and 21 at Les Rocs bleus. At Les Rocs bleus, some species were

specific to this spring: *Amphora copulata*, *Luticola mutica*, *Mastogloia smithii*, *Rhopalodia brebissonii* and different *Nitzschia* such as *N. supralittorea*. *Luticola mutica*, occurred as epiphyte on macrophytes and is a brackish water species (Levkov et al. 2013) as *M. smithii* (Lange-Bertalot et al. 2017).

In all of these springs, *Crenotia thermalis* was observed. This species, occasionally encountered in running waters, is observed in electrolyte-rich inland habitats, particularly thermal and mineral springs such as in Poland, Germany and Italy (Wojtal 2013 ; Lange-Bertalot et al. 2017 ; Lai et al. 2019). *Navicula sanctamargaritae*, described at Tennis spring (Beauger et al. 2015), was observed also in almost all sites of this group of springs (except the drilling), with high mineralization levels of water, mainly in association with *Crenotia thermalis*. *Fragilaria famelica*, present in different springs such as Tambour, Ile, drilling, is known to be typically brackish (Van Dam et al. 1994 ; Rakowska 1997). The observations in springs of Poland suggest that this species is present in water characterized by low dissolved oxygen and low nitrate content (Wojtal 2013) whereas it was observed in this study in more oxygenated water (between 0.71% and 65%) in relation to low nitrate concentration (<3 mg.L<sup>-1</sup>). *Halumphora coffeaeformis* was observed only in 5 springs (drilling, seepage, geyser, Vallois and Grand Saladis). Known to live in naturally salt-rich water with high electrolyte content (Lange-Bertalot et al. 2017), *H. coffeaeformis* is described for the first time in the La Montagne spring (Châteldon) and is therefore a typical species of mineral springs (Levkov 2009). As this species was not observed in all salt-rich springs, as Tennis for example, other chemical factors induced its presence or absence. The springs, where this species was present, were characterized by higher pH (7.09) than the other springs suggesting an alcaliphilous preference as suggested in Van Dam et al. (1994). *Surirella patella* was observed in sites located in the same area, namely the geyser basin, the Vallois spring and *Seminavis pusilla* in Les Rocs bleus, seepage and Grand Saladis springs (only one individual). *Surirella patella* is a mesohalobous species and therefore a brackish water species (Barinova et al. 2011) as *Seminavis pusilla* (Wojtal 2013). In springs of Poland, this last species is associated with calcium-rich waters (Wojtal 2013), as highlighted with the results of the CCA. *P. frequentissimum* was also observed in some of these springs. In the Azores Islands, this taxa is also observed in springs with water temperature above 25°C (Delgado et al. 2019) as in this area of the French Massif central such as in the drilling and Petit Jean springs.

Petit Jean spring was apart on the CA and CCA (with lower calcium concentration than the other springs) and was associated with *Pinnularia kuetzingii*, *Navicula veneta* and *Fallacia pygmaea*. This last species was only present in this spring and is known to occur in brackish water and in very-electrolyte rich water (Lange-Bertalot et al. 2017). *Pinnularia kuetzingii* is known to occur in waters with an average to higher electrolyte content particularly in thermal springs (Krammer 2000). This species was also present in drilling and Grand Saladis springs. At last, *Navicula veneta*, a species more cosmopolitan and common in electrolyte rich to brackish waters (Lange-Bertalot 2001) was well represented in this spring. As *N. veneta* was not observed in all sites, its ecology has to be refined. In Petit Jean spring, the percentage of deformations was high, however, the concentrations of nitrate was low (0.37 mg.L<sup>-1</sup>). The teratological forms should be due to

other physical influence.

Compared to mineral springs of Sardinia, the highly mineralized springs of the area of Sainte Marguerite present different common species such as *Gomphonema parvulum*, *Navicula cincta* (Ehrenberg) Ralfs, *N. veneta*, *N. sanctamargaritae* and *Nitzschia* sp. cf. *liebethuthii* (Lai et al. 2019). In Pełczyska salt spring (Poland), some different species were also common to this group of mineralized springs such as *Halumphora coffeaeformis*, *F. famelica*, *G. parvulum*, *N. cincta*, *N. veneta*, *Nitzschia inconspicua* Grunow, *Nitzschia vitrea* Norman var. *vitrea*, *Surirella brebissonii* var. *kuetzingii* Krammer & Lange-Bertalot. At last, in Azores, even if Henri spring was not thermal, similar species were observed in thermo-mineral springs such as *Cyclotella* sp., *Diadsmis biceps* Arnott and *P. frequentissimum* (Quintela et al. 2013). However, in this area of the world as in Galicia (Spain) and Pełczyska spring (Poland), *C. thermalis* was curiously not present (Rakowska 1997 ; Quintela et al. 2013 ; Leira et al. 2017). It will be interesting to associate all data sets in order to specify the ecology of this species.

## Conclusion

The studied springs showed high species richness with 58 taxa, even though winter is not the most favourable season for growth and diversity of diatom flora. Among the different taxa, two recently described, *Navicula sanctamargaritae* and *Sellaphora labernardierei*, were present in some of the sites. *Crenotia thermalis* was present in all sites except Nid de l'Épervier spring. Multivariate analyses revealed differences in species composition among the different springs due to the physical and chemical characteristics, especially to the presence or absence of nitrate. Petit Jean spring was separate and characterized by lower calcium concentration than the other springs. In this site, *Pinnularia kuetzingii*, *Navicula veneta* and *Fallacia pygmaea* were the dominant species. Overall, this study allowed to have a better knowledge of the algal biodiversity of mineral springs and of the ecology of each species present.

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