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Alpine freshwater ecosystems in a protected area: a source of diatom diversity

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"This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to Hydrobiologia."

Abstract

The “All Taxa Biodiversity Inventories” (ATBIs) project coordinated by the European Distributed Institute of Taxonomy (EDIT) aims to achieve a baseline biodiversity assessment of flora and fauna in those regions characterized by a lack of knowledge and a high potential for biodiversity. Within the framework of the ATBIs, the aim of the present study was to analyse the diatom flora and ecology of a complex of freshwater ecosystems in the Maritime Alps Natural Park (Italy), designated as a Special area of Conservation under the European Habitat Directive. We sampled epilithic and epiphytic diatoms in different habitats in 24 sites: shallow lake, springs and streams. Our analysis resulted in a list of 138 diatom taxa, highlighting the great biodiversity and the complex structure of the investigated diatom communities. The taxa list included a wide range of uncommon species, including some recorded for the first time in North-Western Italy. Among the different habitats the highest level of diversity was found in the more lentic waters, in particular in limnocrene springs. These results show that the diatom communities of pristine and undisturbed high mountain environments are rich and complex, despite the severe environmental conditions.

Introduction

The European Water Framework Directive (2000/60) recognizes the biological and ecological value of freshwater ecosystems, with the view of promoting the sustainable use of these resources and their preservation. Over the last years, an increasing awareness of the importance of biodiversity has led governmental agencies and the scientific community to improve the knowledge and management of these ecosystems in order to preserve their integrity.

Even in high mountains, where species richness tends to be limited due to extreme environmental conditions (Chapin & Körner, 1995), headwaters are characterized by high levels of biodiversity in relatively small areas, reflecting their high habitat heterogeneity. High mountain springs must be considered as fragile ecosystems. At a local level, the main threats include: habitat reduction due to the increase of pastures, establishment of artificial snow basins, and construction of roads. From a global point of view, an increase in temperature, lengthening of the growth season, habitat fragmentation and alteration of the snow free-period duration are the most important ecological factors affecting high mountain ecosystems. As far as climatic warming and global changes are concerned, mountain regions are among the most potentially vulnerable areas, thus being strongly affected by species loss (Körner, 1995).

Epilithic diatom assemblages in Alpine lacustrine ecosystems have been rarely studied despite their key role in inorganic nutrient cycling, e.g. phosphorus and silica (Loeb et al., 1983; Sánchez-Castillo et al., 2008). High mountain lakes are generally considered as pristine environments, although, human influence has become more and more important in recent years (Cantonati et al., 2006). Several studies on the diatom flora colonizing springs have been carried out in the Eastern Alps, in particular in the Trentino region (Cantonati, 1998a, 1999, 2001; Cantonati & Ortler, 1998; Cantonati & Pipp, 2000) and in the Apennines (Dell'Uomo, 1986; Dell'Uomo & Torrisi, 2000). Due to their pristine conditions, geochemical factors are considered as the driving force influencing diatom assemblages (Cantonati, 1998a; Rimet et al., 2007). However, stream origin and anthropogenic activity also must be considered as an important component (Robinson et al., 2010). Spring diatom samples are often characterized by a high number of uncommon or rare taxa, whose ecological preferences are in part still unknown (Reichardt, 1994, 1995; Cantonati, 1998b), and which often present a non-cosmopolitan distribution (Sabater & Roca, 1992). Moreover, due to the peculiar environmental conditions, the finding of new diatom taxa are common (Cantonati, 1998b; Cantonati & Lange-Bertalot, 2006, 2009).

In the Italian Alps, high mountain rivers are directly affected by human impacts. Morphological alterations, such as channelization, flow regulation, bank modification and construction of artificial riparian areas represent threats to the physical integrity of high-altitude streams, with important consequences for biological communities, and especially diatom assemblages (Bona et al., 2008). Local alterations in rivers lead to the loss of species (Maddock, 1999), and communities inhabiting such modified stretches are mainly young and pioneering, lacking the late successional components (Bona et al., 2008).

In order to increase the knowledge about the biodiversity in “focal points”, the “All Taxa Biodiversity Inventories” (ATBIs) project, coordinated by the European Distributed Institute of Taxonomy (EDIT), is carrying out research in the Maritime Alps Natural Park (North-Western Italy), an area protected by the European Habitat Directive. The ATBIs have been established with the aim of achieving a baseline biodiversity assessment of flora and fauna in those regions characterized by both lack of knowledge and high potential for biodiversity. Water bodies of a protected Alpine region correspond well to these criteria. Besides the important taxonomic value of the project, ATBIs will provide important ecological information on the distribution, abundance and biology of the species recorded, through the collaboration of 27 European scientific institutes.

Within the ATBIs project, we analysed the diatom communities of Italian freshwater ecosystems located in two different valleys in the Maritime Alps Natural Park. We focused on one permanent freshwater lake, permanent rivers, and springs, the latter belonging to rheocrene, limnocrene and helocrene typologies as reported by Cantonati (1998b). This study is the first research analysing and comparing diatom biodiversity in lotic and lentic ecosystems in the Italian Western Alps. The aims of our research were i) to analyse diatom communities colonizing different water bodies of two valleys in the Maritime Alps Natural Park from a taxonomic and ecological point of view; ii) to assess and compare biodiversity in different freshwater ecosystems (lake, rivers and springs) and iii) to highlight the presence of rare and endemic species.

Materials and methods

Sampling sites

The Maritime Alps Natural Park (North-Western Italy) (Figure 1a) has been twinned with the French Mercantour National Park since 1987. These Parks enclose an Alpine area of 100,000 ha, recognised to preserve an inestimable natural heritage in terms of flora and fauna.

In this research, we focused on two valleys in the Italian Park (Figure 1b): Gesso della Valletta and Lourousa valleys, both located in the Argentera Cristalline Massif and characterized by metamorphic rocks. The vegetation of the two valleys is composed of broad-leaved (*Acer pseudoplatanus* L., *Alnus viridis* (Chaix) DC., *Fagus sylvatica* L.), coniferous Alpine species (*Larix decidua* L., *Pinus cembra* L.) and shrubs (*Rhododendron ferrugineum* L., *Vaccinium vitis-idaea* L.). The vegetation surrounding the sampled wetlands was mainly hygrophilous, composed of *Alchemilla vulgaris* L., *Dactylorhiza maculata* (L.) Soó, *Eriophorum latifolium* Hoppe, and *Polygonum bistorta* L.

Due to the lack of data about diatoms in the Maritime Alps Natural Park, we chose a systematic sampling with 40 m minimal distance between transects. In particular, we followed a modified stratified-random design as proposed by Croft & Chow-Fraser (2007). At each sampling site, we identified and sampled all the freshwater habitat zones present in order to have a complete view of species inhabiting the site, thus highlighting their biodiversity and richness. Distances between transects were established by means of a telemeter Bushnell Yardage Pro compact 800. Sampling sites in Gesso della Valletta valley (Figure 1c) were located in Pian della Casa (around 1700 m a.s.l.) and have a sampling area of ca.14 ha. Within this area, we completed 12 transects at 100 m intervals, sampling all water bodies along the track. In total, we collected 15 samples: 10 from the main river (Gesso della Valletta River) and 5 from the surrounding springs (1 limnocrene, 2 helocrene and 2 rheocrene springs).

Sampling sites in Lourousa Valley (Figure 1d) were located nearly 1950 m a.s.l. and the sampling area cover 5.33 ha. Within this area, we completed 8 transects, 40 m apart one from the other, sampling all freshwater bodies along the track. In total, we collected 9 samples: 2 from the main river (Lourousa River), 3 from the littoral zone of the lake (Lagarot di Lourousa) and 4 from the surrounding springs, all limnocrenes.

Physical and chemical factors

At each sampling site, we recorded the main physical and chemical parameters. In the field, we recorded UTM coordinates (ED50) by means of a GPS Etrex Garmin. We measured pH, water temperature (T), conductivity (COND), turbidity (TURB), and dissolved oxygen (DO) by means of the probe Hydrolab Quanta. We also estimated water depth (DEPTH) at each sampling site. Chemical analysis of water samples was performed in the laboratory by means of a Lasa 100 spectrophotometer. In particular, we determined calcium ion concentration (Ca), hardness (HARDNESS), nitrates ($N\text{-}NO_3$), soluble reactive phosphorous (SRP), and biological oxygen demand (BOD_5).

Diatom sampling

At each site, epilithic and epiphytic diatoms were collected in one composite sample following the standardized procedure of Kelly et al. (1998) by scraping cobbles or brushing and squeezing macrophytes. We identified diatoms with the help of light and scanning electron microscopy. Each sample was analysed, identifying each species recorded on the glass slide and, after that, counting 400 valves per sample. Species were identified in accordance with Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b), Simonsen (1987), Lange-Bertalot (1993, 2001), Lange-Bertalot & Metzeltin

(1996), Krammer (1997, 2000, 2002, 2003), Reichardt (1999), Werum & Lange-Bertalot (2004), Metzeltin et al. (2005), and Levkov et al. (2007).

Statistical analysis

We estimated diatom diversity using the Shannon diversity index and evenness. We applied the non-parametric Kruskal-Wallis test to highlight statistic differences among physico-chemical parameters and Spearman correlation to underline correlations among them. To analyse diatom community composition in relation to physical and chemical parameters, we performed Twinspan analysis (Hill, 1979; Gauch & Whittaker, 1981) and Canonical Correspondence Analysis (CCA) (ter Braak, 1986) by means of PC-ORD software (McCune & Mefford, 1999). For the multivariate analysis, we selected the most abundant diatom taxa (taxa showing at least 2% of total abundance, at least once in the whole collected samples), and the most variable environmental parameters (7VAR, DEPTH, BOD_5, HARDNESS, SRP). Note that 7VAR grouped under one parameter Altitude, Ca, COND, DO, $N\text{-}NO_3$, pH and TURB because they were highly correlated.

Results

Physical and chemical factors

According to water quality standards proposed by Italian law (D. Lgs. 152/99), the water quality of the three habitat typologies is considered as good (Table 1). In particular, pH ranged from near neutral (6.9) to slightly basic values (8.4) in springs. Conductivity values were low in all the samples, never exceeding 48 µS/cm. The low calcium concentrations confirmed the siliceous character of the waters of the two valleys. Ortho-phosphates were always very low, with mean values below 0.005 mg SRP/L in the lake, 0.018 mg SRP/L in the rivers, and 0.007 mg SRP/L in the springs. Nitrates were slightly higher, with mean values of 0.709 mg N/L in the lake, 0.424 mg N/L in the rivers and 0.462 mg N/L in the springs. In general, BOD_5 results indicated low organic loading. Altitude ranged from 1700 to 1959 m a.s.l., respectively corresponding to Gesso della Valletta and Lourousa.

With regard to physico-chemical parameters, we found significant correlations between altitude and conductivity (ρ = 0.693 ***), altitude and dissolved oxygen (ρ = -0.783 ***), altitude and nitrates (ρ = 0.767 ***), altitude and calcium (ρ = 0.663 ***), and altitude and turbidity (ρ = 0.631 **), altitude and pH (ρ = 0.482 *).

Using CCA, we graphed all correlated variables under the vector (7VAR). The correlation between altitude and nitrates reflects the fact that the lake, characterized by higher levels of nitrates, was located at a higher altitude than the other water bodies.

The Kruskal-Wallis test showed differences between the lake, the rivers and the springs in dissolved oxygen, turbidity, hardness and calcium ($p < 0.05$).

Community composition

In total, we identified 138 diatom taxa. The complete list of taxa is shown in Annex 1. Several plates illustrating the most representative diatom species are provided in Figures 2-152.

A Kruskal-Wallis test highlighted significant differences in evenness values (Table 2) of the two sampled valleys ($p = 0.033$). Moreover, the number of species ($p = 0.015$) and genera ($p = 0.033$) identified during the counting was significantly higher in springs than in the lake and the rivers (springs > lake > rivers). The most frequent and abundant diatom collected in the rivers and springs was *Achnanthidium minutissimum*, which on average represented 40% of the communities. Besides *Achnanthidium pyrenaicum* and *A. minutissimum*, the most abundant taxa in the lake were *Denticula tenuis*, *Encyonema minutum*, *Cymbella excisa*, *Amphora inariensis* and *Gomphonema pumilum* var. *elegans* (in order of relative abundance). River diatom communities were generally composed of rheophilous taxa, such as *A. pyrenaicum*, *Diatoma mesodon*, *Fragilaria arcus*, *Diatoma hyemalis* and *E. minutum* (in order of relative abundance). Besides *A. minutissimum*, springs were dominated by *E. minutum*, *D. mesodon*, *D. hyemalis*, *D. tenuis*, *A. pyrenaicum*, *Achnanthidium daonense*, *Nitzschia* cf. *fonticola* and *Encyonema silesiacum*. An interesting result of the diatom community analysis was the record of uncommon taxa, especially in springs. In particular, we found several species belonging to the genera *Eunotia* and *Pinnularia* as well as the uncommon species *Aulacoseira alpigena* and *Stauroforma exiguiformis*.

Diatom community in relation to environmental variables

Twinspan analysis highlighted 4 different site groups on the basis of diatom composition (Figure 153). In general, the Twinspan dendrogram showed a first separation representing the different geographical locations of the two valleys, at least for 83% of the cases. The separation is particularly evident for spring sites, although some exceptions. The only limnocrene spring in Gesso della Valletta Valley proved to be closer to the limnocrene springs in Lourousa than to the rheocrene and helocrene ones in its own valley. Moreover, the community composition of the rheocrene spring in Lourousa valley was closer to those of the rivers than to those in the other springs, as a result of its lotic character.

On the one hand, spring diatom communities of the two valleys showed a number of differences. Thus, some species such as *D. hyemalis* and *Fragilaria rumpens* were by far more abundant in Gesso della Valletta Valley, while *E. minutum*, *D. tenuis*, *N.* cf. *fonticola* and *Meridion circulare* were mostly restricted to Lourousa Valley. On the other hand, the composition of the communities colonizing river stretches in the two valleys was quite similar. Indeed, we can easily group river sites together in the second group of the Twinspan analysis (Figure 153).

The main summary statistics of CCA are shown in Table 3. The cumulative explained variance was 28.9%. Axes 1 and 2, evaluated with a Monte Carlo test with 1000 permutations, were highly significant ($p < 0.01$). 7VAR and HARDNESS were strongly related to Axis 1, while water depth was correlated to Axis 2 (Table 3). Ordination of the sites based on species composition (Figure 154) showed three separate groups generally overlapping with the three sampled ecosystems (lake, rivers and springs).

Species ordination showed that *C. excisa*, *Cymbella excisiformis, Encyonema ventricosum*, *N.* cf. *fonticola*, and *Staurosira pinnata* were typical of sites presenting high levels of pH, conductivity, dissolved oxygen, turbidity, nitrates, calcium and hardness. On the other side of the CCA biplot, we found *A. alpigena*, *Brachysira neoexilis*, *Cocconeis pseudolineata*, *Diatoma* sp., *F. rumpens*, *Fragilaria vaucheriae*, *Navicula cryptocephala*, *Nitzschia* cf. *acidoclinata*, *Reimeria sinuata* and *Tabellaria flocculosa*. Species characteristic of greater depth (mainly river sites) were *Gomphonema calcifugum*, *C. pseudolineata,* whereas *A. alpigena*, *B. neoexilis* and *M. constrictum* were typical of more shallow sites, mainly springs.

Discussion

Physico-chemical factors indicated a good water quality in all the sampled areas, as expected by the pristine nature of the two valleys. Even though pasture in Pian della Casa could be considered as a factor potentially affecting the water bodies, no differences in nutrient concentrations have been highlighted in the two valleys. As a consequence, diatom communities were mainly composed of oligotrophic and oligosaprobic taxa, characteristic of the subalpine zone. The most frequently recorded species (i.e. present in at least 50% of all samples) were *A. minutissimum*, *E. minutum*, *A. pyrenaicum*, *D. mesodon*, *F. arcus*, *E. silesiacum*, *A. inariensis*, *D. hyemalis*, *D. tenuis*, *G. pumilum* var. *elegans,* and *A. daonense* (in order of percentage of occurrence in the samples). In agreement with some authors (Cantonati & Spitale, 2009; Kawecka & Robinson, 2008; Robinson et al., 2010), *A. minutissimum* was the most recorded species, being found in 100% of the samples. Also, *E. minutum* was usually found in our samples (97% of occurrence), while it seems to be less frequent in the Dolomiti Bellunesi National Park (Cantonati & Spitale, 2009), even though it was the dominant taxon in one of the studied springs. In agreement with Cantonati (1998a, b), Kawecka & Robinson (2008) and Rimet et al. (2007), we highlighted the presence of *D. mesodon* and *D. hyemalis* in most of the samples (85.3% and 61.8% of occurrence, respectively) and the rheophilous species *F. arcus* in rivers. These taxa are common in streams with intermediate conductivity and alkalinity (Robinson et al., 2010). *D. tenuis* was mainly recorded in lentic habitats (lake and limnocrene springs) where it represented a consistent percentage of the community, up to 21.5%. In agreement with previous findings *D. tenuis* seems to have a clear preference for shallow water and it is probably tolerant to occasional exposure to air (Cantonati et al., 2009; Robinson et al., 2010).

In general, the recorded species were adapted to low Ca levels, typical of siliceous substrates; for this reason, species typical of carbonate substrates, such as the recently described *Achnanthidium dolomiticum* Cantonati et Lange-Bertalot (Cantonati & Lange-Bertalot, 2006), were not found. Even *Geissleria gereckei* Cantonati et Lange-Bertalot was not detected at all, confirming its preference for limestone and for highly shaded habitat (Cantonati & Lange-Bertalot, 2009). The mean values of abundance of the most frequent diatom taxa in rivers and springs do agree with the percentages found by Cantonati & Spitale (2009). *A. minutissimum* was the most abundant species in the samples, representing respectively 40% and 42% of the river and spring communities (mean values).

A total of 138 diatom taxa were identified. In general, we recorded the highest number of taxa in springs (62 in total) and, in particular, in limnocrene typology, in agreement with Cantonati (1998b). The faster flow of the rheocrene springs and of the rivers could select for rheophilous taxa, leading to a loss in species number and diversity (Sabater & Roca, 1992; Maier, 1994; Cantonati, 1998a, 2001; Ghosh & Gaur, 1998). Moreover, more lentic waters create suitable habitats for macrophyte growth, leading to the colonization of epiphytic and epilithic taxa. According to previous studies carried out in Piedmont and Aosta Valley (Bona et al., 2007, 2008), we observed that such a small sampling area enclosed a wide range of species, even those never recorded in North-Western Italy, thus confirming that samples from siliceous areas are very species rich (Cantonati, 1998b).

As already pointed out in other studies (Cantonati & Spitale, 2009; Bertrand et al., 1999), some species seem to be restricted to a certain microhabitat or even to a unique sampling site (see Annex 1). In our research ca. 38% of the recorded species are limited to a unique typology of microhabitats (lake, rivers or springs).

By comparing diatom communities of high mountain streams of Piedmont and Aosta Valley (Battegazzore et al., 2004; Bona et al., 2008) flowing on siliceous substrate, we noticed the peculiarity of taxa recorded in the Maritime Alps Natural Park. The sampled springs surrounding the rivers allowed the development of huge populations of centric diatoms, which often drift into the Gesso della Valletta and Lourousa rivers, increasing their biodiversity. *A. alpigena*, for instance, is an oligosaprobous species that requires high oxygenation, low conductivity and slightly acid conditions of water (Van Dam et al., 1994). This species had already been recorded in Adamello Park (Cantonati, 1998b) and was very abundant in the Macun Catchment, Switzerland (Robinson & Kawecka, 2005), but it represents a new finding for the Piedmont region. Other interesting taxa especially found in helocrene springs, and rarely recorded during previous surveys, belong to the genera *Eunotia* and *Pinnularia*; i.e. *Pinnularia borealis* var. *tenuistriata*, *Pinnularia sinistra* and *Pinnularia subcapitata*.

In general, all taxa were typical of oligotrophic, oxygen-rich waters with low electrolyte levels and are characteristic of swamps and moors. According to the “red list” diatoms established by Lange-Bertalot & Steindorf (1996) for Germany, we can consider 56% of the recorded species as endangered at various levels. Moreover, we found some threatened taxa important to be protected, in particular extremely rare species (ca. 3%) such as *Amphora fogediana*, *Craticula dissociata*, *Navicula tridentula* and *Stauroneis agrestis*, and the highly endangered species *Eunotia circumborealis* and *Navicula detenta.* It is important to focus the attention on *A. inariensis,* a species considered as endangered in the red list but particularly frequent in our samples (ca. 61% of occurrence) even though with low percentages of abundance (0.5% in the river typology, 1.2% in the springs and 2.2% in the lake). This emphasises the importance of preserving the integrity of this protected area and its naturalistic value. Lange-Bertalot’s red list refers to the German diatom flora and ca. 27% of the taxa recorded in our research were not included in the catalogue. For this reason, further research specific to the Italian Alpine region is needed.

Multivariate analysis allowed pointing out local and landscape influences on diatom assemblages. On the basis of the community composition and through Twinspan Analysis, we can confirm that the three typologies of sampled habitats were well differentiated. Rivers had similar communities despite their location in different valleys of the Maritime Alps Natural Park. On the other hand, spring diatom communities presented peculiar species composition depending on location. The spring communities are different because of the nature of the two valleys. Lourousa Valley shows a more incised profile in comparison to Gesso della Valletta Valley. Springs were mainly closer to a glacier and exposed to the North in Lourousa, while to the South in Gesso della Valletta. The more shaded nature of the valley coupled with glacial alimentation makes Lourousa springs a harsher environment as far as temperature and light supply are concerned (Hieber et al., 2001). Therefore, it is possible to hypothesize that these different environmental conditions have influenced the growth of macrophytes and diatom communities, leading to the differentiation of characteristic assemblages. As a consequence, these natural conditions could influence springs more than rivers. In water bodies of equivalent water quality, local features such as current velocity are the main driving force influencing diatom assemblages. In more lentic habitats, such as springs, microclimatic factors (such as exposure) may play an important role, otherwise neglected in fast flowing systems.

Through the Canonical Correspondence Analysis we showed that altitude, water conductivity, dissolved oxygen, nitrate, calcium, turbidity, pH, hardness and depth are the main variables influencing diatom species distribution. Moreover, the CCA plot, built on the basis of species composition, was effective in discriminating among the different habitats, grouping sites in accordance to the three habitats. Therefore, we can state that there is a good correspondence between the environmental parameters and relative diatom communities in the Maritime Alps Natural Park.

Conclusions

This research allowed us to identify the diatom communities inhabiting a pristine high mountain area, providing important information concerning the ecological preferences of some rare species, their abundance and distribution. This study indicates the importance of mountain springs as an important source of biodiversity and niche for rare species. Moreover, the juxtaposition of lotic and lentic waters (such as lakes or limnocrene springs) increases habitat heterogeneity and the potential biodiversity of the area (Robinson & Kawecka, 2005). Being enclosed in a protected area, Gesso della Valletta and Lourousa valleys should be considered as reference environments, free from any remarkable kind of human pressure. Diatom communities, indeed, have to be considered excellent bioindicators both for long-term changes (such as mountain temperature increase and glacier retreat) and for local human impacts (such as the construction of winter ski resorts and artificial reservoirs). Over the last 20 years, glacier retreat has globally accelerated, with consequences on flow regime and sediment transport. One of the main consequences of global warming in the Alps, will probably be a shift from kryal to rhithral ecosystems with the increase of intermittent and krenal streams (Brown et al., 2007). These will influence the presence, abundance and distribution of the biological communities inhabiting these stretches and the functional characteristics of the Alpine running and standing waters (Robinson et al., 2010).

This research may represent a valid contribution to the use of diatom communities in the environmental assessment of local human impacts. In this context, mountain streams and springs are unusual environments, characterized by atypical environmental features and pressures. Application of common standardized diatom indices appears inadequate to the evaluation of their water quality. For this reason, comparison with a potential reference community is required. In particular, as our research has illustrated, a complex of abiotic parameters, including site exposure, can influence diatom communities colonizing springs.

The recent need for ecosystem management has led the scientific community to consider the intrinsic natural conservation value besides traditional methods. According to Coesel (2001), biodiversity and species rareness, in connection with ecosystem maturity and replaceability (Schroevers, 1973), should be considered as important parameters in ecosystem assessment.

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Annex 1

Taxon name	CODE	RL	River	Lake	Wetland		
					Rheocrene	Limnocrene	Helocrene
Achnanthes exigua var. *elliptica* Hustedt	AEEL	**		+			
Achnanthidium daonense (Lange-Bertalot) Lange-Bertalot, O. Monnier et Ector	ADDA	G	+	+	+	+	+
Achnanthidium minutissimum (Kützing) Czarnecki	ADMI	**	+	+	+	+	+
Achnanthidium pyrenaicum (Hustedt) H. Kobayasi	ADPY	**	+	+	+	+	+
Achnanthidium subatomoides (Hustedt) O. Monnier, Lange-Bertalot et Ector	ASAT	V	+				+
Adlafia bryophila (J. B. Petersen) Gerd Moser, Lange-Bertalot et Metzeltin	ABRY	V	+	+	+	+	+
Adlafia minuscula var. *muralis* (Grunow) Lange-Bertalot	ADMM	NR	+	+	+	+	+
Amphora fogediana Krammer	AMFO	R	+				
Amphora inariensis Krammer	AINA	3	+	+	+	+	+
Aulacoseira alpigena (Grunow) Krammer	AUAL	G	+			+	+
Aulacoseira distans (Ehrenberg) Simonsen	AUDI	NR	+				
Brachysira brebissonii R. Ross	BBRE	*	+			+	+
Brachysira neoexilis Lange-Bertalot	BNEO	3	+			+	+

Caloneis bacillum (Grunow) Cleve	CBAC	**				+	+
Caloneis molaris (Grunow) Krammer	CMOL	V	+				
Caloneis sp.		NR		+			+
Caloneis spec. Nr. 2 in Lange-Bertalot & Metzeltin		NR				+	+
Caloneis tenuis (W. Gregory) Krammer	CATE	G	+		+	+	+
Cavinula pseudoscutiformis (Hustedt) D.G. Mann et Stickle	CPSE	NR				+	+
Cocconeis euglypta Ehrenberg	CPLE	**	+		+	+	+
Cocconeis lineata Ehrenberg	CPLI	**	+		+		+
Cocconeis pseudolineata (Geitler) Lange-Bertalot	CPPL	**	+	+	+	+	+
Craticula dissociata (E. Reichardt) E. Reichardt	CRDI	R		+			
Cyclotella cyclopuncta Håkansson et J.R. Carter	CCCP	D				+	
Cymbella cymbiformis Pantocsek	CCYM	V					+
Cymbella excisa Kützing	CAEX	*		+		+	
Cymbella excisiformis Krammer	CEXF	NR		+		+	
Cymbella helvetica Kützing	CHEL	V				+	
Cymbopleura naviculiformis (Auerswald) Krammer	CBNA	NR	+		+	+	+
Denticula tenuis Kützing	DTEN	*	+	+	+	+	+
Diadesmis laevissima (Cleve) D.G. Mann	DLAE	**				+	

Diadesmis perpusilla (Grunow) D.G. Mann	DPER	**	+	+	+	+	+
Diatoma ehrenbergii Kützing	DEHR	**	+		+		+
Diatoma hyemalis (Roth) Heiberg	DHIE	*	+	+	+	+	+
Diatoma mesodon Kützing	DMES	*	+	+	+	+	+
Diatoma sp.	D sp.	NR	+	+	+	+	+
Diploneis ovalis (Hilse) Cleve	DOVA	V		+			
Encyonema lunatum (W. Smith) Van Heurck	ENLU	NR					+
Encyonema minutum (Hilse) D.G. Mann	ENMI	*	+	+	+	+	+
Encyonema lunatum (W. Smith) Van Heurck	ENNG	NR	+		+		
Encyonema silesiacum (Bleisch) D.G. Mann	ESLE	*	+	+	+	+	+
Encyonema ventricosum (Kützing) Grunow	ENVE	NR		+		+	
Encyonopsis cesatii (Rabenhorst) Krammer	ECES	NR					+
Encyonopsis falaisensis (Grunow) Krammer	ECFA	G	+				
Eolimna minima (Grunow) Lange-Bertalot	EOMI	**			+		+
Eucocconeis laevis (Østrup) Lange-Bertalot	EULA	*	+	+	+	+	+
Eunotia arcus Ehrenberg	EARC	2				+	
Eunotia bilunaris (Ehrenberg) Souza	EUBI	*			+	+	+
Eunotia boreoalpina Lange-Bertalot et Nörpel-Schempp	EBOA	NR				+	+

Eunotia circumborealis Lange-Bertalot et Nörpel	ECIR	1	+				
Eunotia minor (Kützing) Grunow	EMIN	*	+		+	+	+
Eunotia soleirolii (Kützing) Rabenhorst	ESOL	G		+			+
Eunotia subarcuatoides Alles, Nörpel et Lange-Bertalot	ESUB	**				+	
Eunotia tenella (Grunow) Hustedt	ETEN	V				+	
Fragilaria arcus (Ehrenberg) Cleve	FARC	**	+	+	+	+	+
Fragilaria bicapitata Ant. Mayer	FBIC	*				+	
Fragilaria capitellata (Grunow) J.B. Petersen	FCCP	NR			+		
Fragilaria delicatissima (W. Smith) Lange-Bertalot	FDEL	NR				+	
Fragilaria gracilis Østrup	FGRA	*	+			+	+
Fragilaria rumpens (Kützing) G.W.F. Carlson	FRUM	NR	+		+	+	+
Fragilaria vaucheriae (Kützing) J.B. Petersen	FVAU	**	+	+	+	+	+
Fragilariforma virescens (Ralfs) D. M. Williams et Round	FFVI	NR					+
Frustulia rhomboides (Ehrenberg) De Toni	FRHO	V			+	+	+
Gomphonema angustatum (Kützing) Rabenhorst	GANG	*					+
Gomphonema bohemicum Reichelt et Fricke	GBOH	3	+				
Gomphonema calcifugum Lange-Bertalot et E. Reichardt	GCLF	NR	+			+	
Gomphonema cymbelliclinum E. Reichardt et Lange-Bertalot	GCBC	r	+				+

Gomphonema hebridense W. Gregory	GHEB	V			+		+
Gomphonema mexicanum Grunow	GMEX	NR			+		
Gomphonema micropus Fricke	GMIC	*	+	+	+	+	+
Gomphonema minutum C. Agardh	GMIN	**	+				
Gomphonema parvulum (Kützing) Kützing	GPAR	**	+		+	+	+
Gomphonema productum (Grunow) Lange-Bertalot et E. Reichardt	GPRO	D	+			+	
Gomphonema pumilum var. *elegans* E. Reichardt et Lange-Bertalot	GPEL	NR	+	+	+	+	+
Gomphonema sp.	G sp.	NR					+
Gomphonema tergestinum (Grunow) Fricke	GTER	G	+				+
Gomphonema utae Lange-Bertalot et E. Reichardt	GUTA	D				+	
Luticola acidoclinata Lange-Bertalot	LACD	NR				+	
Luticola mutica (Kützing) D.G. Mann	LMUT	D				+	
Luticola sp.	L sp.	NR	+				
Mayamea atomus (Kützing) Lange-Bertalot	MAAT	**				+	
Melosira varians C. Agardh	MVAR	**	+				
Meridion circulare (Greville) C. Agardh	MCIR	**	+	+	+	+	+
Meridion constrictum Ralfs	MCON	**	+		+	+	+
Microcostatus krasskei (Hustedt) J.R. Johansen et Sray	MKRA	NR	+				

Navicula angusta Grunow	NAAN	3				+	
Navicula antonii Lange-Bertalot et Rumrich	NANT	NR	+			+	
Navicula capitatoradiata H. Germain	NCPR	**	+				+
Navicula cryptocephala Kützing	NCRY	**	+		+	+	+
Navicula cryptotenella Lange-Bertalot	NCTE	*	+				
Navicula detenta Hustedt	NDET	1				+	
Navicula gregaria Donkin	NGRE	**	+				
Navicula lundii E. Reichardt	NLUN	D	+		+	+	
Navicula rhomboides Ehrenberg	NRBO	V			+	+	+
Naviculadicta schmassmannii (Hustedt) Werum et Lange-Bertalot	NSMM	2	+	+	+	+	+
Navicula tridentula Krasske	NTRI	R					+
Navicula tripunctata Bory	NTPT	**	+				
Neidium affine (Ehrenberg) Pfitzer	NEAF	V				+	
Neidium bisulcatum var. *subampliatum* Krammer	NBSA	3	+				+
Neidium longiceps (W. Gregory) R. Ross	NLGI	NR	+			+	+
Nitzschia cf. *acidoclinata* Lange-Bertalot	NACD	*	+		+	+	+
Nitzschia dissipata (Kützing) Grunow	NDIS	**	+	+	+	+	+
Nitzschia cf. *fonticola* (Grunow) Grunow	NFON	**	+	+	+	+	+

Nitzschia gracilis Hantzsch	NIGR	*			+	+	
Nitzschia aff. *hantzschiana* Rabenhorst	NHAN	*	+	+	+	+	+
Nitzschia pura Hustedt	NIPR	*		+			
Nupela imperfecta (Schimanski) Lange-Bertalot et Genkal	NUIP	NR	+				
Nupela lapidosa (Krasske) Lange-Bertalot	NULA	NR	+	+			
Orthoseira roeseana (Rabenhorst) O' Meara	OROE	V			+		+
Pinnularia borealis var. *tenuistriata* Krammer	PBTE	NR	+				+
Pinnularia interrupta Rabenhorst	PINT	NR				+	
Pinnularia microstauron (Ehrenberg) Cleve	PMIC	V	+			+	+
Pinnularia microstauron var. *rostrata* Krammer	PMRO	NR				+	
Pinnularia perrirorata Krammer	PPRI	NR	+			+	
Pinnularia rupestris Hantzsch	PRUP	G	+			+	+
Pinnularia silvatica J.B. Petersen	PSIL	V				+	
Pinnularia sinistra Krammer	PSIN	*	+		+	+	
Pinnularia stomatophora (Grunow) Cleve	PSTO	G	+			+	+
Pinnularia subcapitata W. Gregory	PSCA	*					+
Pinnularia viridiformis Krammer	PVIR	G	+			+	
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	PTFR	**					+

Planothidium lanceolatum (Brébisson) Lange-Bertalot	PTLA	**	+	+	+	+	+
Planothidium lemmermannii (Hustedt) E. Morales	ALEM	D		+			
Reimeria sinuata (W. Gregory) Kociolek et Stoermer	RSIN	**	+		+	+	+
Sellaphora pupula (Kützing) Mereschkowsky	SPUP	**				+	+
Sellaphora stroemii (Hustedt) H. Kobayasi	SSTM	3	+		+	+	+
Stauroforma exiguiformis (Lange-Bertalot) Flower, V.J. Jones et Round	SEXG	NR	+			+	+
Stauroneis agrestis J.B. Petersen	STAG	R					+
Stauroneis anceps Ehrenberg	STAN	V			+	+	
Stauroneis phoenicenteron (Nitzsch) Ehrenberg	SPHO	V				+	
Staurosira pinnata Ehrenberg	SPIN	**	+	+	+	+	+
Staurosira venter (Ehrenberg) H. Kobayasi	SSVE	NR	+	+	+	+	+
Staurosirella leptostauron (Ehrenberg) D.M. Williams et Round	SLEP	NR	+				
Surirella angusta Kützing	SANG	*				+	+
Surirella linearis W. Smith	SLIN	*				+	
Tabellaria flocculosa (Roth) Kützing	TFLO	**	+		+	+	+
Ulnaria acus (Kützing) Aboal	UACU	NR					+
Ulnaria ulna (Nitzsch) Compère	UULN	*				+	

Table 1

Parameter	**Lake** (mean ± SD)	**Rivers** (mean ± SD)	**Springs** (mean ± SD)
Altitude (m)	1882 ± 0	1743 ± 100	1786 ± 103
Depth (cm)	31.7 ± 24.7	23.6 ± 10.4	16.0 ± 8.5
Conductivity (µS/cm^2)	42.3 ± 1.2	30.7 ± 6.1	37.7 ± 9.3
Dissolved Oxygen (%)	61.0 ± 2.1	81.3 ± 3.9	72.6 ± 9.4
Dissolved Oxygen (mg/L)	8.0 ± 0.37	10.1 ± 0.63	8.8 ± 0.87
pH	8.0 ± 0.15	7.4 ± 0.21	7.5 ± 0.50
Temperature (°C)	4.5 ± 0.4	6.5 ± 2.0	7.8 ± 3.0
Turbidity (NTU)	5.4 ± 0.5	3.0 ± 1.9	9.7 ± 7.9
BOD_5 (mg/L)	2.15 ± 1.67	1.24 ± 0.88	1.88 ± 1.52
Ca (mg/L)	9.58 ± 2.05	3.85 ± 2.67	6.63 ± 3.00
Hardness °dH	1.34 ± 0.29	0.77 ± 0.39	1.12 ± 0.36
Mg (mg/L)	0.06 ± 0.00	1.09 ± 1.85	0.85 ± 1.65
N-NO_3 (mg/L)	0.709 ± 0.172	0.424 ± 0.115	0.462 ± 0.211
SRP (mg/L)	< 0.005	0.018 ± 0.042	0.007 ± 0.007

Table 2

VALLEY	CODE	SHANNON	EVENNESS
Pian della Casa	R10	1.67	0.45
	R11	2.7	0.75
	R12	2.4	0.61
	R13	2.63	0.69
	R15	2.4	0.58
	R17	2.6	0.67
	R18	2.31	0.62
	R20	2.5	0.56
	R23	2.37	0.52
	R24	2.3	0.62
	S14	2.08	0.45
	S19	3.06	0.66
	S16	3.06	0.62
	S21	2.51	0.61
	S22	2.61	0.63
Lourousa	R26	2.3	0.62
	R27	3.1	0.79
	S25	3.4	0.75
	S28	2.84	0.68
	S29	2.62	0.67
	S30	2.98	0.69

	L31	3.01	0.7
	L32	2.34	0.56
	L33	2.49	0.62

Table 3

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.197	0.141	0.062
Variance in species data			
Cumulative % explained	14.3	24.5	28.9
Pearson correlation, Spp-Envt	0.881	0.851	0.772
Intraset correlation for environmental variables			
ALTITUDE	0.894	-0.055	0.433
DEPTH	0.254	0.938	-0.157
SRP	-0.021	0.163	0.876
BOD5	0.181	-0.227	-0.148
HARDNESS	0.538	-0.068	0.259

Figure captions

Annex 1 Complete list of diatom taxa found in the samples. RL = Red List species (Lange-Bertalot & Steindorf, 1996). 1 = threatened with extinction; 2 = severely endangered; 3 = endangered; V = abundance decreasing; G = presumed endangered; R = extremely rare; D = data scarce; * = at present not considered threatened; ** = surely not threatened; r = taxa supposed to be rare on the basis of experience and / or the literature

Table 1 Mean (±SD) values of physico-chemical parameters analysed in the three typologies

Table 2 Shannon diversity index and evenness of diatoms in sampled sites

Table 3 Canonical Correspondence Analysis (CCA) axes summary statistics

Fig. 1 Sampling sites location. a. Location of the Maritime Alps Natural Park. b. Location of the two sampled valleys. c. Sampling sites location in Gesso della Valletta Valley. d. Sampling sites location in Lourousa. R=river; S=spring; L=lake

Figs 2-44 Micrographs of some common taxa identified in the samples; scale bar LM = 10 µm; scale bars SEM = 5 µm. **Figs 2-8** *Staurosira venter*; **Figs 9-14** *Stauroforma exiguiformis*; **Figs 15-20** *Diatoma mesodon*;

Figs 21-24 *Meridion constrictum*; **Figs 25-28** *Tabellaria flocculosa*; **Figs 29-33** *Meridion circulare*; **Figs 34-44** *Aulacoseira alpigena*

Figs 45-81 Micrographs of some common taxa identified in the samples; scale bar LM = 10 µm; scale bars SEM = 5 µm. **Figs 45-55** *Achnanthidium minutissimum*; **Figs 56-58 (59?)** *Naviculadicta schmassmannii*; **Figs 60-63** *Achnanthidium daonense*; **Figs 64-70** *Eunotia boreoalpina*; **Figs 71-78** *Eunotia minor*; **Figs 79-83** *Eunotia tenella*

Figs 84-125 Micrographs of some common taxa identified in the samples; scale bar LM = 10 µm; scale bars SEM = 5 µm. **Figs 84-99** *Nitzschia* aff. *hantzschiana*; **Figs 100-109** *Nitzschia* cf. *fonticola*; **Figs 110-119** *Nitzschia* cf. *acidoclinata*; **120-125** *Denticula tenuis*

Figs 126-152 Micrographs of some common taxa identified in the samples; scale bar LM = 10 µm. **Figs 126-128** *Encyonema lunatum*; **Figs 129-131** *Encyonopsis falaisensis*; **Figs 132-135** *Amphora inariensis*; **Figs 136-138** *Pinnularia microstauron* var. *rostrata*; **Figs 139-140** *Pinnularia borealis* var. *tenuistriata*; **Fig. 141** *Pinnularia microstauron*; **Figs 142-146** *Caloneis tenuis*; **Figs 147-149** *Pinnularia perrirorata*; **Figs 150-152** *Caloneis* sp.

Fig. 153 Classification of the sampling sites on the basis of diatom community composition using Twinspan analysis

Fig. 154 Canonical Correspondence Analysis (CCA) diagram showing diatom species and sampling sites in relation to environmental variables